MICROBIOLOGICAL PROFILE OF SEPSIS WITH SPECIAL REFERENCE TO TROPICAL SEPSIS

In partial fulfillment of the requirement for the award of

DOCTOR OF PHILOSOPHY
IN
MEDICAL MICROBIOLOGY
(FACULTY OF MEDICINE)

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AUGUST-2017

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I, **Mrs. Radhika Khara,** declare that this thesis titled, **"Microbiological Profile of Sepsis With Special Reference To Tropical Sepsis"** submitted by me for the degree **Doctor of Philosophy (Ph.D.)** in subject of **Microbiology** is the original research work carried out by me during the period from **July 2011 to August 2017**, under the guidance of **Dr. Sucheta J. Lakhani** and has not formed the basis for the award of any degree, diploma, associate ship, fellowship, titles in this or any other University or other similar institution of higher learning.

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I, **Mrs. Radhika Khara**, hereby declare that **Sumandeep Vidyapeeth** has the right to preserve, use and disseminate my thesis entitled **"Microbiological Profile of Sepsis With Special Reference to Tropical Sepsis"** in print or electronic format for Academic / Research Purpose.

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Contents

LIST OF ABBREVIATIONS

The following abbreviations appear in the thesis:

Sr. No.	Abbreviation	Full form
1.	ACCP	American College of Chest Physicians
2.	ALT	Alanine aminotransferase
3.	API	Analytical Profile Index
4.	ARDS	Acute Respiratory Distress Syndrome
5.	AST	Aspartate aminotransferase
6.	AST	Antimicrobial Susceptibility Testing
7.	ATCC	American Type Culture Collection
8.	BAL	Bronchoalveolar Lavage
9.	BHI	Brain Heart Infusion
10.	BP/vBP	Blood Pressure/Ventricular Blood Pressure
11.	CBC	Complete Blood Count
12.	CD	Cluster of differentiation (4, 8, 11, 14, 36, 45, 64 etc.)
13.	CDST	Combined Disc Synergy Test
14.	CKD	Chronic Kidney Disease
15.	CLSI	Clinical Laboratory Standards Institute
16.	CNS	Central Nervous System
17.	CO_2	Carbon dioxide
18.	*CoNS*	*Coagulase Negative Staphylococci*
19.	COPD	Chronic Obstructive Pulmonary Disease
20.	CRF	Chronic Renal Failure
21.	CRP	C-Reactive Protein
22.	CSF	Cerebrospinal Fluid
23.	DAP 12	12kDa transmembrane protein
24.	DB	Direct bilirubin
25.	DDST	Double Disc Synergy Test
26.	DHF	Dengue Hemorrhagic Fever
27.	DIC	Disseminated Intravascular Coagulation
28.	DM	Diabetes mellitus
29.	DNA	Deoxyribonucleic Acid

Sr. No.	Abbreviation	Full form
30.	DSS	Dengue Shock Syndrome
31.	EDTA	Ethylenediaminetetraacetic acid
32.	ELISA	Enzyme-linked immunosorbent assay
33.	ESBL	Extended Spectrum Beta (β) Lactamase
34.	ESR	Erythrocyte Sedimentation Rate
35.	ET	Endotracheal
36.	GB Syndrome	Guillain Barre Syndrome
37.	GCS	Glasgow Coma Scale
38.	GI/T	Gastro-intestinal/tract
39.	GMB	Glucose Methylene Blue
40.	GNB	Gram negative bacilli
41.	GPC	Gram positive cocci
42.	HMGB1	High-mobility group B1 protein
43.	HTN	Hypertension
44.	HAV	Hepatitis A Virus
45.	HBV	Hepatitis B Virus
46.	HCV	Hepatitis C Virus
47.	HEV	Hepatitis E Virus
48.	HIV	Human Immunodeficiency Virus
49.	H_2O_2	Hydrogen Peroxide
50.	H_2S	Hydrogen Sulphide
51.	IB	Indirect bilirubin
52.	ICU	Intensive Care Unit
53.	ICCU	Intensive Critical Care Unit
54.	ICR	Inducible Clindamycin Resistance
55.	IgM	Immunoglobulin M
56.	IgG	Immunoglobulin G
57.	IL	Interleukin (1,2,4,6 etc.)
58.	KOH	Potassium hydroxide
59.	SICU	Surgical Intensive Care Unit
60.	SIRS	Systemic Inflammatory Response Syndrome
61.	LFT	Liver Function Tests

Sr. No.	Abbreviation	Full form
62.	LPCB	Lactophenol cotton blue
63.	LPS	Lipopolysaccharide
64.	MALDI-TOF	Matrix Assisted Laser Desorption/Ionization Time Of Flight
65.	MBL	Metallo-beta (β)-lactamase
66.	MIC	Minimum Inhibitory Concentration
67.	MIF	Macrophage Inhibitory Factor
68.	MHC	Major Histocompatibility Complex (Class I & II)
69.	MHA	Mueller Hinton Agar
70.	MODS	Multiorgan Dysfunction Syndrome
71.	MRCoNS	Methicillin Resistant *Coagulase Negative Staphylococcus aureus*
72.	MSCoNS	Methicillin Sensitive *Coagulase Negative Staphylococcus aureus*
73.	MRSA	Methicillin Resistant *Staphylococcus aureus*
74.	MSSA	Methicillin Sensitive *Staphylococcus aureus*
75.	NAAT	Nucleic Acid Amplification Test
76.	NASBA	Nucleic Acid Sequence Based Amplification
77.	NICU	Neonatal Intensive Care Unit
78.	NO	Nitrous oxide
79.	NS	Non-structural
80.	NT	Non-tropical Infections (due to common bacterial and fungal pathogens)
81.	NF-kB	Nuclear factor kappa-B
82.	*P. falciparum*	*Plasmodium falciparum*
83.	*P. vivax*	*Plasmodium vivax*
84.	PAF	Platelet Activating Factor
85.	PAMPs	Pathogen associated molecular patterns
86.	PCR	Polymerase Chain Reaction
87.	PCT	Procalcitonin
88.	PICU	Paediatric Intensive Care Unit
89.	PMN	Polymorphonuclear Leuckocyte
90.	PRP	Pattern-recongnition-proteins

Sr. No.	Abbreviation	Full form
91.	PUO	Pyrexia of Unknown Origin
92.	qSOFA	Quick Sequential Organ Failure Assessment
93.	O_2	Oxygen
94.	RBC	Red blood cell
95.	RFT	Renal Function Tests
96.	RNA	Ribonucleic Acid
97.	SCCM	Society of Critical Care Medicine
98.	SDA	Saboraud's Dextrose Agar
99.	SDA	Strand-Displacement Amplification
100.	*spp.*	*Species*
101.	SGOT	Serum Glutamic-oxaloacetic transaminase (AST)
102.	SGPT	Serum Glutamic-pyruvic transaminase (ALT)
103.	SOFA	Sequential Organ Failure Assessment
104.	s-TREM	Soluble triggering receptor expressed on myeloid cells
105.	T	Tropical Infections (malaria, dengue, viral hepatitis, enteric fever & leptospira)
106.	TB	Total bilirubin
107.	TCR	T-cell antigen Receptor
108.	TLC	Total Leukocyte Count
109.	TLRs	Toll-like receptors
110.	TNF	Tumor Necrosis Factor
111.	TMA	Transcription-Mediated Amplification
112.	TSI	Triple Sugar Iron
113.	TSS/T	Toxic Shock Syndrome/ Toxin
114.	UI	Unidentified
115.	UTI	Urinary Tract Infection
116.	UV	Ultraviolet
117.	VAP	Ventilator Associated Pneumonia
118.	VRSA	Vancomycin Resistant *Staphylococcus aureus*
119.	WBC	White blood cell
120.	ZN	Ziehl-Neilson Stain

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Acknowledgement

"The Woods Are Lovely, Dark & Deep. And Miles To Go Before I Sleep" – Robert Frost (This is not the End, but the Beginning of a New Phase of Life with More Responsibilities)

According to the Indian tradition & culture, we offer prayers to God & ask for blessings and success at the beginning of any endeavour and at the end thank him; I too Thank God with all my heart & soul for giving me the courage to take up this task and the strength to overcome all the challenges – physical, mental, emotional; and accomplish it in the best capacity.

As rightly said "Though omnipresent, God cannot remain present all the time with everyone, He sent his angels in the form of parents to be with us in the darkest hours of life." No words can express my gratitude towards my parents and in no way can I repay what they have given me. My mother, Mrs. Sajjankunwarba Rana, always encouraged me to achieve what I wanted to achieve in life and be what I wanted to be. My father, Mr. Arun Sinh Rana, who has a great impact on my life, always taught me to stand strong for what I believed without any apprehensions and hesitations thus supported my decisions, of which this PhD is one. I also thank my other family members, who have supported me in one or the other way from time-to-time during these years. Special thanks to Mr. Hariharan Iyer for being the first person to inspire me and encourage me since my childhood.

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you were always there for me; you are very much here for me right now and will always be there for me.

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I am a microbiologist, by qualification & profession, but the value addition as a "Clinical Microbiologist" is added to my portfolio due to unrestrained support of my co-guide Dr. J. D. Lakhani. Thank you Sir for believing in me and constantly enriching my conceptual clinical approach of my topic in specific as well as the subject as a whole. Your way of indepth evaluation of my PhD task and giving me the liberty to work and express it in my own way has molded my thoughts to a great extent which is reflected in my task. Thank you so much Sir for helping me tread onto the path, your sustained support improved my way to focus clinical aspects in microbiology.

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Finally, I would like to thank the participants of my study, my patients, without whom this study would have not been possible.

ABSTRACT

Introduction:

Sepsis, a clinical condition which results in morbidity as well as mortality, happens to be very important clinical condition all throughout the world. It is also important in tropical and developing countries like India, as infective disorders are very common. Etiology and profile of sepsis may be different in our country and of the places where predominantly patients are from under privileged and rural sector. Early recognition and timely treatment may decrease mortality. Microbiologist may play a very distinct role in diagnosis and management of sepsis.

Methods:

With hypothesis that microbial profile of sepsis in our setup is different from the microbial profile of sepsis elsewhere, this study was undertaken. It was aimed at determining the microbiological profile of sepsis patients along with those having tropical sepsis. This study was conducted between September 2012 and December 2015 (study period of 3 years 3 months). These patients were selected from a tertiary care hospital which caters services to rural as well as nearby urban areas. Patients with age more than18 years and diagnosed clinically as sepsis by 1992-2001 definition {Sepsis = SIRS + Suspected/Proven Infection} were included in the study. Cultures of different samples including blood were performed for identification of the possible bacterial and fungal pathogens. Also rapid tests for malaria, dengue, leptospira and viral hepatitis were carried out. Biomarkers of sepsis like CRP and PCT were also determined. Besides these, results of LFT, RFT, CBC and ESR and other investigations needed to find out etiology, host response and management by the clinicians were noted down. Details relevant to the patients and diagnosis were obtained from the case sheets.

Results:

A total of 743 patients clinically diagnosed having sepsis were studied. Of these 743 patients, 61.50% (457/743) were males and 38.49% (286/743) were female patients. Mean age of the study group patients was 48.02 years. Moreover 55.31% (411/743) patients survived the episode of sepsis whereas 38.22% (284/743) could not survive

i.e. non-survivors and 6.46% (48/743) of the patients took discharge from the hospital against the medical advice or were referred to higher centres for further care and the outcome in these was not known. The average age of non-survivors was 50.86 years and the majority i.e. 23.05% (65/743) belonged equally to the age groups of 51years to 60 years and 61 years to 70 years. Amongst non-survivors (284/743) the most important cause of sepsis was bacterial and/or fungal infections in 44.36% (126/284) patients followed by tropical causes i.e. 30.28% (86/284) of which malaria was seen in 14.08% (40/284) patients whereas for 25.35% (72/284) non-survivors, the microbial etiology could not be identified.

Out of the selected patients, the microbial etiology could be established in 81% (599/743) patients and in 19% (144/743) the microbial etiology could not be identified. Of these 599 patients, 514 had monomicrobial etiology whereas 85 had polymicrobial etiology. Thus 47% (349/743) patients had sepsis due to bacteria and/or fungi, 34% (250/743) due to tropical infections (malaria, dengue, enteric fever, viral hepatitis and *Leptospira*) sepsis and the rest 19% (144/743) had sepsis due to unidentified microbial etiology. Out of 349 patients who had bacteria and/or fungi as cause of sepsis, 283 patients had only bacterial infections, 23 had only fungal infections and 43 had bacterial infection associated with fungal infections. Of the 250 patients who were grouped under confirmed tropical infections 122 had only malaria, 58 had only dengue, 17 had only viral hepatitis, 11 had only enteric fever and though only one patient had infection with *Leptospira spp.*, it was with bacterial + fungal isolates together in blood culture and thus was included with the rest 42 who had polymicrobial infections like malaria, dengue, viral hepatitis, enteric fever associated with other infections which includes bacterial, fungal, parasitic and viral in various combinations of two or three microbial agents.

Of the total 743 patients, 85 had polymicrobial etiology of sepsis, of which 43 were in bacterial/fungal infections group and 42 in tropical infections group. Of these 85 patients who had sepsis due to more than one organism, the causes were as follows: 43 had bacterial & fungal combined, 9 had dengue & bacterial, 8 had malaria & bacterial, 4 had malaria & dengue, 3 had malaria + HAV infections, 2 each had dengue & *Salmonella typhi*; HAV+bacteria, HEV+bacteria and 1 each of HAV+fungus, HAV+Dengue, HBV+Bacteria, malaria+*Salmonella paratyphi A*,

Salmonella typhi & *E. coli* whereas 1 each of 3 microbial etiologies together i.e. malaria + dengue+bacteria, malaria + fungus + bacteria, HCV + fungus + bacteria, HAV + fungus + bacteria, HAV + HBV + bacteria, dengue + HEV + bacteria and *Leptospira* + fungus + bacteria.

A total of 2183 samples were tested for determining the microbiological profile. Thus a total of 1136 samples were processed for bacterial cultures which also included 103 fungal cultures. Besides culture and susceptibility testing, 199 samples were tested for malaria (Malarial Antigen & PSMP); 171 for Widal (anti-O and anti-H titres); 128 for dengue (NS1, IgM, IgG); 59 for viral hepatitis i.e. HAV & HEV (IgM & IgG) and 19 for leptospira. Also the tests were carried out for biomarkers like CRP (C-reactive protein) in 183 and PCT (procalcitonin) in 288 samples. In addition, the results for CBC (Complete Blood Count), ESR (Erythrocyte Sedimentation Rate), RFT (Renal Function Test) and LFT (Liver Function Test) were also noted down.

A total of 1136 different samples were cultured for isolation and identification of bacterial & fungal isolates from 619 patients. These included 673 blood samples followed by 167 urine, 80 pus, 75 ET (Endotracheal) Tip/ET Secretion, 73 sputum, 38 CSF, 17 body fluids (Ascitic fluid, Pleural fluid, Peritoneal fluid and bronchoalveolar lavage) and 13 miscellaneous samples. Of the 1136 samples cultured growth was obtained in 57.74% (656/1136) and 42.25% (480/1136) samples were culture negative.

A total of 598 bacteria, 100 *Candida species* and 3 other fungi, making a total of 701 isolates were obtained from 656 samples showing growth. Of the 598 bacterial isolates, 67.22% (402/598) were Gram negative bacilli and 32.77% (196/598) were Gram positive bacteria. *Klebsiella spp.* (n=136) was the most common isolate followed by *Escherichia coli* (*E.coli*, n=120), *Candida species* (n=100), *Staphylococcus aureus* (n=79), *Acinetobacter species* (n=74), *Coagulase Negative Staphylococcus* (*CoNS*, n=60), *Enterococcus species* (n=49), *Pseudomonas species* (n=43), *Salmonella species* (n=15) and a also *Proteus species* (n=7), *Streptococcus pyogenes* (n=6), *Citrobacter freundii* (n=3), unidentified Gram negative bacilli (n=4), Gram positive bacilli (n=2), *Mucor species* (n=2) and *Aspergillus species* (n=1).

Amongst Gram negative bacilli least resistance was observed against Imipenem (9.71%) and Ertapenem/Meropenem (10%) and highest resistance was seen against Cefuroxime (92%) and Cefotaxime (90%) whereas amongst Gram positive cocci least resistance i.e. 0% was seen against Vancomycin followed by Linezolid (4.12%) and highest resistance was seen against Penicillin (97%) followed by Erythromycin (85%).

From blood culture, *Klebsiella spp.* (n=66) was the most common isolate, whereas from urine, *E. coli* (n=50), *S.aureus* (n=17) from pus, *Klebsiella spp.* (n=19) from ET tip/secretion, *Candida species* (n=19) from sputum, *Klebsiella spp.* (n=2) from CSF samples, *E. coli* (n=4) from body fluids and *CoNS* (n=3) from miscellaneous samples were commonly isolated.

A total of 53.98% (217/402) gram negative bacilli exhibited one or the other form of resistance mechanism. ESBL production was the major form exhibited by 44% (45/217) gram negative bacilli with *Klebsiella spp.* (n=41) being the major contributor. Amongst gram positive cocci, methicillin resistance was observed in 64.55% (51/79) *S. aureus* isolates (MRSA) and 40% (24/60) isolates of *CoNS* (MRCoNS). The erythromycin induced clindamycin resistance (ICR) was observed in 22.78% (18/79) *S. aureus* isolates and in none among the isolates of *CoNS*. Also ICR was frequently observed among MRSA isolates as compared to MSSA isolates. ICR was also observed in 18.36% (9/49) isolates of *Enterococcus species*.

Amongst fungal isolates, *Candida species* (n=100) were the frequent isolates, of which *C. albicans* (n=53) was most frequently isolated. These were more commonly isolated from blood (35%) followed by 20% from catheterized urine. The overall antifungal susceptibility pattern showed a higher resistance to most of the antifungal agents tested with 85%, 77%, 63% and 62% against Clotrimazole, Ketoconazole, Fluconazole and Itraconazole respectively whereas 80% susceptibility was seen against Amphotericin B and 80% were susceptible-dose-dependent for Nystatin. The other fungi included *Mucor spp.* (n=2) and *Aspergillus spp.* (n=1).

In patients with indwelling devices like peripheral/IV lines, urinary catheters (n=53), on mechanical ventilation (n=196), hemodialysis (n=53) and those developing

infections post-operatively (n=20) were considered as potential sources of sepsis due to hospital acquired/nosocomial infections. *Candida species* (n=20) were most frequently isolated followed by *E. coli* (n=13), *Acinetobacter spp.* (n=7), *Klebsiella spp*, (n=6), suggesting, these to be the common causes of nosocomial sepsis in our setup.

Majority of the patients (n=168) had PCT values of ≥ 2<10ng/ml. It was noted that though cultures were negative, the PCT values were raised, indicating there was an infection but the microbial etiology could not be established. In malaria patients values of >10 ng/ml were associated with severity and mortality whereas those with dengue had ≤ 0.5ng/ml. The CRP values increased with increasing values of PCT and this rise was stastically significant. Also ESR values increased with increasing PCT values.

The most common comorbid condition was Diabetes mellitus (n=193) followed by hypertension (n=188) and Tuberculosis (n=10). All these were significantly associated with mortality in patients who had these as compared to those who did not have. Also other comorbid conditions like COPD (chronic obstructive pulmonary disease), CRF/CKD (chronic renal failure/chronic kidney disease), liver cirrhosis and sickle cell anemia were observed in 15, 20, 9 and 4 patients respectively. In addition GCS<13, use of mechanical ventilation and dialysis were significantly associated with mortality in these patients. CRP, TLC and Total bilirubin along with SGOT & SGPT were significantly higher in patients with sepsis due to tropical infections as compared to those with sepsis due to bacterial/fungal infections and unidentified etiology, whereas ESR, Urea and Creatinine were higher in those with sepsis due to bacterial/fungal infections with a borderline significance.

A total of 274 patients had sepsis with no organ involvement; 97 with one organ involvement; 320 had sepsis with multiorgan involvement and 52 with septic shock. The kidney was the most frequently involved organ (n=371) followed by liver (n=357) and hemopoetic system with platelets <1, 50,000/µl (n=310). It was also noted that kidney (n=189) and liver (n=173) were frequently involved in patients with sepsis due to bacterial/fungal infections and tropical infections respectively. Thrombocytopenia was most commonly found in those with tropical infections

(n=181) followed by those with bacterial/fungal infections (n=88) and least amongst those with unidentified etiology (n=41).

Conclusion:

Sepsis, a fatal condition in one-third of middle aged adults can have bacterial, fungal, viral, parasitic or polymicrobial etiology. Host response and outcome of the sepsis patients depends upon the infecting microbial agents. Polymicrobial etiology warrants treatment with different group of drugs, one of the findings of the study, highlights the role of microbiologist in management of sepsis patients. Again tropical infections may be the cause of sepsis in one-third of patients in rural setup of a tropical country like ours and of which half of the patients will be having malaria. Thus the microbial etiology established by microscopy, culture or rapid tests, antibiotic susceptibility pattern, detection of antibiotic resistance mechanisms expressed by bacteria and procalcitonin & CRP as a markers of sepsis can facilitate in early diagnosis and timely treatment of sepsis patients.

CHAPTER 1
INTRODUCTION

1 INTRODUCTION

The region of the earth lying between the Tropic of Cancer and Tropic of Capricorn has been referred as "tropics". In the tropics, due to warm & humid climate, poor social, economic and educational status along with poor sanitation facilities provide a favourable environment for pathogens as well a their vectors and intermediate hosts to thrive. [1] *Sepsis* is derived from the Greek word meaning *putrid.* It was believed that putrefaction of a wound was caused by contact with air and that death occurred when the process of putrefaction reached the blood termed as *septicaemia*. The term septicaemia is now considered to be an outdated ambiguous term. Austrian obstetrician Semmelweis and the British surgeon Lister introduced the concept of infection as a cause of sepsis in 19th century. From then on sepsis has always been related to bacterial infection. However, as the understanding of the human immune physiology improved, the importance of the host response to infection in the pathophysiology of sepsis was recognized. [2]

In this context, sepsis was defined "as an invasion of microorganisms or their toxins into the bloodstream, together with the host response to this invasion". Thus, the pathophysiology of sepsis combines the impact of infection with the host response of generalized inflammation, which finally leads to multiorgan dysfunction and death. [2] By definition, infection is a fundamental part of the pathophysiology of sepsis. The bloodstream, the skin, and the respiratory, gastrointestinal, and genitourinary tracts are common sites of infection associated with sepsis. Most common infections that lead to sepsis are due to gram-positive or gram-negative bacteria. Gram-positive bacteria include *Staphylococcus* species, *Enterococcus* spp., and *Streptococcus* spp. Gram-negative bacteria include *Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp., *Proteus* spp. Apart from bacteria, other microorganisms like viruses, protozoa/parasites and also fungi can cause sepsis. Fungemia in immunocompromised patients & those with transplant surgery is now an increasingly common problem. Clinical symptoms of sepsis vary depending on the cause of infection, and may mimic other conditions, which can make sepsis difficult to diagnose. Thus laboratory as well as imaging diagnostic services are utilized to rule out other causes and to determine the cause of infection. [3, 4, 5]

The infections commonly seen in tropical countries like India that often lead to life-threatening multiorgan dysfunction are malaria, dengue hemorrhagic fever, typhoid, scrub typhus, viral hepatitis, influenza etc. requiring intensive care (ICU) like mechanical ventilation, vasopressors, blood and blood products, kidney replacements therapy etc. [6,7]

As inflammation was thought to be prime important pathology, the definition by ACCP/SCCM (The American College of Chest Physicians and Society of Critical Care Medicine) consensus conference in 1991, included SIRS (Systemic Inflammation Response Syndrome) criteria. Category of patients having organ dysfunction was diagnosed as severe sepsis, while septic shock, if hypotension unresponsive to proper fluid resuscitation developed. Next change came in 2001, where expansion of diagnostic criteria was made, but definition remained same. [2, 3, 8] Recent definition of 2016, which was in relation to organ dysfunction by infection which can threaten life, is identified by change in SOFA (Sequential Organ Failure Assessment) score $\geq$2 points, which is also called qSOFA (q for quick) score [9]. Even though sepsis has global importance, community awareness is lacking. With a view to decrease mortality and to improve survival "The Surviving Sepsis Campaign" emerged in 2002. [10]

Clinicians attending to the patients, who might be an intensivist, a physician or a surgeon, make a provisional diagnosis initially and frequently arrive to a differential diagnosis, which might be very broad in case of patients having fever and prescribe a number of investigations, both laboratory & imaging, to establish the diagnosis. The main role of a microbiologist is to help the clinician in decision making. It is mainly identifying the causative agents i.e. bacteria, fungi, parasites or viruses & as early as possible with the available resources, guide in the management in relation to appropriate antimicrobials and methods for infection control. Timely reporting of the microbial identification helps clinician start the right treatment on time, as time is a very critical factor in sepsis patients. [11]

In recent times the inflammatory cascade, procoagulant & coagulant factors responsible for disseminated intravascular coagulation (DIC) in patients of sepsis, other cytokines and inflammatory substances have gained a lot of importance due to

its bearing with management and early diagnosis. It is now very well known that SIRS is the final common pathway of the triggering mechanisms that lead to sepsis. But still the signal transduction pathway from infection to the complex host response differs among the microbiologic stimuli. Induction of innate immune response is triggered by specific microbial molecules (e.g. bacterial cell wall components, exotoxins, bacterial DNA, viral RNA) called pathogen associated molecular patterns (PAMPs). The presence of such patterns is sensed by recognition molecules called pattern-recognition proteins, which are able to initiate host response e.g. Toll-like receptors. These proteins may be present on the cell membrane or inside the cell. These Toll-like receptors (TLRs) represent the membrane-bound pattern-recognition proteins. Signal transductions by other microbes have not been well studied as in bacterial sepsis. In part, this may be due to the fact that induction of cytokine release differs markedly not only among these microbiologic classes but also among species. Nevertheless, the release of proinflammatory mediators has been demonstrated during infections with *Candida albicans*, *Coxsackie B virus* and *Plasmodium falciparum*. [2]

With the better understanding of human immune physiology; especially the inflammatory pathways, its mediators & other markers, newer methods for the early diagnosis of sepsis have been developed which include the detection & measurement of serum levels of novel immunological markers/biomarkers like PCT (procalcitonin), CD64, IL-6 (interleukin), sTREM-1 (soluble triggering receptor expressed on myeloid cells) in adults as well as neonates. These markers help monitor patient condition & treatment and also differentiate sepsis from non-septic inflammatory condition and severe sepsis too. [5, 6, 7] However, microbiologists' contributions in form of understanding & research would open up new vistas.

There is no clear cut distinction between sepsis and tropical sepsis but sepsis occurring due to tropical infections like malaria, dengue, typhoid fever, puerperal sepsis, may be an important cause of multiorgan failure in our set up. Moreover, infections due to these microbes fulfil the criteria for sepsis definition and studying these as a cause of sepsis may be able to give more insight and thus importance is given to this aspect in this study work.

CHAPTER 2
AIM & OBJECTIVES

2 AIM & OBJECTIVES

2.1 Aim

To study the microbiological profile, aetiology, host factors and organ involvement pattern in patients presenting with sepsis, especially those with tropical sepsis, at a rural population catering teaching hospital.

2.2 Objectives

1. To highlight the role of microbiologist in early diagnosis of sepsis, especially tropical sepsis patients, by emphasising onto proper and timely collection of appropriate clinical specimens as well as the timely and appropriate processing of the specimens yielding early & correct results.

2. To study the microbiological aetiology of sepsis including tropical sepsis and the antibiotic resistance pattern of the bacterial isolates.

3. To find out the microbiological determinants in relation with the outcome of patient.

4. To determine the utility of novel biomarkers like PCT for sepsis & their role in early detection of sepsis.

5. To study the correlation between the clinical picture/profile/presentation due to sepsis with microbiological profile e.g. Gram positive bacterial, Gram negative bacterial sepsis or sepsis due to fungi, viruses, parasites or polymicrobial aetiology.

CHAPTER 3
REVIEW OF LITERATURE

3 REVIEW OF LITERATURE

The literature is reviewed under the following headings:

- Definition
- Etiopathogenesis: Etiology & Microbiological Aspects
- Tropical Sepsis
- Host, Environmental & Epidemiological Factors & Their Relation to Sepsis
- Pathophysiology & immunology
- Laboratory Diagnosis
- Management

Definition: Review of various definitions and terms used in this research work

Infection is defined as a "pathologic process caused by the invasion of normally sterile tissue or fluid by pathogenic or potentially pathogenic microorganism." [12] The suffix '–emia' refers to the circulatory system. Bacteraemia, fungemia, viremia & parasitemia are states in which bacteria, fungi, viruses and parasites respectively, circulate through the vascular system. Signs & symptoms may be present, but are not invariable. If patient is not aware of the circulating microbes, the condition is termed "silent" or "subclinical." Bacteraemia may be transient, intermittent, or continuous, reflecting several mechanisms by which bacteria enter the bloodstream. Transient bacteraemia occurs when organisms, often members of normal flora, are introduced into the blood by minimal trauma to membranes (e.g. brushing of teeth, straining during bowel movements, or medical procedures). Intermittent bacteraemia occurs when bacteria from an infected site are periodically released into the blood from extravascular sites like abscesses, spreading cellulitis, or infected body cavities, such as empyema, peritonitis, or septic arthritis. Continuous bacteraemia usually occurs when the infection is intravascular, such as infected endothelium of vessel or heart or arteriovascular fistulas, intra-arterial catheters, or indwelling cannulas. [13]

Despite vast improvements in the understanding of the mechanisms underlying sepsis process, a little progress has been made in establishing universal agreements on the terminology definition of sepsis. One of the reasons for problems in definition is that sepsis is a very complex process; although typical signs and symptoms exist, these

may not occur in all patients, or indeed in the same patient at all times during the sepsis response. [14]

The definition of sepsis has undergone so many changes, in the last two decades, since it was first defined & published in 1992. The ACCP-SCCM (The American College of Chest Physicians and Society of Critical Care Medicine) Second International Sepsis Definition Conference was held in December 2001 as the clinicians felt that 1991 definition was less specific for defining sepsis. The 2001 International Sepsis Definitions Conference focused discussion on whether sepsis should continue to be defined as SIRS plus infection or infection plus SIRS plus signs of organ dysfunction. The participants at this conference concluded that the SIRS criteria were indeed too sensitive and non-specific and that, in preference to the SIRS criteria, an expanded list of signs and symptoms of sepsis should be used to reflect the clinical response to infection. However, there was no change suggested in the definition except for addition of the terms "suspected or proven infection" and "some of the following" for the signs and symptoms including plasma C-reactive protein (CRP) and procalcitonin (PCT) were added to the list of 1991 definition. [8, 15]

The members at the conference also concluded that no marker was 100% specific for sepsis and diagnosis must rely on the presence of a combination of clinical symptoms and signs and available markers. Thus the definition of sepsis should include suspected or proven infection with "some of the following" signs & symptoms like: **(a)** General: hyperthermia or hypothermia, tachypnea or respiratory alkalosis, oedema; **(b)** Inflammatory: increased (sometimes) decreased WBCs, increased CRP, IL-6 & PCT; **(c)** Hemodynamic alterations: tachycardia, arterial hypotension, increased cardiac output, altered skin perfusion, decreased urine output, hyperlactatemia; **(d)** at least one of the following signs of organ dysfunction: altered mental status, hypoxemia, bounding pulses, of increased alteration in renal function, hyperglycemia, thrombocytopenia, DIC (disseminated intravascular coagulation), altered liver function (hyperbilirubinemia) and intolerance to feeding (altered GI motility).Thus with same concept severe sepsis was defined as sepsis complicated by organ dysfunction. Septic shock was defined as the severe sepsis complicated by circulatory failure characterised by persistent arterial hypotension, despite adequate volume resuscitation, and unexplained by other causes. [15]

Recently, the Third International Consensus Definitions for Sepsis and Septic Shock was convened to evaluate and update definitions of sepsis and septic shock (the criteria were released in February 2016). The task force of 19 experts in sepsis pathobiology, clinical trials and epidemiology was appointed. They believed that multiple definitions and terminologies were in use for sepsis, septic shock and organ dysfunction, and were leading to discrepancies in reported incidence and mortality, suggesting the need for revisiting the definition. They believed that the previous definition included an excessive focus on inflammation, the misleading model which said that sepsis followed a continuum through severe sepsis to septic shock and inadequate specificity and sensitivity of the SIRS criteria. Thus sepsis was defined as "life-threatening organ dysfunction caused by a dsyregulated host response to infection". The organ dysfunction should be identified by bedside qSOFA (Quick Sequential Organ Failure Assessment) score of 2 points or more i.e. altered mental status, systolic blood pressure ≤100 mm Hg or respiratory rate ≥22/min. Septic shock should be defined as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality in in-hospital patients than with sepsis alone. Patients with septic shock can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65mm Hg or greater and serum lactate level greater than 2mmol/L (>18mg/dL) in the absence of hypovolemia. In the out-of-hospital, emergency department or general ward settings, adult patients with suspected infection can be rapidly identified as being more likely to have poor outcomes typical of sepsis if they have atleast 2 of the following quickSOFA (qSOFA): respiratory rate of ≥22/min, altered mentation, or systolic blood pressure of 100 mm Hg or less. The task force concluded that the term "severe sepsis" was redundant. [9]

Why is it important to differentiate Sepsis from SIRS?

The SIRS means a systemic inflammatory response that can be triggered by a variety of infectious and noninfectious conditions. Signs of systemic inflammation *can* and *do* occur in the absence of infection among patients with burns, pancreatitis, and other disease states [9]. It is important to differentiate patients with sepsis from those with SIRS, as treatments may be very different, e.g. antibiotics should not be given to all patients with SIRS, but only to those with clinical and/or bacteriological evidence of

infection: also knowing what type of organism is causing the infection certainly matters in terms of both epidemiology and selecting antibiotic therapy and also the clinical picture which might differ in both the community acquired & hospital acquired.[16]

However, in a recent review article by Long B and Koyfman A [17] on Sepsis Mimics in September 2016 suggests that the mimics are often a cause of misdiagnosis in emergency departments as most of these mimics, clinical conditions, produce symptoms and signs that meet the SIRS criteria, obscuring the clinical picture and suggest that addressing resuscitation is much more important before differentiating sepsis and mimics. Once resuscitation is started, appropriate diagnostic tests should be carried out to identify the source which includes laboratory as well as imaging studies. An empirical broad-spectrum antibiotic and fluid bolus should also be started. However, in cases where no source is found, the patients should be carefully evaluated for response with a systematic approach and treating any entity detected or diagnosed.

Phua J et al [18] report 41.5% patients with sepsis having negative cultures/no microbial etiology identified in their study on determining characteristics and outcome in patients with culture negative versus culture positive severe sepsis. They concluded that a larger proportion of patients with severe sepsis were culture negative and have low mortality, fewer comorbidities, lower severity of illness and short hospital stay duration.

Etiopathogenesis: Etiological agents & Microbiological aspects

Although there is considerable laboratory evidence that micro-organisms initiate sepsis in different ways, the clinical consequences are usually indistinguishable as all these pathways finally converge to exaggerated inflammation & MODS. Knowing whether the infecting organism is *Streptococcus pneumoniae* or *Escherichia coli* quite clearly matters when it comes to prescribing antibiotics. It has been noted that patients who are treated with antibiotics that are effective against the causative organism are more likely to do well than been treated with an ineffective agent. [2,16] Also, that it is not only the choice of antibiotics but also the speed with which they are given is crucial; delaying to start the treatment, even by as little as 1 hour, increases the chance

of a poor outcome. [16] It clearly matters whether the patient's wound abscess is caused by a methicillin-sensitive *Staphylococcus aureus* (MSSA) or a methicillin-resistant *S. aureus* (MRSA), not just because antibiotic therapy (if indicated) would be different, but it will also the clinician to decide whether to isolate the patient with methicillin-resistant *S. aureus* or at least use enhanced infection control practices.[12] *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococci*, *E. coli*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, *Proteus* species, *Salmonella* species, are the common pathogens; while *Coagulase Negative Staphylococcus (CoNS)*, *Acinetobacter spp.*, *Citrobacter spp.* and other gram negative bacilli, earlier said to be rare, have now been implicated in sepsis. Fungal causes include *Candida species*, *Mucor species* and *Aspergillus species*.[19] The recent increase in the number of medical facilities and the unrestricted access to medical care for the majority of the population in most developed countries have played a major role in the prescription of antibiotics very early in the course of most infections. In addition, the widespread use of broad spectrum antibiotics, either for therapy or for surgical prophylaxis, may be partially responsible for the increase in the relative proportions of *Coagulase Negative Staphylococci* and *Enterococci*. The proportion of *Candida species* has considerably increased in many institutions as well. Prolonged treatment with multiple antibiotics, the use of indwelling intravascular devices, and prolonged neutropenia in patients with cancer have been demonstrated to be independent risk factors for the acquisition of nosocomial candidemia.[2]

Tropical Sepsis: [20]

Besides bacterial infections as seen world over as cause of sepsis, the infections of tropical and developing countries that can cause MODS are *Plasmodium falciparum* infections, dengue hemorrhagic fever, viral hepatitis, salmonella infections, tetanus, disseminated haematogenous tuberculosis, *Bacillus typhosus* and Gram-negative infections caused by ingestion of contaminated food, fulminant amoebic infections, severe leptospiral infections and hyperinfection due to strongyloidiasis. A few basic aspects of important fulminant tropical infections that can lead to MODS have been well explained by Udwadia FE in his article as under:

The important cause of the life-threatening complications of malaria is the intra-erythrocytic asexual forms of *Plasmodium falciparum* parasite. Almost every organ in the body can be affected, due to: **(a)** Partial or complete blockage of vessels that

results into ischaemia and ultimately dysfunction of various organ systems because of cytoadherence of RBCs to the endothelial cells of the capillary and post-capillary venules in all tissues and organs of the body. The parasitised red blood cells induce the formation of protein-rich 'knobs' on the cell surface which in turn promote adherence to microvascular endothelial cells, through proteins such as thrombospondin, intracellular adhesion molecules and CD36. **(b)** A number of potentially toxic or pharmacologically active compounds (induced by parasitised RBCs) are produced and released for e.g. tumor necrosis factor alpha and interleukins, that damage endothelial cells, and lead to increased capillary permeability which in turn damages other tissue cells and produces various toxic effects. **(c)** The complement system in turn gets activated and leads to disseminated intravascular coagulaopathy. [20]

Hemmer CJ et al [21], from their in vitro study on endothelial apoptosis, suggest that there are similar mechanisms of inflammation involved in endothelial apoptosis. The secretory products of neutrophils and their ability to bind intercellular adhesin molecule-1 present on endothelial cells damage endothelial cells and cause apoptosis. Moreover, addition of ascorbic acid and ulinastatin in presence of neutrophils reduced the rate of apoptosis and addition of anti-intercellular adhesion molecule-1 antibodies augmented the rate of apoptosis.

Fulminant *Plasmodium falciparum* infection like bacterial sepsis initially results in a hyperdynamic circulatory state, characterized by tachycardia, high cardiac output, hypotension and a reduced systemic vascular resistance. In very fulminant cases, the haemodynamic state is characterized by a high systemic vascular resistance, a low exactly as in terminal bacterial sepsis. The central nervous system is most frequently involved in about > 70% of patients. This takes the form of cerebral encephalopathy, manifested by disturbed consciousness, hyperpyrexia, coma, seizures. Death may result from *Plasmodium falciparum* infection because of severe involvement and dysfunction of other organ systems with less or without involvement of the CNS. The severe acute respiratory distress syndrome (ARDS) and severe disseminated intravascular coagulopathy turn out to be lethal. The notable features with reference to *Plasmodium falciparum* infections are as follows: A high parasitic index (> 20%) in the peripheral blood often indicates a severe infection, the most fulminant forms are

sometimes characterized by few or undetectable parasites in the peripheral blood. Such patients are generally in severe shock - cold, clammy, pulseless with a barely recordable blood pressure. The parasites in these patients are sequestrated within the capillaries and post-capillary venules of different organ systems. A definite diagnosis may be impossible but an empiric treatment with quinine is mandatory when suspicion of fulminant malaria is strong in an endemic area. The next important point to note is that, in some patients, organ system dysfunction may develop and evolve even after parasites have been eradicated from the blood by specific therapy. It appears that the immunoinflammatory processes set into motion by the fulminant infection may at times be self-perpetuating even when parasites have been destroyed by treatment. The scenario in fulminant *Plasmodium falciparum* infection can be complicated further by secondary bacterial infection which adds both to morbidity and mortality [(20)]. Hoque AEMM et al [(22)] report a case of severe sepsis due to *P. falciparum* malaria with fever, jaundice, acute renal failure and aspiration pneumonia i.e. signs of multiorgan failure. Ojukwu JU et al [(23)] found malaria in 32 neonates suspected of sepsis which were blood smear positive with 4 neonates having both malaria and septicaemia. With the clinical signs of malaria being similar to bacterial neonatal sepsis they suggest a workup for malaria in malaria endemic areas with a high suspicion index.

Fulminant amoebic infections are due to single or multiple hepatic abscesses that leak into the pleural space, the lung or into the peritoneal cavity. Severe hepatic dysfunction with deep jaundice can result from multiple hepatic abscesses, particularly in malnourished individuals or in alcoholics. Multiple organ dysfunctions generally arise in those diagnosed late or in those with a poor response to specific therapy. Septic shock due to severe cardiovascular dysfunction is the usual cause of death. Ulcerative amoebic colitis can cause paracolic abscesses or a peritonitis which again leads to MODS. Associated bacterial sepsis is an important contributory factor. [(20)]

Strongyloidosis, a nematode infection, is endemic in tropical Asia, Africa and Latin America. Hyperinfection in immunocompromised individuals (such as HIV positive patients) can lead to MODS with lesions in the lungs, liver, colon and other organs. ARDS is the presenting feature. Severe lung injury can be caused directly by the

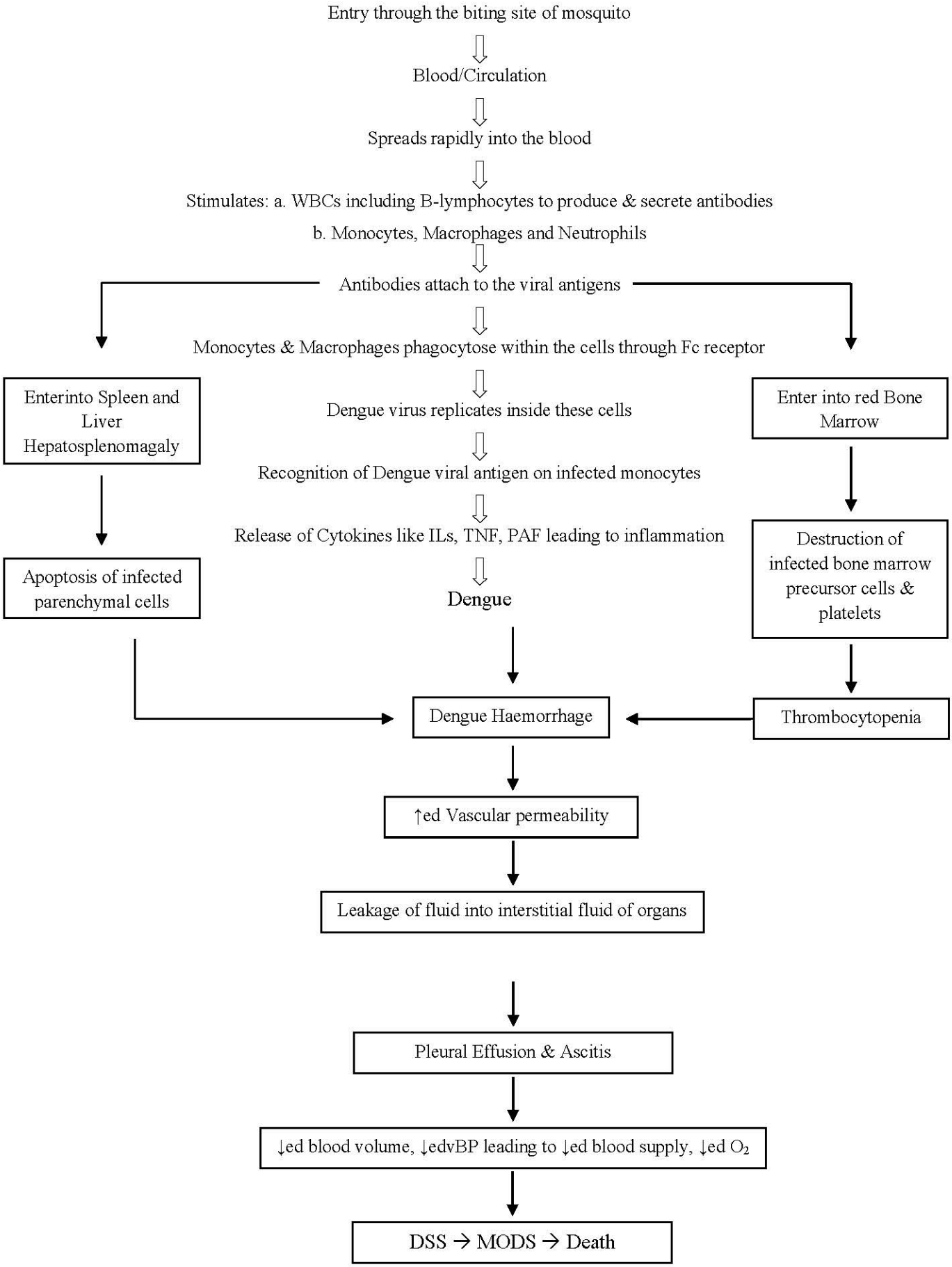
parasites, by associated bacterial sepsis, and by the inflammatory response triggered by the destruction of parasites following the use of specific therapy. Mortality is high in spite of specific treatment and support to all organ systems. [20]

Fulminant infection with *Bacillus typhosus* or *Salmonella* can cause multiple organ dysfunction. This can set in with frightening abruptness and intensity. ARDS is the most common extra-intestinal manifestation. Hepatic dysfunction, shock, renal dysfunction and even CNS dysfunction with low Glasgow coma scores can occur. Again prompt diagnosis, use of specific antibiotic therapy, ventilator support and support to all other organ systems sharply reduces mortality. Also tuberculsosis, especially, disseminated hematogenous tuberculosis and miliary tuberculosis, is an important cause of morbidity and mortality in tropical countries. [20]

CNS dysfunction, liver cell dysfunction and renal failure are commonly observed with severe leptospiral infections. In addition acute lung injury can also occur and worsen prognosis. Lung involvement can lead to an extensive intra-alveolar bleed that can cause death from overwhelming hypoxia. [20]

Haemorrhagic dengue can cause death from uncontrollable bleeding or from multiorgan dysfunction caused by bleeds into various organs of the body. The pathophysiology of Dengue, Dengue Hemorrhagic Fever and Dengue Shock Syndrome are schematically represented in the following Chart 1. (Adopted from 24)

Chart 1: Pathophysiology of Dengue, DHF and DSS [24]



Severe haemorrhagic fevers like the Ebola virus and the Hanta virus fevers are confined to specific areas in Africa which cause fulminant infections resulting in death from widespread tissue necrosis, haemorrhage and severe organ dysfunction. [20] The pathogenesis of MODS due to sepsis caused by bacterial infections observed all over the world is probably very similar to MODS caused by infections in the tropical regions. MODS results from immunoinflammatory, metabolic, vascular, neural, endocrine responses to a causative agent. These responses are dynamic, variable, interconnected and according to current concepts, perhaps non-linear in nature. They vary not only in different hosts, but probably vary at different time intervals in the same host. The responses form an ever-changing, interconnecting network or non-linear system, so that an analytical approach would fail to evaluate the emergent properties of this network or system. To summarize, the overall host response in MODS, whatever the cause, is non-coordinated, poorly directed and characterized by an exaggeration of the proinflammatory cytokines, mediators and mediator products, so that more harm than benefit is caused to the host. It appears that organs do not work in splendid isolation. They 'communicate' to each other, and cells within them do likewise at the molecular level. It is also likely that they communicate in more than one language. It is probably this communication system which preserves the balance between health and disease. There is a need to decipher this communication system in order to comprehend and find means to arrest the frequent downward spiral of severe multiple organ dysfunctions. [20]

Fevers without distinguishing symptoms in persons, who have visited tropical or subtropical areas, is an increasing health problem in most western countries. The condition may be caused by several different microbes, but among cases diagnosed in Norway five infections dominate: *P.falciparum* malaria, *P. vivax* malaria, typhoid fever, paratyphoid fever and dengue fever. [25]

As the infections, commonly seen in ‘tropics,’ are not seen in the developed countries, the people travelling from such places to ‘tropics’ are said to have “imported fever” and are screened for infections which they could have acquired during their travel to tropical countries. [26] With the same concern Johnston V, et al have proposed the recommendations for a pragmatic approach to the initial management of fever in

returned travellers, based on evidence where it is available & on consensus of expert opinion where it is not. [27]

Thus the studies do make it clear that sepsis & its causes in tropical countries are different from those seen in the western countries. Hence, carrying out such a study in a tropical country like ours, would lead to a better understanding of the causes, course, laboratory diagnostic methods & management of sepsis.

Host, Environmental & Epidemiological Factors And Their Relation To Sepsis:

The environmental and genetic factors associated with reduced immune function are the predisposing factors to the development of sepsis & septic shock. These factors include age (prematurity, neonate, age<1year, and advanced age), cancer and immunosuppressive chemotherapeutic agents, transplantation, primary immunodeficiency disorders (e.g. hypogammaglobulinemia, chronic granulomatous disease), acquired immunodeficiency disorders (neutropenia, lymphocytopenia, monocyte deactivation), malnutrition, and also the extended hospitalization, surgery, prolonged use of invasive catheters, ventilators (>48 hours) and also the factors associated with the lifestyle (e.g. alcoholism) may affect the course of septic shock.[2] The incidence and mortality of sepsis and severe sepsis are seasonal and consistently highest during the winter, predominantly related to respiratory sepsis. Seasonal changes in sepsis incidence vary according to geographic region. The mechanisms underlying these differences require further investigation. [28] The results of one of the studies carried out in mice suggest that seasonal changes of the host's hypothalamus-pituitary-adrenal axis response influence the risk of infection and the susceptibility to stress, which interferes with the outcome after infection. [29]

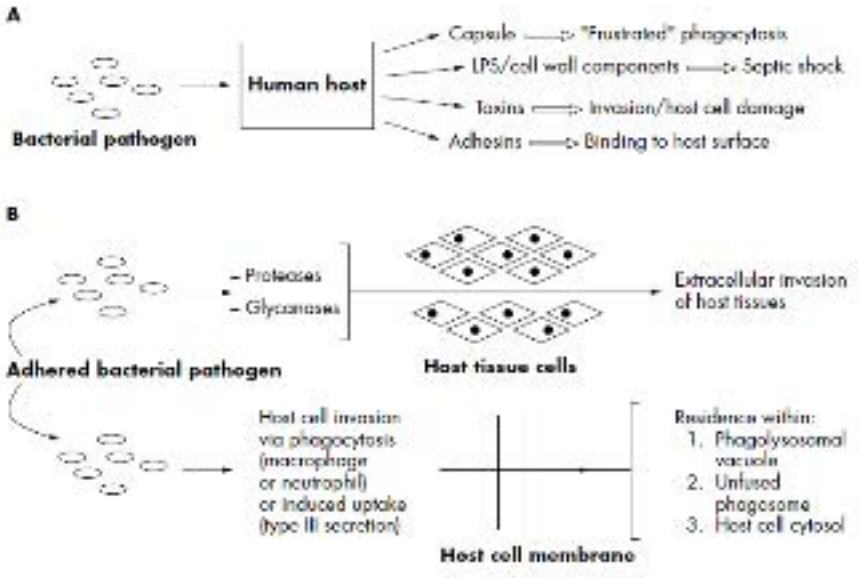
Pathophysiology & Immunology:

Though there have been modifications in the definition of sepsis in the last two decades except the 2016 definition [9], sepsis represents the body's systemic response to severe infection, and thus true sepsis can only be present if there is an associated infection. The normal host response to infection is a complex process that serves to localize and control bacterial invasion and to initiate repair of injured tissue. This inflammatory process is normally accompanied by activation of circulating and fixed phagocytic cells and by generation of proinflammatory and anti-inflammatory

mediators. [19] The concept that mortality in sepsis is due to an uncontrolled hyperinflammatory response of the host has recently been questioned. It is now widely thought that the host response to sepsis involves many, concomitant, integrated, and often antagonistic processes that involve both exaggerated inflammation and immune suppression. Several novel mediators and pathways have been shown to play a part. Moreover, evidence is accumulating that microbial virulence and bacterial load contribute to the host response and the outcome of severe infections. A complex and dynamic interaction exists between pathogens and host immune-defence mechanisms during the course of invasive infection. Some pathogens have acquired the capacity to communicate with each other and sense the host's vulnerabilities. Bidirectional signals are detectable at the critical interface between the host and microbial invaders. The outcome of this interaction determines the fate of the host at the outset of the septic process. A formidable array of innate and acquired immune defences must be breached if a pathogen is to successfully disseminate and cause severe sepsis and septic shock. [20]

Pathogenic bacteria utilise a number of mechanisms to cause disease in human hosts (**Figure 1**). Bacterial pathogens express a wide range of molecules that bind host cell targets to facilitate a variety of different host responses. The molecular strategies used by bacteria to interact with the host can be unique to specific pathogens or conserved across several different species. [31]

Figure 1: An overview of bacterial mechanisms of pathogenicity [31]



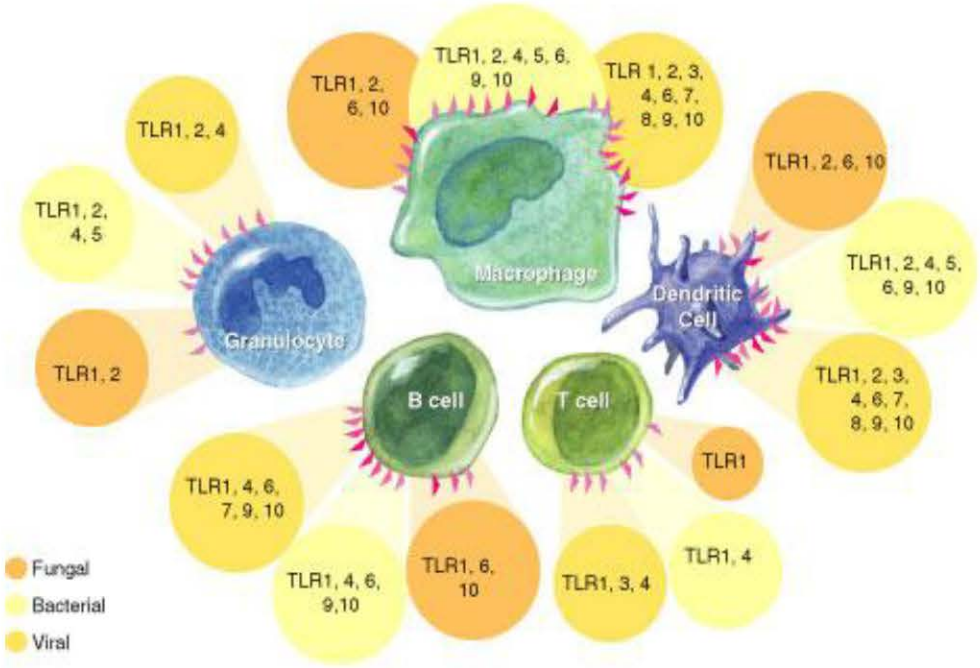
(A) Upon encountering a human host, the bacterial pathogen may illicit several host responses and uses a variety of mechanisms to evade human host defences. The bacterial components that interact with the host include: (a) Capsules that act to frustrate phagocytosis and protect the pathogen from macrophage and neutrophil engulfment, (b) LPS and cell wall components which can cause septic shock, (c) toxins that can serve to damage host cells and aid invasion, and (d) adhesins which facilitate binding of the pathogen to the host surfaces. The degree to which these various mechanisms play a part in the pathogenesis of an infection depends on the bacterial species or strain, the site of pathogen entry, the immune status, and other similar factors. (B) Once adhered to a host surface, a bacterial pathogen may further invade host tissues. Pathogens may burrow further into a tissue by expressing & secreting proteases and glycanases that digest host extracellular matrix proteins and polysaccharides. In addition pathogen may also invade the host tissue cells and gain access to the intracellular environment. This can be facilitated by the natural phagocytosis mechanisms of macrophages & neutrophils or by induced uptake where the pathogen signals the host cell to engulf adhered bacteria. A common strategy for pathogens to induce uptake is the use of a type III secretion system which injects bacterial signalling proteins into the host cell. Within the host cell, the pathogen may reside within a phagolysome (a phagosome which has fused with a lysosome), a phagosome which has not fused with a lysosome, or within the host cell cytosol.[31]

Immune Response in Gram Negative Bacteraemia:

In Gram negative bacteraemia, initiation of the immune response is mediated primarily by lipopolysaccharide (LPS), a bacterial cell wall product. In plasma, LPS is bound to the receptor CD14, which is located on several cell membranes, especially on monocytes. A soluble form of CD14 interacts with CD14-negative cells (e.g. dendritic cells). However, CD14 alone cannot explain the actions of LPS, because CD14 does not have an intracellular tail. Another binding site of LPS is the transmembranous (toll-like receptor) TLR4, which exists in combination with the accessory protein MD2. [2] Human Toll-like receptors, like their homologues in insects and other mammalian species, are type I transmembrane proteins with an extracellular leucine rich repeat domain and an intracellular domain homologous to the interleukin 1 receptor. Each of the TLRs is expressed on a different subset of

leukocytes and each of the TLRs detects different subsets of pathogens allowing vigilant surveillance by the immune system [32] **(Figure 2 adopted from [32])**.

Figure 2: Expression patterns of TLRs on leukocyte and PAMP specificities [32]



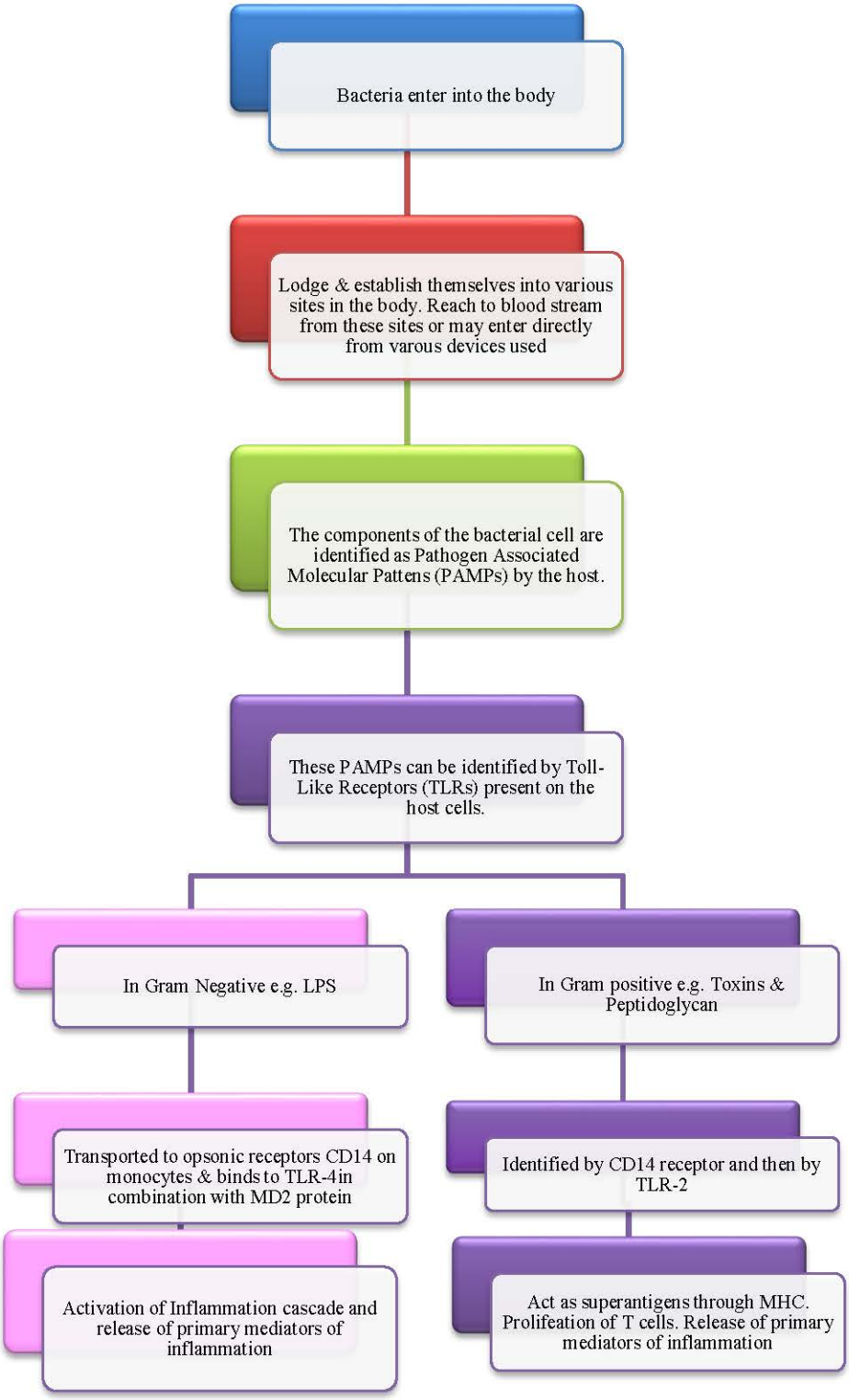
The binding of LPS to CD14 and TLR4 induces, via other molecules, activation of nuclear factor kappa-B (NF-kB). Activated NF-kB migrates into the nucleus and activates gene promoter, which results in the transcription of cytokines and other proinflammatory mediators **(Chart 2)**. In monocytes LPS also induces cytokine transcription via the triggering receptor expressed on myeloid cells-1 and the myeloid DAP12- associated lectin. Intracellular pattern-recognition proteins in monocytes for LPS have recently been identified as another pathway of cytokine expression, such as nucleotide-binding of oligomerization domain 1 and 2 as binding sites.[2]

Gram Positive Bacteria & Role of Toxins In Sepsis:

Whereas, until the early 1980s, Gram-negative bacteria were the predominant organisms that caused sepsis, the incidence of Gram-positive sepsis has steadily increased. In a large survey done in 2000 in the USA, Gram-positive bacteria accounted for 52.1% of sepsis cases, Gram-negative bacteria 37.6%, polymicrobial infections 4.7%, anaerobes 1.0%, and fungi 4.6%; the greatest relative changes were

seen in the incidence of Gram-positive and fungal infections.[30] Gram positive organisms can cause sepsis by at least two mechanisms: by producing exotoxins that act as superantigens and by components of their cell walls stimulating immune cells. Superantigens are molecules that bind to MHC class II molecules of antigen presenting cells and to Vβ chains of T cell receptors. In doing so, they activate large numbers of T cells to produce massive amounts of pro-inflammatory cytokines. Staphylococcal enterotoxins, toxic shock syndrome toxin-1, and streptococcal pyrogenic exotoxins are examples of bacterial superantigens. [33] The initial recognition of gram positive bacteria involves the CD14 receptor. TLR-2 has been identified as a pattern-recognition protein for gram positive bacteria. The platelet activating factor receptor also plays a role of signal transduction in some gram positive bacteria such as *Streptococcus pneumoniae*. Both CD14 and platelet activating factor receptor are capable of initiating cytokine production via the NF-kB pathway. Some exotoxins cause a special type of septic shock called the toxic shock syndrome (TSS). TSS may be caused by the *Staphylococcus aureus*, or streptococcal pyrogenic exotoxins. These toxins deploy their effects via the T-cell antigen receptor (TCR). The T cell activation causes release of several cytokines, such as interferon-gamma, interleukin (IL)-2, and tumour necrosis factor (TNF) from T cells as well as IL-1 and TNF from macrophages. **(Chart 2)** Thus, the presence of superantigens ends in release of cytokines, similar to Gram-negative sepsis. [2]

Chart 2: Immune Response in Gram Negative & Gram Positive Bacterial Infections [2]



Inflammatory Pathways & Mediators of Inflammation:

The primary mediators induce the release of several secondary mediators that amplify the signal of inflammation. An important step in signal amplification is the activation of complement system. The complement system may be stimulated by bacterial surface sugars and endotoxin. The complement fragment C5a, a cleavage product of the complement cascade, is a strong chemo-attractant. Another mediator that amplifies the immune response is the macrophage migration inhibitory factor, which is produced by T cells, macrophages, and pituitary cells in response to an infectious stimulus. About 24 hours after the initiation of sepsis, levels of high-mobility group B1protein (HMGB-1) increase and appear to play a role in endotoxin-related sepsis. High-mobility group B1 protein is a nuclear binding protein that, among other things, is capable of activating NF-κB. As a rather late mediator in sepsis, it is produced by macrophages and neutrophils and stimulates other phagocytic cells. Normally, the inflammatory process is well balanced and is necessary for the host to overcome the infectious impact. However, under certain conditions, the amplification process of the inflammation is not limited to the site of infection and becomes generalized. This phenomenon is known as systemic inflammatory response syndrome (SIRS). SIRS is not restricted to infectious stimuli; it is present in a variety of other conditions, such as pancreatitis, burns, multiple traumas, and in patients undergoing heart surgery with cardiopulmonary bypass. [(2)]

Neutrophil (PMN) CD64 is one of the many activation-related antigenic changes manifested by neutrophils during the normal pathophysiological acute inflammatory or innate immune response. PMN expression of CD64 is up-regulated under the influence of inflammatory related cytokines such as interleukin 12 (IL-12), interferon gamma (IFN-γ) and granulocyte colony stimulating factor (G-CSF). The up-regulation of CD64 in response to infection is only one of many changes related to neutrophil activation that have been elucidated in the last few decades. However, PMN CD64 expression differs from the parallel changes of increased CD45RA, CD66b and CD11b/18 expression in that the normal baseline PMN CD64 expression is negligible, and is stable in *in vitro* stored blood samples. Thus CD64 appears uniquely suited as a surrogate marker of neutrophil activation or systemic acute inflammatory response as its expression starts from less than 2,000 sites per cell and

becomes up-regulated in a graded fashion depending upon the intensity of stimulation by cytokines. [34]

Anti-Inflammatory Response:

The immune response in sepsis does not involve only pro-inflammatory mediators. Pro-inflammatory mediators are counteracted by anti-inflammatory molecules such as IL-4 and IL-10, because CD4 T cells can switch from the production of inflammatory cytokines (type 1 helper T cells) to the production of anti-inflammatory cytokines (type 2 helper T cells). [2]

SIRS, Sepsis & Coagulation Factors:

Sepsis is accompanied by activation of the coagulation cascade via the tissue factor and factor VII-dependent generation of thrombin. Tissue factor is capable of activating factor VII, which activates further steps in the extrinsic coagulation pathway. Activate factor VI of the extrinsic pathway activates factor XI of the intrinsic pathway (cross-talk), and thrombin is capable of activating factors VIII & XI of the intrinsic pathway (feedback). Thus, whereas extrinsic pathway initiates coagulation, the intrinsic pathway serves as an amplifier of coagulation. Under normal conditions, the vascular luminal surface has anticoagulant properties. However, several cytokines as well as acute-phase proteins such as C-reactive protein induce tissue factor expression on monocytes, neutrophils and endothelial cells. This induces intravascular thrombin formation initiated by the extrinsic pathway. Further thrombin formation is maintained by the intrinsic pathway due to cross-talk & feedback. Because this process is not restricted to a local area, it is called disseminated intravascular coagulation. [2]

MODS, SIRS/Sepsis: [2]

The embolization of microvesels by continuous and latent coagulation is believed to be more important, because it may be relevant in development of microcirculatory dysfunction and organ failure. Multiorgan dysfunction is the parallel or sequential failure of atleast two organs. It is a frequent complication of sepsis. Clinically, multiorgan dysfunction is termed the multiorgan dysfunction syndrome (MODS). MODS can involve any organ or a critically ill patient, even a remote organ that was not originally affected by the underlying disease. The development of MODS

significantly contributes to ICU mortality. Scoring systems such as Sequential Organ Failure Assessment (SOFA) or the Multiorgan Dysfunction Score, which assess the severity of MODS, correlate well with mortality.

Some major pathways and components of the pathophysiology of MODS are depicted in the following **Chart 3** [35], **Figure 3** [2] and Table 1 [2]

Chart 3: Major Pathways and components of Pathophysiology of MODS

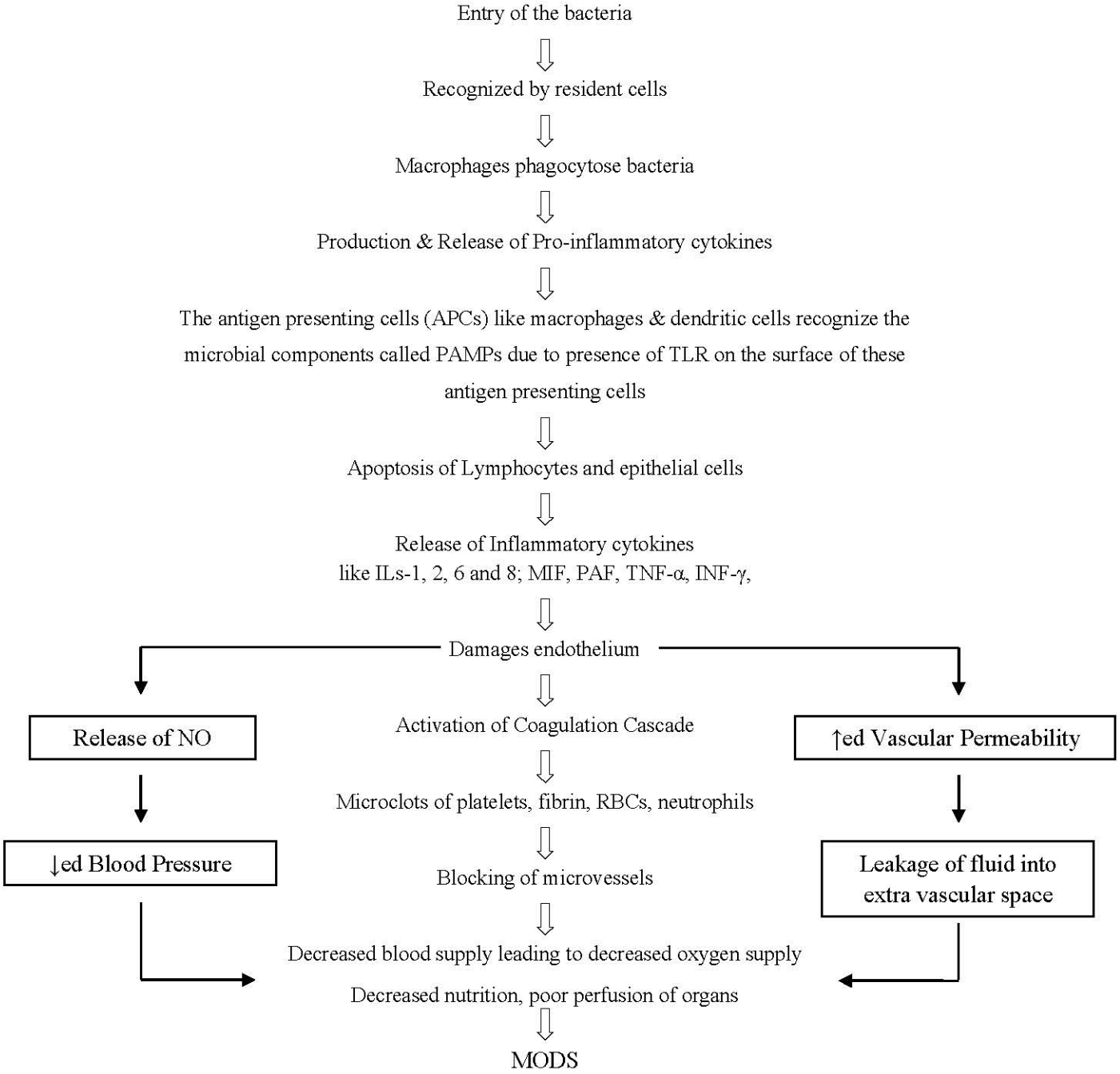
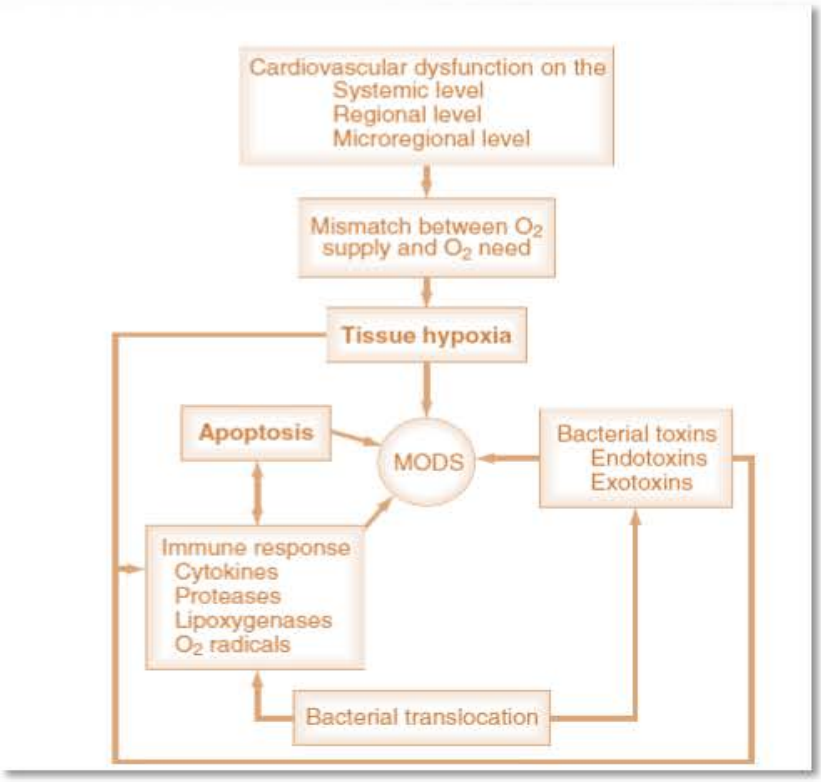


Figure 3: Pathophysiology of MODS [2]



The development of MODS includes a complicated network of inter- and intracellular actions. Because MODS can involve a variety of pathophysiologic changes, different concepts of the pathophysiology of MODS have generated (**Refer Table: 1**) [2]

Table 1: Conceptual models of multiple organ dysfunctions [2]

Pathologic Process	Manifestation
Uncontrolled infection	Persistent infection, nosocomial acquired infection, endotoxemia
Systemic inflammation	Cytokinemia (IL-6, IL-8, TNF), leukocytosis, increased capillary permeability
Immune paralysis	Nosocomial infection, increased anti-inflammatory cytokine levels (IL-10), decreased HLA-DR expression; shift from type 1 to type 2 helper T cells
Tissue hypoxia	Increased lactate, low central venous O_2 saturation
Microvascular coagulopathy	Increased procoagulant activity, decreased anticoagulant acitivity (anti-thrombin III ↓, protein C ↓), high levels of fibrin derivatives, ↑ed von Willebrand's factor, soluble thrombbomodulin.
Endothelial dysfunction	↑ed capillary permeability

Pathologic Process	Manifestation
Dysregulated apoptosis	↑ed epithelial and lymphoid apoptosis, decreased neutrophil apoptosis
Gut-liver axis	↑ed infection with gut organisms, endotoxemia, Kupffer cell activation

Laboratory diagnosis:

Sepsis is primarily a clinical diagnosis, supported by laboratory investigations and imaging. Culture of specimens from a sterile site is the gold standard microbiological investigation and the key to successful diagnosis. [36] The diagnostic cycle starts with clinician attending the patient and investigations she/he prescribes. It starts with appropriate sample with proper collection followed by gross examination, microscopy, culture & antibiotic susceptibility testing, serological investigations for antigen or antibody detection and finally molecular biology techniques by detecting DNA or RNA or gene sequences of relevance. Finally the report is given to the clinician, which helps in deciding the exact treatment, or may be stopping or deescalating the drug, etc. Even before the organism grows in culture, identified and reported; a great deal of information that can help in early provisional diagnosis can be obtained from microscopic examination of the clinical specimens, whether unstained (for e.g. CSF, urine, body fluids and stool) or stained (sputum, peripheral smears of blood. It not only unveils the mystery regarding a particular microbial type i.e. bacteria, fungi, viruses or parasites, but also helps to determine (a) whether the sample is representative of the site of infection for e.g. sputum; (b) number & percentage of neutrophils, indicating the magnitude and type of inflammatory response as well as validating the quality of specimen. [13] Blood for culture is the most important specimen submitted to the microbiology laboratory for examination. It helps in specific etiological diagnosis. The presence of living microorganisms in blood suggests active and possibly spreading infection of tissues. [19] Blood culture plays an integral role in the evaluation of sepsis. As bacteraemia is often associated with fever, clinicians are encouraged to obtain blood samples for culture from febrile patients. The indications for blood culture are broad and ill-defined. The indications of blood culture include: sepsis (with clinical features), suspicion of infective endocarditis, pyrexia of unknown origin (PUO), unexplained leukocytosis or leucopenia, systemic and localised infections including suspected meningitis,

osteomyelitis, septic arthritis, acute untreated bacterial pneumonia or other possible bacterial infection. The goal of culturing blood is to determine whether a pathogenic microorganism(s) is responsible for the patient's clinical presentation. Media used in blood culture bottles support the growth of most medically important bacteria and fungi, including anaerobes, which grow adequately in the aerobic blood culture bottle. [36] The critical factors that should be considered for blood culture include the type of collection, number and timing of blood cultures, the volume of blood to be cultured, the amount and composition of the culture medium, also when and how frequently to subculture. Every precaution should be taken to minimize the contamination of blood cultures, especially by coagulase negative staphylococci (*CoNS*), because these species are increasingly causes of true bacteraemia but are also indigenous flora of the skin. A variety of techniques have been proposed for determining whether an isolate of *CoNS* represents contamination but unfortunately none works well. [13] In a recent study by Singh S et al [37] from North India, reported 2.8% isolates being *CoNS* from blood stream infections with a prevalence of 1.4/1000 blood cultures and accounted for 61.5% of the total staphylococcal isolates. They report 57.6% prevalence of *MRCoNS* showing a high level of resistance to non-beta lactam antibiotics as compared to *MSCoNS* (methicillin sensitive *Coagulase Negative Staphylococcus*). They also reported prevalence of 49.2% multidrug resistance of the *CoNS* isolates. They suggest molecular typing and establishing a correlation with antibiotic resistance to identify these organisms as a cause of infections.

The number of specimens collected is less important than total volume of blood cultured. Most of the studies report an increase in yield by 30% by increasing the volume of blood cultured. Traditional guidelines were that multiple blood cultures must be drawn at different times if intravascular infection and continuous bacteraemia was suspected but spacing time between blood cultures has no additional advantage. Thus 2-3 sets, each containing 2 bottles can be collected at one time. [13]
Blood cultures should be drawn preferably before starting the antimicrobials. However, if the treatment has been started, cultures should still be obtained, but negative results must be interpreted with circumspection. [13]

Most commercially available blood culture media contain the anticoagulant SPS (sodium polyanetholsulfonate) in varying concentrations from 0.025% to 0.05%

because certain bacteria do not survive in the clot, where phagocytosis by neutrophils and macrophages remains active. In addition to anticoagulant properties SPS also inactivates neutrophils and certain antibiotics. But it may inhibit the growth of certain bacteria like *Peptostreptococcus anaerobius*, *Neisseria gonorrhoea* and *N. meningitidis*. Many currently available blood culture bottles incorporate synthetic antibiotic-removing resins which have greatly improved the recovery of pathogens. Traditionally, blood culture sets consist of a bottle designed to recover aerobic and anaerobic bacteria. But it has been suggested that anaerobic bottles be limited to situations in which anaerobes might be expected. Moreover, *Candida species* are recovered effectively in systems designed for bacteria. [13]

Automated and Computerized Blood Culture Systems: The automated blood culture systems employ equipment that automatically detects an early sign of bacterial growth in a special blood culture bottle and media can be chosen on the basis of gram reaction and morphology of microbes e.g. (1) Roche Septicheck: A commercial system containing a clear plastic screw-capped bottle with an internal paddle or dipslide holding sterile medium. This assembly is placed on the blood culture bottle in which patient's blood has been collected. The bottle is inverted and the whole assembly is incubated. (2) Signal system: this uses pressure of gas produced by bacterial action to drive liquid medium into an upper chamber and signal growth. But it does not detect the non-gas producing bacteria. (3) BacT/Alert detects CO_2 production by colorimetric method etc. [38] (4) BACTEC system consists of a self-contained incubator, agitator, and detection system that detects CO_2 produced by bacterial metabolism through fluorescence [13] and (5) Vitek from bioMérieux. [38] API & Vitek systems contain dried substrates in cupules/cards for identification. The results are obtained in the form of codes or as complete result on the reading device. [39] Besides blood culture, cultures of the intravenous catheter tips; swabs from infected burns, wounds or abscesses; CSF in meningitis; urine in UTI; sputum in respiratory tract infections and stool in intestinal infections.[19]

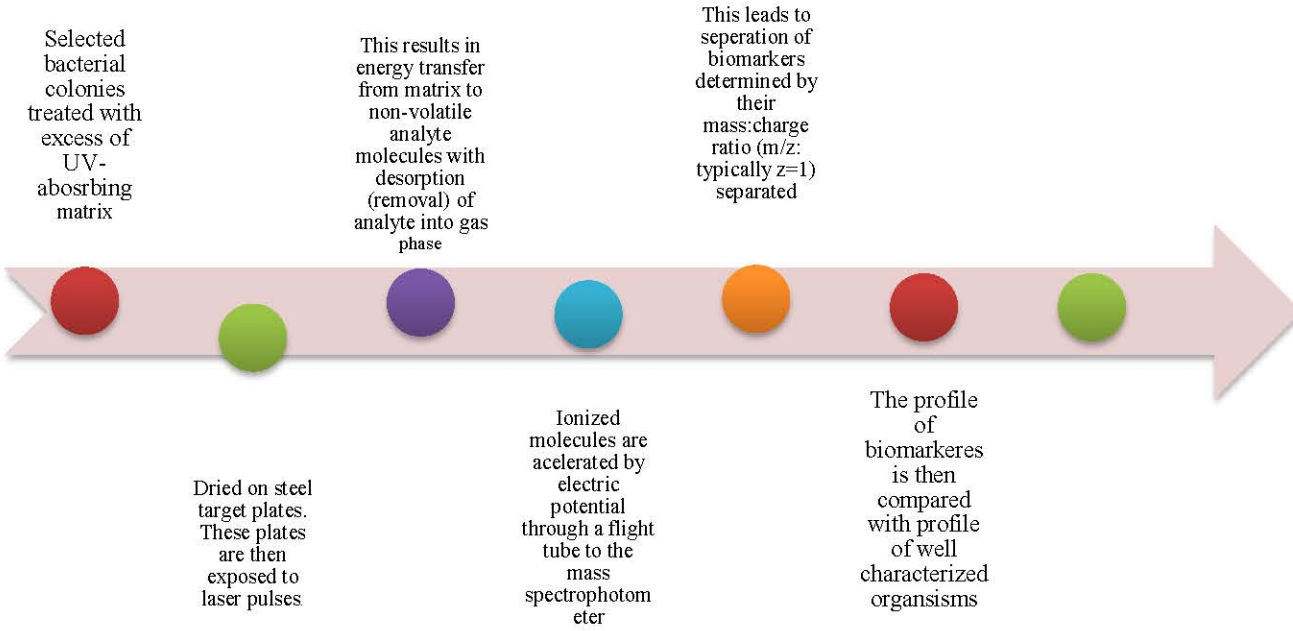
In addition to culture, through conventional, semi-automated or a completely automated system, or may be when it is negative, the other methods like Nucleic Acid Amplification tests (NAAT), PCR ((Polymerase Chain Reaction) & MALDI-TOF (Matrix Assisted Laser Desorption/Ionization Time of Flight) may be useful in early

as well as specific diagnosis by identifying the microbial etiology responsible for the condition.

Polymerase chain reaction (PCR), first described by Kary Mullis in 1983, was a great transformation in the way the diagnostic laboratories were identifying the microbes. Its main objective was to amplify a specific portion of DNA which was useful for identification of microbial etiology. It helps in the identification of those organisms which cannot be cultured as well as those which are difficult to culture. The initial methods were much demanding on time and labour. However, with the availability of semi-automated and automated PCR, it made PCR easier to use and since then has been used world over for identification of an array of pathogens. The modification in the form of Reverse-transcriptase PCR allowed researchers as well as laboratories to identify/detect RNA virsues. Thus several commercial diagnostic kits for identification of microbial pathogens are available. The other non-PCR methods are also available which includes NASBA (Nucleic Acid Sequence Based Amplification), transcription-mediated amplification (TMA) and strand-displacement amplification (SDA). (Koneman) [13]

Murray PR [40] in his review article quotes the study of Cain et al, reported in 1994, suggesting MALDI-TOF to be useful in differentiating bacterial species by analysing protein profiles from disrupted cells which was almost a decade after the introduction of PCR. However, it took a few more years to be finally introduced to the diagnostic laboratories from research laboratories as the newly found method of identification of microbes. It has completely revolutionized the well established methods of diagnosis like biochemical testing, the complex algorithms of identification and even the gene sequencing techniques like PCR or NAAT used in the most of Clinical Microbiology labs. MALDI-TOF requires preanalysis preparation of the samples and schematic presentation of its working is explained in the diagram below:

Figure 4: Working of MALDI-TOF [40]



It helps in identification of almost all bacteria to the genus as well as species level; yeasts especially of the *Candida species* and also *Mycobacteria spp.* alongwith other acid-fast organisms in 90 minutes, which is quite less than days & weeks needed for its culture & reporting. Singhal N et al [41] in their review article have mentioned its use in identification of a variety of bacteria causing infections like UTI, meningitis, respiratory infections and diarrhoea from various samples like urine, body fluids and blood. It has also been proved to be useful in identification of microbes from food, water and environment as well as those used in bioterrorism. Besides its use in identification of the microbes from various samples, it has also proved to be useful in typing of microbial strains as well as antimicrobial susceptibility testing especially the resistance patterns expressed by bacteria. [40, 41]

A number of adjunctive tests, including measurements of serum interleukin-6 (IL-6) IL-8, procalcitonin and CRP levels, have been studied for their ability to predict sepsis with clinical signs and symptoms of infection. Although serum cytokine levels may rise 12 to 48 h prior to the onset of sepsis symptoms and have been found to be highly predictive of neonatal sepsis, cytokine testing is not routinely performed in most clinical microbiology or biochemistry laboratories. Serial measurements of CRP levels have proved to have the best discriminatory value for predicting septicemia

among the tests currently available in many hospital laboratories, and the ability to produce CRP appears to be unaffected by gestational age. [5, 42] The other markers like CD64 [34, 43] and s-TREM-1(soluble triggering receptor expressed on myeloid cells) [44] too have been tested for their ability to diagnose sepsis early and have been appreciated by authors in the literature.

PCT (Procalcitonin) is a 116 amino acid protein with a sequence identical to that of the prohormone calcitonin (32 amino acids). PCT can be produced by several cell types and many organs in response to pro-inflammatory stimuli, in particular by bacterial products. The exact biological role of PCT is largely unknown; however, the experimental studies suggest that PCT may play a pathogenic role in sepsis. The protein carries leukocyte chemo-attractant and modulates the production of NO (nitrous oxide) by endothelial cells. In healthy individuals, PCT concentrations are found to be below 0.05ng/ml. Viral infections, bacterial colonization, localized infections, allergic disorders, autoimmune diseases, and transplant rejection do not usually induce a significant PCT response (values<0.5 ng/ml). PCT values between 0.5 ng/ml and 2 ng/ml represent a grey zone with uncertainity of diagnosis of sepsis. [14, 45]

Management

Though the role of microbiologists in management of sepsis case is indirect, their role is very much important. The further research in management of SIRS/sepsis may depend upon active involvement of microbiologist, as new direction in immune mechanisms, cytokine activation, diagnosis and newer drug modalities other than antibiotics would come under the domain of a primary researcher like a microbiologist.

As MODS leads to mortality & morbidity, not only managing the infecting agent is important, the other aspects like coagulation factors, various cytokines and inflammatory process leading to development of SIRS and MODS are important for research in newer management strategy.

Early diagnosis is very important for early management and prevention of multiorgan failure. The concept of 'golden hour' and 'silver day' is coming up. Management approach can be divided broadly into (1) Identification and control of source of infection and (2) resuscitation. [46]

Thus for identification of focus of infection the relevant cultures should be taken including blood cultures before administration of antibiotics. The two main components of infection control include removal of an infected focus and appropriate antimicrobial therapy. It is important to identify any focus and remove it, if necessary, even with surgical intervention. The focus of initial search should focus on the lungs, abdomen, urine, wounds and catheters. Before antibiotics are started, all appropriate cultures should be taken. One should bear in mind that a delay in initiation of antimicrobial therapy may lead to worse outcomes. Recent guidelines suggest an intravenous antibiotic therapy to be started as early as possible and within the first hour of recognition of septic shock. The empiric antibiotics with a spectrum covering any likely infectious agents as determined by the likely source(s) in that patient and local patterns of microorganism prevalence and antimicrobial resistance should be started. The use of corticosteroid in patients with sepsis is a debatable subject, as the adverse effects of these agents are well known, including neuromyopathy. [46, 47]

CHAPTER 4
MATERIALS
&
METHODS

4 MATERIAL & METHODS

This prospective study was carried out at the Microbiology Department of SBKS Medical Institute & Research Centre affiliated to Sumandeep Vidyapeeth. Through Clinical Microbiology Laboratory, the department caters microbiological services to the patients coming to Dhiraj General Hospital, which is a 1100 bedded tertiary care, multispecialty teaching hospital having general wards, special wards, casualty, state in art 28 bedded ICU and ICCU, PICU (6 bedded), NICU (12 bedded) as well as ICUs for other specialties. It also has 19 OTs, 6 bedded SICU as well as a 20 bedded postoperative surgery recovery room. This teaching hospital caters services to urban as well as rural patient population of Vadodara district and nearby districts as well as the neighbouring states like Madhya Pradesh, Maharashtra and Rajasthan. This study was carried out after taking the approval from the Institutional Ethical Committee (Ref: SVIEC/ON/MEDI/RP/1293)

4.1 Inclusion Criteria

Adult patients with age ≥18 years with:

Two or more of the following conditions along with a proven or suspected microbial aetiology: [9, 15]

1. Fever (oral temperature >38°C/100.4^{0}F) or hypothermia (<36°C/96.8^{0}F);
2. Tachypnea (>20 breaths/min);
3. Tachycardia (heart rate >90 beats/min);
4. Leukocytosis (>12,000/mm^3) or leukopenia (<4,000/mm^3)

4.2 Exclusion Criteria:

1. Patients with age <18 years
2. Patients NOT having 2 or more signs of the following: tachycardia, tachypnea, leukocytosis or fever/hypothermia

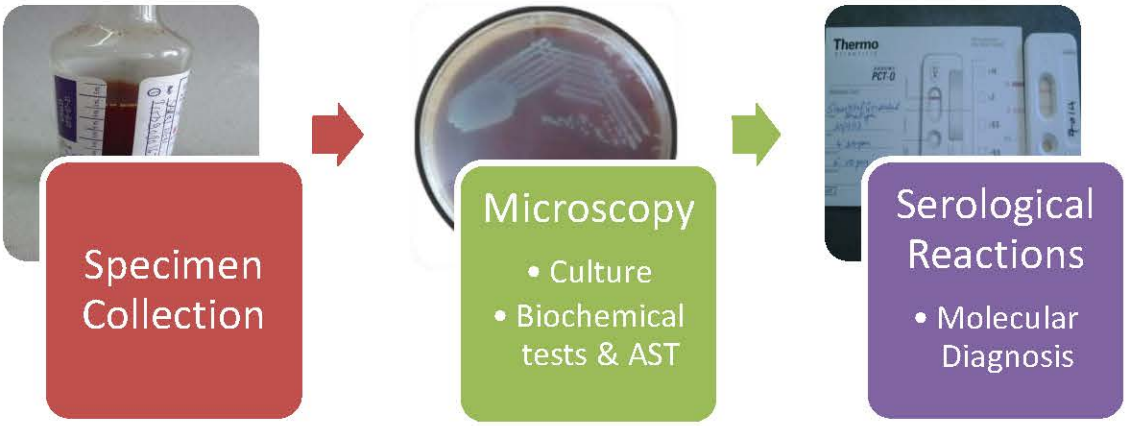
A detailed history and clinical findings were recorded for each patient. Patients' age, sex, other epidemiological and biological data, presenting complaints, comorbid conditions and risk factors were documented in a Proforma.

Laboratory Diagnosis

The microbiological profile of the specimens for diagnosis of sepsis was carried out in the department of Microbiology. The specimens were received for the laboratory diagnosis of suspected sepsis patients. Specimens were collected after informed consent from the patient or patient's relative where patient was not able to give consent for herself/himself.

Though sepsis is primarily a clinical diagnosis, supported by laboratory investigations and imaging, culture of specimens from a sterile site is the 'gold standard' microbiological investigation and the key to successful diagnosis.[36] Thus blood culture was taken as the 'gold standard' for diagnosis of sepsis. Tropical sepsis being the area of interest of this study considering primarily malaria, dengue, viral hepatitis and enteric fever as the cause, appropriate specimens were collected and tested/processed. The process of determining the microbial etiology is not a single step method but is a multistage process beginning with the collection of appropriate and proper sample collection, microscopic examination, culture & susceptibility testing, serological tests and use of molecular biology techniques.

Figure 5: Process of identifying microbial etiology



SPECIMENS:

A total of 2183 specimens were collected for culture as well as for serological investigations in Microbiology.

A total of 1136 different samples from 619 patients were collected for culture i.e. isolation and identification of bacteria and fungi as well as susceptibility testing i.e. antibiotic for bacteria and anti-fungal for *Candida species*. The details of which are as follows:

1. A total of 673 blood specimens were cultured from 619 patients diagnosed clinically as sepsis and for 124 patients who had anyone or two of the causes of tropical sepsis established i.e. malaria, dengue or viral hepatitis, the cultures were not done. Thus an average of 0.90 blood samples was cultured per patient.

The following specimens were also collected and processed for microbiological profile as and when indicated/required:

2. Urine (n=167)
3. Pus (n=80) i.e. swabs/discharge from infected burns, wounds, abscesses etc
4. Endotracheal (ET) secretions/ ET Tip (n=75)
5. Sputum (n=73)
6. CSF (n=38)
7. Body fluids (n=17) like ascitic fluid, peritoneal fluid, pleural fluid and bronchioalveolar lavage
8. Other samples (n=13) like vein-flow tips, central line tips, nasal swabs, throat swab, mitral valve, vitreous fluid and stool.

Also blood samples were collected for performing rapid tests for diagnosis of malaria and examination of peripheral smears. In addition serum samples were collected for carrying out rapid tests for dengue, viral hepatitis and leptospira and also for determining PCT and CRP levels in patients.

Collection of Specimens for Culture: [13, 38, 39, 48]

A proper collection and transport of the specimens to the laboratory is a very important step leading to final identification of the microbial eitology responsible for an infectious clinical condition and in the same manner an improperly collected sample may lead to misleading reports resulting in faulty treatment or an ineffective treatment, causing more harm than benefit to the patient. Specimen collection

encompasses of the following aspects related to collection: (a) the specimen should be collected from the exact site of infection, with a negligible contamination with the commensals from the adjoining tissues or organs; (b) the optimal timing for collection of sample, which could be the time of the day or night, or number of days or week depending on the incubation period, natural history and pathogenesis of the infections; (c) the sufficient quantity of specimen should be provided for carrying out the investigations prescribed and the standard guidelines should be followed. When the specimen is very less, it is difficult to perform all the tests with a very less amount of specimen and in such cases the clinician must be informed so as to decide the priority of the investigation; (d) the use of appropriate containers for culture (sterile containers) and serological diagnosis; and (e) preferably before starting the antibiotic. (13)

Blood:

Blood for culture is the most important specimen submitted to the microbiology laboratory for examination. It helps in specific etiological diagnosis. The presence of living microorganisms in blood suggests active and possibly spreading infection of tissues. (19) Blood culture plays an integral role in the evaluation of sepsis.

For blood culture, blood samples were collected before administering antibiotics at our hospital. However, for patients referred from private hospitals, already treated with antibiotics, were still considered for blood culture and blood samples were collected before starting antibiotic in our setup. Hence 5-7ml of blood sample was collected by venepuncture taking all aseptic precautions. For this a tourniquet was tied around the arm tightly and the patient was asked to make fist. The vein was palpated and located. Isopropyl alcohol (70%) or Povidone Iodine (10%) was applied with a help of sterile cotton over the cubital aspect of the forearm. Depending on the case, the samples were collected in BACTEC bottles (from BD Diagnostics - 30 ml of broth) or in commercially available conventional Monophasic Brain Heart Infusion (BHI) Broth bottles (HiMedia-70ml broth) and also in the Biphasic Medium (HiMedia) containing BHI broth 40ml and BHI slope 20ml. The BACTEC bottles **(Figure 6)** after collection were placed in BACTEC 9050 machine **(Figure 7)** while the Monophasic and Biphasic BHI broth bottles **(Figure 8 &9)** were placed in incubator at 37^0C for 24 hours before processing.

Figure 6: BACTEC Aerobic Culture Bottle

Figure 7: BACTEC 9050

Figure 8: Biphasic Blood Culture Bottle

Figure 9: Biphasic Blood Culture Bottle

Urine:

The urine samples were collected before administering antibiotics. For the patients who were mobile, were instructed to collect first morning urine sample. In order to decrease the chances of contamination with the commensal flora the patients were explained to cleanse the genitalia properly with soap and water and allow the area to dry before collecting urine. They were instructed to allow the first part of urine to be voided and then to collect the midstream in a sterile, wide-mouth, leak-proof

container provided to them. The patient were instructed not to touch the inside or rim of the container to avoid contamination.

At times the urine samples were collected as random samples at the time of admission of patient and the above mentioned points were explained to ensure proper collection with no contamination.

For catheterised patients, the area over the collecting tube was cleaned and a puncture was made with the help of sterile needle and the sample was drawn in sterile syringe. The sample so collected was transferred to sterile container and sent to laboratory.

Endotracheal Tip/Secretion/Aspirate (ETA):

Endotracheal aspirate was collected using suction catheter taking all aseptic precautions. For this the suction catheter was gently introduced through the endotracheal tube, gentle aspiration was then performed and then the catheter was withdrawn from the endotracheal tube. The aspirate and also the ET tips were sent in sterile container to the laboratory.

Sputum:

Patients were instructed to take a deep breath and collect 'Coughed up'/expectorated sputum sample in a sterile, wide-mouthed, leak- proof container.

Cerebrospinal fluid (CSF):

The CSF specimens were collected by the physician. A lumbar puncture was performed taking all aspetic precautions and the specimens were collected in sterile, leak-proof containers.

Pus/Wound Swabs/Pus discharge:

The wound/pus swabs were collected before using any disinfectant or dressing of the wound. The wound was cleansed with sterile water or saline to remove accumulated drainage and transient skin flora thus preventing the contamination with skin commensal. The surface lesion was opened and the sample was collected firmly from the lesion. In case the wound was dry, the swab was moistened with sterile normal saline and swab was collected. At times when the specimen seemed to be less on first

swab, a second swab was collected. The swabs so collected were placed back in the sterile test tube and sent to the laboratory.

In case of frank thick pus discharge/serous discharge the sample was collected by the sterile needle and syringe. The sample collected was transferred in the sterile container and sent to the laboratory.

Processing of Specimens: [13, 38, 39, 48]

Microscopy: [13]

As a known fact, microscopic examination provides a great deal of information about the specimen in terms of microbial type, inflammatory response and quality of specimen; it was done for every specimen received. It aids in early presumptive diagnosis and hence the findings were reported and communicated to the clinician at the earliest. The smears were made on clean, grease free slides and processed according to the need. Under this heading, (a) direct wet mount preparations of CSF, urine, body fluids, and stool were examined; (b) Gram stained smears of all the samples were examined; (c) Acid-fast stained smears of CSF, sputum, urine and body fluids were also examined; (d) peripheral smears of blood – thick and thin, mainly for malaria, were examined too (detailed method is described under malaria); (e) KOH mounts for fungal elements in suspected cases were also examined.

In the similar manner Gram stain was performed, for all the colonies isolated, to determine whether they are the colonies of Gram positive or Gram negative bacteria – bacilli or cocci and to choose the set of biochemical tests required for their identification. Also a hanging drop preparation for motility was examined mainly for bacilli, especially, Gram negative.

Aerobic Culture:

Blood Culture:

BACTEC:

The BACTEC bottles once received in the laboratory were immediately kept in the BACTEC (9050) machine. The machine detects the presence of the organism by sensing the presence of CO_2 produced by the organisms when they grow in the broth. It indicates Growth/Positive and No Growth/Negative by sounding a 'beep' and

flashing a 'light' on the screen. The bottles were then removed from the machine and processed appropriately.

After the bottles were removed from machine direct smears were prepared and stained by Gram stain to detect presence of organism in the bottles. Thereafter the samples were sub-cultured on Mac Conkey's agar, Blood agar and also on Saboraud's Dextrose Agar (SDA) (all media from HiMedia) if fungal elements were seen on direct smear. Inoculated plates were incubated for 18-24 hours at 37^0C. The plates were observed for the formation of colonies.

For the bottles showing no growth the BACTEC machine indicated negative result on the 5^{th} day. The specimens from these bottles were also subcultured to ensure that no organism is missed. After 24 hours if no growth was found on the plates, it was reported as negative i.e. "No Organism Isolated" finally on the 6^{th} day.

Conventional Blood Culture using Monophasic Medium:

In case of conventional BHI broth bottles, after adding blood samples, the bottles were sent directly to Clinical Microbiology Lab. The bottles were placed inside incubator at 37^0C for 24 hours. Bottles were observed daily for turbidity. The first sub-cultures were done on day 2 of receiving the samples and direct smears stained with Gram stain were also observed. These cultures were declared negative after 3 sub-cultures on alternate days were done. However, if any growth was found in any of the 3 sub-cultures, they were considered as positive for growth.

Conventional Blood Culture using Biphasic Medium:

Blood culture was also done using Biphasic Medium (HiMedia). Here 4ml of blood was collected for 40 ml of broth. The medium consisted of Brain Heart Infusion (BHI) Broth (40 ml) and BHI agar slope (20 ml). After adding blood to it, the bottle was kept in upright position and sent to the laboratory. Once received the bottle was kept in the incubator at 37^0C for 24 hours. On day 2 the bottle was tilted so that the broth flows over the surface of the slope and gets inoculated. Again the bottle was kept in the incubator in upright position at 37^0C for 24 hours. Next day the bottle was observed for growth on the slope and turbidity in the broth. If there was no growth the process was repeated on the day after again for a 2^{nd} subculture and again a 3^{rd}

subculture i.e. 3 subcultures on alternate days. If there was any growth, the colonies were identified by standard biochemical tests.

All plates showing growth, either from BACTEC or conventional BHI bottles with monophasic or biphasic medium, were subjected to identification using standard methods. The Gram stain reports of the smears prepared directly from the samples were communicated to the clinician as early as possible.

Urine Culture:

The urine specimens were examined grossly for colour and turbidity. Wet mount preparations were made by placing a drop of urine on the slide and then covering with a cover glass. These were observed under 10X first and then under 40X. The smears were then prepared by transferring loop full of sample on the clean, grease free slide. The smears were then fixed and stained by Gram stain. The smears were then observed under microscope for presence of pus cells, bacteria and fungal elements (if any). The specimens were then inoculated on the Mac Conkey's agar, and Saboraud's Dextrose agar (SDA) and incubated aerobically overnight at 37^0C. For colony count the samples were inoculated on blood agar by standard semi-quantitative loop method. The organisms were identified as commensal or pathogen on the basis of colony count. Thus $<10^5$ CFU/ml were considered as commensal or contaminant.[39] Single or mixed growth isolates were identified by observing colony characteristics on the blood, Mac Conkey agar plate, and biochemical reactions using standard microbiological methods.[39]

Pus/Wound Swabs:

Smears were prepared directly from the swabs/pus discharge by rolling the swabs/or smearing the loopful on the clean, grease free slides and heat fixed. The smears were then stained by Gram's stain and observed for the presence of pus cells, bacteria and fungal elements, if any. The swabs were then inoculated on blood agar, Mac Conkey's and SDA. Inoculated plates were incubated at 37^0C for 18-24 hours.

Sputum/ETA Culture:

The specimens were examined grossly for the presence of purulent sputum, blood and colour of the sputum. Smears were then prepared from the specimens by taking a

loop-full of specimen with the sterile loop on a grease free slide. The smears were heat fixed. Then the slides were stained with Gram stain. The sputum specimens were stained with Acid Fast Stain/Ziehl Neilson stain too for detecting acid fast bacilli. The slides were then observed under the microscope for presence of pus cells, epithelial cells, bacteria and fungal elements, if any. Moreover, the specimens showing about 10 squamous epithelial cells/ low power field (LPF) and 25 leukocystes/ LPF i.e. Group 5, were considered clinically relevant and those with ≥ 25 squamous epithelial cells/LPF with10 leukocytes /LPF i.e. Group 1 were considered inappropriate for processing as per the Murray & Wahington Grading system **(Table 2)**

Table 2: Murray and Washington Grading System for Sputum [13]

Group	Epithelial cells/LPF	Leukocytes/LPF
Group 1	25	10*
Group 2	25	10-25
Group 3	25	25
Group 4	10-25	25
Group 5	10	25**
*Inappropriate		
** Appropriate		

The clinically relevant specimens were inoculated on the Mac Conkey's agar, blood agar (HiMedia) and Saboraud's Dextrose agar and incubated aerobically overnight at 37^0C. For sputum, tracheal or bronchial aspirates, the organism were identified as commensal or pathogen on the basis of colony count. Thus $<10^5$ CFU/ml for tracheal aspirate & sputum while $<10^4$CFU/ml in bronchial aspirate were considered as commensal or contaminant.[34] Single or mixed growth isolates were identified by observing colony characteristics on the blood, Mac Conkey agar and SDA plates, and biochemical reactions using standard microbiological methods.[39]

CSF Culture:

The CSF specimens were always processed immediately on receiving. The samples were observed grossly for turbidity and colour. The specimens were first centrifuged. Wet mount preparations were made by placing a drop of CSF sediment on the clean,

grease free slides and covered with cover glasses and observed for pus cells and organisms. The smears were prepared from the deposit and stained by Gram stain and ZN (Acid Fast) stain and were observed under microscope for pus cells, bacteria and any fungal elements and acid-fast bacilli respectively. The deposit was transferred with a sterile loop for inoculation on chocolate agar, blood agar and Mac Conkey's agar. The inoculated chocolate agar plates were placed in candle jar with a lighted candle and lid closed tightly which was incubated aerobically at 37^0C for 18-24 hours.

Identification of Bacteria: [13, 38, 39, 48, 49]

Colony characters:

The plates were observed for different colony characters and were utilised for identification for e.g.

1. Lactose fermenting pink coloured or non-lactose fermenting pale coloured colonies on Mac Conkey's agar;
2. Presence or absence of hemolysis and type of hemolysis on blood agar;
3. Type of odour - curdy, fishy, and earthy;
4. Consistency - Mucoid, moist or dry etc.
5. Margins – irregular, complete or indented etc.

Culture Smears:

The smears from colonies were then prepared by placing drop of normal saline on clean, grease free slides. With the help of sterile straight wire the growth from the colony was emulsified in the normal saline. The smears so prepared were air dried and then heat fixed. The smears were stained by Gram stain and observed to identify the organism as Gram positive or Gram negative; cocci or bacilli; type of arrangement; if any yeast-like cells with budding and pseudohyphae etc.

Biochemical tests: [13, 38, 39, 48, 49]

The set of standard biochemical tests were chosen according to the type of organism in the colonies and appropriate control strains were used for each of the tests. Commonly isolated organisms like Gram positive cocci (GPC), Gram negative bacilli (GNB) and Candida species were identified.

Set of Biochemical Tests for Identification of Gram positive cocci (GPC):

1. **Catalase test:**

The colonies suspected to be of GPC, as seen on gram stain, were picked up with the help of sterile sticks and applied on clean slides. To which a drop of 3% H_2O_2 was added. It was observed for the production of bubbles or effervescence.

Observation & Interpretation:

Production of bubbles/effervescence: Positive test.

Positive test indicates that the organism is producing catalase enzyme and breaks down H_2O_2.

Absence of production of bubbles/effervescence: Negative test.

2. **Coagulase test:**

The colonies whose gram stain smear showed presence of GPC in cluster and whose catalase test was positive were further tested for production of coagulase enzyme and identification of *Staphylococcus aureus*. Coagulase enzyme is present in two forms, bound and free, each having different properties. The detection of these two requires 2 separate testing procedures:

i) Slide coagulase test for detection of bound coagulase:

A drop of normal saline was taken on each end of a slide. A colony from the growth on the inoculated plate of nutrient agar or blood agar was picked up and emulsified to make a milky suspension. A drop of plasma was added to the drop on one side and slide was rocked back and forth, observing for agglutination of the colonies.

Observation & Interpretation:

Clumping/agglutination visible to naked eye: Positive

No clumping/agglutination: Negative

No change in the control drop – Non-autoagglutinable

Clumping/agglutination in control drop - Autoagglutinable

ii) Tube coagulase test:

One or two colonies from the growth were picked up and emulsified in a test tube containing normal saline/peptone. Plasma was added to the test suspension and not in the control suspension. The ratio of plasma: test broth was 1:4. It was mixed well and incubated at 37^{o} C for 4 hours. The mixture

was examined at 1 hour and 4 hours for coagulum formation. If no clot was seen, it was incubated at room temperature for 18 hours or overnight and observed for clot formation.

Observation & Interpretation:

Clotting of the plasma which does not flow on tilting the test tube: Positive

No clotting as indicated by the flowing of plasma on tilting the test tube: Negative

No clotting and flowing of plasma on tilting the control test tube.

3. Mannitol Salt Agar test: (MSA)

Those colonies which tested positive for both catalase and coagulase were tested for MSA test as an additional test for identification of *Staphylococcus aureus*. Mannitol salt agar slants/slopes, containing 1% mannitol and 7.5% sodium chloride (HiMedia), were prepared according to the manufacturer's instruction and were used. A colony from the growth was picked up and inoculated on the slant/slope of a dried mannitol salt agar medium using a stroke method with a sterile straight wire. The inoculated medium was then incubated at 37^0 C overnight and observed next day for the colour change.

Observation & Interpretation:

Colour of the slant changes from red to yellow with a growth on it: Positive

No colour change and no growth: Negative

4. Bile Esculin Test:

Those colonies, whose gram stain showed GPC in pairs and catalase test was negative, were tested for bile esculin test. A small portion of the growth from the colonies was picked up using an inoculating wire and emulsified in a test tube containing sterile peptone water. Sterile bile esculin disc (HiMedia) was added to the suspension with the help of sterile forceps. The suspension was incubated at 37^0 C and development of black colour was observed after 4 hours. If no colour change was observed, suspension was further incubated at 37^0 C overnight. This test was used for identification of *Enterococcus species*.

Observation & Interpretation:

Colour of the suspension turns to black: Positive

No colour change: Negative

5. **Bacitracin sensitivity:**

Those colonies with beta hemolysis on SBA, whose gram stain showed GPC in pairs/short chains and catalase test was negative, were tested for bacitracin sensitivity. Using an inoculating wire the colonies were picked up and emulsified in sterile normal saline. With the help of the sterile cotton swab, dipped in suspension, a lawn culture was prepared on sheep blood agar plate. A sterile bacitracin disc (0.04 Units) (HiMedia) was placed with the help of sterile forceps. The plate was placed in candle jar for 5-10% CO_2 at 37^0C for 18-24 hours and observed for formation of zone of inhibition around the bacitracin disc.

Observation & Interpretation:

Zone of inhibition of ≥15mm or any zone size: Sensitive & suggestive of β-hemolytic-Group A Streptococci i.e. *Streptococcus pyogenes*

No Zone of inhibition/Growth on the edge of the disc: Resistant & suggestive of β-hemolytic-Group B Streptococci.

A. Set of biochemical tests for identification of Gram negative bacilli:

1. **Catalase test:** Same as described in the catalase test for Gram positive cocci.

2. **Oxidase test:**

This was mainly done to rule out members of Enterobacteriaceae (usually negative) and identify the colonies suspected of belonging to other genera like *Pseudomonas species*, *Neisseria species*, etc. For this test, oxidase discs (HiMedia), impregnated with the reagent were used. The disc was first moistened with sterile normal saline and the suspected colonies were picked up with sterile sticks and rubbed on the disc.

Observation & Interpretation:

The purple/violet colour at the site of application of colonies: Positive

No colour: Negative

3. **Indole:**

The colonies were picked up and emulsified in sterile peptone water and incubated at 37^0C for 18-24 hours. At the end of this time, 15 drops of Kovac Reagent were

added drop-by-drop down the inner wall of the tube and observed for ring formation at the interface of the peptone water and reagent.

Observation & Interpretation:

Development of bright fuchsia red colour at the interface within seconds of adding reagent: Positive

No bright fuchsia red colour: Negative

4. **Methyl Red test:**

Glucose phosphate broth was prepared according to manufacturer's instructions and filled in the test tubes. Colonies were inoculated in glucose phosphate broth and incubated at 37° C for 48-72 hours. Some amount of broth was transferred to another test tube and few drops of methyl red indicator were added in it.

Observation & Interpretation:

Development of red color in 3-5 minutes: Positive

No change in the colour: Negative

5. **Voges-Proskauer test:**

Glucose phosphate broth was prepared according to manufacturer's instructions and filled in the test tubes. Colonies were inoculated in glucose phosphate broth and incubated at 37^0C for 48-72 hours. Some amount of broth was transferred to another test tube and 1 ml of Barrit's reagent A containing 30% potassium hydroxide and 3ml of Barrit's reagent B containing 5% α-naphthol were added.

Observation & Interpretation:

Development of pink colour in 2-5 minutes: Positive

No change in colour: Negative

6. **Citrate:**

Simmons citrate medium (HiMedia), was prepared according to manufacturer's instructions, poured in the form of slant/slope, was used for this test. The medium was inoculated by stroke method using a sterile straight wire and incubated at 370C for 24 hours.

Observation & Interpretation:

Development of blue colour with a visible growth on the line of inoculation: Positive

No blue colour, but a visible growth on the line of inoculation, were incubated for further 24 hours and if the blue colour developed: Positive

No blue colour and no growth: Negative

7. **Urease:**

For this test Christensen's Urea agar base (HiMedia) slant/slope, prepared according to manufacturer's instructions, was used. The surface of the agar slant was inoculated by stroke method using sterile straight wire and was incubated for 24 hours at 37^0C and observed for the colour change.

Pink colour: Positive

No pink colour: Negative

8. **PPA/PDA:**

This test was mainly done for non-lactose fermenting, pale coloured, oxidase negative and urease positive colonies of gram negative bacilli to confirm that the organism is belonging to Proteae tribe under family Enterobacteriaeceae. For this test the PPA (HiMedia) slant/slope, prepared according to manufacturer's instructions, was used. The surface of the agar slant was inoculated using sterile straight wire. After incubating for 24 hours at 37^0C, 4-5 drops of ferric chloride reagent were added directly to the surface of the agar and tubes were rotated while adding the reagent covering the entire surface of the slant.

Observation & Interpretation:

Immediate appearance of deep green colour: Positive

No green colour: Negative

9. **Motility test:**

This was done mainly for colonies of gram negative bacilli, to see whether the organisms are motile or non-motile. For this hanging drop method was used. A small amount of petroleum jelly was applied to the 4 corners of the coverslip. A small drop of 3-4 hours bacterial suspension was placed, with the help of inoculating loop, on the centre of the coverslip. A concavity slide was inverted and

pressed over the coverslip. The slide was carefully brought to an upright position and observed under 10X and later 40X by first focusing the edge of the drop underneath the coverslip. The motility and types of motility were observed and documented.

Observation & Interpretation:

Bacteria seen moving and changing its position: Motile

Bacteria not seen moving and do not change its position: Non-motile

10. Triple Sugar Iron Agar (TSI):

The TSI medium was prepared according to manufacturer's instructions. Colonies were picked using a sterile straight wire and inoculated the medium by stabbing the 3/4ths of the butt and while withdrawing the straight wire the slope/slant was inoculated by stroke method. It was then incubated at 37^0C for 18-24 hours.

Observation & Interpretation:

The changes in the colour of slant & butt as well as formation of gas & H_2S were observed and interpreted in the following manner. Depending on the type of reaction shown by each organism, these were used for the identification.

Yellow colour in the slant/butt: Acidic reaction in the slant/butt

Pink colour in the slant/butt: Alkaline reaction in slant/butt

Black colour in the butt: Production of H_2S

Bubbles/cracks in the medium or raising up of the medium: Production of gas

11. Sugar Fermentation:

Different sugars like glucose, sucrose, lactose and mannitol were tested according to the bacteria in consideration for identification. For this the sugar solutions were prepared from the powder (HiMedia) as per the manufacturer's instructions. Durham's tubes were added to each tube. An inoculum was prepared by emulsifying the colonies in peptone water and incubating it for about 2 hours at 37^0C. A loopful was transferred for inoculating the sugars. These were then incubated at 37^0C for 18-24 hours and observed for change in the colour of sugar solution and production of gas in the Durham's tube.

Observation & Interpretation:

Colour changes to yellow from red: Sugar is fermented

No change in the colour: Sugar has not been fermented.

Bubble at the top/ or length of Durham's tube: Production of gas

No bubble in the Durham's tube: No gas is produced

Antimicrobial Susceptibility Testing (AST): [13, 38, 39, 50]

For all bacterial isolates AST was performed by modified Kirby-Bauer method and according to the CLSI guidelines. In addition to the routine AST, the following were detected by phenotypic methods:

1. Methicillin Resistant *Staphyloccocus aureus* (MRSA) and *Coagulase Negative Staphylococci* (MRCoNS) [51]
2. Vancomycin Resistant *Staphylococcus aureus* (VRSA) [52 & 53]
3. Erythromycin Induced Clindamycin Resistance (ICR) in *Staphylococcus aureus* isolates [51]
4. Extended Spectrum Beta Lactamase (ESBL) [50 & 51]
5. AmpC-beta lactamase producers [54 & 55]
6. Metallo-Beta-Lactamase (MBL) producers [56]

} Gram Negative Bacilli

AST was performed by modified Kirby-Bauer disk diffusion method. A well isolated colony or morphologically similar colonies were picked up with the help of sterile wire and mixed with sterile normal saline to prepare an inoculum of 0.5 McFarland. The mixture was then vortexed for homogenous turbidity. A sterile swab was dipped into the prepared inoculum and it was ensured that extra solution was drained by rolling the swab against the wall of the tube. Using this swab, a lawn culture was prepared on Mueller Hinton Agar (HiMedia). By rotating the plate thrice at 60^0 it was ensured that the whole surface is covered and uniform lawn culture is made. The antibiotic discs were placed on the plate within 15 minutes of inoculation and then the plates were incubated for 24 hours at 37^0C. The following antibiotic discs were used depending upon the isolate type:

For Gram Positive Cocci:

Penicillin G (10 units), Erythromycin (15µg), Levofloxacin (5µg), Gentamicin (10µg), Cotrimoxazole (25µg i.e.1.25/23.75), Vancomycin (30µg), Linezolid (15µg) and Doxycycline (30µg) were tested.

Clindamycin (2µg) for detection of Erythromycin Induced Clindamycin Resistance (ICR)

For Gram Negative Bacilli:

Imipenem (10µg), Amikacin (30µg), Gentamicin (10µg), Cefepime (30µg), Cefuroxime (30µg), Ceftazidime (30µg), Cefotaxime (30µg) Ciprofloxacin (5µg), Amoxycillin+Clavulanic Acid (30µg i.e 20/10 µg) and Cotrimoxazole (25µg i.e. 1.25/23.75)) were tested.

Ceftazidime+Clavulanic Acid (30µg+10µg) for ESBL detection

Cefotaxime +Clavulanic Acid (30µg+10µg) for ESBL detection

For *Pseudomonas spp.*:

Imipenem (10µg), Aztreonam (30µg), Piperacillin (100µg), Piperacillin+Tazobactam (100/10µg), Ceftazidime (30µg), Amikacin (30µg), Gentamicin (10µg) and Cefipime (30µg) were tested.

Imipenem + EDTA (10µg+ 750 µg) were used for MBL detection.

The observations were made by measuring the sizes of zones of inhibition around each disc using the zone scale (HiMedia) for each isolated organism. These zone sizes were then interpreted as Susceptible/Sensitive (S), Resistant (R) and Intermediate (I) according to the manufacturer's zone size interpretative chart which is as per the CLSI guidelines.

Detection of MRSA & MRCONS: [51]

The staphylococci isolated and identified as *Staphylococcus aureus* as well as *Coagulase Negative Staphylococcus* were tested for resistance to methicillin. As per CLSI guidelines this test was carried out using Cefoxitin disc (30µg). A lawn culture was made on MHA plates using an inoculum of 0.5 McFarland turbidity. The plates were incubated in ambient air at 33^0C for 18 hours. The control strains used were *S. aureus* – ATCC 25923 (mec-A negative and zone size 23-29 mm) and ATCC 43300 – mec-A positive and zone size ≤21mm)

The plates were observed for zones of inhibition. The zones were measured using the zone scale (HiMedia).

Zone size of ≤21mm was considered as Resistant to methicillin/mec-A positive i.e. MRSA

Zone size of ≥ 22 mm was considered as Sensitive to methicillin/mec-A negative i.e. MSSA

For detection of resistance to oxacillin in *Coagulase Negative Staphylococcus* (*CoNS*) the procedure, media, temperature etc. remained the same as that for *Staphylococcus aureus*. However, zone sizes were interpreted as per CLSI guidelines for *CoNS*. Thus zone size of ≤24mm was considered as Resistant to methicillin/mec-A positive i.e. *MRCONS* and ≥ 25 mm was considered as Sensitive to methicillin/mec-A negative i.e. *MSCONS*.

Cefoxitin is a surrogate for mec-A mediated oxacillin resistance. Therefore resistance to Cefoxitin was reported as Oxacillin resistance and not as Cefoxitin resistance.

Detection of VRSA: [52 & 53]

As per CLSI, disc diffusion testing is not a reliable method for detection of vancomycin resistance. Hence MIC should be performed for all isolates which show no zone of inhibition around the disc before reporting it as resistant. Thus for detection of VRSA, MIC was performed using Vancomycin Ezy MIC strip (HiMedia) according to the manufacturer's instructions.

For this the colonies isolated and identified as *Staphylococcus aureus* were picked and inoculum of 0.5 McFarland turbidity was prepared. A lawn culture was prepared using the above inoculum. With the help of the applicator stick provided with the package, the strip was placed on the centre of the plate and the plate was incubated for 18-24 hours. The control strains used: *Enterococcus faecalis* ATCC 29212 – Susceptible *E. faecalis* ATCC 51299 – Resistant as well as *S. aureus* ATCC 25923

The MIC was read where the ellipse intersected the MIC scale on the strip **(Figure 10)**. These strips have a continuous gradient and thus MIC values may fall "in-between" two fold dilutions. In such cases the values were rounded up to the next two fold dilution before categorization as per the manufacturer's instructions.

According to CLSI the MIC values of ≤2µg/ml as well as ≤4 µg/ml should be considered as susceptible while those with ≥8 µg/ml should be sent to the reference laboratory for confirmation before reporting as resistant.

Figure 10: Vancomycin MIC-E-test

Detection of Inducible Clindamycin Resistance due to Erythromycin: [51]

Disc diffusion method as recommended in CLSI guidelines was used for detection of resistance to Clindamycin induced by erythromycin. Accordingly, a 2µg Clindamycin disk was placed 15mm away from the edge of a 15µg Erythromycin disk on Mueller Hinton agar plate and *S. aureus* ATCC 25923 was used as control strain. Plates were observed for flattening of clindamycin zone after 16-18 hours of incubation at 35 ± 2^0C in ambient air. Thus flattening of clindamycin zone adjacent to erythromycin disk i.e. forming a D-zone was considered to be exhibiting inducible clindamycin resistance **(Figure 11)**. Appearance of hazy growth within the zone of inhibition around clindamycin was considered as resistant to clindamycin. Those organisms which did not show a D- zone were reported as susceptible if found susceptible and resistant if found resistant on the individual observations of testing each drug.

Figure 11: D Zone-test Positive – Erythromycin-induced-Clindamycin Resistance

Detection of ESBL (Extended Spectrum β-lactamase) Producers: [50& 51]

For detection of ESBLs in gram negative isolates, phenotypic screening and confirmatory tests recommended by CLSI 2011 were used. For screening test, Ceftazidime disk (30 µg) and Ceftazidime-clavulanic acid disk (30 µg /10 µg) were placed on surface of MHA plate and incubated at 35±2^{0}C in ambient air for 16-18 hours. A >5mm increase in zone diameter of cetazidime-clavulanic acid as compared to ceftazidime alone was considered positive for ESBL production. For confirmatory testing both cefotaxime (30 µg) and ceftazidime (30 µg) were placed on the surface of MHA along with their clavulanic combinations (30 µg /10µg) and incubated at 35±2^{0}C for 16-18 hours. A >5mm increase in zone diameter of either of the antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone was considered ESBL producer **(Figure 12)**.

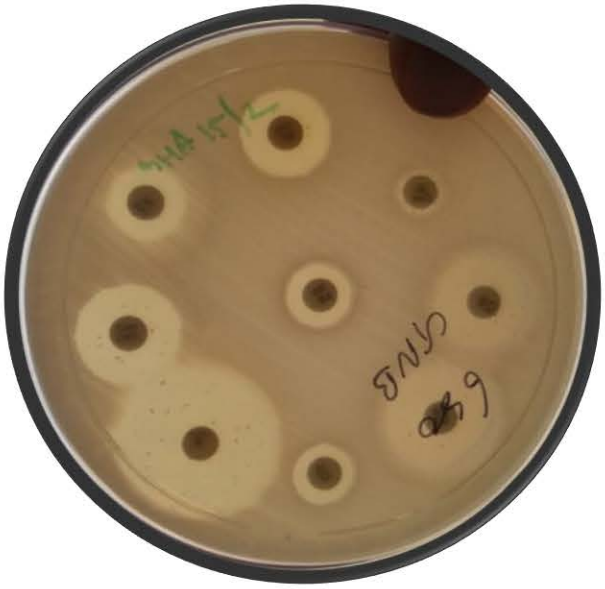
Control Strains used: When performing the ESBL confirmatory tests, *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were tested. *E. coli* ATCC 25922: ≤ 2-mm increase in zone diameter for antimicrobial agent tested alone vs. its zone when tested in combination with clavulanic acid. *K. pneumoniae* ATCC 700603: ≥ 5-mm increase in ceftazidime clavulanic acid zone diameter; ≥ 3-mm increase in cefotaxime clavulanic acid zone diameter.

Figure 12: ESBL Production

Detection of AmpC-β-lactamase producers: [53, 54 & 55]

There are no CLSI-validated phenotypic tests to confirm the presence of plasmid-encoded AmpC β-lactamases in clinical isolates. [53] Tan TY et al evaluated various phenotypic screening methods and concluded that method using cefoxitin-cloxacillin disk combination accurately detected AmpC-positive isolates. Hence the same method developed by Tan TY et al in 2009 was used. All gram negative isolates were tested. A lawn culture was made on MHA plate with 0.5 McFarland. A cefoxitin (30 µg) disc was first placed with a sterile forceps on the surface of agar. Then a cefoxin-cloxacillin (30µg) disc was placed 20mm away from cefoxitin disk. The plate was incubated for 16-18 hours at 35^0C. The plate was then observed for zone of inhibition. A difference of ≥4mm in zone size of cefoxitin-cloxacillin (30µg/200µg) disk compared to cefoxitin alone was considered positive for AmpC-β-lactamase production/activity **(Figure 13)** and less was considered to be negative for the same [54 & 55]

Figure 13: Detection of AmpC-β-lactamase production

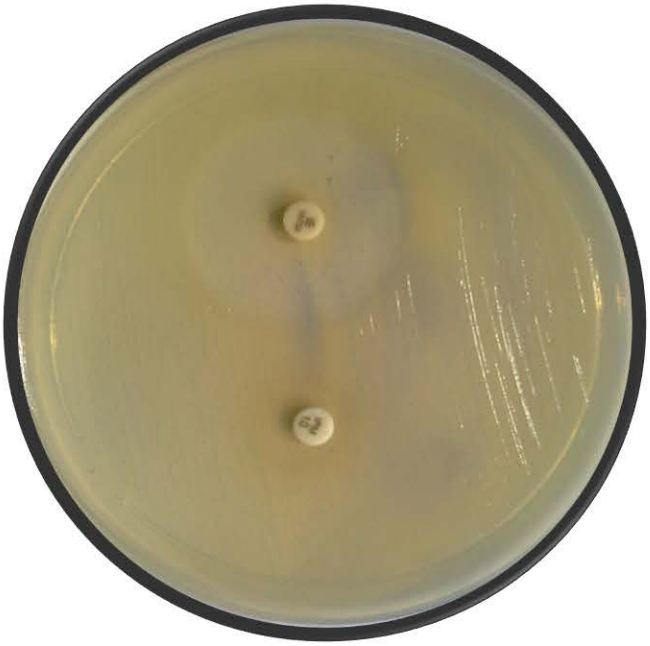


Detection of MBL (Metallo-beta-lactamase) Producers: [53 & 56]

All gram negative bacterial isolates, whether sensitive or resistant to Imipenem, were tested for MBL production. Isolates were classified as Susceptible (zone size of ≥16 mm), Intermediate (zone size of 14-15mm) and Resistant (zone size of ≤13 mm).

Since there is no CLSI validated standard guideline for phenotypic detection of MBL production [53], different authors have used different methods like PCR, double disc synergy test (DDST), combined disc synergy test (CDST) and E-Test for detection of MBL. Of the phenotypic methods for MBL detection, combined disk synergy (CDST) test was found to be better test over the other tests. Thus MBL detection was carried out by CDST method as described by Yong D et al. Hence a lawn culture of the isolate was made on MHA using inoculum of 0.5 McFarland turbidity. On the surface of agar, an Imipenem disc (10 µg) and other Imipenem+EDTA (10µg+ 750 µg) were placed at a centre to centre distance of 30 mm. After incubation of 16-18 hours at 35^0C in ambient air, the plate was observed for zone sizes above a black non-reflecting background and read under reflected light. An increase in the zone size of ≥7mm than Imipenem alone was considered positive for MBL production [56] **(Figure 14)**.

Figure 14: Detection of MBL Production



Fungal Culture: [13]

The specimens whose Gram stained smears showed any fungal elements like hyphae, yeast cells with budding with or without pseudohyphae etc. were inoculated on Sabourad's Dextrose Agar (SDA) as well as on blood agar and Mac Conkey's agar. Plates were incubated aerobically at 37^0C. The colonies of *Candida species* were seen after overnight incubation whereas for other fungi, the plates were kept at room temperature for 1 week and observed daily for growth.

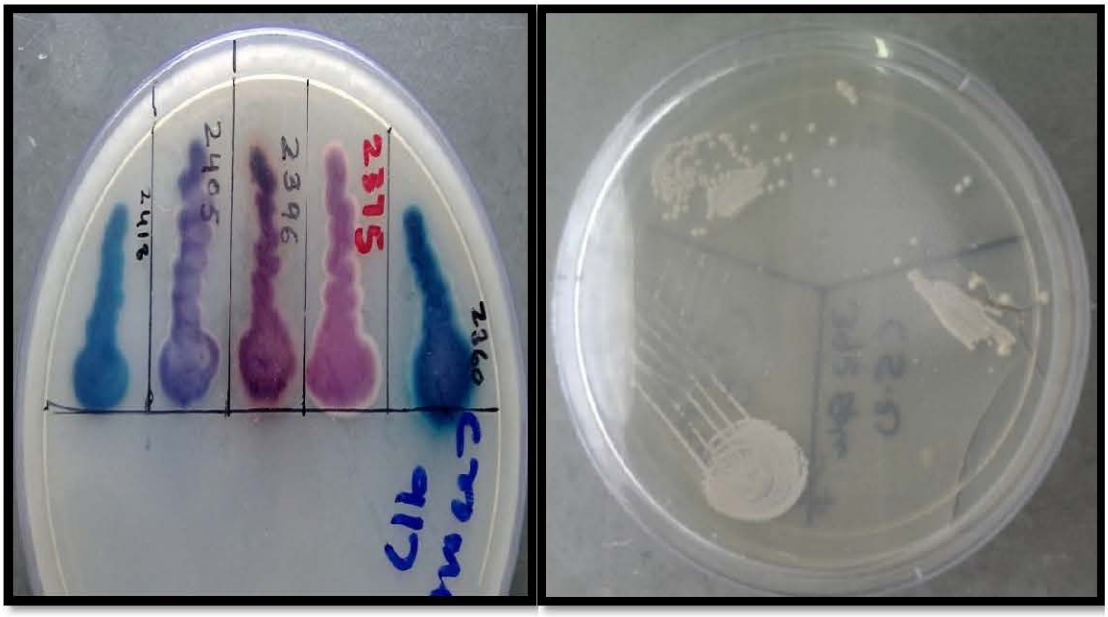
Identification of yeasts & yeast-like fungi: [13]

Colonies of white, creamy white or yellow-white with smooth, pasty consistency on SDA after overnight incubation are suggestive of yeast or yeast-like fungi. In addition (1) the presence of pseudohyphae in yeasts is suggestive of *Candida species* and (2) growth on Mac Conkey's agar showing light pink, small colonies is also suggestive of *Candida species*. The gram stain of the colonies was also performed. Well isolated colonies suggestive of *Candida species* from SDA were picked up with the sterile inoculating loop and streaked onto HiCrome from HiMedia for species identification. HiCrome has been found to be equally good when compared to CHROME agar, Nested PCR and standard biochemical methods for identification of *Candida species*. [57, 58, 59] Different species of *Candida* grow with different coloured colonies on this medium. The colonies were identified

according to colour and interpreted as shown in the **Table 3** below & **Figure 15 & 16**.

Figure 15: Colonies of *Candida species* on HiCrome agar

Figure 16: Colonies of *Candida species* on SDA



Those which could not be identified as one of the species mentioned in the Table 3 were labelled as *Candida non-albicans*.

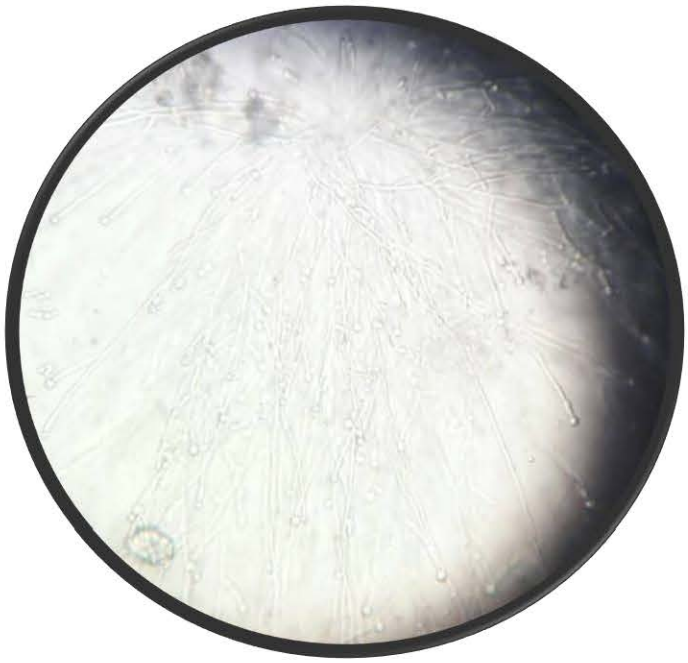
Table 3: Interpretation of the colour of the colonies: [57, 58, 59]

Colour of the colony	Species identified
Light green	*Candida albicans*
Cream to White	*Candida glabrata*
Purple, fuzzy	*Candida krusei*
Blue purple & Bluish green	*Candida tropicalis*

In addition to the colour of the colonies on HiCrome, a germ tube test and observation of chlamydospore formation on cornmeal agar were carried out for identification of *Candida albicans*. For germ tube test, a well isolated colony from SDA was emulsified in 0.5 ml o human serum using sterile straight wire. The test tubes were incubated at 35^0C and no longer than 2 hours. A drop of serum sample was place on a clean, grease free slide and a coverslip was place over it. This slide was then observed first under 10X and then under 40X objective lens of microscope for the presence of germ tubes. Germ tube is a filamentous extension

from yeast cell without constriction at the neck (true germ tube), is seen in *C. albicans*. For inoculation of corn meal agar, 3 parallel cuts about 1 cm apart were made on the surface of the agar holding the sterile straight wire at about 45^0 angle. A cover slip was placed over the inoculated areas. The plates were then incubated for 24-48 hours at 30^0 C. The cover slips were then observed under the microscope for chlamydospore. [13]

Figure 17: Chlamydospore formation under 40X objective



For Filamentous Molds: [13]

These fungi were identified by macroscopic and microscopic observations. Thus the colony morphology was observed on the obverse and reverse of the SDA plates. For microscopic examination, transparency tape mount preparations were observed using lactophenol cotton blue (LPCB). This method allows better observation of hyphae, spore morphology and arrangement. For this method the sticky side of the unfrosted, clear cellophane tape was gently but firmly pressed against the surface of the colony, picking up the aerial mycelium with spores/conidia. A drop of LPCB was place on the slide. Then one of cellophane tape was stuck on the slide near the drop of stain and then gently and slowly lowering it, the tape was stretched and the other end was stuck to the slide. This was then observed under the microscope 10X and 40X objectives.

Antifungal Susceptibility Test: [60, 61, 62]

Antifungal Susceptibility test was carried out only for *Candida species* according to CLSI-M44-A2 guidelines for testing anti-fungals for yeasts. In order to carry out antifungal susceptibility testing, an inoculum of 0.5McFarland was prepared from a well isolated colony from SDA. A lawn culture was made on Mueller-Hinton Agar + Glucose-Methylene-Blue (GMB) medium from the above inoculums using a sterile swab. The GMB was prepared according to the method described in the guidelines [60]. Thus according to the manufacturer's instructions, MHA was prepared first. To this 2% of glucose (final concentration) and 0.5 µg/ml methylene blue was added. A hexadisc (Hexa-Antimyco -01/HX104 from HiMedia) containing Amphotericin B (100 units), Clotrimazole (10 µg), Fluconazole (25 µg), Itraconazole (10 µg), Ketoconazole (10 µg) and Nystatin (100 units) was used. The plates were then incubated at 37^0C for 24 hours. Zones of inhibition for antifungals tested for *Candida species* were measured **(Figure 18)** & interpreted according to the Mahmoudabadi AZ et al [61]. For Amphotericin B and Clotrimazole, the zones were interpreted according to the manufacturer's manual [62]

Figure 18: Antifungal Susceptibility Test on GMB Medium

Serological Investigations:

Procalcitonin Test: [63, 64 & 65]

Principle:

Detection & measurement of PCT from serum samples was carried out using PCT-Q kit (CPC Diagnostic Pvt. Ltd.) It is a semi-quantitative test based on immunochromotographic method. The test uses a monoclonal mouse anti-catacalcin antibody conjugated colloidal gold (tracer) and a polyclonal sheep anti-calcitonin antibody (solid phase). After the test sample has been applied to test strip, the tracer binds to the PCT in the sample and a marked antigen-antibody complex forms. This complex moves by capillary action through the test system and, in the process passes through the area containing the test band. Here, the marked antigen-antibody complex binds to the fixed anti-calcitonin antibodies and forms a sandwich complex.

Procedure: The test was performed according to the manual as follows:

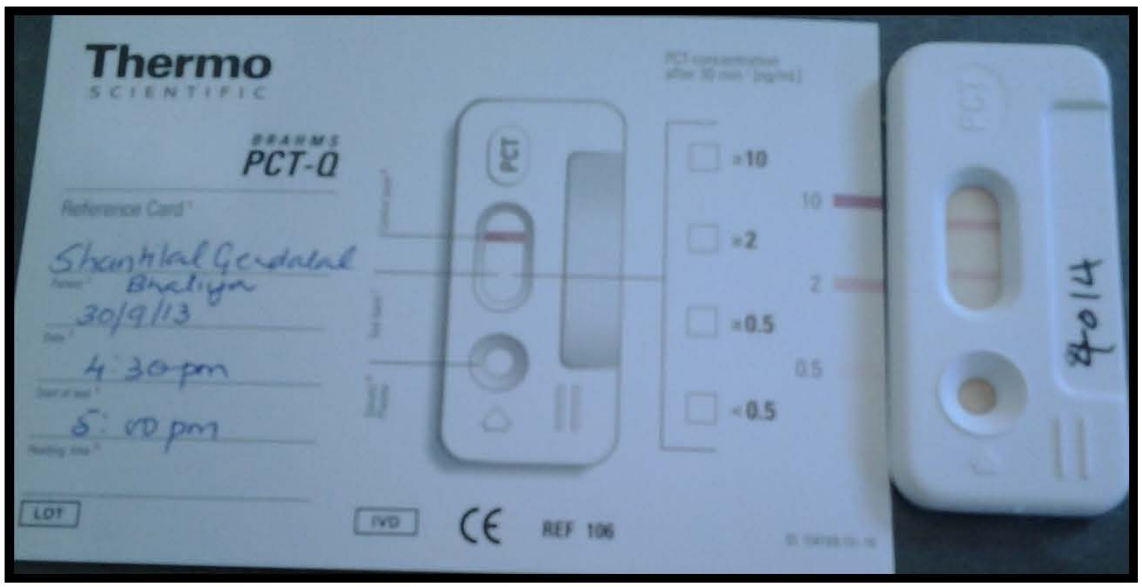
First, a 200 µl of the patient's serum sample was added, using the dropper provided in the kit, into the cavity of the card & the card was kept for 30 minutes. After 30 minutes the results were read. A coloured band is formed at "C" region and also at "T" region. The intensity of the colour of the band at "T" region is compared with colour on the reference card. The colour corresponds to the value of PCT concentrations. The interpretation of the results is shown below:

Table 4: Interpretation of PCT Levels: [63, 64 & 65]

Concentration	Interpretation	Diagnostic utility
≤ 0.05 ng/ml	Normal value	Healthy individuals
0.05-0.5 ng/ml	Local infection is possible	Without systemic signs it may be associated with low PCT levels. Also if PCT is done during early phase <6 hrs; reassessed again after 6-24 hours Low risk of progression to severe systemic infection (severe sepsis)
≥0.5 and < 2.0 ng/ml	Systemic infections/ Sepsis is possible	Moderate risk for progression to severe systemic infection (severe sepsis) The patient should be closely monitored both clinically and by re-assessing PCT within 6-24 hours
≥2.0 and < 10 ng/ml	Systemic infections/	High risk for progression to severe systemic infection (severe sepsis)

Concentration	Interpretation	Diagnostic utility
	Sepsis is possible	
≥10.0ng/ml	SIRS due to bacterial sepsis or Septic Shock	High likelihood of severe sepsis or septic shock

Figure 19: PCT Test Kit & Card



CRP (C - reactive protein): [66]

Principle:

CRP is a cytokine induced acute phase protein that increases in concentration as a result of inflammation. CRP levels in the body have been used as a marker or indicator of infections and inflammation. The assay of CRP is more sensitive than erythrocyte sedimentation rate (ESR) and the levels return to reference ranges more rapidly after the disease subsides.

The test was carried out using the kit and nephelometer (MISPA- *i*) by AGAPPE Diagnostic Ltd. This kit is latex enhanced turbidimetric immunoassay. CRP present in the patient's sample bind to anti-CRP antibodies (rabbit polyclonal antibody), which have been adsorbed to latex particles and agglutination occurs. The agglutination is directly proportional to the quantity of CRP in the sample and is measured using nephelometer. The test was carried out as per the manufacturer's instructions.

The measuring range of the assay was 0.5-320mg/L. The result was obtained in the form a value on the display screen and print out. Values of ≥ 6mg/L (as per the manufacturer's instructions) were considered significant.

Figure 20: CRP test using MISPA-*i*

Malarial Antigen Test & Peripheral Smear for Malarial Parasite: [67, 68]

Principle: [67]

Detection of malarial antigen was carried out using Accucare One Step Malarial Antigen Test kit. This test is based on the principle of immunochromatography. This kit qualitatively determines the presence of *Plasmodium falciparum* specific histidine rich protein-2 (Pf HRP-2) and pan specific lactate dehydrogenase (pLDH) antigens for *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* in human blood. The test kit contains a nitrocellulose membrane strip coated with specific monoclonal antibodies for *P. falciparum* HRP-2 at region "T1" and another monoclonal antibody that is pan specific to the lactate dehydrogenase of *Plasmodium species (P. falciparum, P. vivax, P. malariae* and *P. ovale)*.

Procedure:

For this test whole blood was collected by venipuncture using aseptic precautions in an EDTA vaccutainer before administering any anti-malarial or antibiotic. After bringing the reagents and kit to room temperature, 5µl of blood was added to a well marked "S" for sample on the test cassette using dropper provided with the kit. Then 4 drops of buffer solution (provided with the kit) were added to the well marked "A". After 20 minutes the results were read.

Observation & Interpretation:

Presence of only 1 colour band i.e. at region C: Negative

Presence of 2 colour bands i.e. first at region C and second at region 1: Positive for *P. falciparum*

Presence of 2 colour bands i.e. first at region C and second at region 2: Positive for *P. vivax* or other *Plasmodium species* **(Figure 21)**

Presence of 3 colour bands i.e. first at region C, second at region 1 and third at region 2: Positive for both *P. vivax* and *P. falciparum* **(Figure 22)**

Figure 21: Malarial Antigen test – *P. vivax*

Figure 22: Malarial Antigen test _ *P. falciparum and P. vivax*

Peripheral Smear for Malarial Parasite: [68]

Peripheral smear for detection of malarial parasite was prepared from the same blood sample which was collected for the malarial antigen test. Thick and thin blood smears were prepared and stained by Giemsa stain.

The smears were then examined under oil immersion objective lens (100X) of microscope for detecting morphological forms of malarial parasite. Thick smears were examined for presence/absence of malarial parasites and thin smears for identification of *Plasmodium* species. Smears were carefully examined for presence of ring forms of *P. falciparum* as well as *P.vivax*; gametocytes of *P.falciparum* and *P. vivax* (Figure 23, 24, 25, 26 & 27) and schizonts of *P. vivax mainly* and *P. falciparum*, if any, were found.

Figure 23: Thick Smear (Giemsa stain, 1000X) of *P. falciparum* showing ring forms and gametocyte

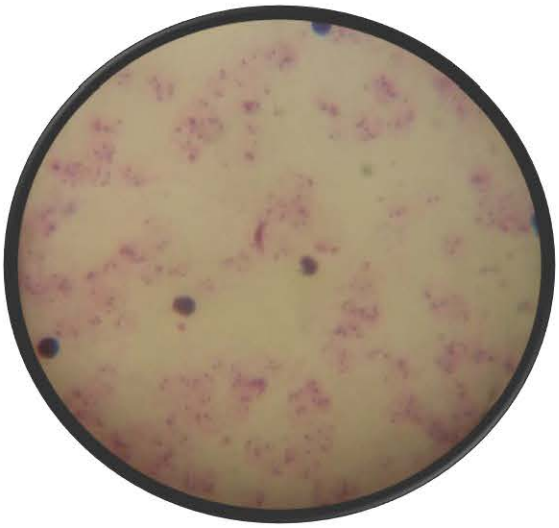


Figure 24: Thin Smear (Giemsa stain, 1000X) of *P. falciparum* showing plenty of ring forms and also accole forms and RBCs with 2 rings

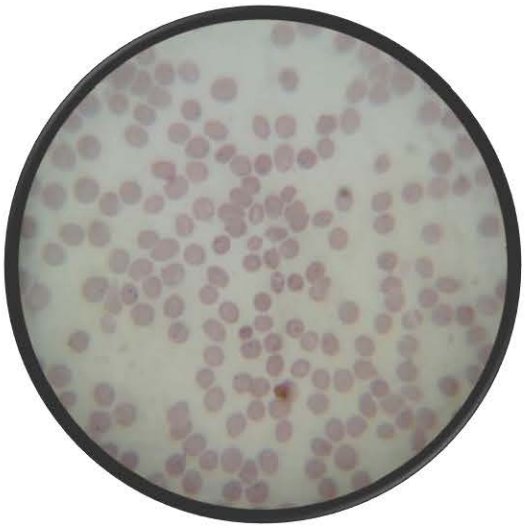


Figure 25: Thin Smear (Giemsa stain, 1000X) of *P. falciparum* showing gametocyte forms

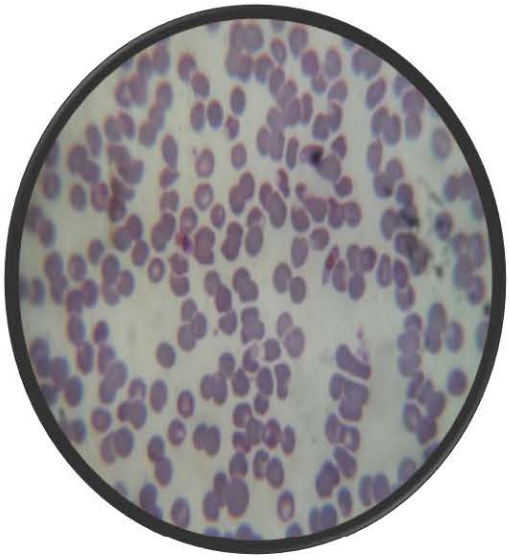


Figure 26: Thick Smear (Giemsa stain, 1000X) of *P. vivax* showing ring forms in ghost RBCs

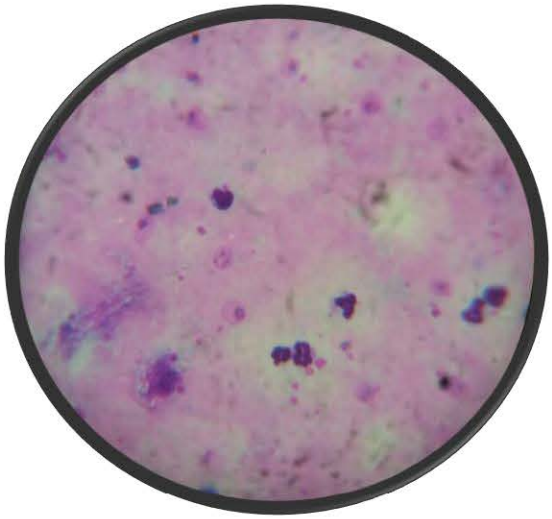
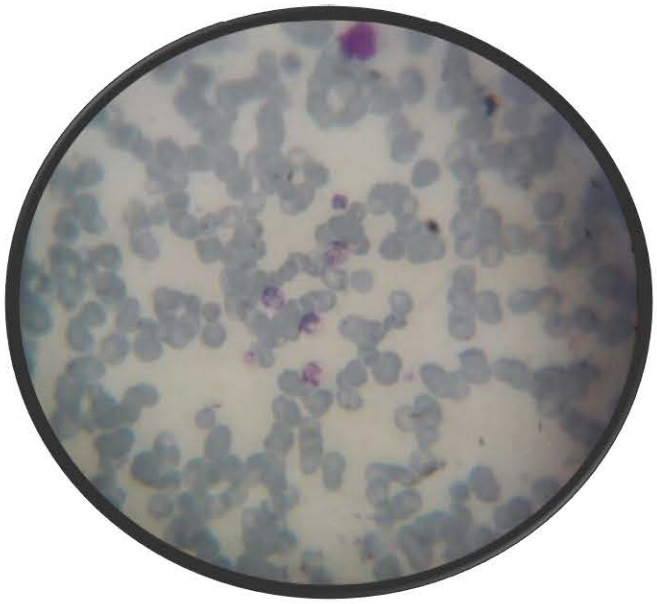


Figure 27: Thin Smear (Giemsa stain, 1000X) of *P. vivax* showing few ring forms



Dengue: [69]

Principle:

For diagnosis of Dengue infection caused by Dengue virus (types 1, 2, 3 and 4), presence of NS1 antigen and IgM/IgG antibodies were detected in patients' serum using SD BioLine Dengue Duo (SD Bio Standard Diagnostics, Inc.)

This is an immunochromatography based rapid test kit for detection of both the NS1 antigen of the virus and differential IgG/IgM antibodies to dengue virus in human serum or plasma or whole blood. NS1 is a highly conserved glycoprotein that is present at high concentrations in the sera of the dengue infected patients during the early clinical phase of the disease. It is found from the first day and up to 9 days after onset of fever in sample of primary or secondary dengue infected patients. Usually IgM does not become detectable until 5 to 10 days after onset of illness in primary dengue infection and until 4-5 days after onset of illness in secondary infections. In primary infection IgG appears on the 14^{th} day and persists for life. In secondary infections, IgG rises within 1-2 days after onset of symptoms and induce IgM response after 20 days.

Observation & Interpretation:

NS1 Ag. Result Window:

Appearance of only one colour line in "C" region: Negative Result

Appearance of two colour lines in "C" region and "T" region: Positive Result

No line on "C" region/No line in any region: Invalid Result

IgG/IgM Result Window:

Appearance of only one colour line in "C" region: Negative Result

Appearance of two colour lines, in "C" and "IgM" regions: Positive for IgM antibodies indicating primary dengue infection.

Appearance of two colour lines, in "C" and "IgG" regions: Positive for IgG antibodies indicating secondary or past dengue infection.

Appearance of three colour lines, in "C", "IgM" and "IgG" regions: Positive for both the IgM and IgG antibodies indicating late primary or early secondary dengue infection.

No colour in "C" region/No line in any region: Invalid result.

Leptospira: [70]

Principle:

For detection of antibodies against *Leptospira interrogans* in suspected patients, SD BioLine Leptospira IgG/IgM (SD Bio Standard Diagnostics Pvt. Ltd) kit was used. It is a solid phase immunochromatographic assay for the qualitative and differential detection of IgM and/or IgG antibody to *Leptospira interrogans* in human serum or plasma. It has 3 pre-coated lines, "G" (Leptospira IgG test line), "M" (Leptospira IgM Test line) and "C" (Control Line) on the surface of the strip. Add 10µl of serum or plasma in the sample well. Read the results within 15-20 minutes.

Observation & Interpretation:

Only one pink line in "C" region: Negative result

Two pink lines, in "C" and "M" regions: Positive for IgM antibodies

Two pink lines, in "C" and "G" regions: Positive for IgG antibodies

Three pink lines, in "C", "M" and "G" regions: Positive for IgM and IgG both the antibodies.

No colour line in "C" region/No colour line in any region: Invalid result

Viral Hepatitis: [71, 72, 73, 74]

Hepatitis A Virus: (HAV) [71]

For detection of IgM and IgG antibodies against Hepatitis A virus in the suspected cases of viral hepatitis, SD Bioline HAV IgG/IgM One Step Antibody Test kit was used. It is a rapid, solid phase immunochromatographic assay for the qualitative and differential detection of IgM and /or IgG antibody to Hepatitis Virus in human serum or plasma. It has 3 pre-coated lines, "G" (HAV IgG test line), "M" (HAV IgM test line) and "C" (Control Line) on the surface of the strip.

Hepatitis E Virus: (HEV) [72]

For detection antibodies to Hepatitis E virus in suspected cases of viral hepatitis, INSIGHT HEV IgM test kit (Tulip Dianostics Pvt. Ltd.) was used. It is a rapid, self performing, immunochromatographic assay for detection of IgM antibodies to Hepatitis E virus in human serum or plasma.

INSIGHT HEV-IgM is based on the principle of agglutinating sera on membrane and utilizes the technique of immunochromatography.

Hepatitis C Virus: (HCV) [73]

As a protocol for ICU admission as well as in patients suspected of acute/chronic hepatitis, HCV testing was done using HCV TRI-DOT by Diagnostic Enterprises. It is a 4th generation, rapid, visual, sensitive and qualitative diagnostic test for detection of antibodies to Hepatitis C Virus in human serum or plasma. It utilizes a combination of structural i.e. putative core antigens and non-structural NS3, NS4 and NS5 antigens of HCV.

Hepatitis B Virus: (HBV) [74]

As a protocol for ICU admission as well as in patients suspected of acute/chronic hepatitis, HBsAg testing was done using SD BIOLINE HBsAg One Step test. It is rapid, immunochromatographic, one step test for qualitative determination of HBsAg in human serum/plasma. The test cassette contains a membrane strip, which is precoated with mouse monoclonal anti-HBs-capture antibody on test band region.

HIV: [75 & 76]

According to NACO (National AIDS Control Organization) guidelines 2007, serum samples should be tested by any one of the kits approved by NACO. First by screening tests and if found positive then by other two tests based on different principles. Hence, the serum samples were first tested by 3rd Generation ELISA using ErbaLisa HIV Gen 3 from TRANSASIA BIOMEDICALS Ltd. If found reactive then they were tested with two other kits based on different principles i.e. SD BioLine HIV-1/2-3.0, a 3rd generation one step anti-HIV1/HIV 2 test from Alere Medical and Signal HIV 3D, an Immunodot test kit from ARKRAY Healthcare Pvt. Ltd. Also at times COMBAIDS kits have been used.

Other Investigations:

The reports of other investigations like CBC, ESR, LFT, RFT, etc. were recorded from patients' files and documented in the Proforma.

CBC (Complete Blood Count) was done using the Sysmex-KX-21N Automated Hematology Analyzer, a flow cytometry, from Sysmex Corporation which included the total count of white blood cells, red blood cells, platelets and haemoglobin.

ESR (Erythrocyte Sedimentation Rate) was determined by Westergren's method.

LFT (Liver Function Test) included total serum bilirubin with direct and indirect bilirubin, serum glutamic oxalacetic transaminase (SGOT) also called AST i.e. aspartate transaminase and serum glutamate pyruvate transaminase (SGPT) also called ALT i.e. alanine transaminase. RFT (Renal Function Test) included serum urea, creatinine and electrolytes i.e. sodium (Na^+), Potassium (K^+) and Chloride (Cl^-). These were done on ERBA-EM-200, a fully automated biochemistry analyzer in the biochemistry section of the Central Diagnostic laboratory in the hospital.

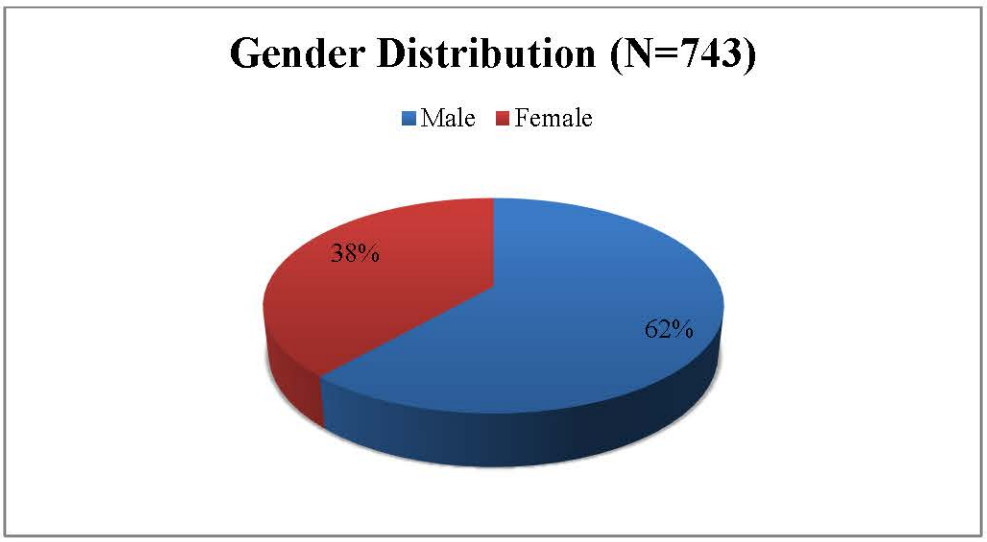
5 RESULTS

This study was carried out in the rural setup of a tropical, developing country where we see sepsis not only due to the common bacterial and fungal pathogens but also due to pathogens which are peculiar to the tropics and are usually not the common cause of sepsis in countries/regions not under the tropical region. Hence sepsis due to infections like malaria, dengue, viral hepatitis, enteric fever and leptospira was considered to be tropical sepsis whereas that with common bacteria & fungi was considered to be non-tropical sepsis.

Patients:

A total of 743 patients diagnosed clinically as sepsis were included in the study from September 2012 to December 2015 (3 years 3 months). A set of diagnostic tests were used to identify the probable causative agents of sepsis. Of the total 743 patients, 61.50% (457/743) were male and 38.49% (286/743) were female patients **(Chart 4).** The mean age of the patients was 48.02 years.

Chart 4: Gender Distribution among Sepsis Patients

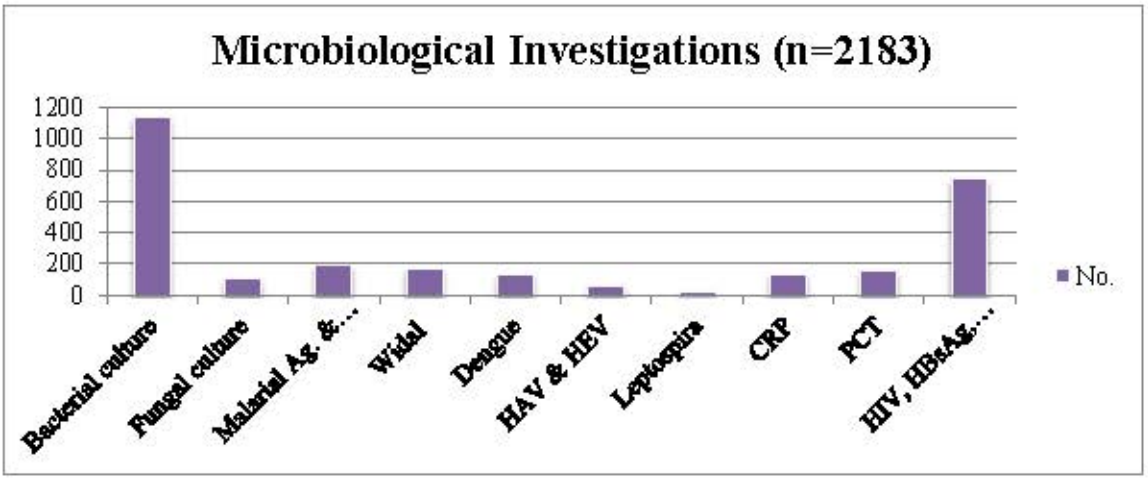


Samples:

A total of 2183 samples were processed/tested for establishing microbial etiology. A total of 1136 samples were processed for bacterial cultures which also included 103 fungal cultures. Besides culture and susceptibility testing, 199 samples were tested for malaria (Malarial Antigen & PSMP); 171 for Widal (anti-O and anti-H titres); 128 for

dengue (NS1, IgM, IgG); 59 for viral hepatitis i.e. HAV & HEV (IgM & IgG) and 19 for leptospira. Also the tests were carried out for CRP (C-reactive protein) in 183 and PCT (procalcitonin) in 288 samples. In addition to the above 2183 samples, all the 743 patients were tested, for HIV, HBsAg and HCV, as per the protocol of ICU admission. Moreover, the results for CBC, ESR, RFT and LFT were noted down. **(Chart 5)**

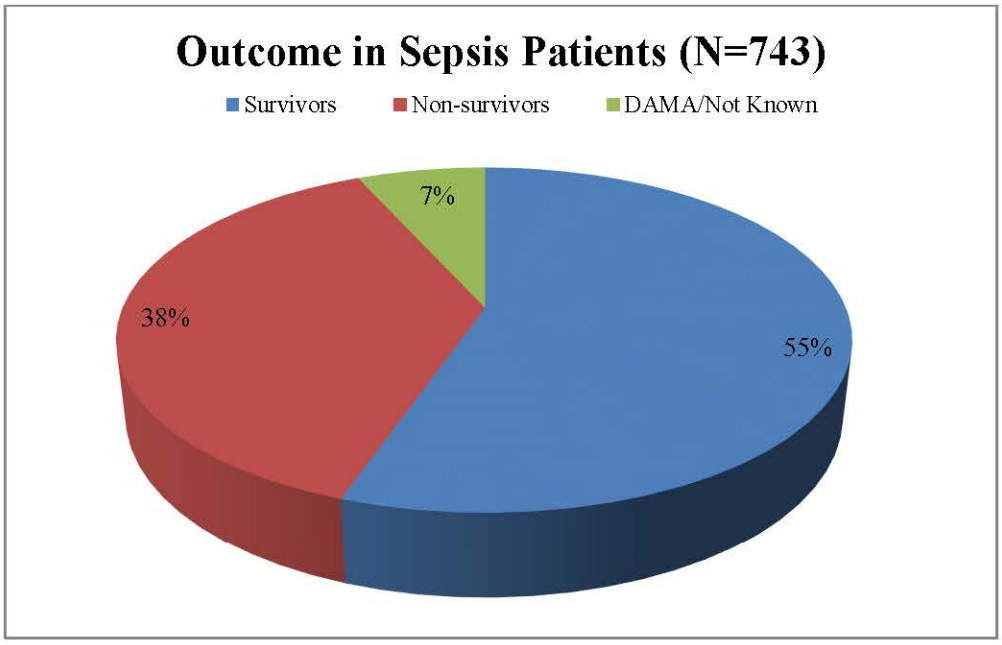
Chart5: Total Number of Microbiological Investigations done/tested/processed (n=2183)



Outcome:

A total of 55.31% (411/743) patients survived the episode of sepsis whereas 38.22% (284/743) could not survive i.e. non-survivors and the outcome for 6.46% (48/743) of the patients was not known as they took discharge from the hospital against the medical advice (DAMA) or were referred to other/higher centres for further care **(Chart 6)**. The average age of non-survivors was 50.86 years and the majority i.e. 23.05% belonged equally to the age group of 51years to 60 years and 61 years to 70 years i.e. 65 each in both the age groups. The average of the survivors was 46.05 years.

Chart 6: Outcome in Sepsis Patients (N=743)



It was also noted that the mortality was significantly higher in patients with unidentified etiology as compared to those having bacterial/fungal infections and tropical infections. (p=0.005)

Microbial Profile:

The microbial etiology could be identified and established in 599/743 (81%) patients and in 144/743 (19%) no pathogen could be established as a cause of sepsis. Of the total 599, 514 had monomicrobial cause whereas 85 had polymicrobial cause. Thus a total of 47% (349/743) patients had sepsis due to bacteria and/or fungi also referred here as non-tropical, followed by 34% (250/743) due to tropical (malaria, dengue, enteric fever, viral hepatitis and leptospira) infections and the rest 19% (144/743) had sepsis due to unidentified microbial aetiology. **(Chart 7 & 8)**

Chart 7: Microbial Profile of Sepsis (N=743)

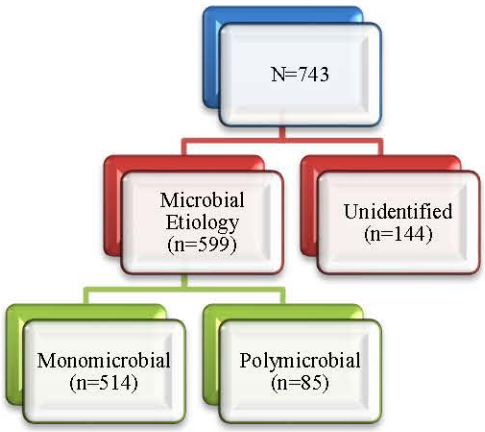
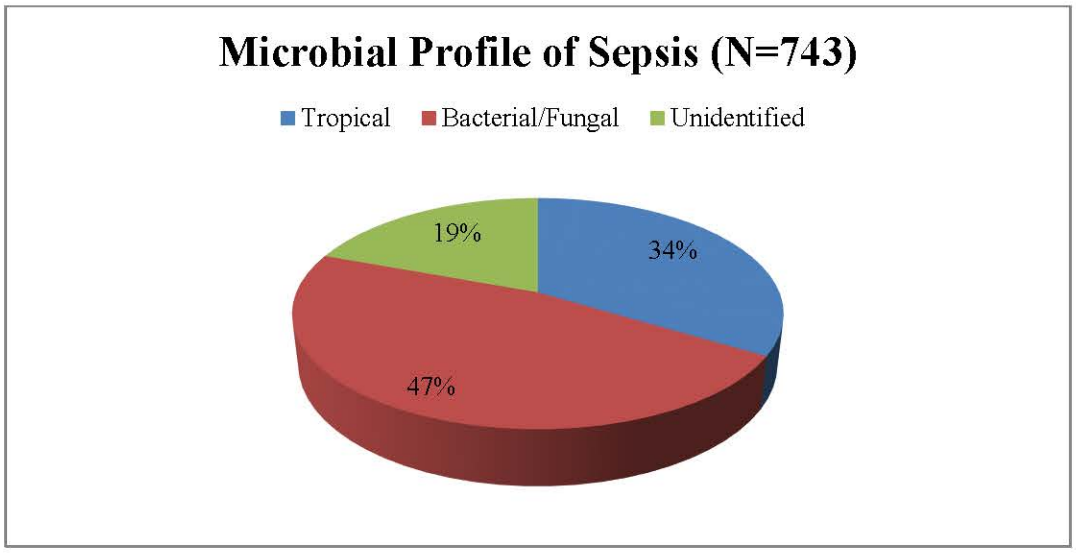


Chart 8: Microbial Profile of Sepsis (N=743)



From a total of 743 patients 38.09% (283/743) patients had only bacteria as a cause of sepsis, 16.41% (122/743) had only malaria, 7.80% (58/743) had only dengue, 3.10% (23/743) had only fungal, 2.29% (17/743) had only viral hepatitis, 1.48% (11/743) had only enteric fever whereas the rest 85 had more than one microbial etiology as shown in **(Chart 9 & 10)**.

Chart 9: Mono-Microbial Etiology amongst Sepsis Patients (n=514)

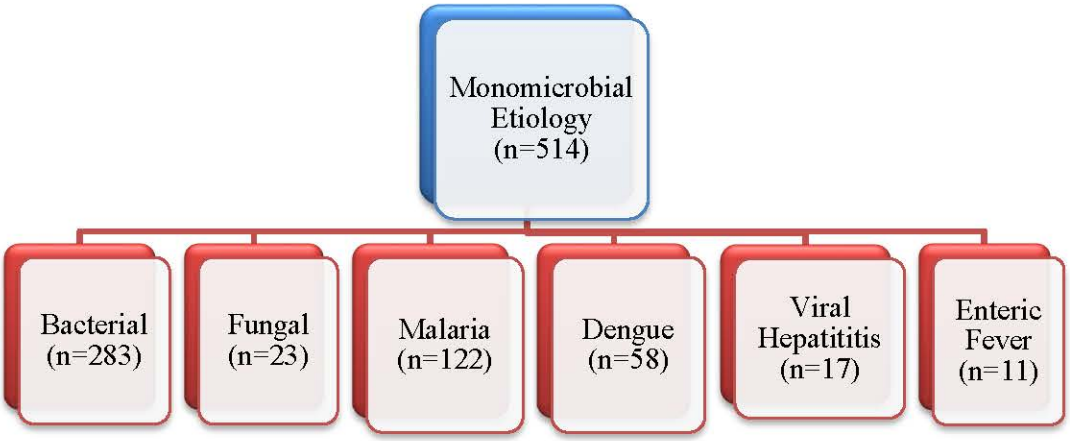
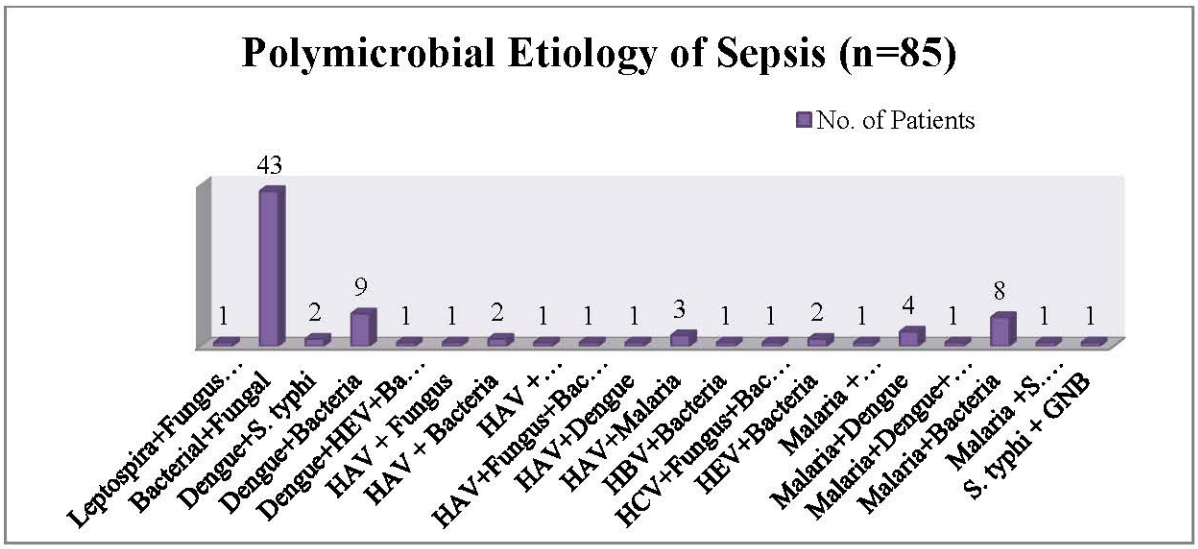


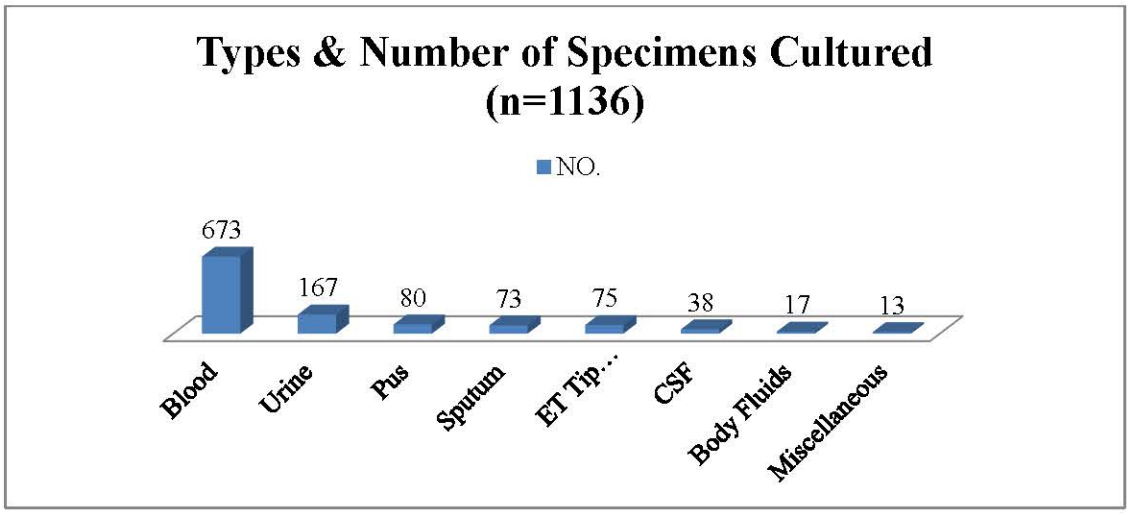
Chart 10: Polymicrobial Etiology Amongst Sepsis Patients (n=85)



A total of 1136 different samples were cultured for isolation and identification of bacterial & fungal isolates from 619 patients. These included 673 blood samples followed by 167 urine, 80 pus, 73 sputum, 75 ET Tip/ET Secretion, 38 CSF, 17 body fluids (Ascitic fluid, Pleural fluid, peritoneal fluid and broncheoalveolar lavage) and

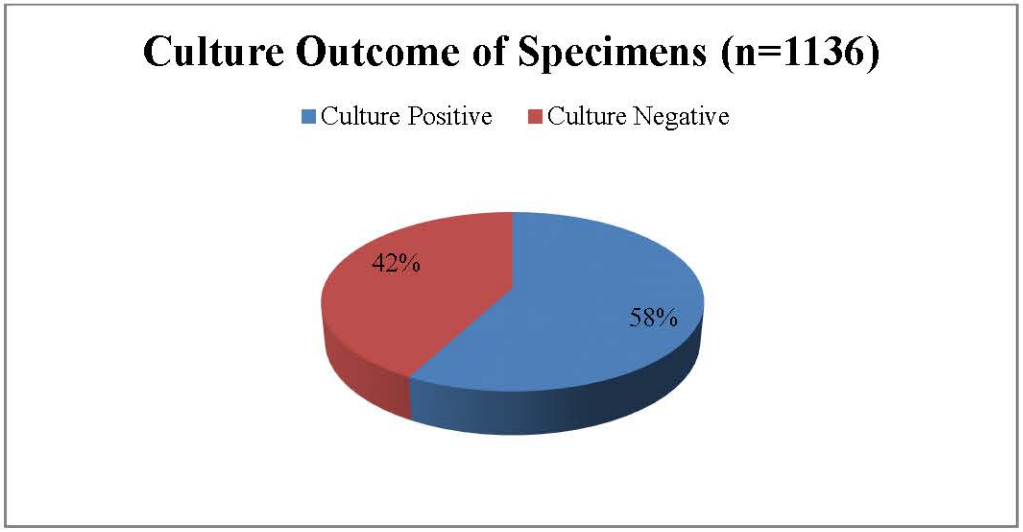
13 miscellaneous samples which included IV flow tip, central line tips, mitral valve, stool, nasal swabs, throat swabs and vitreous fluid as shown in the **(Chart 11)**.

Chart 11: Types & Number of Specimens Cultured (n=1136)



A total 57.74% (656/1136) samples were positive for cultures yielding 701 isolates whereas 42.25% (480/1136) samples did not yield any bacterial or fungal isolate i.e. they were culture negative **(Chart 12)**.

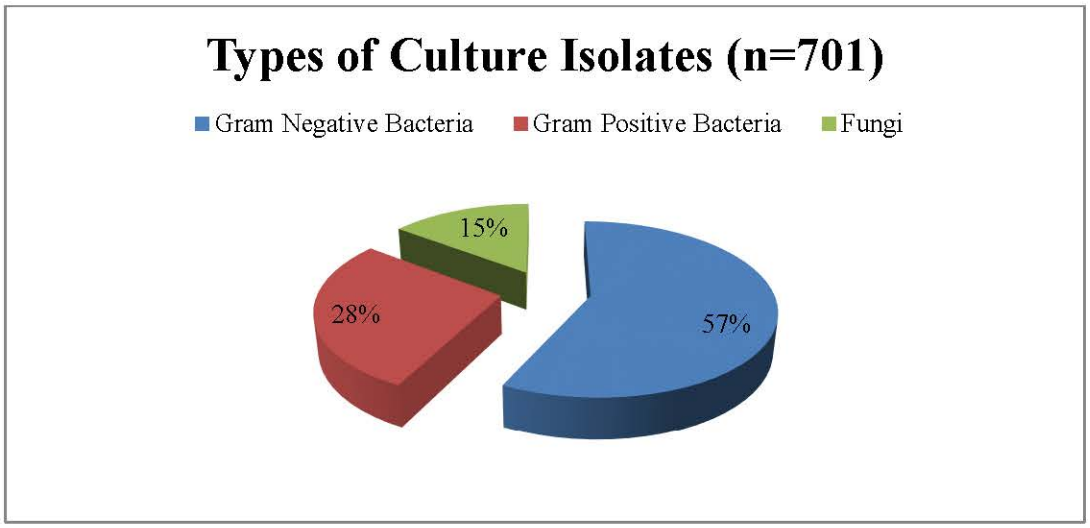
Chart 12: The Culture Outcome of Samples (n=1136)



A total of 598 bacteria [402(57%) + 196 (28%)] and 103 fungi were isolated making a total of 701 isolates from 657 cultures positive specimens. Of the 598 bacterial

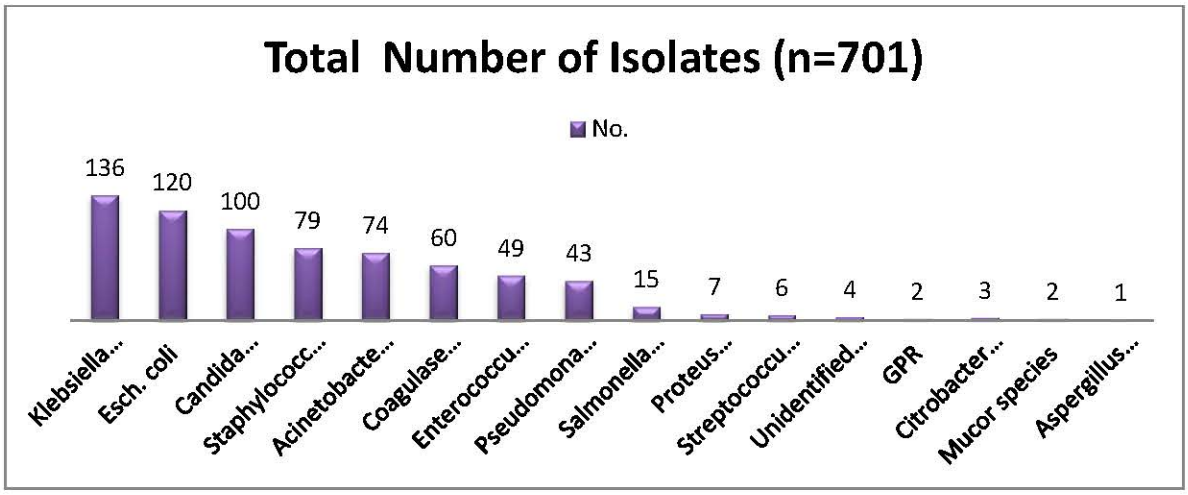
isolates, 67.22% (402/598) were Gram negative bacilli and 32.77% (196/598) were Gram positive bacteria **(Chart 13)**.

Chart 13: Types of Culture Isolates (n=701)



The total number of each type of bacterial and fungal isolates is shown below **(Chart 14)**:

Chart 14: Total Number & Type of Isolates (n=701)



As shown (in the **Chart 14**) above the most common isolate was *Klebsiella species* (n=136) followed by *E. coli* (n=120), *Candida species* (n=100), *Staphylcoccus aureus* (n=79), *Acinetobacter species* (n=74), *Coagulase Negative Staphylococcus* i.e. *CoNS* (n=60), *Enterococcus species* (n=49), *Pseudmonas species* (n=43) and *Salmonella*

species (n=15); these been the major isolates with a smaller number of other isolates like *Proteus species* (n=7), *Streptococcus pyogenes* (n=6), *Citrobacter freundii* (n=3), unidentified GNB (n=4), Gram positive bacilli (n=2), *Mucor species* (n=2) and *Aspergillus niger* (n=1).

Antibiotic Resistance Pattern:

The overall antibiotic resistance pattern of the major Gram negative isolates, excluding *Pseudomonas species* and unidentified GNB is shown below in **Table 5 & Chart 15,** that of *Pseudomonas spp.* in **Chart 16** while that of Gram positive cocci is shown in **Chart 17 & Chart 18** below.

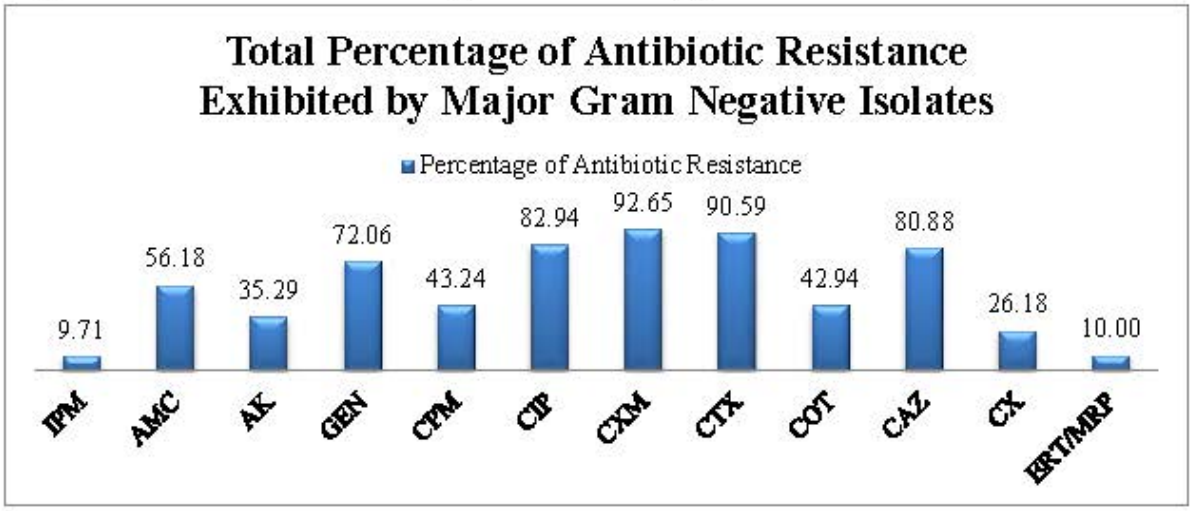
As shown below (in the **Table 5**), least resistance was seen against Imipenem and Ertapenem/Meropenem. Amongst all Gram negative bacilli, *Acinetobacter species* showed the maximum resistance to Imipenem i.e. 19% and 20% to Ertapenem/Meropenem. A slightly higher resistance was observed against Cefipime i.e. about 57.35%, 30.83% and 43.24% by *Klebsiella species*, *E. coli* and *Acinetobacter species* respectively. On the other hand a very high resistance was exhibited against Cefuroxime, Cefotaxime and Ceftazidime by *Klebsiella spp.* (92.65%, 93.38% and 94.12%), *E. coli* (92.50%, 90.83% and 77.50%) and *Acinetobacter spp.* (97.30%, 93.24% and 68.98). Overall, *Salmonella spp.* showed least resistance against most of the antibiotics while *Citrobacter freundii* exhibited a slightly higher resistance as compared to *Salmonella spp.*

Table 5: Antibiotic Resistance Pattern of Major Gram Negative Bacilli (excluding *Pseudomonas spp.*, & Uidentified GNB): (Expressed in percentage)

Isolate (n=355)	IPM	AMC	AK	GEN	CPM	CIP	CXM	CTX	COT	CAZ
Klebsiella spp. (n=136)	8.09	77.94	50.74	86.03	57.35	83.09	92.65	93.38	62.50	94.12
E. coli (n=120)	6.67	42.50	24.17	62.50	30.83	87.50	92.50	90.83	30.00	77.50
Acinetobacter spp. (74)	18.92	45.95	29.73	70.27	43.24	81.08	97.30	93.24	32.43	68.92
Citrobacter freundii (n=3)	0	0	0	0	0	33.33	33.33	33.33	33.33	33.33
Proteus	0	0	0	14.2	0	42.8	71.42	28.5	-	28.57

species (n=7)				8		5	8	7		
Salmonella spp.	0	6.66	0	0	0	6.66	20	0	0	0

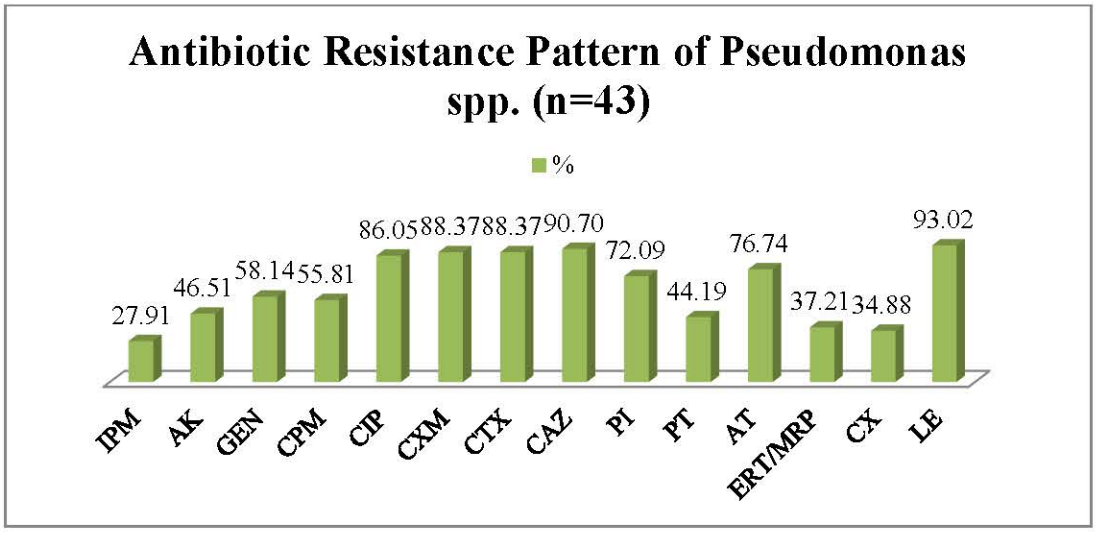
Chart 15: Total Percentage of Antibiotic Resistance Exhibited by Major Gram Negative Bacilli (n=355) excluding *Pseudomonas spp.* & Unidentified GNB



As shown in **(Chart 15)** above, an overall resistance of 92.65%, 90.59%, 82.94%, 80.88%, 72.06%, 56.18%, 43.24%, 42.94%, 35.29%, 26.18%, 10% and 9.71% was shown majority of gram negative isolates, against Cefuroxime, Cefotaxime, Ciprofloxacin, Ceftazidime, Gentamicin, Amoxycillin-clavulanic acid, Cefepime, Cotrimoxazole, Amikacin, Cefoxitin, Ertapenem/Meropenem and Imipenem respectively.

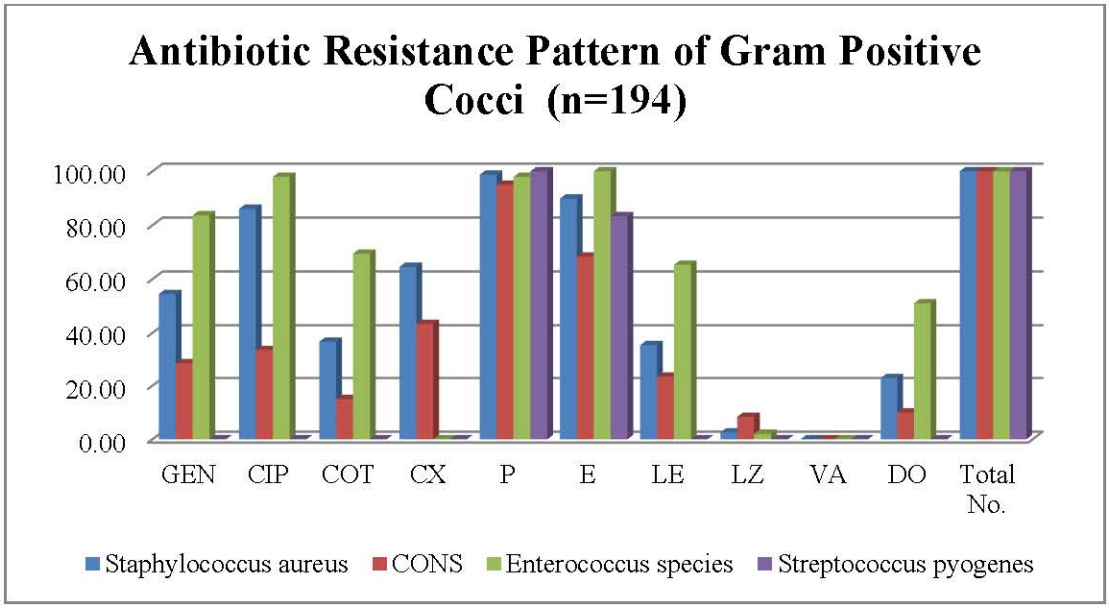
The antibiotic resistance pattern of *Pseudomonas spp.* **(Chart 16)** though showed least resistance towards Imipenem (27.91%) and Ertapenem/Meropenem (37.21%) as compared to other antibiotics but higher as compared to all the other gram negative bacilli including *Acinetobacter spp.* It showed a higher resistance to levofloxacin and ceftazidime followed by equal percentages to cefuroxime and cefotaxamie and then ciprofloxacin with a lesser resistance to Amikacin, Gentamicin and Cefipime.

Chart 16: Antibiotic Resistance Pattern of *Pseudomonas spp.*



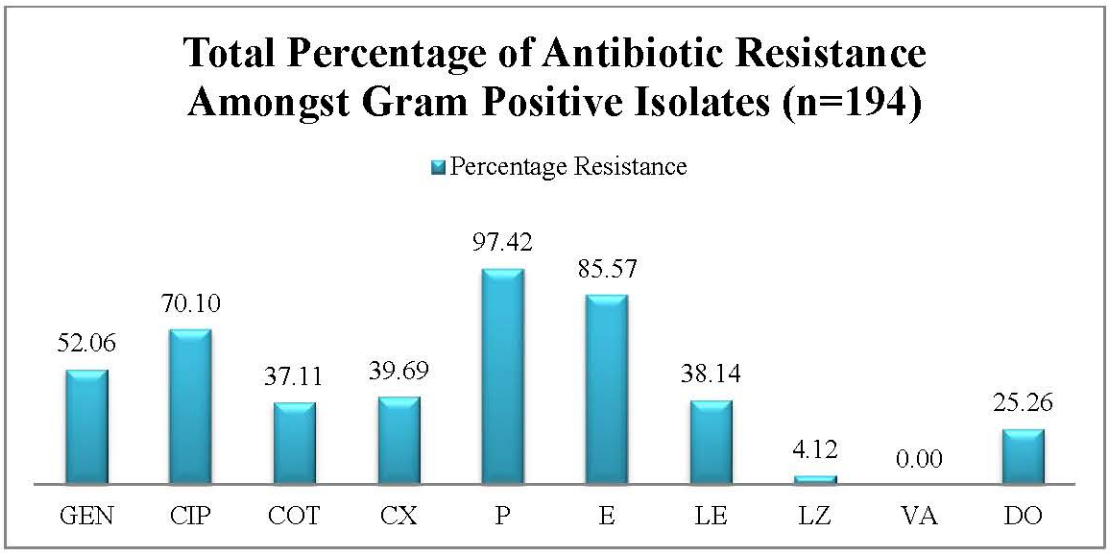
No resistance was observed against Vancomycin i.e. all the isolates were completely susceptible to Vancomycin, whereas against Linezolid - 2%, 2.5% and 8.33% resistance was shown by *Enterococcus species*, *S. aureus* and *CoNS* respectively. A slightly higher resistance was observed against Doxycycline i.e. 22.78%, 10% and 51% by *S.aureus*, *CONS* and *Enterococcus species* respectively as shown below (in the **Chart 17**) excluding 2 Gram positive bacilli.

Chart 17: Resistance Pattern of Gram Positive Cocci (n=194)



A very high percentage of resistance was seen against Penicillin (almost 98% to 100%) and Eryhtromycin (68% to 100%) by almost all the isolates. Thus, a total of 97.42%, 85.57%, 70.10%, 52.06%, 38.14%, 37.11%, 25.26%, 4.12% and 0% resistance were exhibited against Penicillin, Erythromycin, Ciprofloxacin, Gentamicin, Levofloxacin, Doxycycline, Linezolid and Vancomycin respectively by total 194 Gram positive cocci isolates **(Chart 18)**

Chart 18: Total Percentage of Antibiotic Resistance Exhibited by Gram Positive Cocci



Blood Culture:

A total of 673 blood cultures were performed from 619 patients and for the rest 124 patients, in whom one or more causes of tropical infections had been established, none of the cultures were performed. Of the 619 patients single blood cultures were done in 570 patients, while in 44 patients 2 blood specimens were cultured and for 5 patients 3 blood specimens were cultured. Of the total 619 patients, blood cultures of 49.16% (308/619) patients did not show any growth whereas 50.24% (311/619) patients showed growth. Of the 311 patients, 275 with single blood cultures showed the growth whereas the rest 36 showed growth in one or more samples.

Of the total 673 blood samples cultured, 50.96% (343/673) samples did not yield any bacterial/fungal growth whereas the rest 49.03% (330/673) showed growth **(Chart 19)**. A total of 339 isolates were obtained from 330 (49.18%) blood culture positive

samples. The majority samples had single bacterial isolate i.e. 292 (88.48%) whereas 12 (3.64%) had polymicrobial i.e.>1 type of bacteria or bacteria plus fungus together from a single sample and 26 (7.90%) samples had only fungal isolates **(Chart 20).**

Chart 19: Culture Outcome of Blood Specimens (n=673)

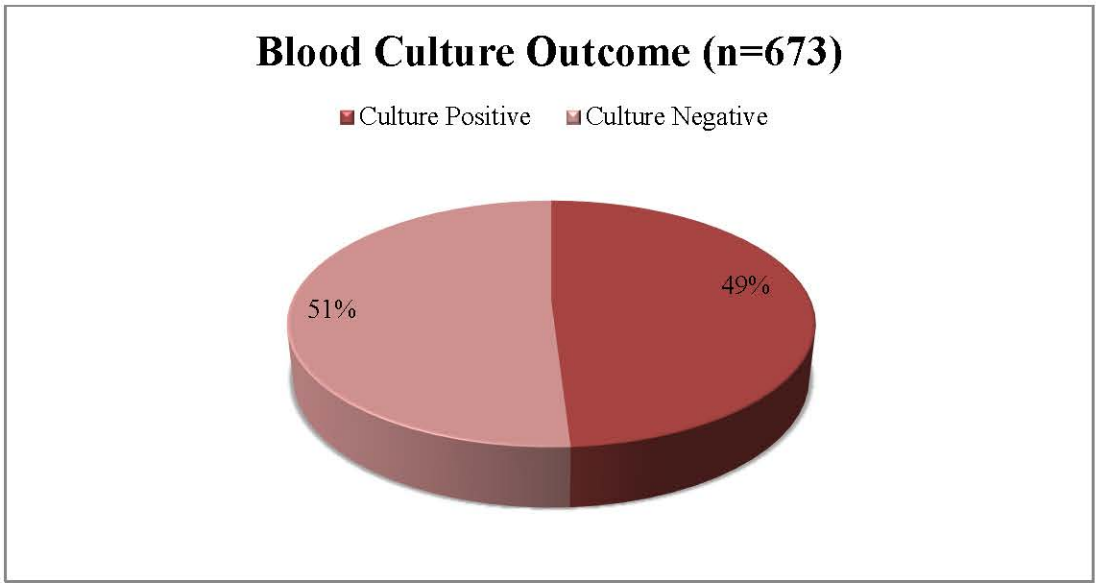
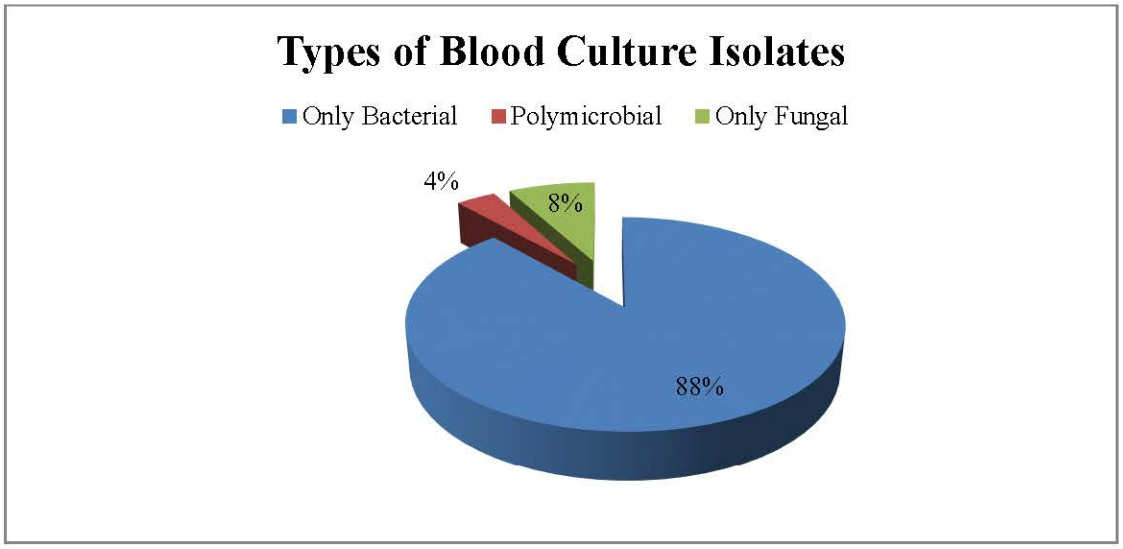
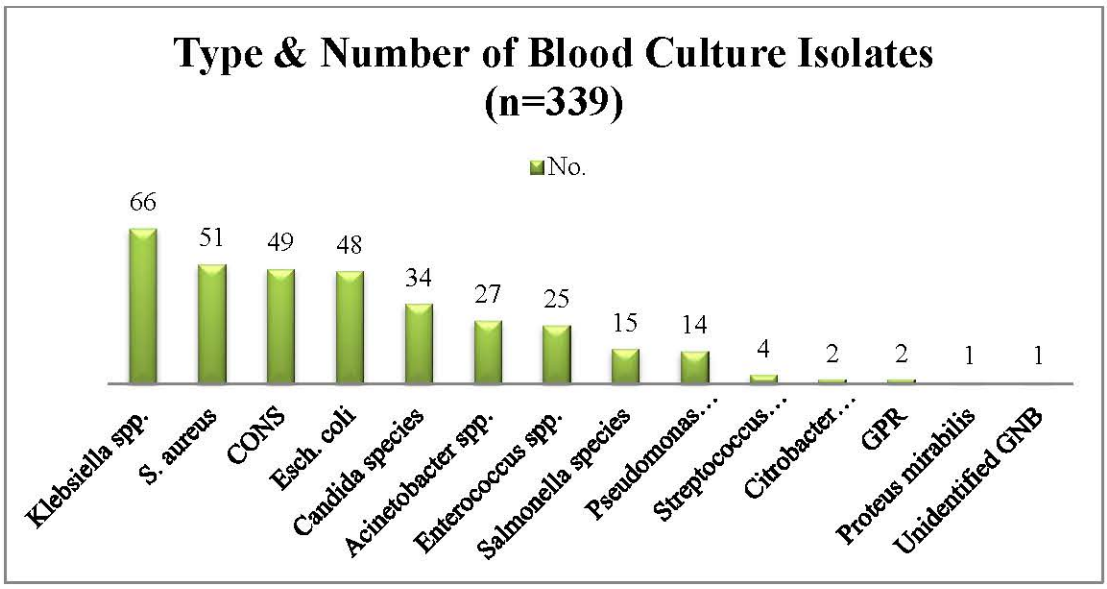


Chart 20: Types of Isolates obtained from Blood Culture Positive Specimens (n=330)



The following **Chart 21** shows number of isolates (n=339) and its types obtained from blood culture positive specimens (n= 330).

Chart 21: Number & Type of Isolates (n=339) Obtained from Blood Culture Positive Specimens (n=330)



Of the 339 isolates, 51.32% (174/339) were Gram negative bacilli, 38.64% (131/339) were Gram positive bacteria and 10.02% (34/339) *Candida species*. As shown (in the **(Chart 21)** predominantly Gram negative bacteria were isolated. In our study *Klebseilla spp.* - 19.46% (66/339) was the most frequent isolate from blood culture followed by *Staphyloccoccus aureus* - 15.04% (51/339), *CoNS* - 14.45% (49/339), *E. coli* 14.15% (48/339), *Candida species* 10.02% (34/339), *Enterococcus species*

7.37% (25/339), *Acinetobacter species* 7.96% (27/339), *Pseudomonas spp.* 4.13% (14/339) and a small percentage of each of *Streptoccus pyogenes* (4/339), *Citrobacter freundii* (2/339), *Proteus spp.* (n=1), an unidentified GNB (1/339) and contaminant GPR (2/339) as shown in the chart below. The blood culture isolates also included 15 *Salmonella species* contributing to 4.42% of the total number of blood culture isolates.

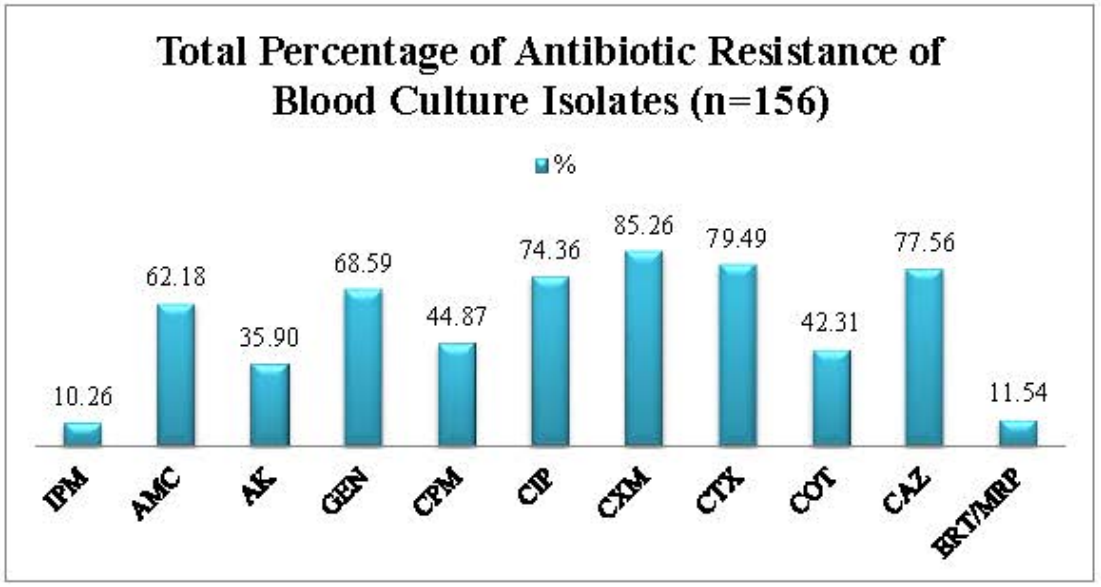
The antibiotic resistance pattern of the major gram negative bacilli isolated from blood excluding *Pseudomonas species*, *Proteus species*, *Citrobacter freundii* and unidentified GNB is shown (in the **Table 6 & Chart 22**) below i.e. (n=174-18=156). Least resistance was observed against cabapenems i.e. 21.8% with 10.26% against Imipenem and 11.54% against Ertapenem & Meropenem with *Acinetobacter spp.* showing the highest resistance i.e. 29.63% whereas the most was seen against Cefuroxime, Cefotaxime and Ceftazidime.

Table 6: Antibiotic Resistance Pattern of Major Gram Negative Isolates of Blood (%):

Blood Cultures Isolates (n=174-18=156)	IPM	AMC	AK	GEN	CPM	CIP	CXM	CTX	COT	CAZ	CX	ERT/MRP
Klebsiella spp. (n=66)	7.58	89.39	57.58	84.85	65.15	78.79	93.94	92.42	69.70	93.94	22.73	9.09
E. coli (n=48)	6.25	45.83	22.92	64.58	31.25	83.33	87.50	85.42	20.83	79.17	37.50	6.25
Acinetobacter spp. (n=27)	29.63	55.56	25.93	74.07	44.44	85.19	96.30	81.48	37.04	77.78	40.74	33.33
Salmonella species (n=15)	0.00	6.67	0.00	0.00	0.00	6.67	20.00	0.00	0.00	0.00	0.00	0.00

A total of 89.39%, 55.56% and 45.83% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance to amoxycilin-clavulanic acid. A total of 57.58%, 25.93% and 22.92% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance against Amikacin. Moreover, 93.94%, 96.30% and 87.50% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance to Cefuroxime and almost a similar percentage of resistance against Cefotaxime.

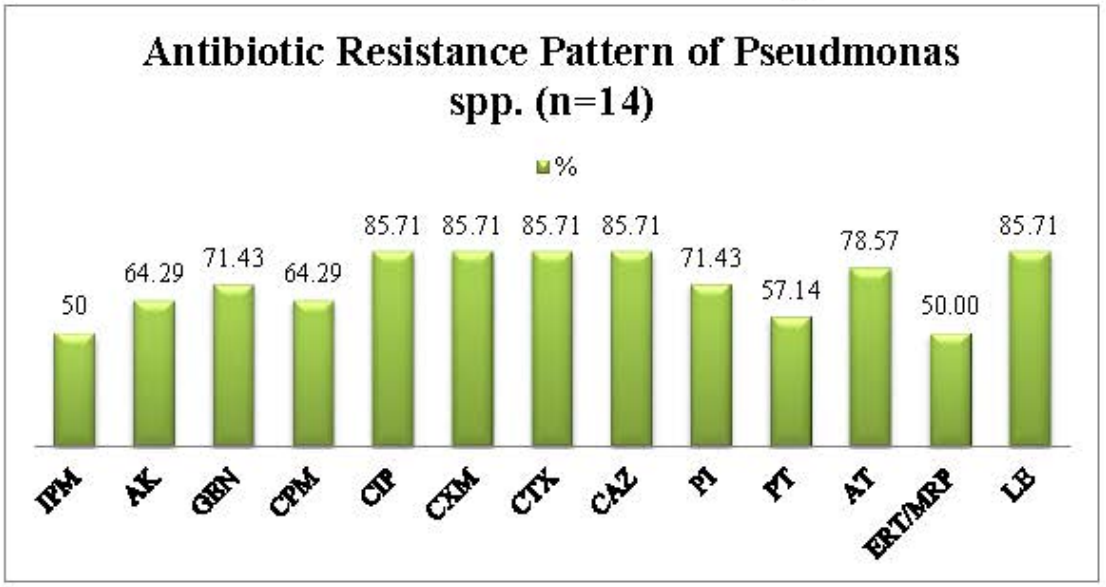
Chart 22: Total Percentage of Resistance Exhibited by Major Gram Negative Bacilli:



Thus overall, least resistance was noted against imipenem (10.26%) and ertapenem/meropenem (11.54%) whereas highest was seen against cefuroxime (85.26%) followed by cefotaxime (79.49%), ceftazidime (77.56%), ciprofloxacin (74.36%) and gentamicin (68.59%) as shown **(in the Chart 22)** above.

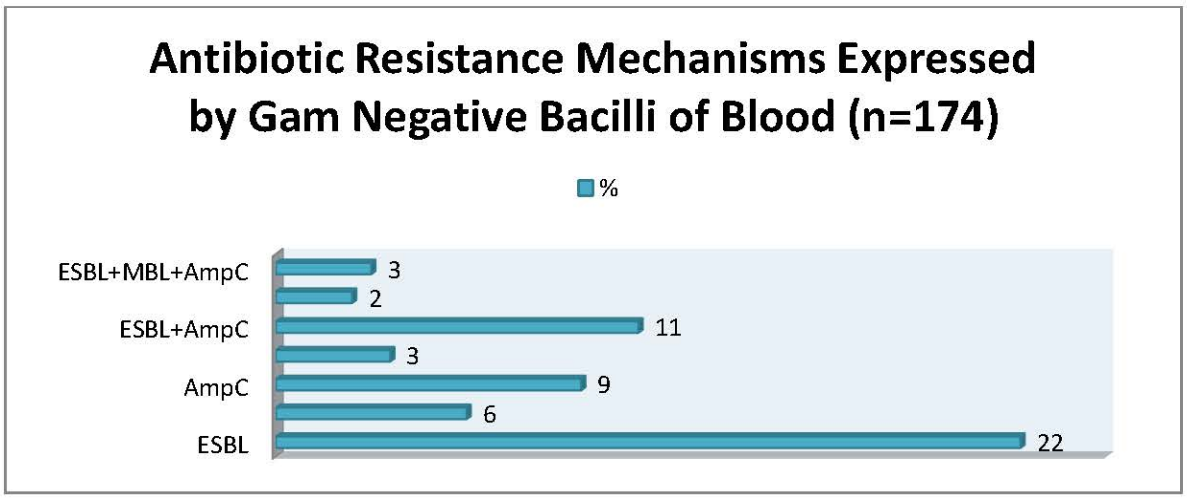
Pseudomonas spp. from blood showed much higher resistance to most of the antibiotics as compared to all other gram negative bacilli with as high as 50% resistance to Imipenem and Ertapenem/Meropenem both as shown **(in the Chart 23)** below.

Chart 23: Antibiotic Resistance Pattern of *Pseudomonas spp.* (n=14)



Of the total 174 Gram negative bacilli, 22.41% (39/174) isolates showed only ESBL production as a major form of mechanism resistance to which 56.41% (22/39) was contributed by *Klebsiella spp.*, followed by *E. coli* – 33.33% (13/39) and smaller percentage of each of 5.12% (2/39) *Acinetobacter spp.* and *Pseudomonas spp* and only AmpC-Beta lactamase 9.19% (16/174) was next in the sequence with *E. coli* been the major contributor followed by *Acinetobacter spp.* and finally only MBL production 5.74% (10/174) with maximum number of *Acinetobacter spp.* (40%). A combination of 2 or more mechanisms was also seen as shown **(in the Chart 24)** below.

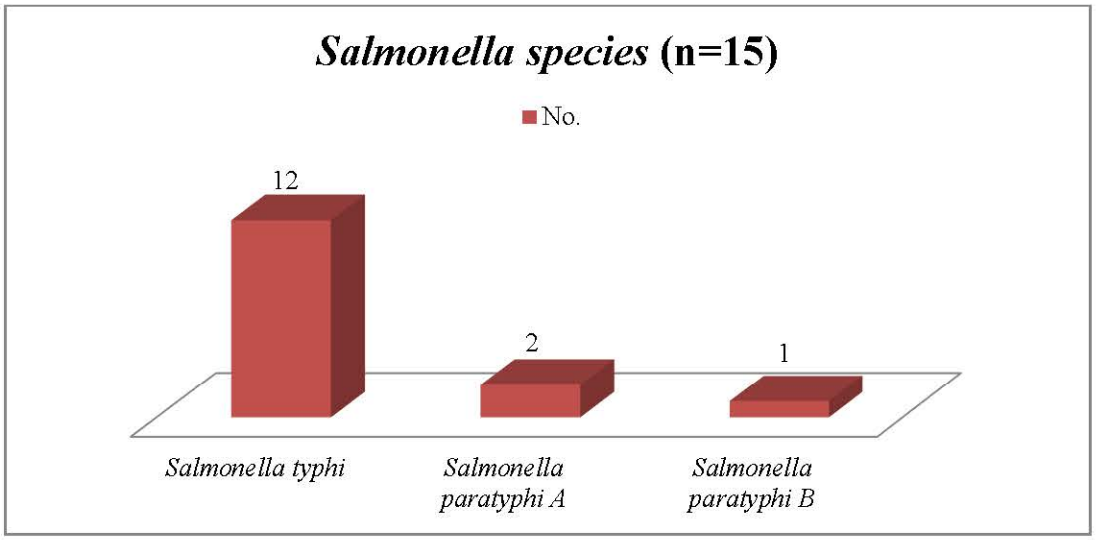
Chart 24: Antibiotic Resistance Mechanisms Expressed by Gram Negative Bacilli of Blood (n=174)



Enteric Fever:

Of the 673 blood samples cultured, 2.22% (15/673) samples yielded *Salmonella species.* It contributed to 4.42% (15/339) of the total blood culture isolates. However, only *Salmonella species* as a cause of sepsis was found in 1.48% (11/743) patients. The distribution of *Salmonella species* is shown (in **Chart 25)**

Chart 25: Distribution of *Salmonella species* (n=15)



Thus, twelve isolates of *Salmonella typhi* and one each of *Salmonella paratyphi A* and *Salmonella paratyphi B* were isolated. Of these 15 patients, 2 had concomitant dengue, 1 had *P.falciparum* malaria and 1 had *E.coli* as the 2nd isolate. Two patients did not survive the episode of enteric fever as one was complicated with concurrent dengue and other with *P.falciparum* malaria; 12 survived and one patient took discharge against medical advice.

The *Salmonella* species *showed* 100% susceptibility to Imipenem, Ertapenem/Meropenem, , Gentamicin, Amikacin, Cefotaxime, Ceftazidime, Cefoxitin and Cefepime; but exhibited 20% resistance towards Cefurroxime and 6.66% resistance to Amoxycillin-Clavulanic acid and Ciprofloxacin each as shown in **(Table 6)** above.

A total of 38.64% (131/339) isolates were Gram positive bacteria including 2 Gram positive bacilli. Thus a total of 38.05% (129/339) were Gram positive cocci and of which 39.53% (51/129) were *S. aureus*, 37.98% (49/129) *CoNS*, 19.37% (25/129) *Enterococcus species* and 3.1% (4/129) were *Streptococcus pyogenes*. As shown (in the **Chart 26**) the maximum resistance was observed against Penicillin and Erythromicin whereas least was against Linezolid and Doxycycline. All the isolates showed 100% susceptibility to Vancomycin. Moreover, the maximum resistance against most of the antibiotics was shown by *Enterococcus species* isolates. Total of 52.94% (27/51) *S. aureus* were found to be *MRSA* and 34.69% (17/49) were found to

be *MRCONS*. The antibiotic resistance pattern of the gram positive culture isolates of blood is as shown below (in the **Chart 26)**

Chart 26: Antibiotic Resistance Pattern of Gram Positive Cocci from Blood Culture

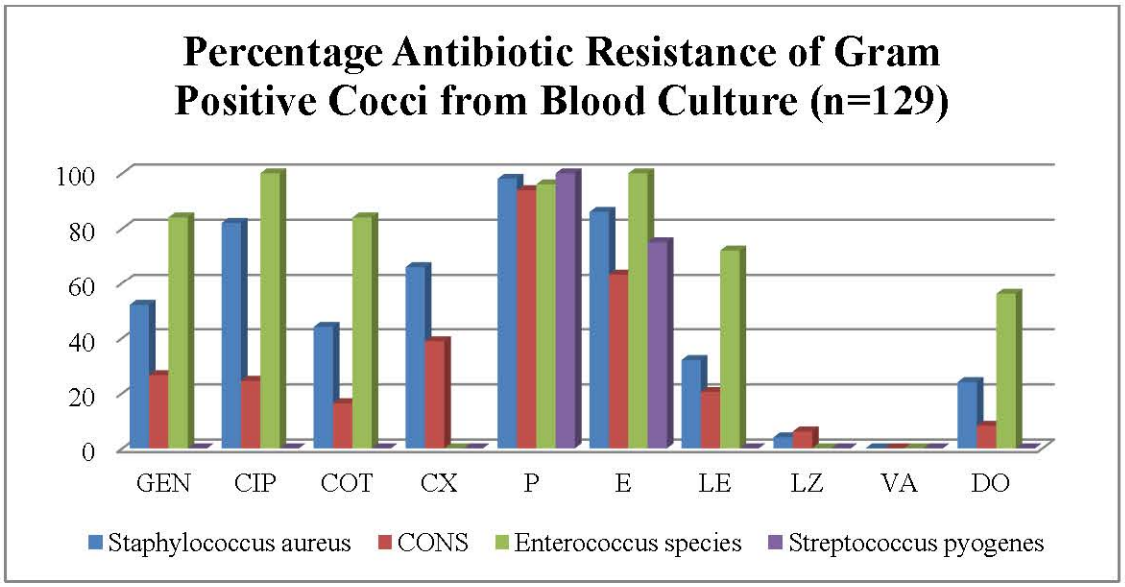
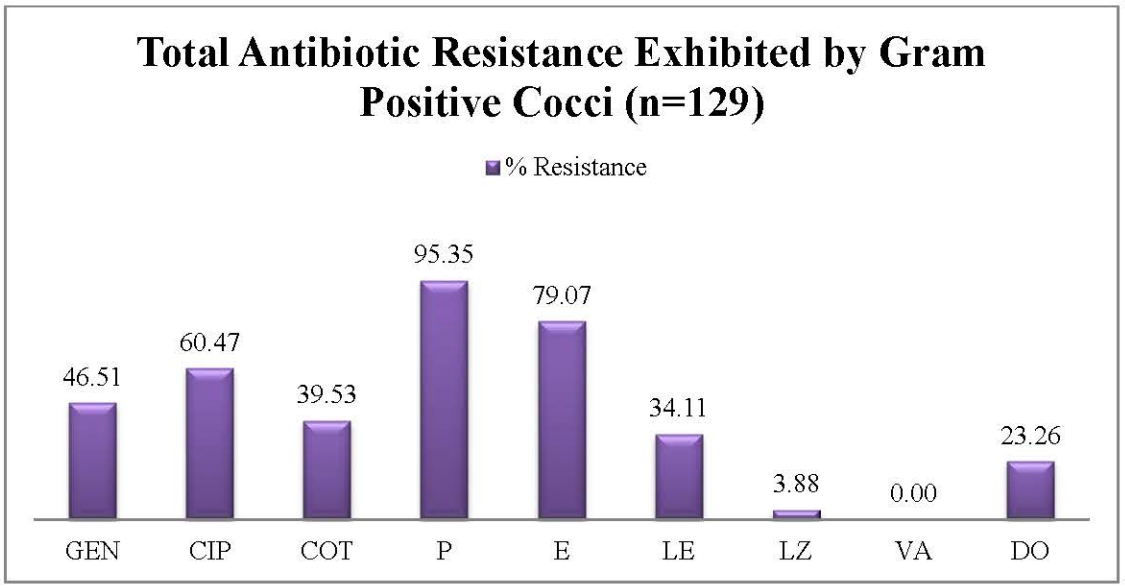


Chart 27: Total Percentage of Antibiotic Resistance Exhibited by Gram positive cocci (n=129)



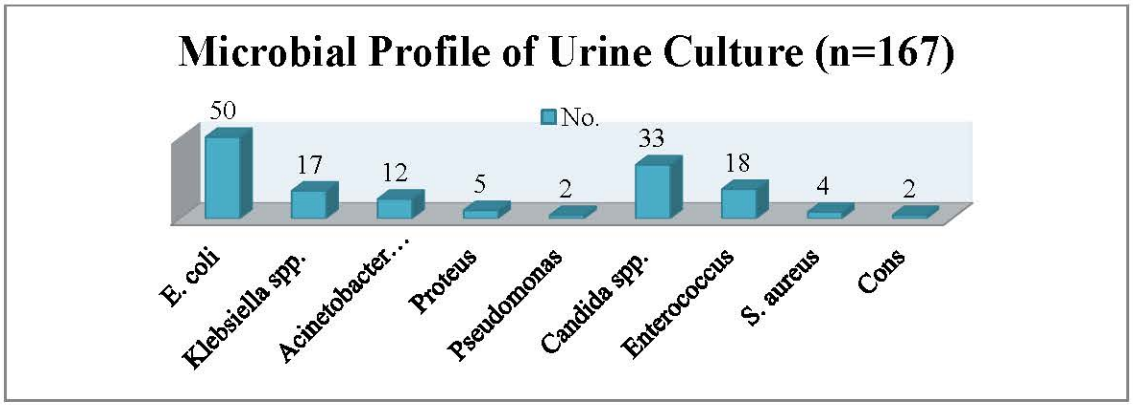
The overall resistance against antibiotics tested for gram positive cocci reveals 0% resistance towards vancomycin and 95.35% towards penicillin with varying resistance towards other antibiotics tested as shown **(in the Chart 27)** above.

Urine Culture:

A total of 167 urine samples were cultured from 143 patients with suspected urinary tract infections or it as source of infection leading to sepsis; 122 patients with single specimens, 18 with 2 and 3 patients with 3 specimens. Growth was observed in 134/167 (80.24%) samples yielding 143 isolates and in the remaining 33 (19.76) there was no growth. Of the total 143 isolates, 86 (60.13%) were Gram negative bacilli, 33 (23.07%) *Candida species* and 24 (16.78%) were Gram positive cocci. The most common isolate was *E. coli* (50/143) followed by *Enterococcus species* (18/143), *Klebsiella species* (17/143), *Acinetobacter species* (12/143) and also other isolate types as shown in the **(Chart 28)**.

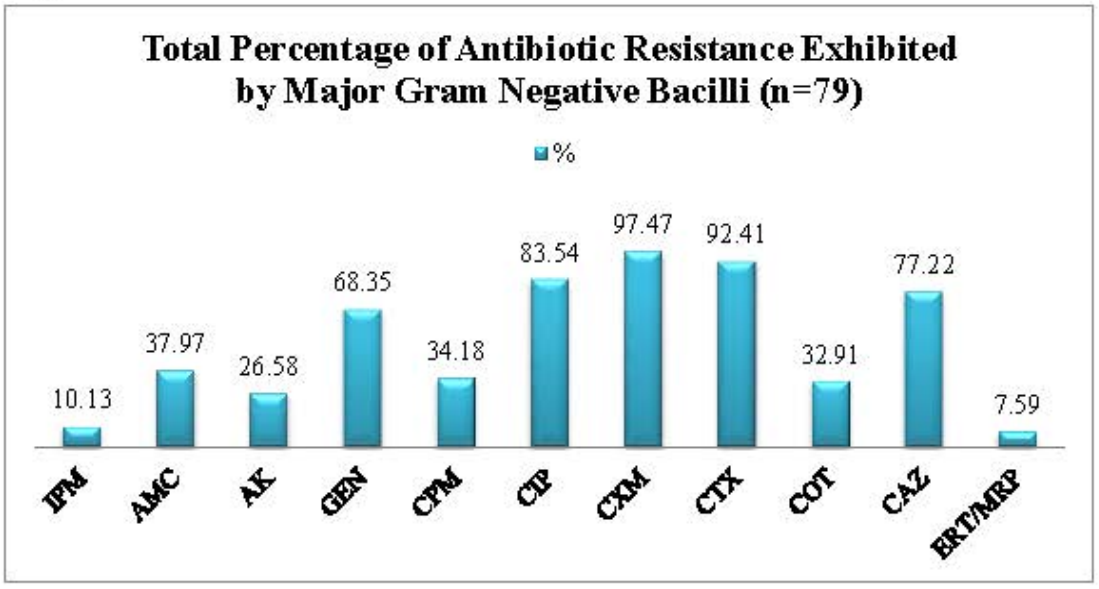
Of the 167 samples 58 (34.73%) samples were catheterized samples obtained from 53 patients (49 patients with single specimens, 3 with 2 and 1 with 3 specimens). Amongst the catheterized samples, growth was obtained in 52 (89.65%) samples yielding 58 isolates with 6 specimens yielding 2 isolates from same sample. Here *Candida species* (20/58) were the major isolate followed by E.coli (13/58) and equal numbers (7/58) of *Acinetobacter spp.* and *Enterococcus species* and then *Klebsiella spp.* (6/58), *S. aurues* (2/58), *Pseudomonas spp.* (2/58) and *CoNS* (1/58) in the order. Of the total 33 isolates of *Candida species*, 20 were identified as *C. albicans*, 12 as *C. non-albicans* and one as *C. tropicalis*.

Chart 28: Microbial Profile of Urine Culture



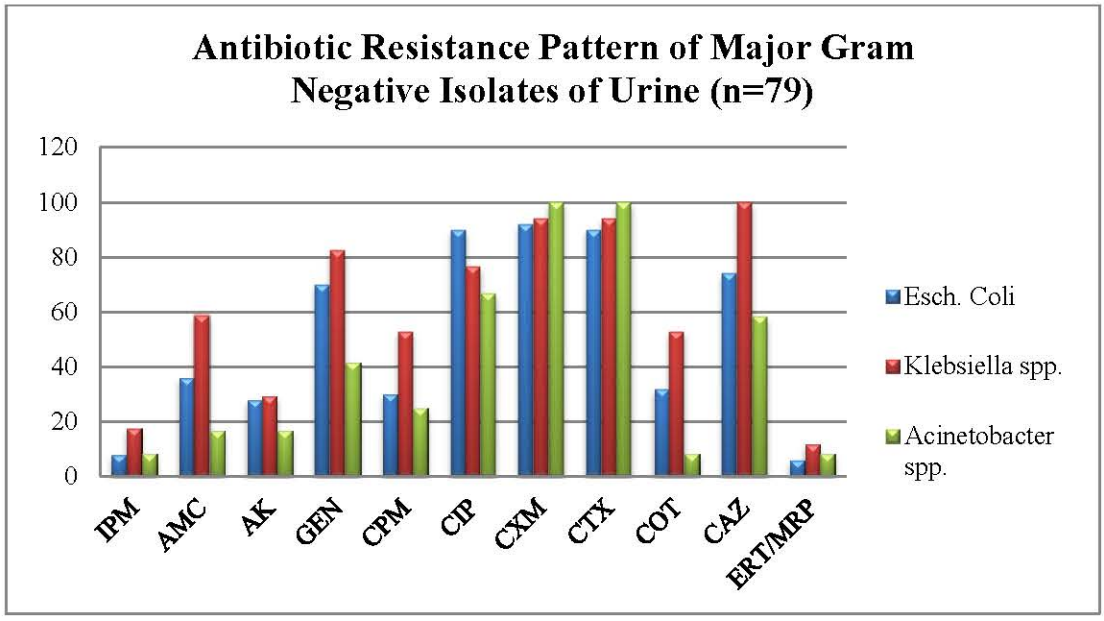
The overall antibiotic resistance pattern of the major Gram negative bacilli excluding *Proteus spp.* (n=5) and *Pseudomonas spp.* (n=2) showed a higher resistance towards Cefuroxime, Cefotaxime and Ceftazidime i.e. 97.47%, 92.41% and 77.22% respectively. On the other hand less resistance was seen against Imipenem, Ertapenem, Amikacin and Cefipime i.e. 10.03%, 7.59%, 26.58%, and 34.18% respectively as shown in the **(Chart 29)**.

Chart 29: Total Percentage of Antibiotic Resistance Exhibited by Major Gram Negative Bacilli (n=79)



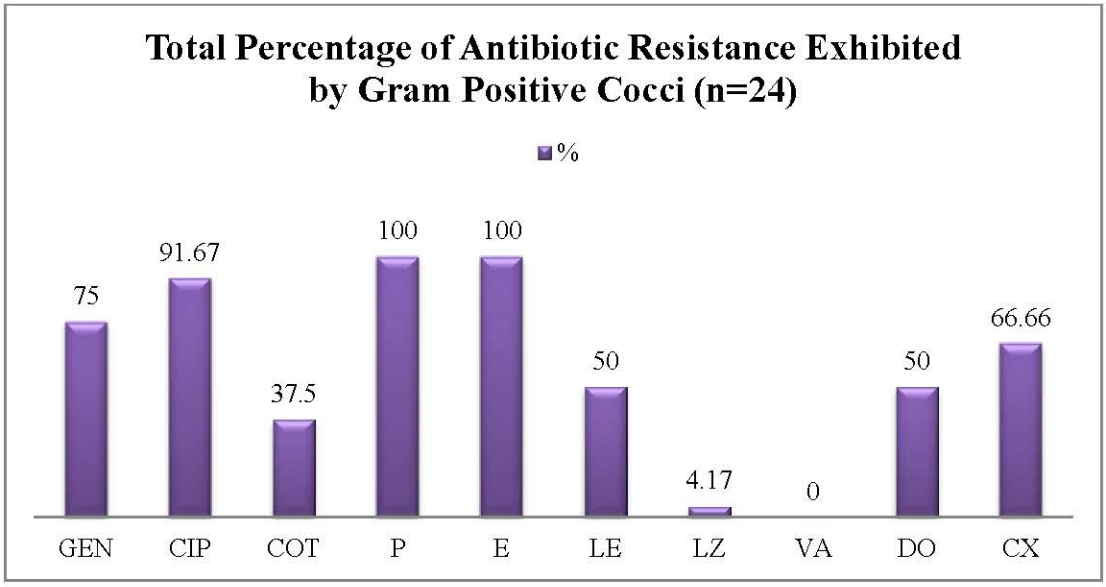
The individual resistance pattern reveals that *Klebsiella spp.* was more resistant to most of the antibiotics followed by *E. coli* and finally *Acinetobacter spp.* as shown in the **Chart 30**.

Chart 30: Antibiotic Resistance Pattern of Major Gram Negative Bacilli (n=79) in %



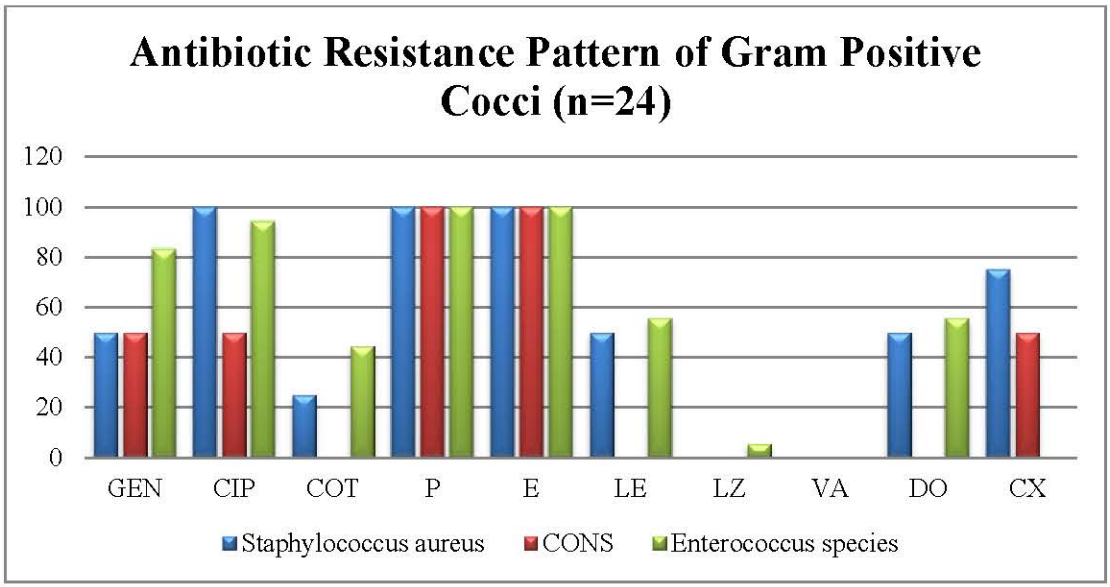
Amongst gram positive cocci, all the isolates were 100% susceptible to vancomycin whereas 100% resistance was seen against Penicillin and Erythromycin as shown in the **(Chart 31)**.

Chart 31: Total Percentage of Antibiotic Resistance Exhibited by Gram Positive Cocci (n=24)



The major isolate showing a higher resistance to most of the antibiotics amongst gram positive cocci was *Enterococcus spp.* (n=18) followed by *S. aureus* (n=4) and *CoNS* (n=2) as shown in the **(Chart 32)**. Also MRSA was found to be 75% (3/4) and MRCoNS 50% (1/2). Inducible Clindamycin Resistance (ICR) was observed in 16.66% (4/24) gram positive cocci.

Chart 32: Antibiotic Resistance Pattern of Gram positive cocci (n=24)

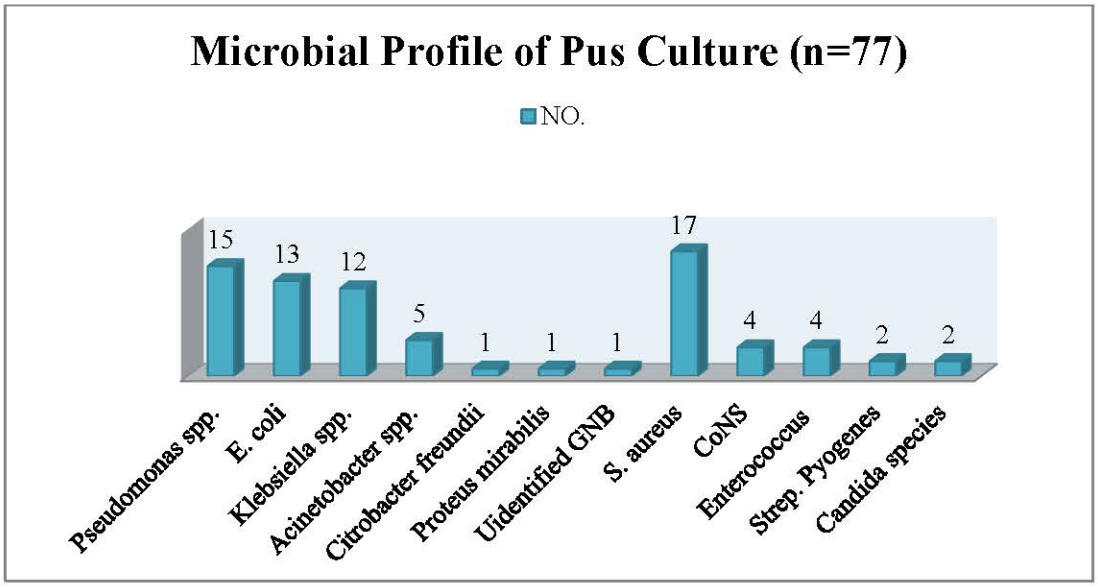


Also an important observation was that the same organism type with the similar antibiotic susceptibility pattern were isolated from blood as well as urine cultures from samples of 40 patients whereas in the remaining103 patients either the blood cultures were negative or an isolate type was different from that of urine.

Pus Culture:

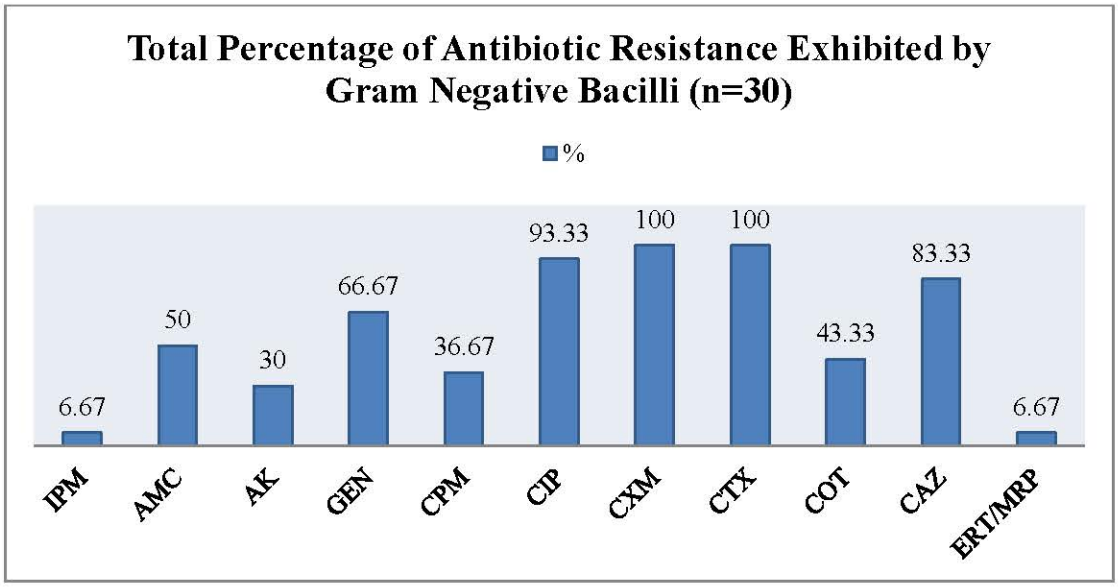
A total of 80 pus samples were cultured from 68 patients; with single specimens from 59 patients, 2 from 7 patients, 4 from 1 patient and 3 from 1 patient. The growth was obtained from 70 samples (87.5%) of 60 patients yielding 77 isolates and 10 (12.5%) samples did not yield any growth (8 single samples from 8 patients + 2 samples from a total of 4 samples from 2 patients). Of the total 77 isolates, 48 were Gram negative bacilli, 27 were gram positive cocci and 2 were *Candida species*. The most common isolate was *S. aureus* (n=17) followed by *Pseudomonas spp.* (n=15), *E. coli* (n=13) and *Klebsiella spp.* (n=12) as shown in the **(Chart 33)** along with other isolates.

Chart 33: Microbial Profile of Pus culture (n=80)



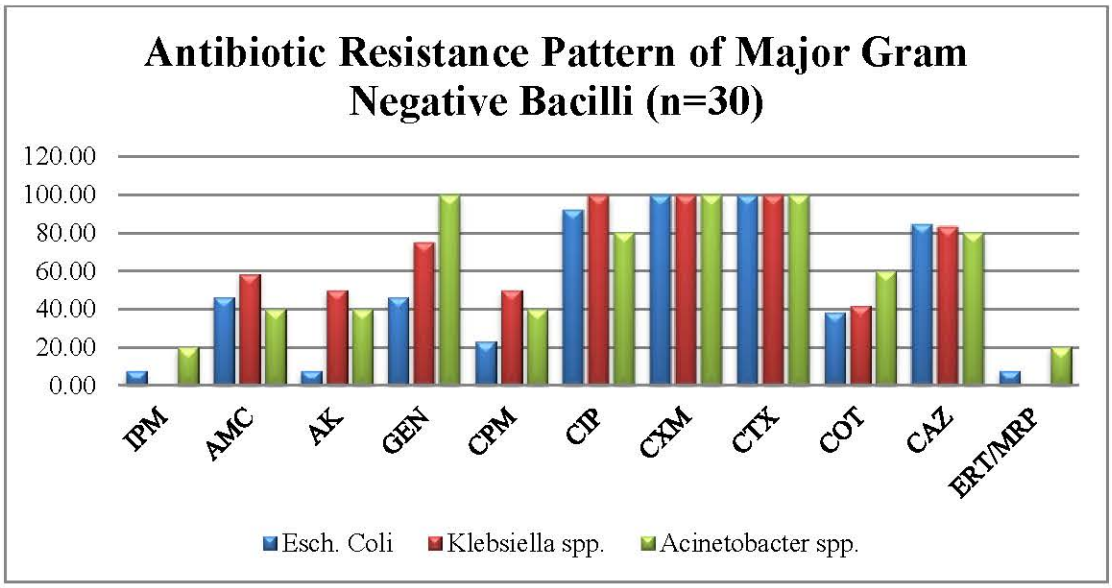
The overall antibiotic resistance pattern of gram negative bacilli revealed a higher percentage of resistance against Cefuroxime, Cefotaxime, Ciprofloxacin and Ceftazidime with least resistance against Imipenem and Ertapenem as shown in the **Chart 34** (excluding *Pseudomonas spp.*, *Citrobacter freundii*, *Proteus mirabilis* and unidentified GNB)

Chart 34: Total Percentage of Antibiotic Resistance Exhibited by Major Gram Negative Bacilli.



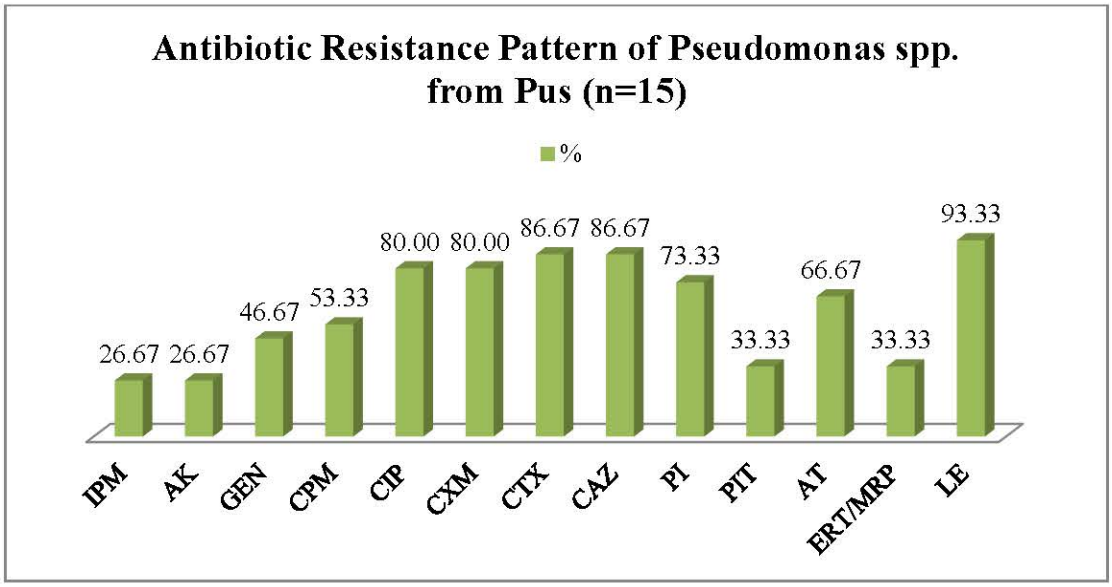
As shown in the **Chart 35** below, the highest resistance was shown by *Acinetobacter spp.* followed by *Klebsiella spp.* and finally *E. coli*. However, least resistance was seen against Imipenem and Ertapenem, especially *Klebsiella spp.* exhibited 100% susceptibility to both these antibiotics whereas *Acinetobacter spp.* showed higher resistance (20%) compared to both *E. coli* (7.69%) and *Klebsiella spp.* (0%)

Chart 35: Antibiotic Resistance Pattern of Major Gram Negative Bacilli



The antibiotic resistance pattern exhibited by *Pseudomonas spp.* is shown below (in the **Chart 36**). It shows that highest resistance was seen against Levofloxacin, Cefotaxime, Ceftazidime, Ciprofloxacin, Cefuroxime and Piperacillin. When compared to other gram negative bacilli, it showed a higher resistnace to Imipenem (26.67%) and Ertapenem (33.33%).

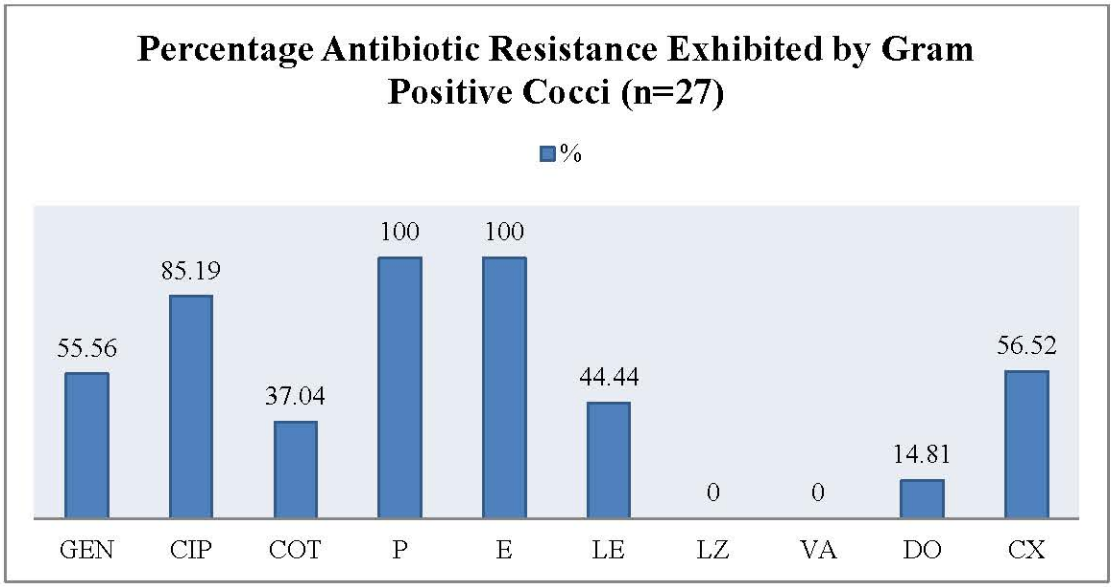
Chart 36: Antibiotic Resistance Pattern of *Pseudomonas spp.* (n=15)



Amongst the gram negative bacilli, ESBL and MBL production was the most common form of resistance mechanism.

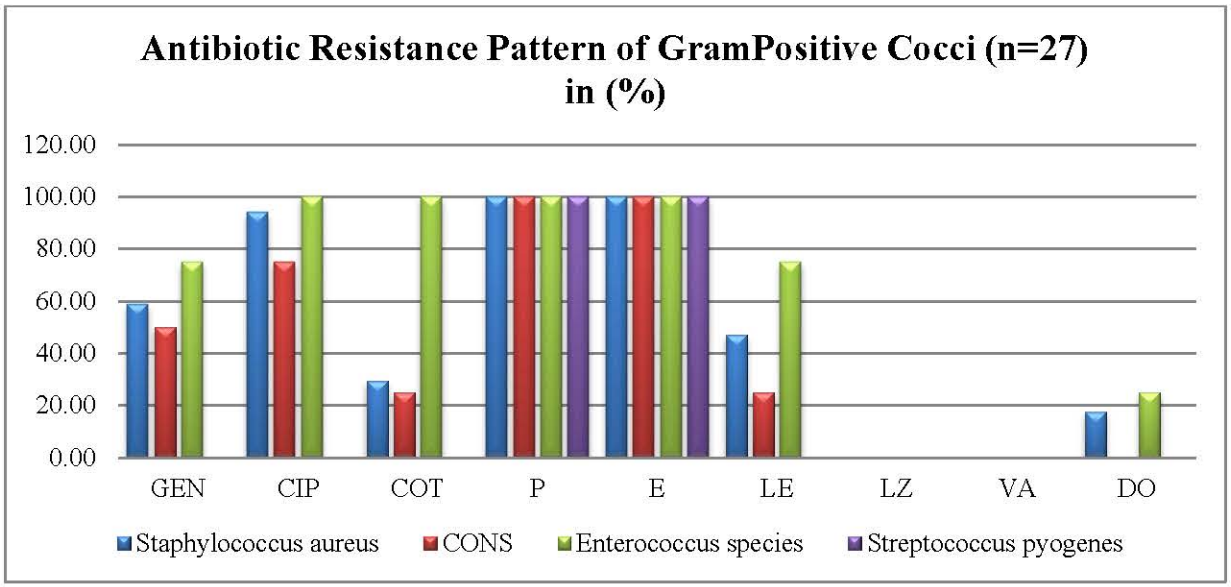
The overall antibiotic resistance pattern of the Gram positive cocci is shown in the **Chart 37** below which shows that all the isolates were 100% susceptible to vancomycin and linezolid whereas least resistance was seen against Doxycycline and cotrimoxazole and 100% resistance towards Penicillin and Erythromycin and slightly lesser to Ciprofloxaxin.

Chart 37: Total Percentage of Antibiotic Resistance Exhibited by Gram Positive Cocci



Looking to the individual pattern as shown (in the **Chart 38)** below, a higher resistance was exhibited by *Enterococcus species* followed by *S. aureus* whereas least resistance towards most of the antibiotics tested was by *Streptococcus pyogenes*, except for Pencillin and Erythromycin, to which 100% resistance was observed. Methicillin resistance was observed in 58.82% *S. aureus* and 75% *CoNS* isolates.

Chart 38: Antibiotic Resistance Pattern of Gram Positive Cocci

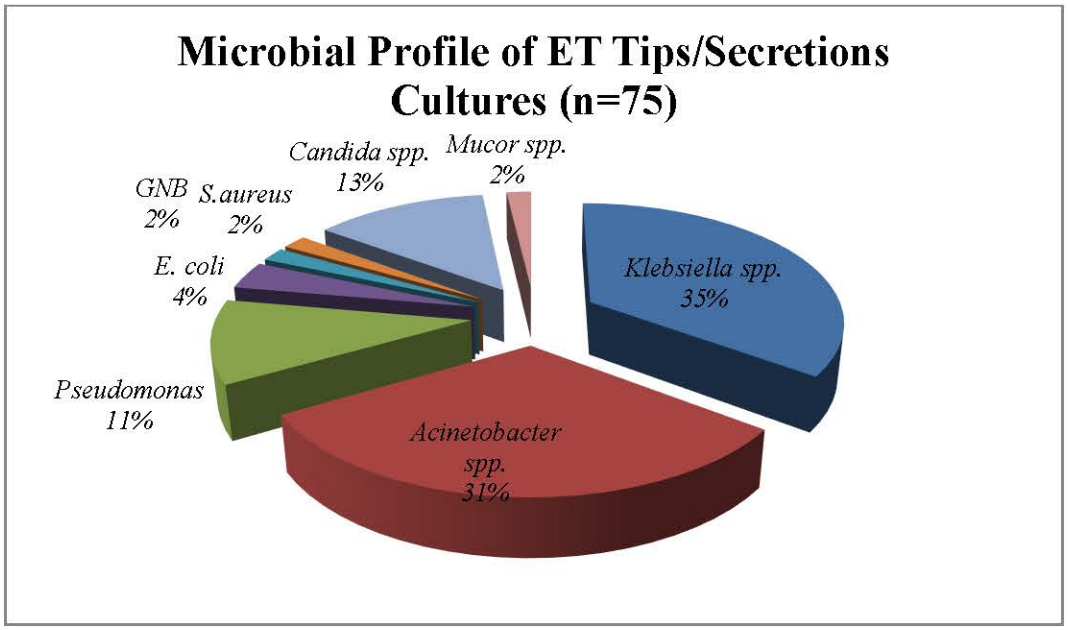


Of the total 68 patients, 30 patients' blood and pus cultures yielded the same organism whereas in the remaining patients either the blood cultures were negative or yielded a type of bacteria/fungi different from that of pus culture.

Endotracheal Tips/Secretions/Aspirates Culture:

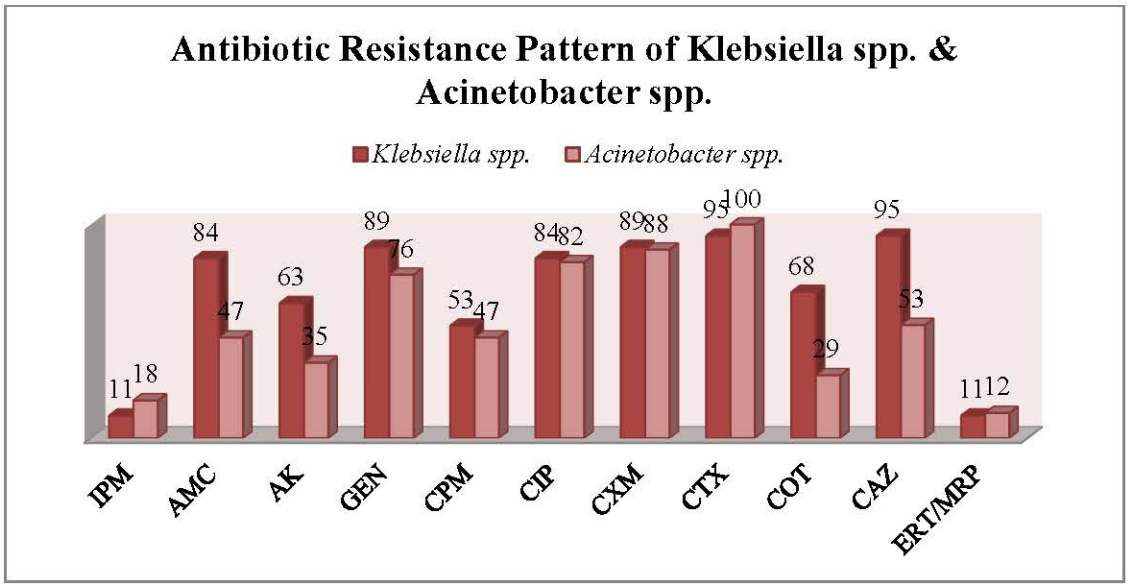
A total of 75 ET tips/secretions/aspirates were cultured from 73 patients who were intubated for mechanical ventilation. Of these, total 46 samples (61.33%) showed growth yielding 54 isolates and 29 samples (38.67%) were culture negative. From 46 culture positive ET samples, 54 isolates were obtained with eight samples showing polymicrobial growth. The gram negative bacilli were predominant isolates with *Klebsiella spp.* (35%) being the most common isolate, *Acinetobacter spp.* (31%) the second, *Pseudomonas spp.* (11%) and *E. coli* (4%) in the sequence; followed by fungi with *Candida spp.* (13%) and only one isolate of gram positive cocci i.e. *S. aureus* (2%) as shown in the **Chart 39**.

Chart 39: Microbial Profile of ET Tips/Secretions/Aspirates Culture (n=75)



The antibiotic resistance pattern of *Klebsiella spp. and Acinetobacter spp.* is as shown (in the **Chart 40**) below. *Klebsiella spp.* showed a higher resistance to most of the antibiotics as compared to *Acinetobacter spp.* However, *Acinetobacter spp.* showed a higher resistance 29.51% to carbapenems. Also a higher resistance to cefotaxime and cefuroxime was observed. Thus overall least resistance was seen against Imipenem and Ertapenem whereas highest was seen against cefotaxime and cefuroxime.

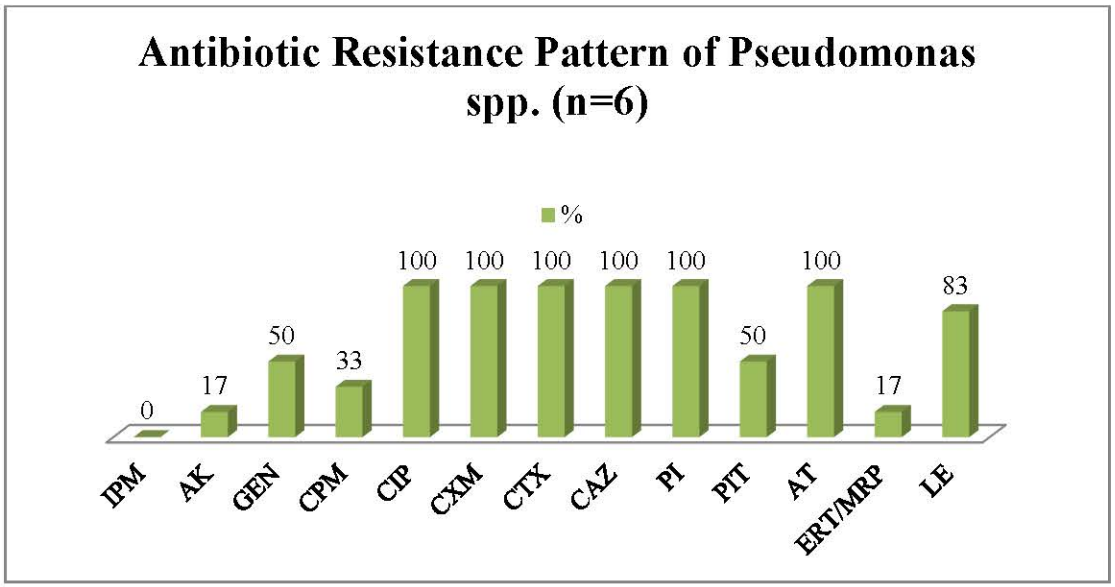
Chart 40: Antibiotic Resistance Pattern of *Klebsiella spp.* & *Acinetobacter spp.*



Moreover, ESBL production was the most common mechanism of resistance followed by AmpC-beta-lactamase production.

Pseudomonas spp., as shown below (in the **Chart 41),** exhibited a higher resistance to most of the antibiotics tested but with no resistance against Imipenem and least against Ertapenem.

Chart 41: Antibiotic Resistance Pattern of *Pseudomonas spp.* (n=6)

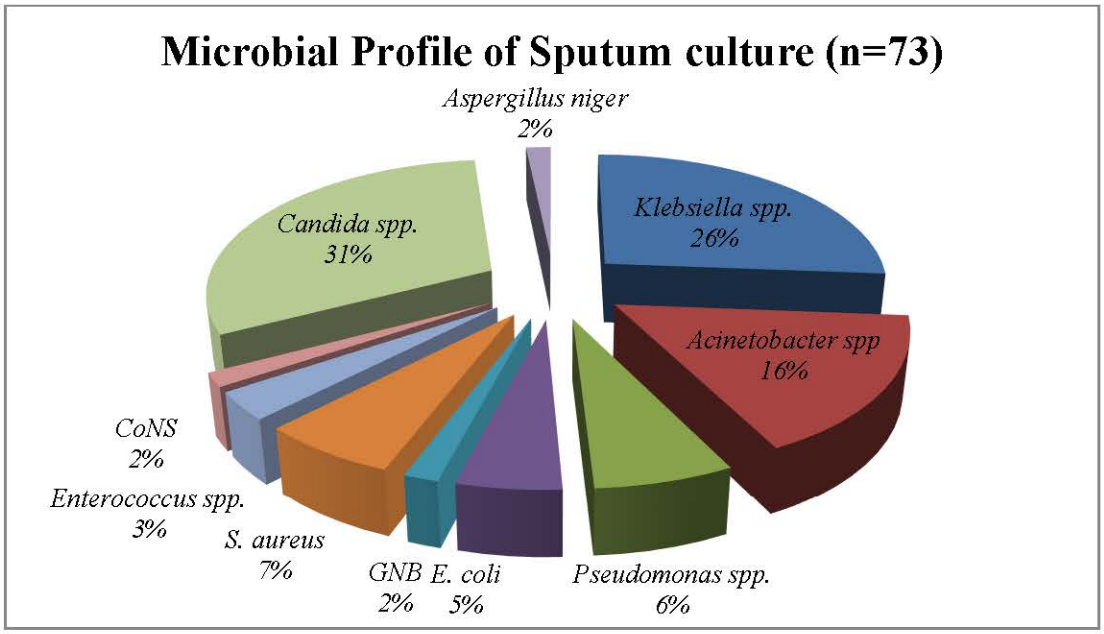


Only one *S. aureus* was isolated which was sensitive to all the antibiotics tested except Penicillin. It was methicillin sensitive. In 40 patients' both the blood and ET tips cultures yielded the same organism type and in the remaining either the blood cultures were negative or yielded a different type of isolate.

Sputum Culture:

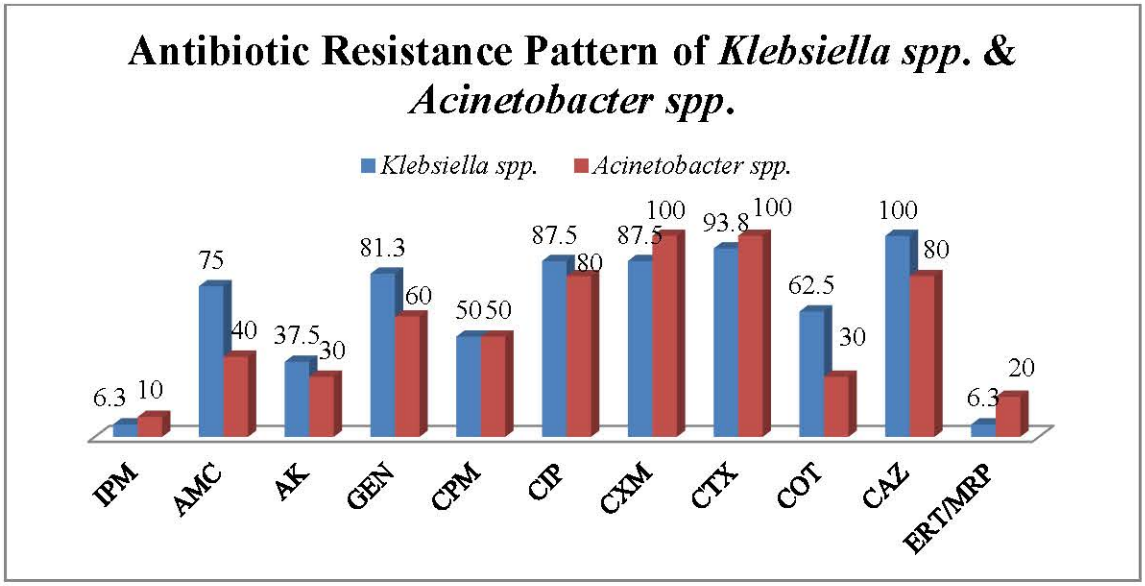
A total of 73 sputum samples were processed from 68 patients; single specimens from 63 patients and 2 specimens from 5 patients. Growth was obtained in 56 samples yielding 61 isolates and no growth in 17 samples. As shown (in the **Chart 42**) below, *Candida spp.* (31%) was the most common isolate followed by *Klebsiella spp.* (26%), *Acinetobacter spp.* (16%), *S. arueus* (7%), *Pseudomonas spp.* (6%) and also other isolates. The sputum samples were processed only when they were found to be appropriate as per the sputum score. Thus *Candida species* isolated from such samples and showing pseudohyphae were considered significant pathogens in this study.

Chart 42: Microbial Profile of Sputum Culture (n=73)



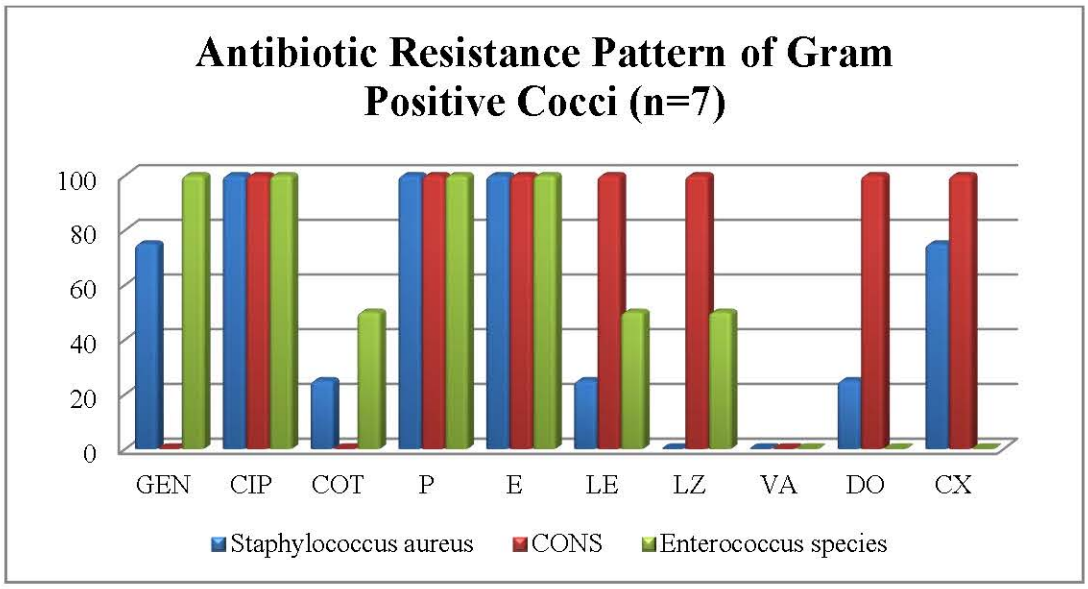
Klebsiella spp. showed a higher resistance against Amoxycillin-clavulanic acid, Amikacin, Gentamicin, Ciprofloxacin, Cotrimoxazole and Ceftazidime whereas *Acinetobacter spp.* showed a higher resistance towards Imipenem, Ertapenem, Cefuroxime and Cefotaxime **(Chart 43)**. Also ESBL production was the major form of resistance mechanism followed by MBL production.

Chart 43: Antibiotic Resistance Pattern of *Klebsiella spp.* & *Acinetobacter spp.*



Amongst gram positive cocci, the higher resistance was exhibited by *Enterococcus* spp. towards most of the antibiotics tested as compared to *S. aureus* which exhibited higher resistance towards doxycycline and cefoxitin. (Shown in the **Chart 44** below) MRSA was found to be 75%.

Chart 44: Antibiotic Resistance Pattern of Gram Positive Cocci (n=7)



Additionally, 23 patients had same organism type from both the blood and sputum cultures whereas in rest the blood cultures were either negative or had a different type of isolate.

CSF Culture:

A total of 38 CSF samples were cultured from 38 patients. The following **Chart 45** shows the outcome of CSF culture. A total of 95% samples were culture negative and only 5% (2/38) were positive. Both these samples yielded *Klebsiella spp.*, whose antibiotic susceptibility pattern is shown below (in the **Chart 46)** revealing 100% susceptibility to most of the antibiotics tested and 50% to Amikacin, Cefuroxime, Ceftazidime and 0% to Gentamicin and Ciprofloxacin. None of them were ESBL producers and neither did they exhibit any other resistance mechanism.

Chart 45: CSF Culture Outcome (n=38)

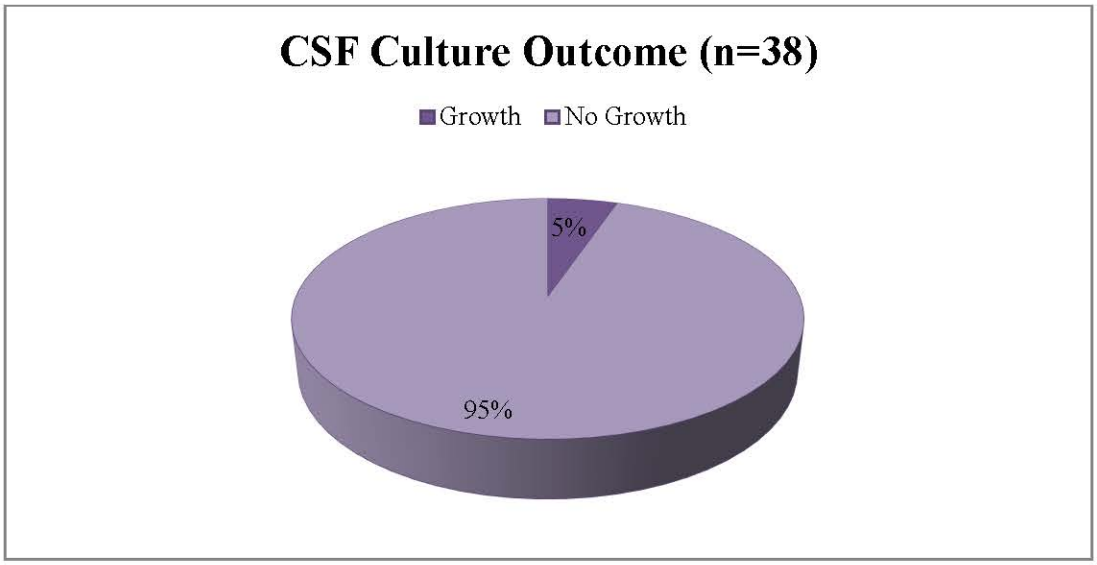
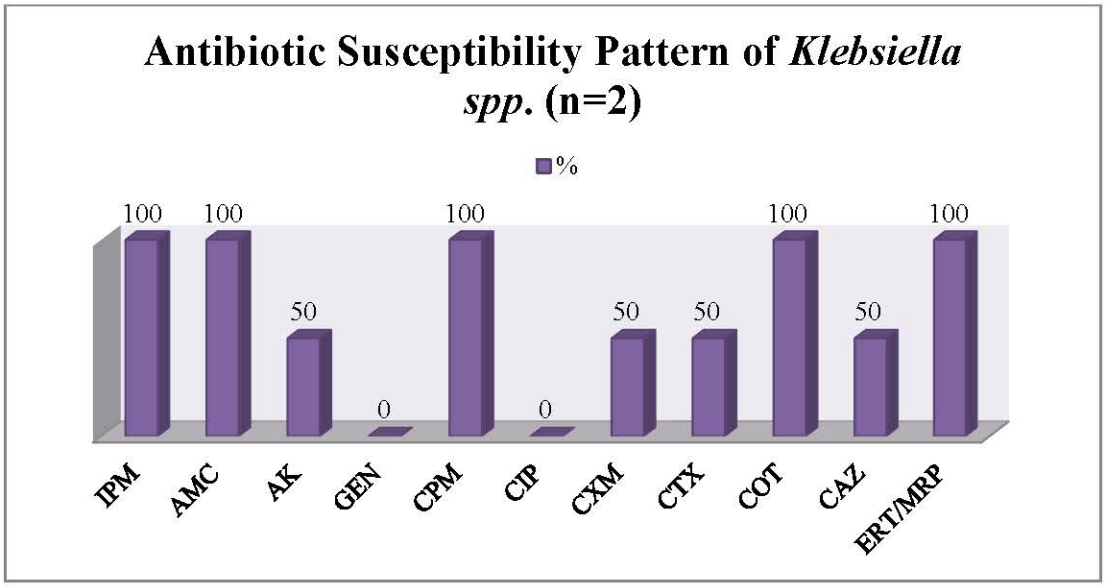


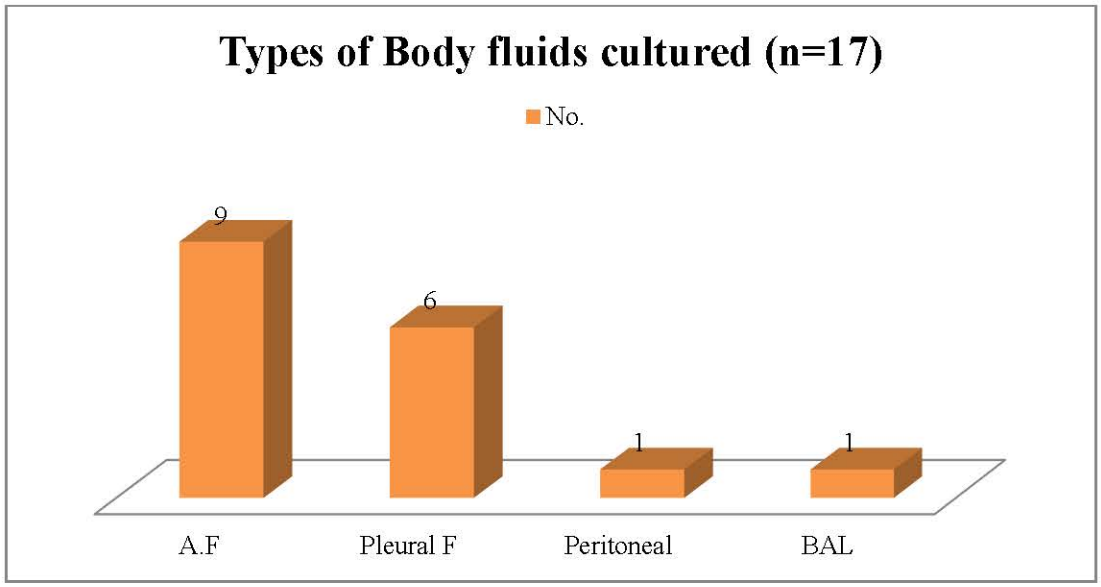
Chart 46: Antibiotic Susceptibility Pattern of *Klebsiella spp.* from CSF



Body Fluids

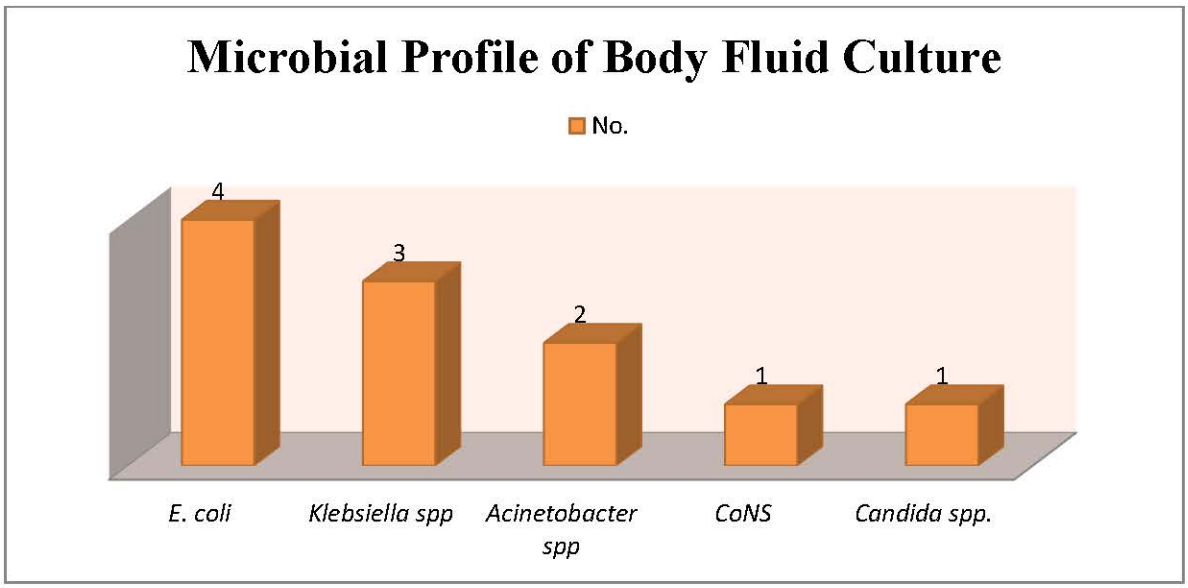
A total of 17 body fluid samples were cultured. The following **Chart 47** shows types & number of body fluid samples with maximum been ascitic fluid (AF).

Chart 47: Types of Body Fluids Cultured (n=17)



A total of 41% (10/17) showed growth and *E. coli* (4/10) was the major isolate followed by *Klebsiella spp.*, *Acinetobacter spp.*, *CoNS* and *Candida spp.* as shown in the **Chart 48** below.

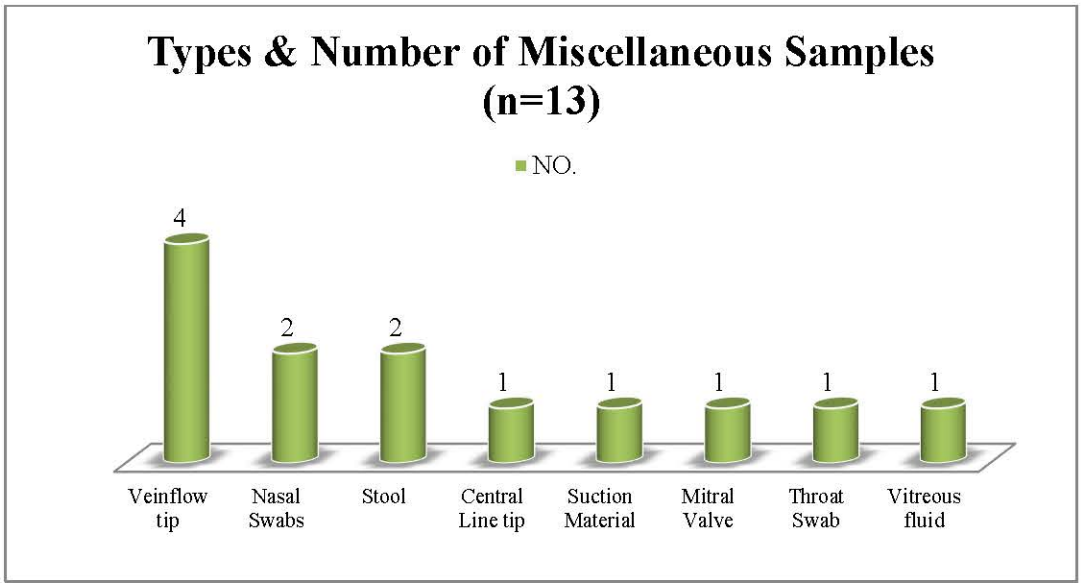
Chart 48: Microbial Profile of Body Fluid Cultures (n=17)



Miscellaneous Samples:

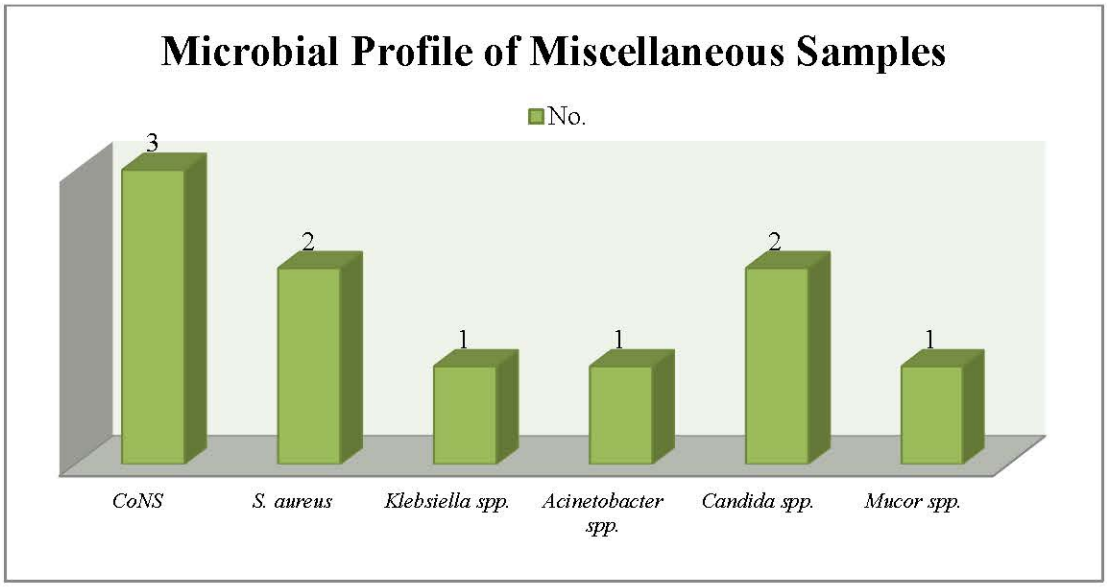
A total of 13 different miscellaneous samples considered as a source of infection were cultured as shown in the **Chart 49** below.

Chart 49: Types & Number of Miscellaneous Samples (n=13)



Growth was obtained in 38% (8/13) of the samples with *CoNS* (3/8) been the major isolate followed by equal numbers of *S. aureus* (2/13) and *Candida spp.* (2/8) and 1 each of *Klebsiella spp.*, *Acinetobacter spp.* and *Mucor spp.* as shown in the **Chart 50** below.

Chart 50: Microbial Profile of Miscellaneous Samples

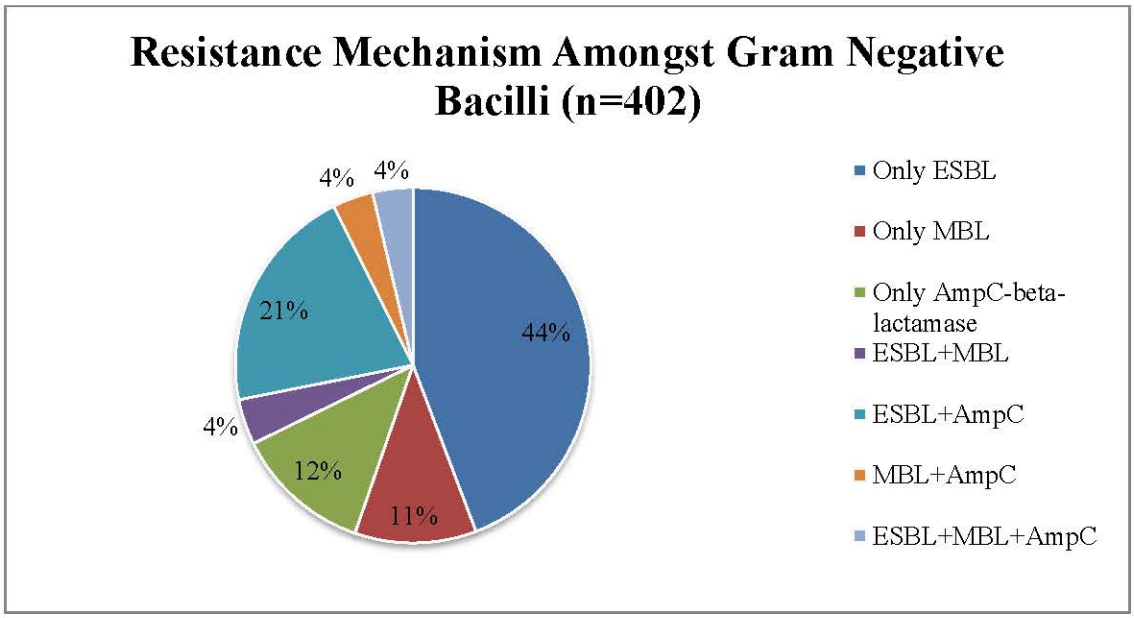


Mechanisms of Resistance Expressed by the Bacterial Isolates: (ESBL, MBL, AmpC, MRSA, MRCoNS, ICR)

All the bacterial isolates including gram negative bacilli and gram positive cocci were tested for antibiotic resistance as well as detection of various mechanisms that lead to this resistance amongst them. Thus for gram negative bacilli, detection of ESBL, MBL and AmpC-beta-lactamase production and for gram positive cocci Methicillin Resistance and erythromycin-induced-Clindamycin resistance were detected.

Thus a total of 53.98% (217/402) gram negative bacilli isolated from various samples exhibited different mechanisms of resistance. Of which ESBL production was the major form – 44% (96/217) followed by 21% (45/217) of ESBL+AmpC-beta-lactamase combination, 12% (27/217) of AmpC-beta-lactamase production, 11% (24/217) of MBL production and 4% each of the combinations of ESBL+MBL (n=9), MBL + AmpC-beta-lactamase (n=8) and finally all the three mechanisms i.e. ESBL+MBL+AmpC-beta-lactamase production together (n=8) as shown in the **Chart 51**.

Chart 51: Types of Resistance Mechanisms Expressed by Gram Negative Bacilli (n=217)



ESBL production was most frequently seen in isolates of *Klebsiella spp.* (n=41) followed by *E. coli* (n=33), *Acinetobacter spp.* (n=11), *Pseudomonas spp.* (n=7) and least by others (n=4). MBL production was seen more commonly in *Acinetobacter spp.* (n=8) followed by *Klebsiella spp.* (n=7), *Pseudomonas spp.* (n=5) and *E. coli*

(n=4). AmpC-beta-lactamases were most commonly produced by *E. coli* (n=13), *Acinetobacter spp.* (n=6), *Pseudomonas spp.* (n=5) and least by *Klebsiella spp.* (n=1). Of the combination of resistance mechanisms, ESBL+ AmpC-beta-lactamase was the most frequent mechanism (n=45) of which *Klebsiella spp.* (n=21) was the major exhibitor followed by *E. coli* (n=15), *Acinetobacter spp.* (n=7) and least was *Pseudomonas spp.* (n=2). The other mechanism and contributing isolates are shown in the table below:

Table 7: Resistance Mechanisms Expressed by Gram Negative Bacilli (n=217)

Mechanism	***Klebsiella spp.***	***E. coli***	***Acinetobacter spp.***	***Pseudomonas spp.***	***Proteus spp.***	**GNB**	**Total No.**
Only ESBL	41	33	11	7	2	2	96
Only MBL	7	4	8	5	0	0	24
Only AmpC-beta-lactamase	1	13	6	5	0	2	27
ESBL+MBL	2	1	1	5	0	0	9
ESBL+AmpC	21	15	7	2	0	0	45
MBL+AmpC	0	1	3	4	0	0	8
ESBL+MBL+AmpC	2	3	2	1	0	0	8
Total No.	**74**	**70**	**38**	**29**	**2**	**4**	**217**

Overall, *Klebsiella spp.* (n=74) exhibited almost all forms of resistance mechanisms followed by *E. coli* (n=70), *Acinetobacter spp.* (n=38), *Pseudomonas spp.* (n=29) whereas unidentified GNB (n=4) exhibited only 2 mechanisms i.e. ESBL & AmpC-beta-lactamase and *Proteus spp.* (n=2) exhibited only ESBL production. As shown (in the **Table 6)** above, 57.74% (41/74) isolates of *Klebsiella spp.* exhibited ESBL production whereas 47.14% (33/70) of *E. coli*, 28.94% (11/38) of *Acinetobacter spp.* and 24.13% (7/29) of *Pseudomonas spp.* were ESBL producers.

Resistance mechanisms observed in each isolate type out of the total number of 402 isolates of each type of gram negative bacilli is shown below (in the **Table 8)**

excluding *Citrobacter spp.* (n=3) & *Salmonella spp.* (n=15) who did not exhibit any of the mechanisms of resistance tested. Thus 30.14% (41/136) of total isolates of *Klebsiella* spp. were ESBL producers, 5.14% (7/136) were MBL producers and 15.44% (21/136) were ESBL + AmpC-beta-lactamase producers.

Table 8: Resistance Mechanisms Detected in Each Isolate Type of Gram Negative Bacilli (n=402)

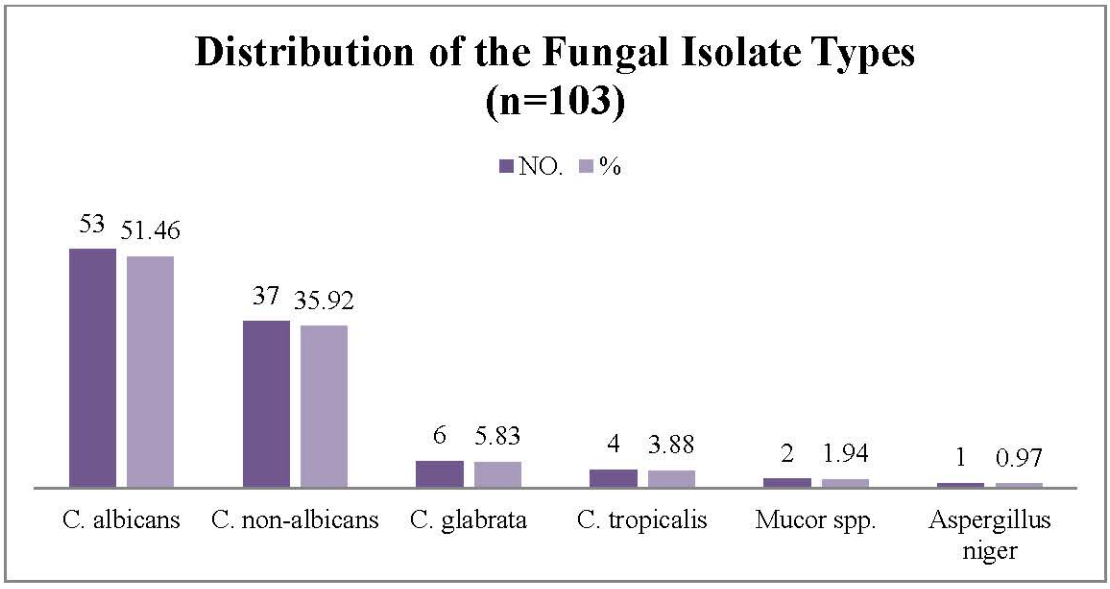
Mechanism/Isolate Types (n=402)	***Klebsiella spp. (n=136)***	***E. coli (n=120)***	***Acinetobacter spp. (n=74)***	***Pseudomonas spp. (n=43)***	***Proteus spp. (n=7)***	**GNB (n=4)**	***Citrobacter spp. (n=3)***	***Salmonella spp. (n=15)***	**Total No. (n=402)**
Only ESBL	41 (30.14%)	33 (27.5 %)	11(14.86%)	7 (16.27%)	2 (28.57 %)	2 (50 %)	0	0	96
Only MBL	7 (5.14%	4 (3.33 %)	8 (10.81%)	5 (6.75%)	0	0	0	0	24
Only AmpC-beta-lactamase	1 (0.7%)	13 (10.83 %)	6 (8.10%)	5 (6.75%)	0	2 (50 %)	0	0	27
ESBL+MBL	2 (1.47%)	1(0.83 %)	1(1.35%)	5 (6.75%)	0	0	0	0	9
ESBL+AmpC	21 (15.44%)	15 (12.5 %)	7 (9.45%)	2 (4.65%)	0	0	0	0	45
MBL+AmpC	0 (0%)	1 (0.83 %)	3(4.05%)	4 (9.30%)	0	0	0	0	8
ESBL+MBL+AmpC	2 (1.47%)	3 (2.5%)	2 (2.70%)	1 (2.32%)	0	0	0	0	8
Total No.	**74 (54.41%)**	**70 (58.33 %)**	**38 (51.35%)**	**29 (67.44%)**	**2 (28.57 %)**	**4 (100 %)**	**0**	**0**	**217**

Amongst gram positive cocci, methicillin resistance was observed in 64.55% (51/79) *S. aureus* isolates (MRSA) and 40% (24/60) isolates of *CoNS* (MRCoNS). The erythromycin induced clindamycin resistance (ICR) was observed in 22.78% (18/79) *S. aureus* isolates and in none among the isolates of *CoNS*. It was also noted that of the 18 isolates with ICR, 11 isolates actually exhibited both methicllin resistance as well as ICR together. Thus single mechanism was observed in 13 with MRSA & 7 with ICR amongst isolates of *S. aureus*. Also ICR was frequently observed among MRSA as compared to MSSA isolates. ICR was also observed in 18.36% (9/49) isolates of *Enterococcus species*.

Fungal Culture & Anti-fungal Susceptibility:

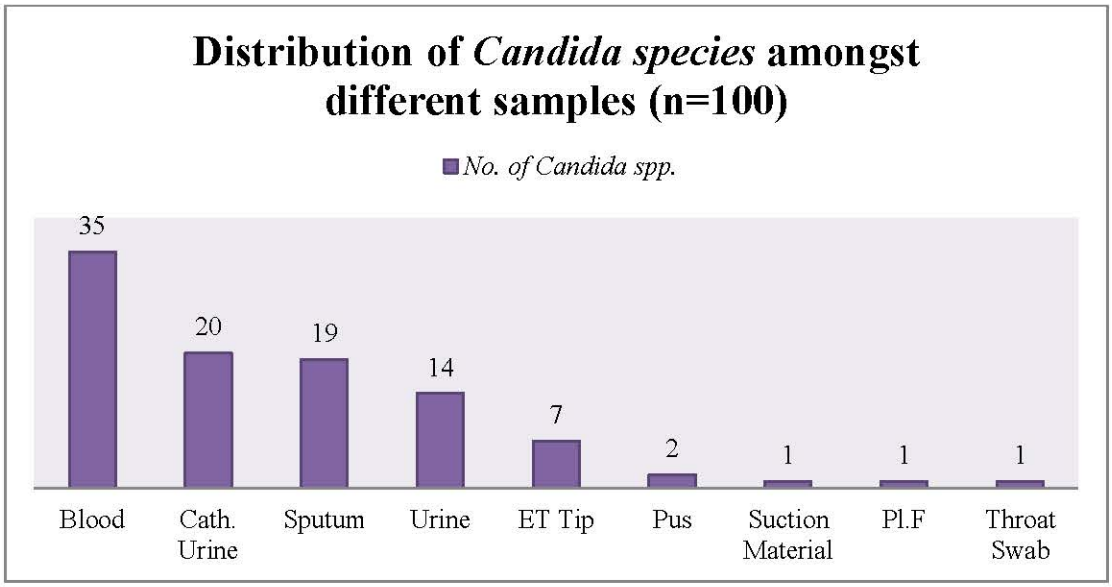
A total of 103 fungal isolates were obtained from 1136 different samples cultured. Of these, 100 were *Candida species*, 2 were *Mucor species* and 1 *Aspergillus species*. *Aspergillus spp.* was obtained from sputum sample of one patient alongwith *Klebsiella spp.* whereas 2 *Mucor spp.* were obtained one each from ET tip and nasal septum samples of the another patient. A total of 14.26% (100/701) isolates of *Candida species* were obtained from a total of 701 bacterial & fungal isolates put together. However, 10.60% (35/330) were obtained from blood cultures with 7.90% (26/330) as single isolate and 2.92% (9/330) with another bacterial isolate contributing as the 5th most common blood culture isolate. The distribution of different fungal isolates is as shown in the **Chart 52**. Thus *Candida species* were the frequent isolate amongst the fungi contributing to 97.08% (100/103) and other filamentous fungi to 2.91% (3/103). Amongst *Candida species*, the most frequent isolate was *C. albicans*, followed by *C. non-albicans*, *C.glabrata* and *C. tropicalis*.

Chart 52: Distribution of Fungal Isolate Types (n=103)



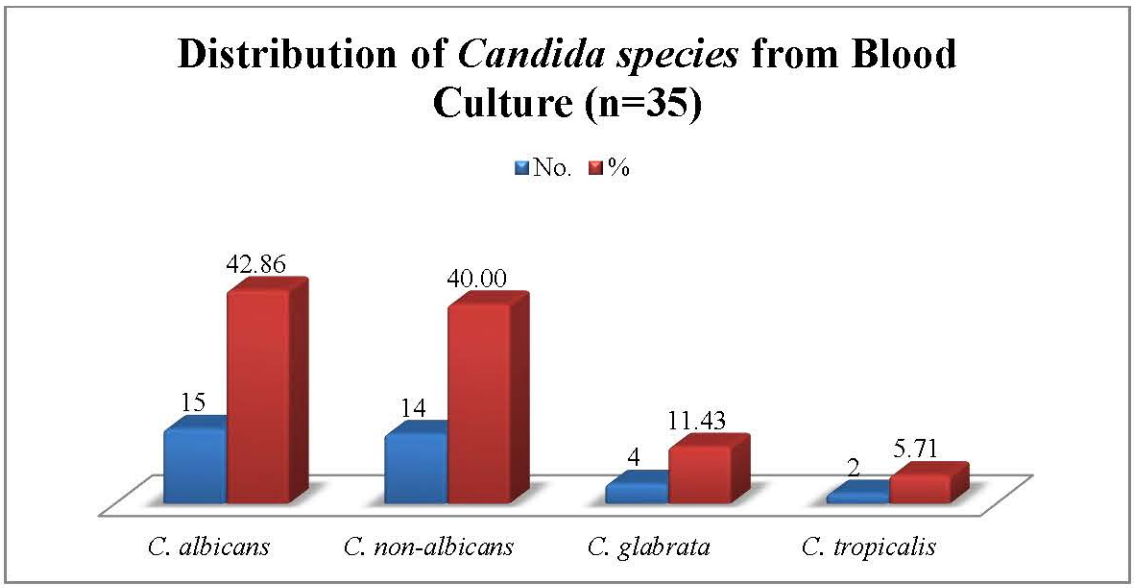
The following **Chart 53** shows the number & type of specimens from which these *Candida species* were obtained. They were frequently isolated from blood (35%) followed by catheterized urine (20%), sputum (19%), non-catheterized urine (14%), ET tips/secretions (7%) and also in small percentages from other samples.

Chart 53: Distribution of *Candida species* amongst different samples (n=100)



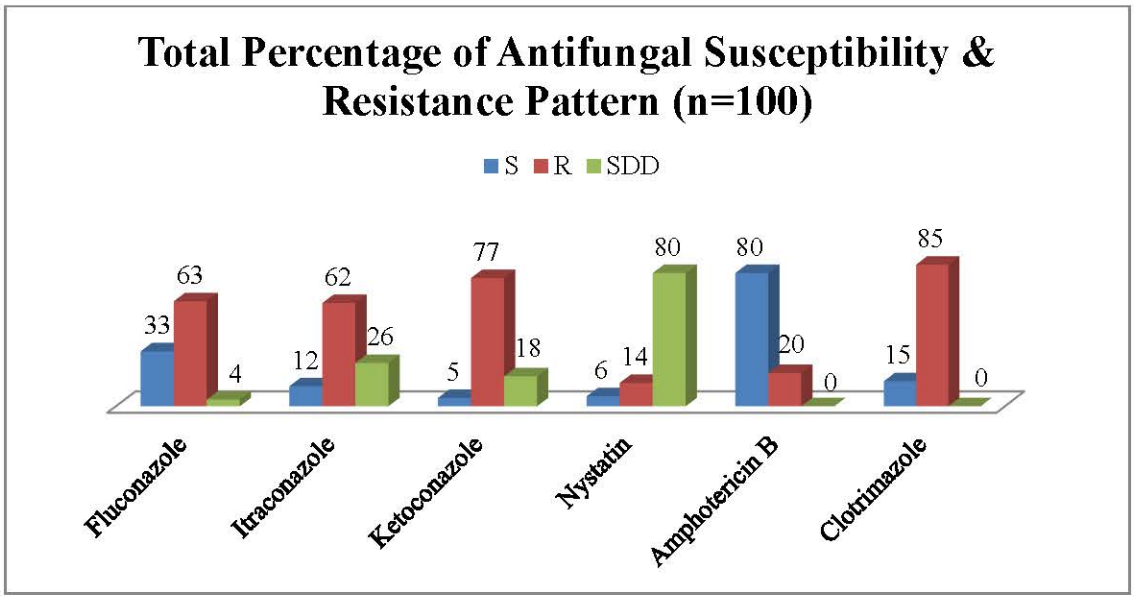
From a total of 35 isolates (10.32% i.e. 35/339) obtained from blood culture, 26 (7.66% i.e. 26/339) were obtained as single isolates whereas 9 (2.72% i.e. 9/330) were obtained with another bacterial isolate. Of the total 35 isolates obtained from blood, *C. albicans* was the most common isolate 42.86% (15/35) followed by *C. non-albicans*, 40% (14/35), *C. glabrata*, 11.43% (4/35) and *C. tropicalis*, 5.71% (2/35) as shown (in the **Chart 54**) below.

Chart 54: Type & Number of *Candida species* from Blood Culture (n=35)



The antifungal susceptibility testing was carried out for all the *Candida* isolates using Fluconazole, Itraconazole, Ketoconazole, Nystatin, Amphotericin B and Clotrimazole. The overall susceptibility pattern of the antifungal tested is as shown below in the **Chart 55**.

Chart 55: Total Percentage of Antifungal Susceptibility & Resistance Pattern of *Candida species* (n=100)



Susceptibility to Fluconazole:

Of the isolates tested 33%, 63% and 4% were sensitive, resistant and dose dependent respectively. The maximum resistance was exhibited by *C. albicans* followed by *C. non-albicans, C. tropicalis* and least by *C.glabrata.*

Susceptibility to Itraconazole:

As shown in the chart 62%, 26% and 12% were resistant, dose dependent and susceptible to Itraconazole respectively. The maximum resistance was shown by C. albicans followed by C. tropicalis and least but almost equally by *C.non-albicans* and *C.glabrata.*

Susceptibility to Ketoconazole:

It showed that 77%, 18% and 5% were resistant, dose dependent and susceptible to Ketoconazole respectively. The maximum yet equally the dose dependence were shown by *C. glabrata* and *C. tropicalis* followed by *C. non-albicans* and least by *C. albicans*, which also showed a maximum resistance.

Susceptibility to Nystatin:

Of the total isolates 80%, 14% and 6% were dose dependent, resistant and sensitive to Nystatin respectively. The maximum dose dependence was shown by *C. albicans*, *C. glabrata*, *C. non-albicans* and finally *C. tropicalis*.

Susceptibility to Amphotericin B:

Candida species were 80% and 20% sensitive and resistant respectively to Amphotericin B. The SDD could not be reported for this category as the interpretation was not available for this category from either the guidelines or the literature. The maximum resistance was shown by *C. tropicalis* followed *C. glabrata*, *C. non-albicans* and least by *C. albicans*.

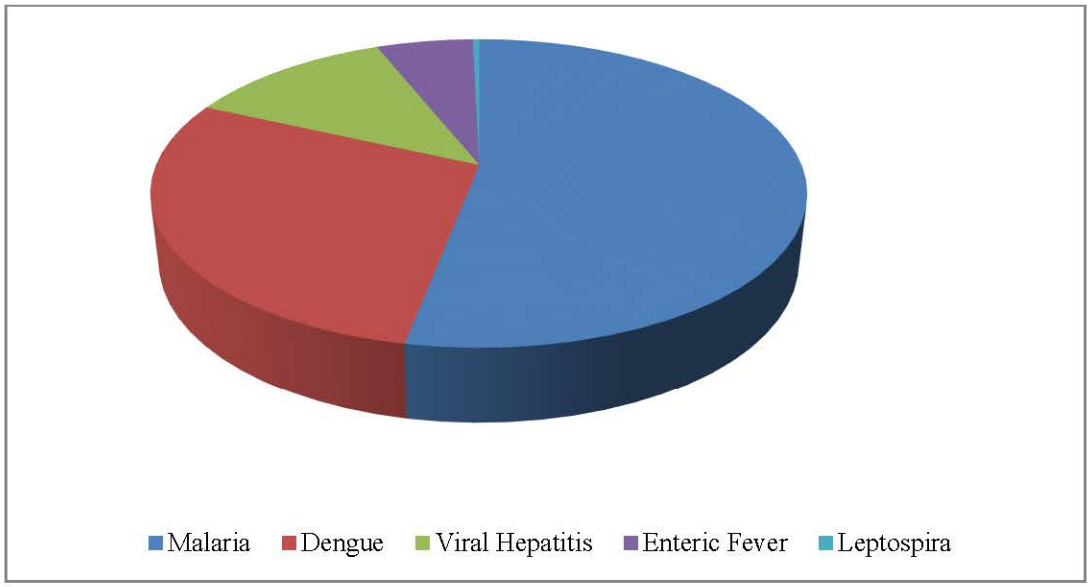
Susceptibility to Clotrimazole:

Here also no SDD could be reported but 85% and 15% resistance and susceptibility were seen against Clotrimazole respectively. The maximum resistance was shown by C. albicans followed equally by *C. non-albicans* and *C. glabrata* and least by *C. tropicalis*.

Tropical Sepsis:

A total of 250 patients i.e. 34% had sepsis due to malaria, dengue, viral hepatitis, enteric fever and leptospira. Of the 250 patients who were grouped in tropical infections 122 (48.8%) had only malaria, 58 (23.2%) had only dengue, 17 (6.8%) had only viral hepatitis, 11 (4.4%) had only enteric fever and rest 42 (16.8%) had polymicrobial infections like malaria, dengue, viral hepatitis, enteric fever associated with other mixed infections which includes bacterial, fungal, parasitic and viral in various combinations of two or three microbial agents; including one patient with *Leptospira* spp. infection. Thus of the total 34% patients with tropical sepsis, total malaria contributed (56%), total dengue (30.4%), total viral hepatitis (12.40%), total enteric fever (6%) and leptospira contributed 0.40% as shown in **(Chart 56).** In our study malaria was the major cause followed by dengue, viral hepatitis and enteric fever Chart). Of the total tropical patients 65.2% (163/250) were males and 34.8% (87/250) were females.

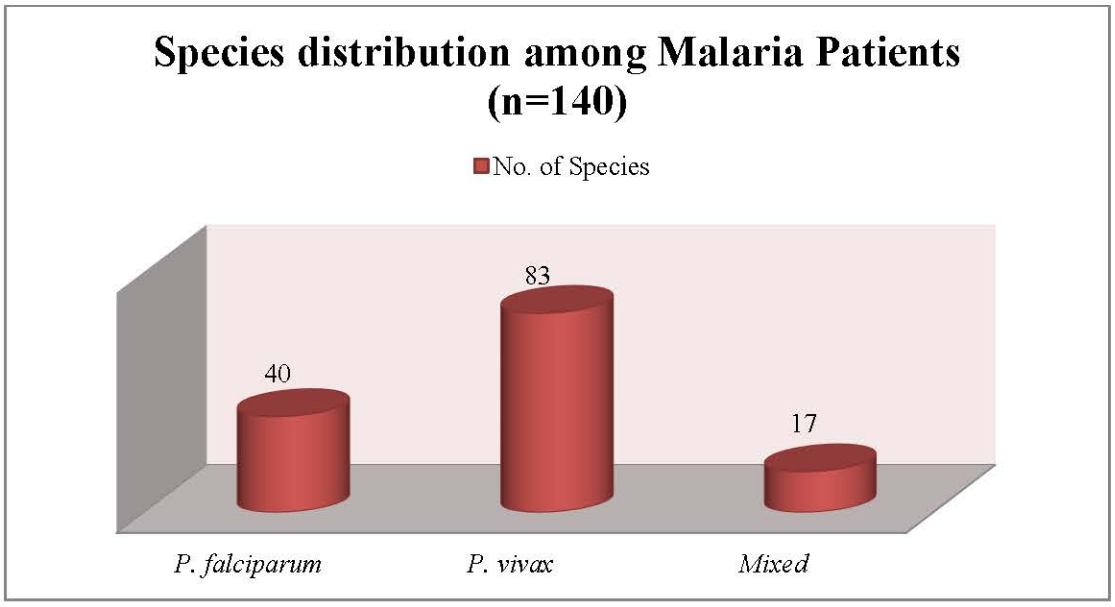
Chart 56: Microbial Profile of Tropical Sepsis (n=250)



Malaria:

A total 140 patients from 743 (18.84%) and 56% (140/250) of the 250 patients with tropical sepsis had malaria. Of the 140 patients, 42 were females and 98 males. The average age of female patients was 36.69 years whereas that of male patients was 40.27 years. A total of 65% survived, 31.42% did not survive and a small percentage i.e. 3.57% got discharged against medical advice. Most common cause of malaria among 140 malaria positive patients was *Plasmodium vivax* in 59.29% (83/140) patients followed by 28.57% (40/140) *Plasmodium falciparum* and least i.e. 12.14% (17/140) due to mixed infections with *P.faclicparum* and *P. vivax*. **(Chart 57)**

Chart 57: Distribution of *Plasmodium species* among Malaria Patients



Also 18 (12.85%) patients had concomitant infections with other microbes. Thus of the 18 patients, 10 had bacterial, 5 had dengue and 3 had hepatitis A infections as shown in the **(Chart 58)**. In addition to these 18 patients one patient was HIV seropositive. The Gram positive bacteria predominated over Gram negative bacteria amongst the patient with concurrent bacterial infection. **(Chart 59)**

Chart 58: Coinfections in Malaria Patients

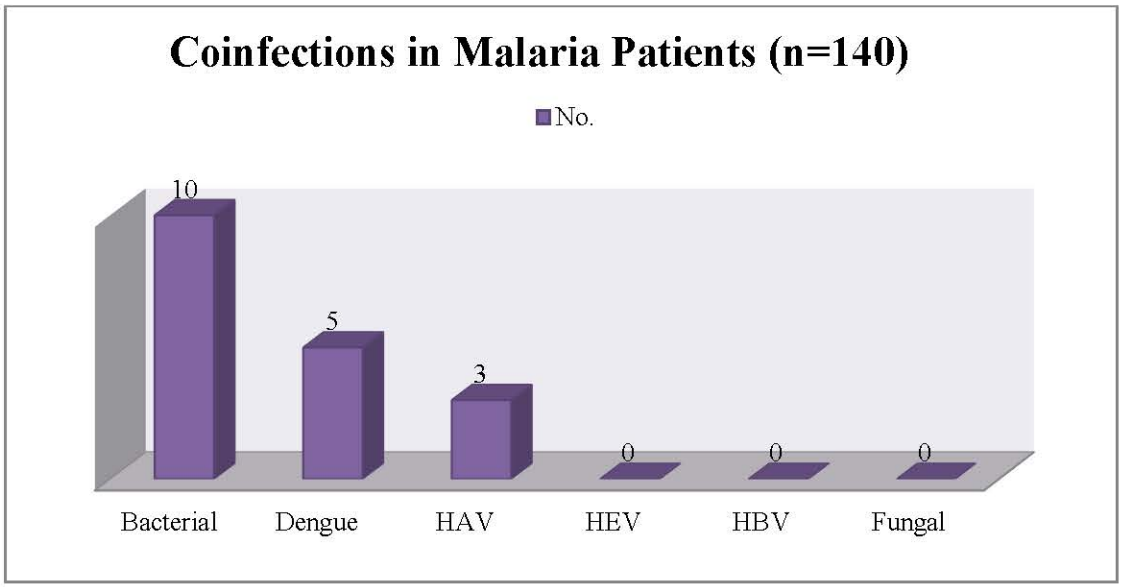
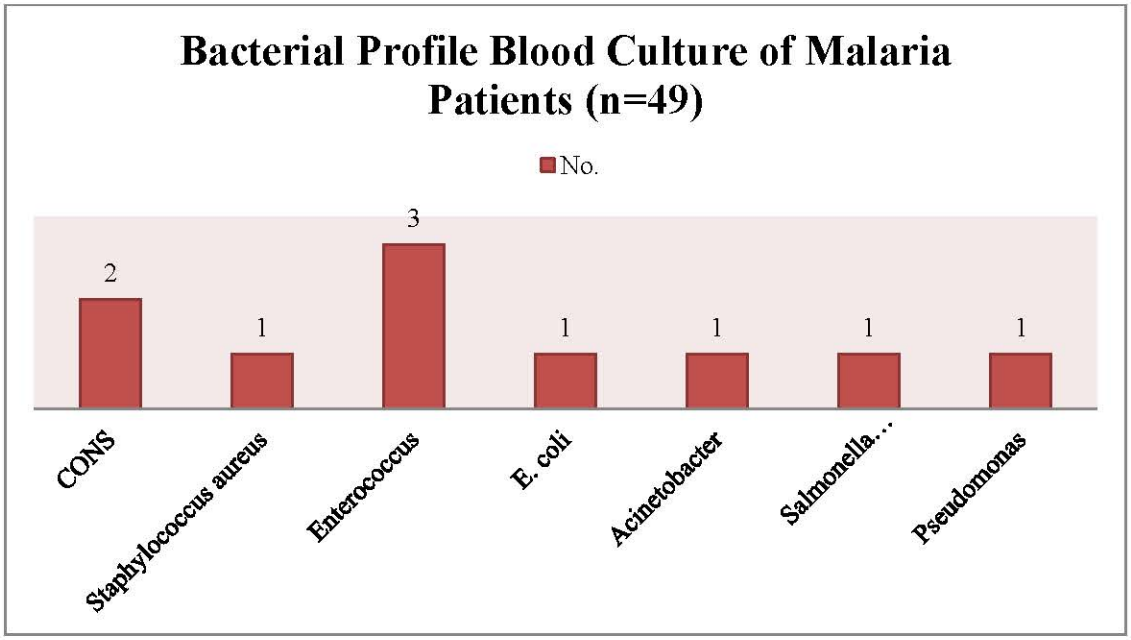


Chart 59: Bacterial Profile of Blood Culture of Malaria Patients (n=49)



A total of 65% patients with malaria survived whereas 31.43% were non-survivors and a small percentage i.e. 3.5% were DAMA patients **(Chart 60)**. Amongst 44 non-survivors the major cause of death was *P. falciparum* infection i.e. 43.18% followed by *P. vivax* 34.09% and 22.73% due to mixed infections **(Chart 61)**. The 44 non-survivors included 1 patient with HIV infection, 3 with concurrent HAV infection, 1 with Dengue and 3 with bacterial infections.

Chart 60: Outcome in Malaria Patients

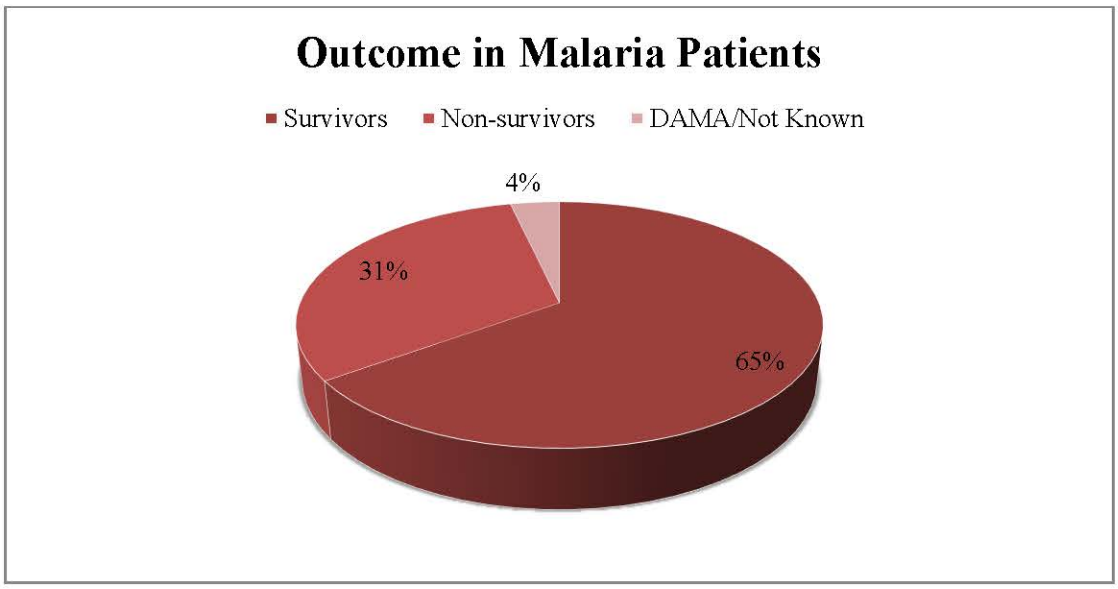
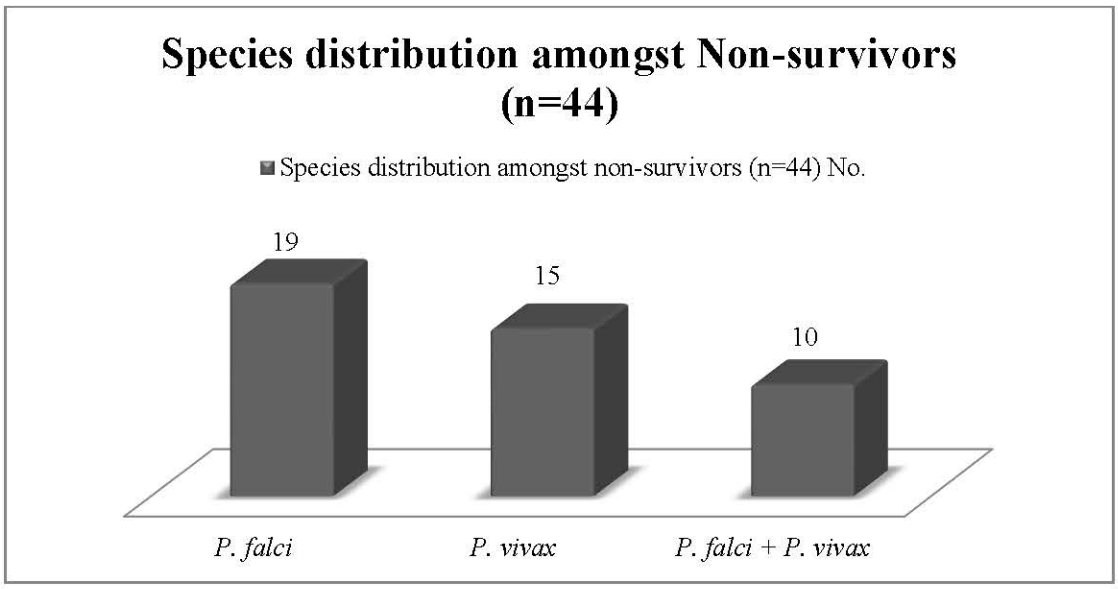


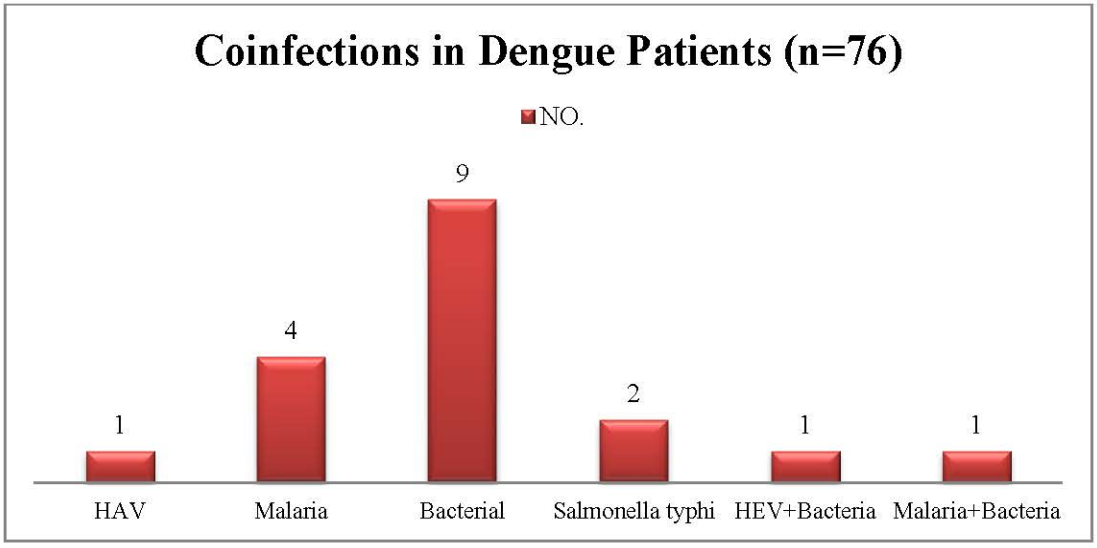
Chart 61: Distribution of *Plasmodium species* amongst Malaria Non-Surviviors



Dengue:

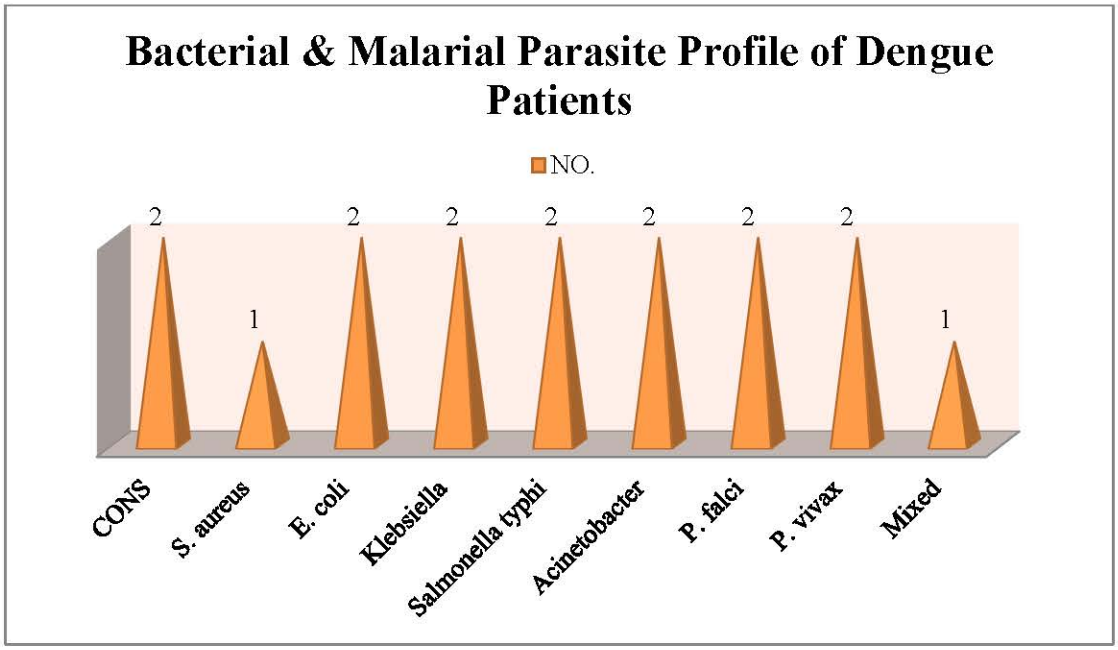
A total of 128 patients were tested for dengue infection by detecting NS1 antigen and IgM, IgG antibodies in patients' serum samples. Of these, 76 (59.37%) tested positive for an antigen or antibody or both. A total of 30.4% (76/250) had dengue as cause of the tropical sepsis and 10.22% (76/743) of the total 743 patients with sepsis, whereas only dengue as a cause of sepsis was seen in 7.80% (58/743) patients whereas the rest 18 had concomitant infections viz. malaria 4, bacterial 9, *Salmonella typhi* – 2, 1 each of HAV, HEV+Bacteria, Malaria + Bacteria **(Chart 62)**. It included samples from 48 males and 28 females.

Chart 62: Coinfections in Dengue Patients



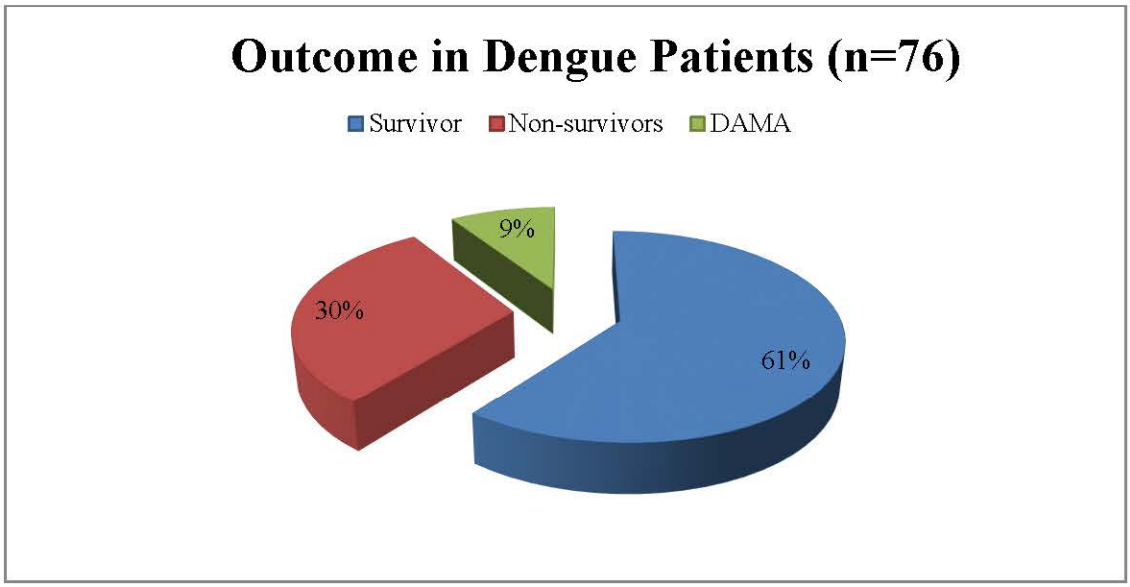
The bacterial profile of blood culture of dengue patients showed gram negative bacterial predominance over gram positive bacterial isolates. Amongst those with concurrent infection with malaria, 2 each had *P. falciparum* and *P. vivax* and 1 had mixed infections with both the species as shown in the **Chart 63** below:

Chart 63: Profile of Coinfections with Bacterial & Malarial Parasite in Dengue Patients



Amongst the 76 patients tested, 60.52% (46/76) survived, 30.26% (23/76) did not survive the episode of dengue while 9.21% (7/76) were DAMA patients. **(Chart 64)**

Chart 64: Outcome in Dengue Patients (n=76)



It was also noted that 5 out of 23 non-survivors had concomitant bacterial infections in 3 patients, malaria in 1 patient and 1 patient had CoNS+HEV together.

Viral Hepatitis:

Hepatitis either due to viruses or alcoholism or any other pathological conditions, is an important cause of sepsis-related MODS and sepsis as well. As a protocol, all the patients admitted to ICU, during the course of hospital stay, were tested for Hepatitis B and Hepatitis C. Hence, all the 743 patients were tested for HBsAg for Hepatitis B and antibodies against Hepatitis C. However, with a clinical suspicion of hepatitis due to faeco-oral route, 59 serum samples were tested for Hepatitis A and Hepatitis E virus antibodies. Thus a total of 4.17% (31/743) tested positive for viral hepatitis **(Chart 65).** Of the total patients with hepatitis, hepatitis due to single viral type was observed in 29 patients, of whom HAV (13/31) was the most common cause followed by HEV (10/31), HBV (4/31) and least by HCV (2/31). Infection with more than one type of hepatitis viruses was seen in two patients i.e. one with HAV+HEV and one with HAV+HBV with *E.coli* isolate obtained from the blood culture **(Chart 66).** Thus viral hepatitis as single entity was seen in 17 patients. Concurrent infections with other microbes were observed in 14 (45.16%) out of total 31 patients i.e. 5 with bacterial, 3 with malaria , 2 with dengue, 1 with HAV+ *Candida spp.*, 1 with HCV+*Candida spp.*+ *CoNS* and 1 with HAV+*Candida spp.*+*E.coli* and HAV+HBV+*E. coli*. **(Chart 66)**

Chart 65: Hepatitis Viruses causing Viral Hepatitis

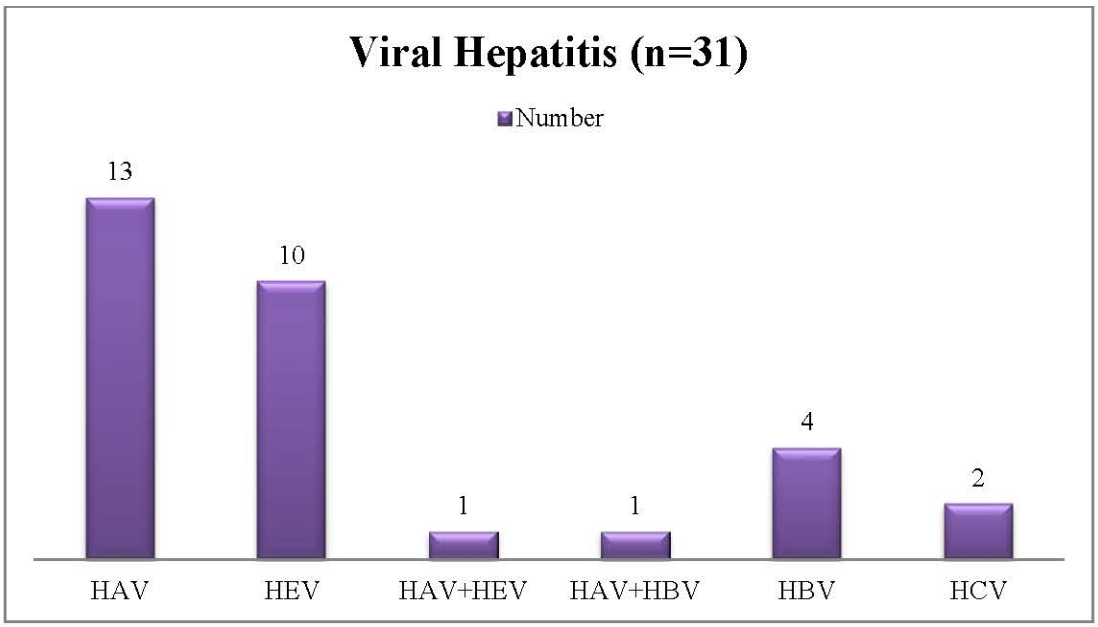
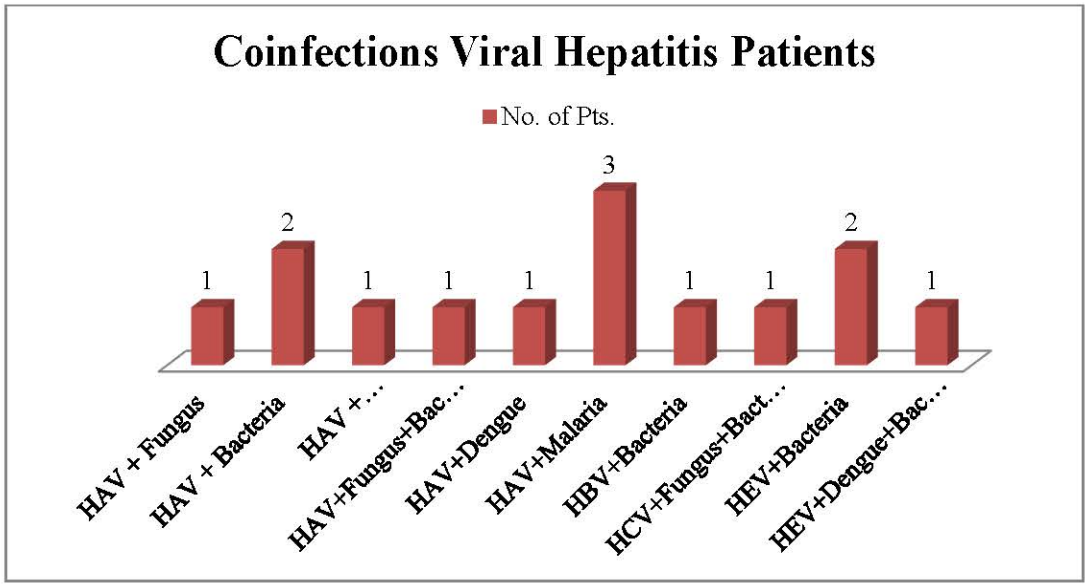
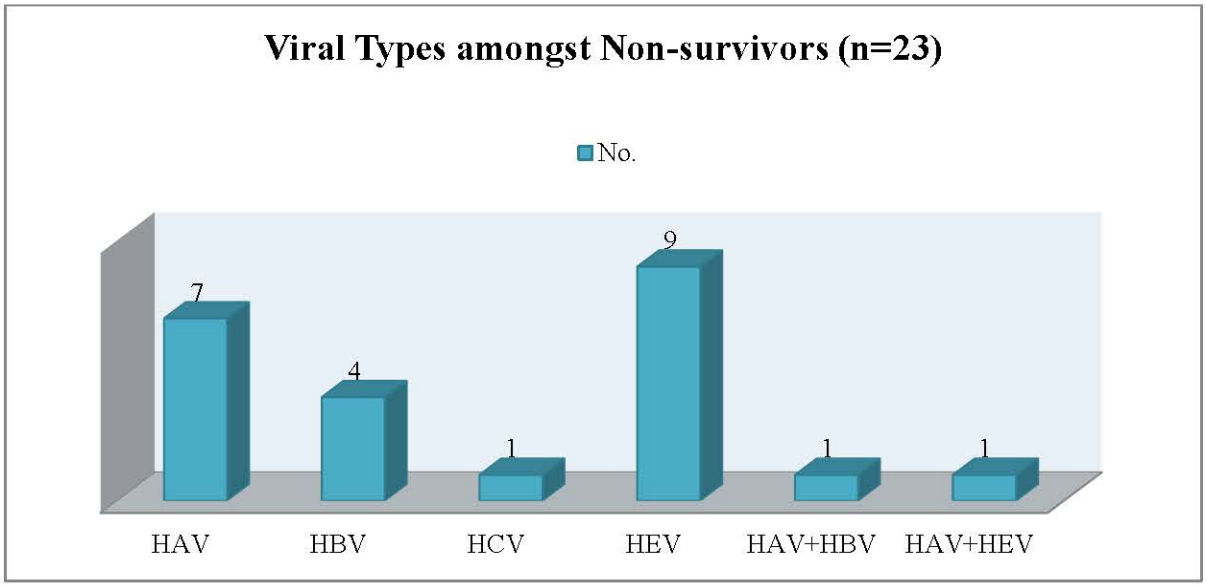


Chart 66: Coinfections in Viral Hepatitis Patients



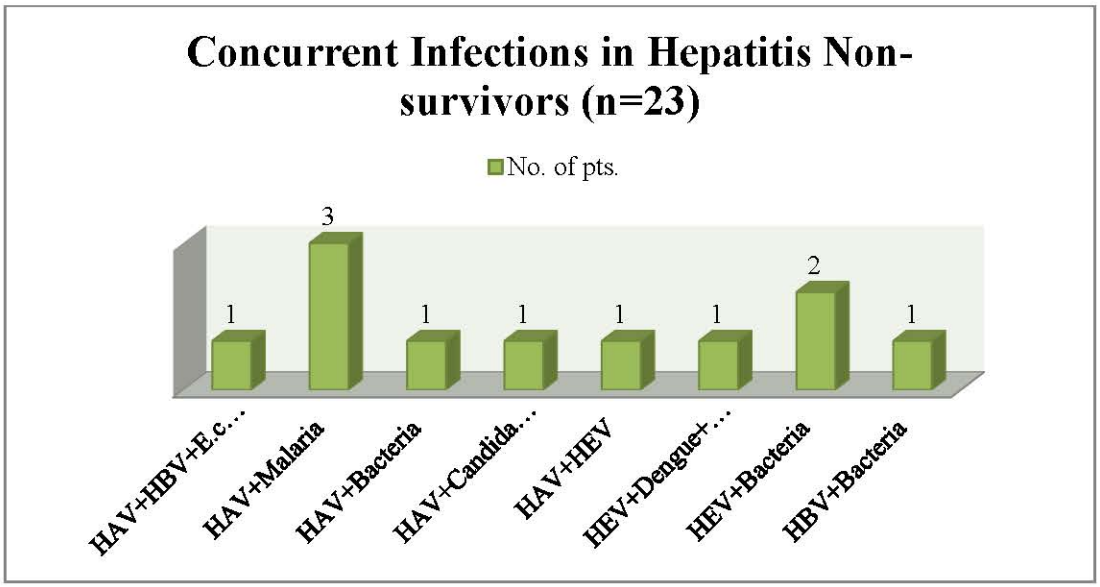
Of the total 31 patients with viral hepatitis, only 25.81% (8/31) patients survived whereas 74.19% (23/31) did not survive sepsis due to viral hepatitis. The mortality was much higher in this group when compared to malaria and dengue group of patients. Amongst the non-survivors the fatality was 100% (5/5) among patients with hepatitis B followed by 91% (10/11) in HEV patients, 60% (9/15) in HAV patients and 50% (1/2) in patients with hepatitis C virus infection as shown (in **Chart 67**) below.

Chart 67: Viral Types Amongst Non-Survivors of Viral Hepatitis



Concurrent infections amongst non-survivors were seen in 11 patients. The details are shown in the **(Chart 68)**

Chart 68: Concurrent infections in Non-survivors of Viral Hepatitis (n=23)



***Leptospira*:**

Of the total 743 patients, 19 patients with clinical suspicion of *Leptospira* infection were tested for IgM and IgG antibodies against *Leptospira interrogans* antigens. Only one patient tested positive for the IgM antibodies representing 5.26% of the total 19 tested; 0.13% (1/743) of the total patients as cause of sepsis and 0.4% (1/250) as cause of tropical sepsis. The patient was a 57 year old male who did not survive and was diagnosed with septic shock with MODS. He had history of diabetes mellitus, smoking, alcoholism and also dialysis and blood transfusion. He tested negative for dengue, HIV, HAV, HBV, HCV, and HEV. His PCT was 10ng/ml and CRP was 110 mg/dL. His platelet counts were 22,000/cumm and his serum SGOT, SGPT, bilirubin, amylase and lipase were all raised. Also his blood culture yielded *Candida non-albicans* and *CoNS* together.

Polymicrobial Etiology:

Of the total 743 patients, 85 had polymicrobial etiology of sepsis, of which 43 were in non-tropical (bacterial/fungal) and 42 in tropical groups. The most common combinations of two microbes are shown in Charts 69, 70 and 71, whereas of the 3 microbes together are shown in 72 & 73.

Chart 69: Bacterial & Fungal Infections together (n=43)

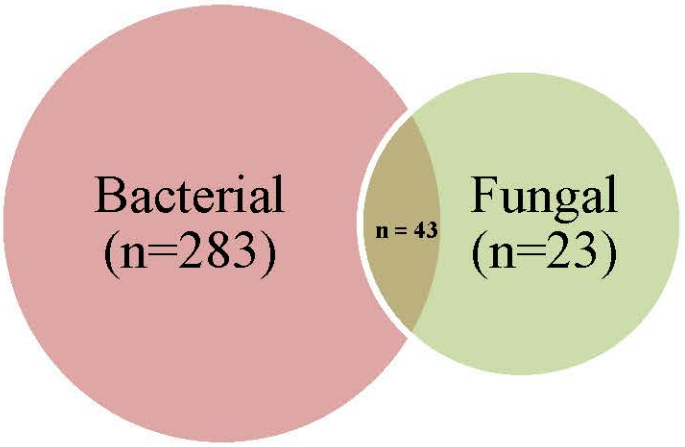


Chart 70: Bacterial & Malaria Infections together (n=8)

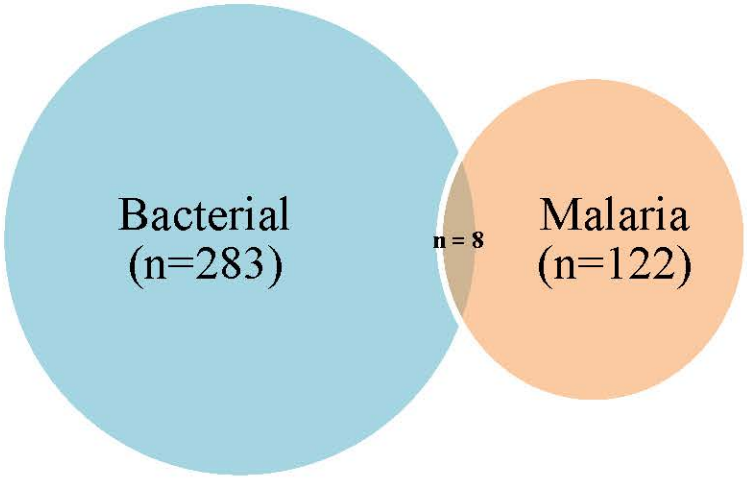


Chart 71: Bacterial & Viral Infections together (n=16)

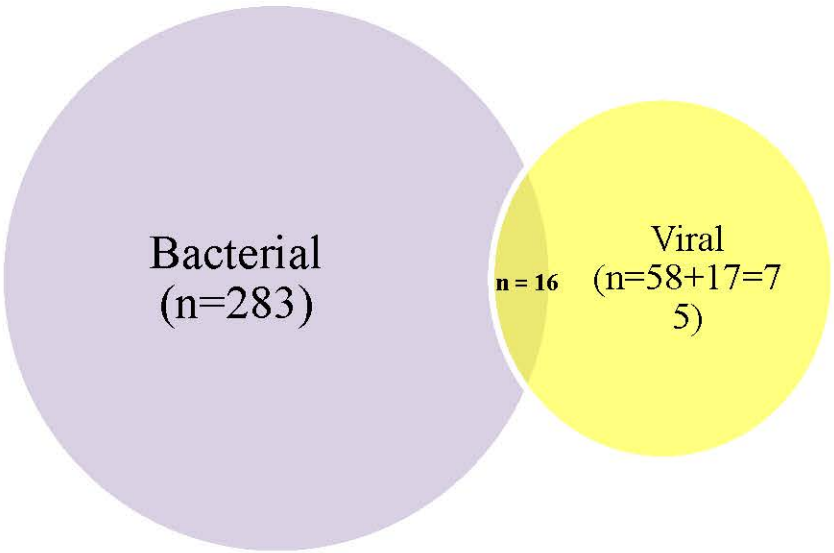


Chart 72: Combination of Bacterial, Viral & Fungal Infections together

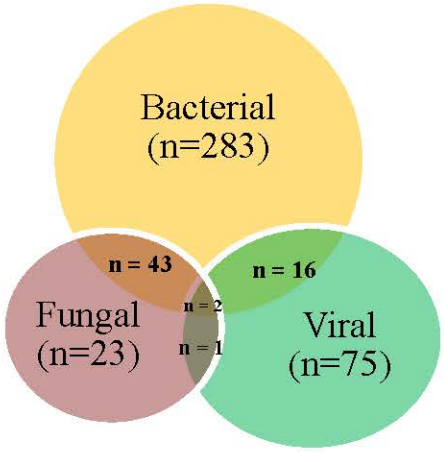
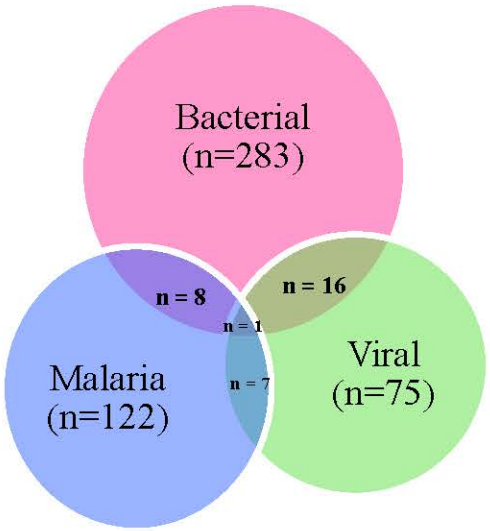
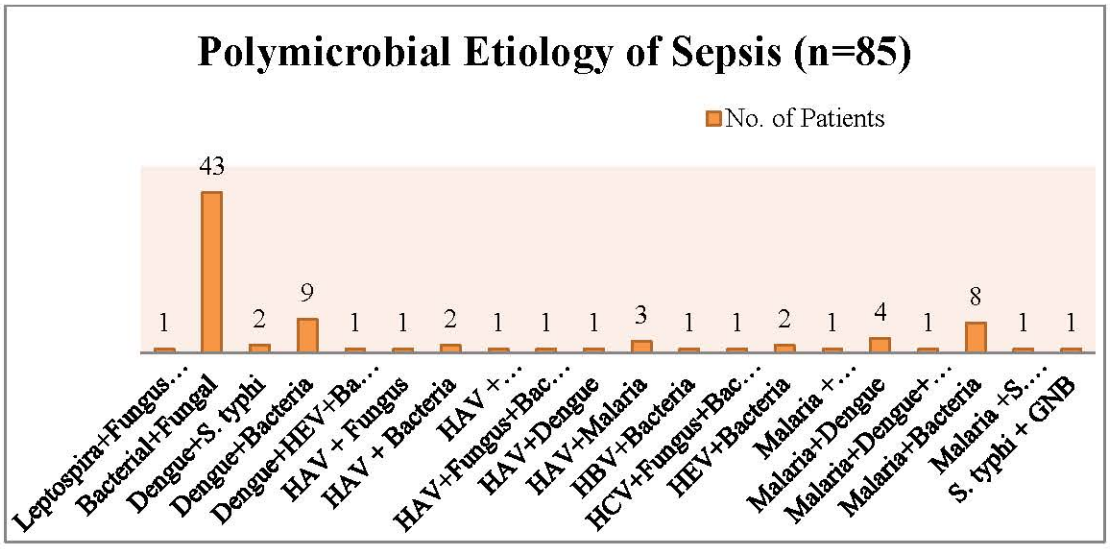


Chart 73: Combination of Bacterial, Viral & Malaria Infections together



Of these 85 patients who had sepsis due to more than one organisms, the causes were as follows: 43 had bacterial & fungal combined, 9 had dengue & bacterial, 8 had malaria & bacterial, 4 had malaria & dengue, 3 had malaria + HAV infections, 2 each of dengue & Salmonella typhi; HAV+bacteria, HEV+bacteria and 1 each of HAV+fungus, HAV+Dengue, HBV+Bacteria, malaria+*Salmonella paratyphi A*, *Salmonella typhi* & *E. coli* whereas 1 each of 3 microbial aetiologies together i.e. malaria+dengue+bacteria, malaria+fungus+bacteria, HCV+fungus+bacteria, HAV+fungus+bacteria, HAV+HBV+bacteria, dengue+HEV+bacteria and dengue+fungus+bacteria **(Chart 74)**

Chart 74: Polymicrobial Etiology of Sepsis

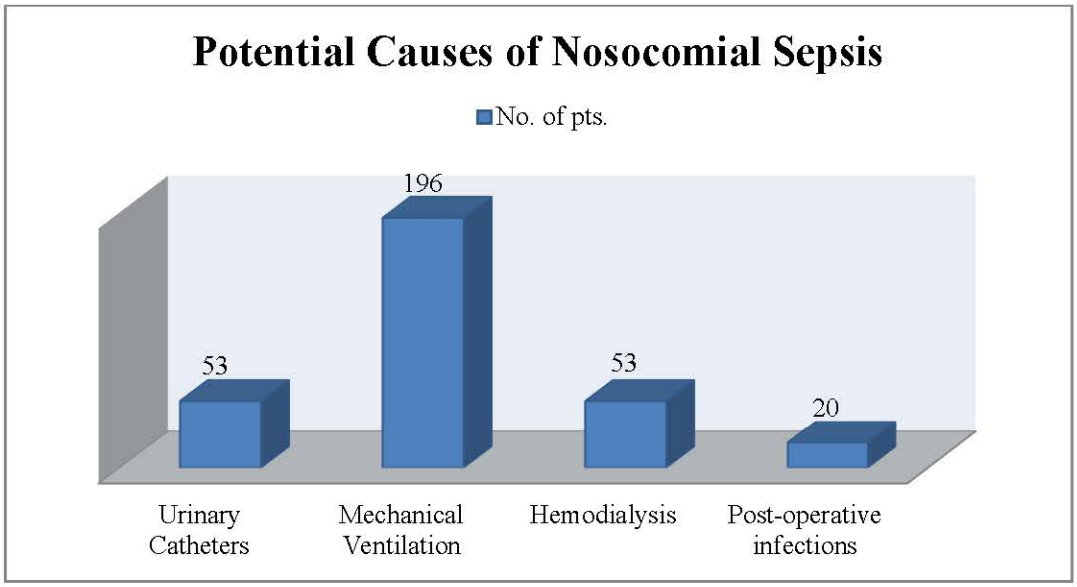


In addition to the above findings, in 132 (21.32%) patients out of 619 patients in whom cultures were performed, besides blood culture second/other samples cultured yielded the same bacterial and/or fungal isolate type with the simiar susceptibility and resistance patterns, thus proving infections at those sites as the cause of sepsis in these patients. In the rest 487 either second/other sample was not cultured or it yielded a different bacterial/fungal isolate or they were culture negative.

Sepsis due to Hospital Acquired/Nosocomial Infections:

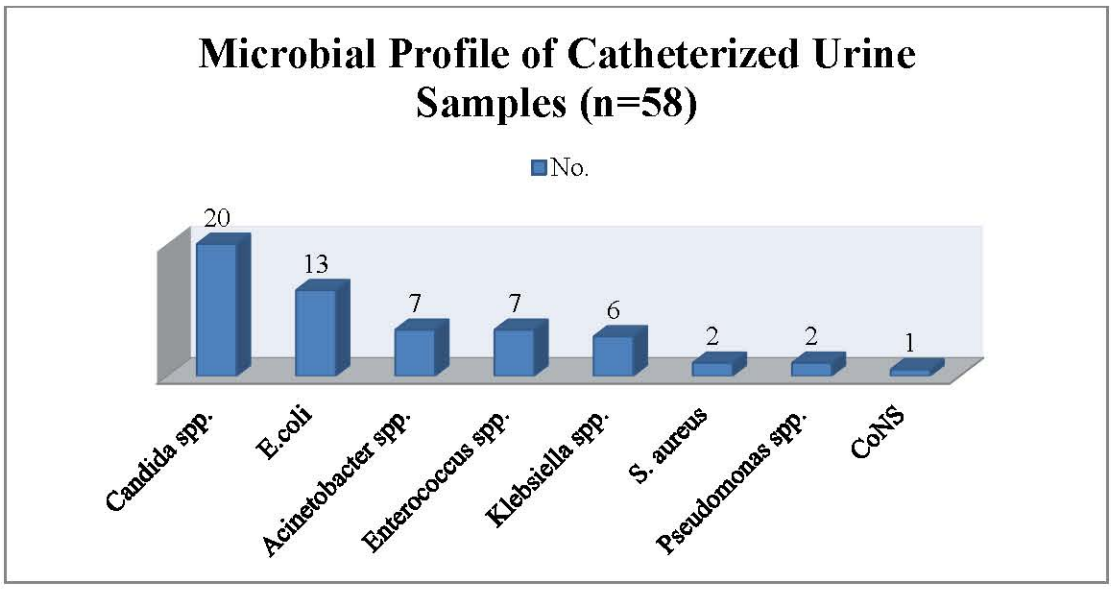
A single value in terms of number or percentage to indicate the sepsis acquired during ICU or hospital stay could not be obtained, as all the patients presenting with sepsis on admission, with or without the history of previous hospitalization, surgery or treatment for any condition as well as those who developed it later during the stay were included. Moreover, infections like malaria, dengue and leptospira are mainly community acquired infections. However, indwelling devices like peripheral lines were placed in almost all the 743 patients at any one given point of time during ICU stay for parenteral nutrition or administration of drugs, urinary catheters in 53 patients, 196 patients on mechanical ventilation; 53 patients who underwent hemodialysis and post-operative infections in patients having undergone surgery were found in 20 patients. Thus these can be considered as potential sources of nosocomial infections leading to sepsis in our setup as shown in the **Chart 75** below.

Chart 75: Potential Causes of Nosocomial Sepsis



From a total of 53 catheterized patients 58 (34.73%) urine samples were obtained. Of the 58 samples, growth was obtained in 89.65% samples yielding 58 isolates. Here *Candida species* (20/58) were the major isolate followed by *E.coli* (13/58) and equal numbers (7/58) of *Acinetobacter spp.* and *Enterococcus species* and then *Klebsiella spp.* (6/58), Pseudomonas spp. (2/58), *S. aurues* (2/58), and *CoNS* (1/58) in the sequence as shown in the **Chart 76**.

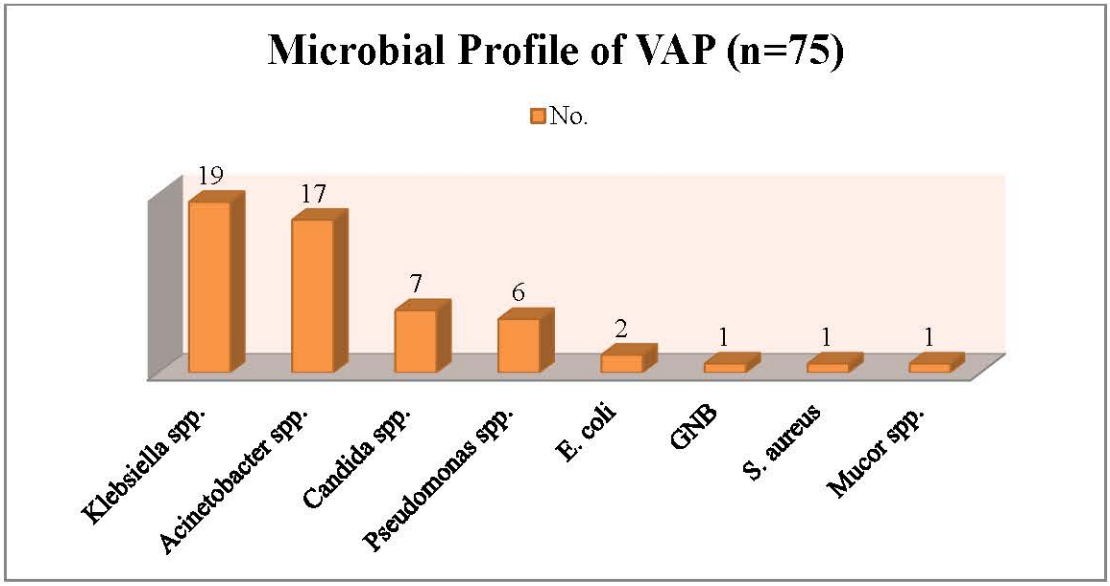
Chart 76: Microbial Profile of Catheterized Urine Samples (n=58)



Similarly from 73 patients, out of 196 on ventilation, with clinically suspected VAP (Ventilator associated pneumonia), 75 ET tips were cultured yielding 54 isolates from

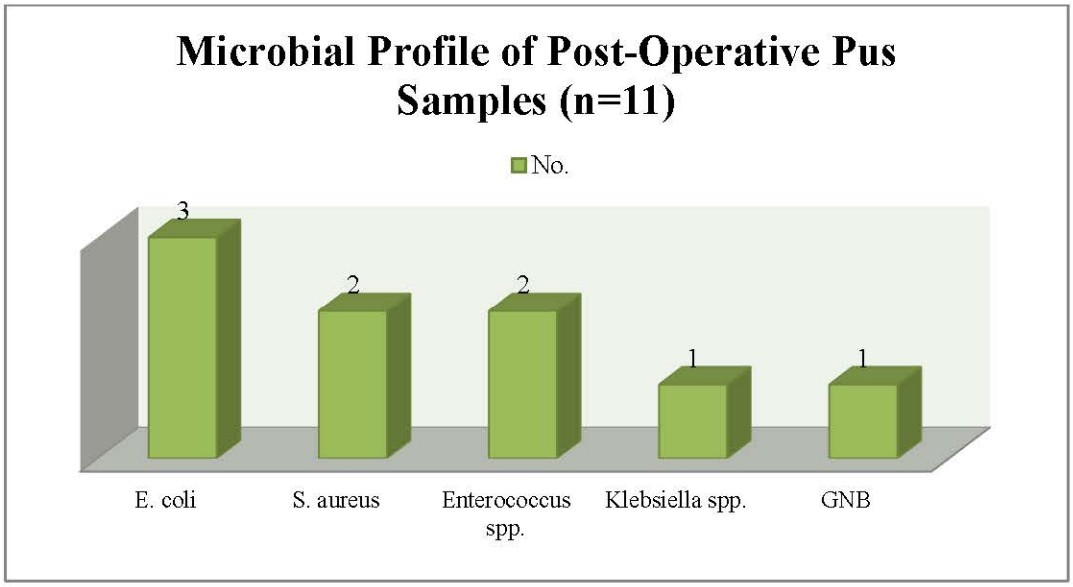
46 samples of 46 patients. Thus 6.19% (46/743) can be labeled to have developed bacteriologically proven VAP whereas 3.63% (27/743) had VAP with an unproven microbial etiology. Eight samples showed polymicrobial growth. Of the total isolates, 45 were gram negative bacilli, 8 were fungi with 7 *Candida species* and 1 *Mucor spp.* and only one isolate of *S. aureus* (2%-1/54)). The most common isolate was *Klebsiella spp.* 35% (19/54), followed by *Acinetobacter* spp. 31% (17/54), *Candida spp.* 13% (7/54), *Pseudomonas spp.* 11% (6/54) and *E. coli* 4% (2/54), 2% each of unidentified GNB (1/8) and *Mucor spp.* (1/54) as shown in the **Chart** 77.

Chart 77: Microbial Profile of Ventilator Associated Pneumonia (n=75)



Of the total 80 pus samples, 20 patients were suspected with infection at the site of surgery performed but pus samples for culture & susceptibility were obtained from only 11 patients who had undergone surgery. The cultures were positive in 8 samples yielding 9 isolates; one sample having 2 isolates together. *E. coli* (3/9) was the most common isolate followed by *S. aureus* & *Enterococcus spp.* in equal numbers i.e. 2 each, *Klebsiella spp.* (1/9) and unidentified GNB (1/9) as shown in **Chart 78**.

Chart 78: Microbial Profile of Post-operative Pus Samples (n=11)



The overall microbial profile of 144 (11+75+58=144) different specimens, from 137 (53+73+11=137) patients, processed for identifying the source of nosocomial infections is shown below in the **Chart 79**. A total of 121 isolates were obtained of which *Candida spp.* with 22.3% (27/121) was most common isolate followed by *Klebsiella spp.* - 21.5% (26/121), *Acinetobacter spp.* 19.8% (24/121), *E. coli* – 14.9% (18/121), *Enterococcus spp.* – 7.4% (9/121), *Pseudomonas spp.* - 6.6% (8/121), *S. aureus* – 4.1% (5/121) and a smaller percentage of unidentified GNB, *CoNS* & *Mucor* spp.

Chart 79: Microbial Profile of Nosocomial Sepsis

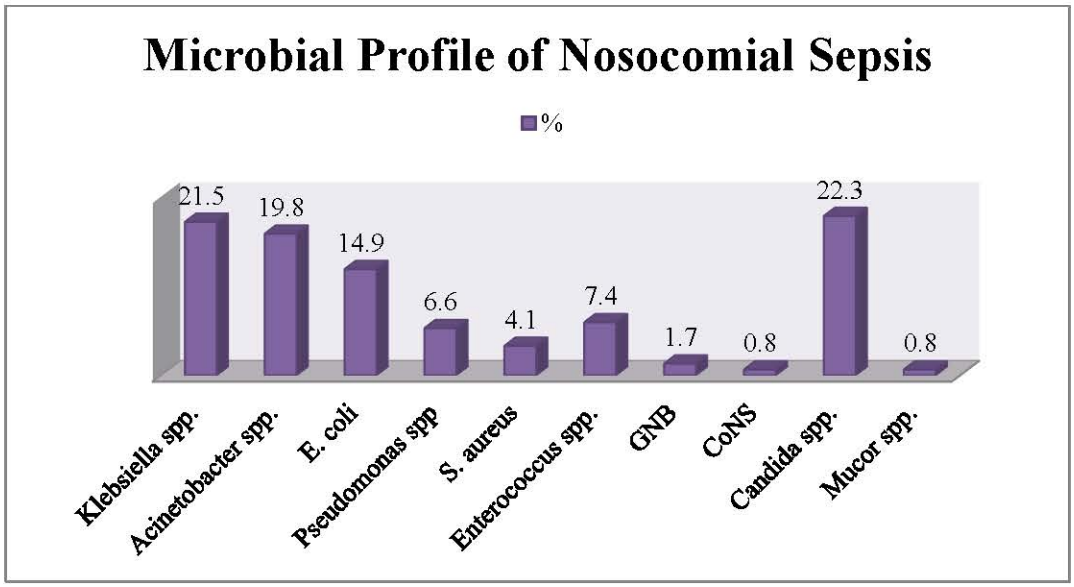
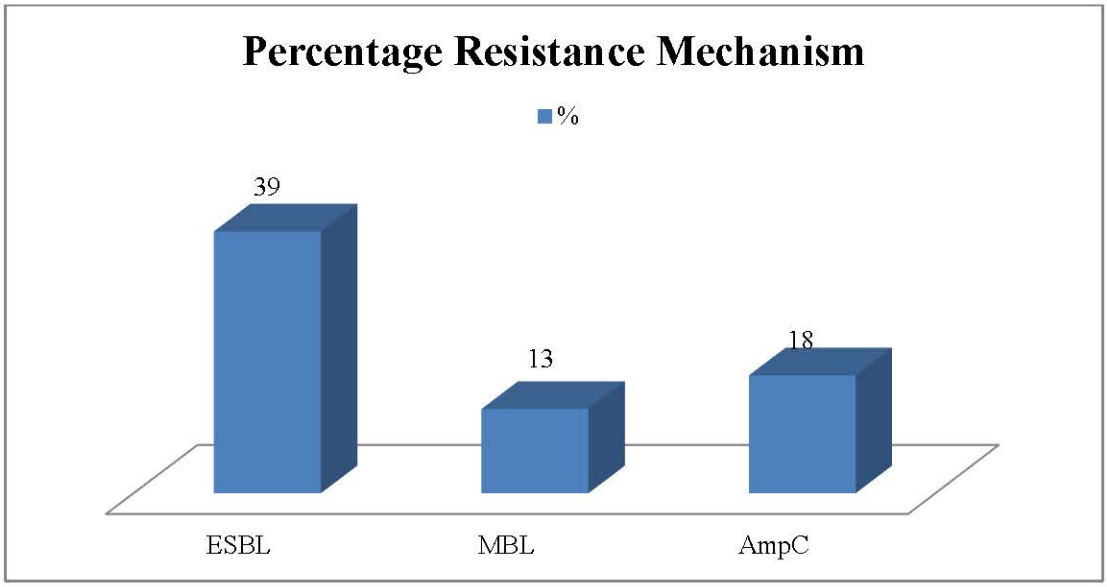


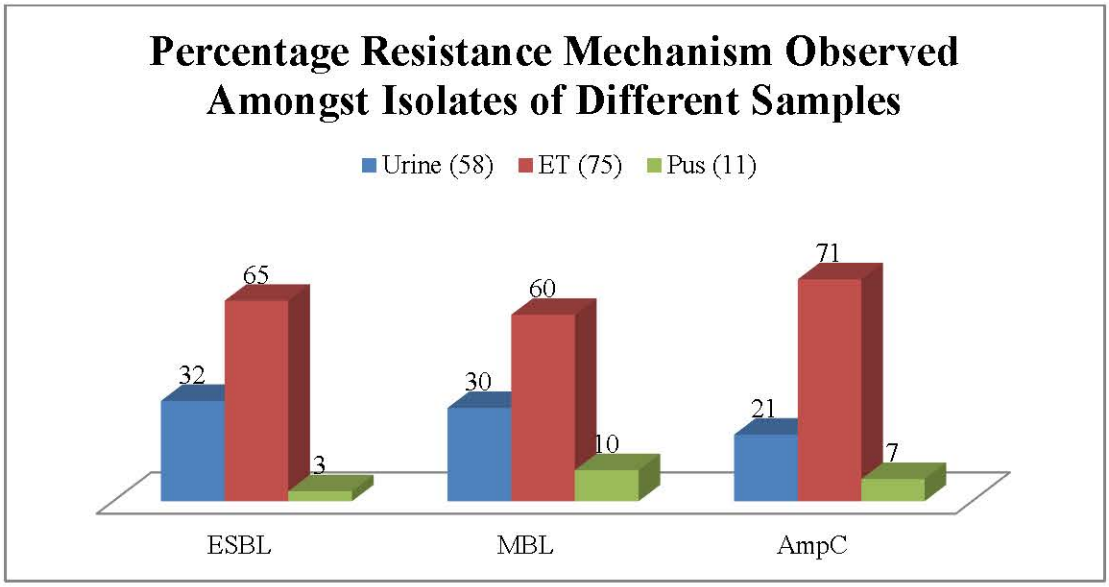
Chart 80: Percentage of Each Resistance Mechanism Type Exhibited by Gram Negative Nosocomial Pathogens (n=78)



Of the total 78 GNB, ESBL production was seen in 39% (31/80) isolates, MBL in 13% (10/80), AmpC in 18% (14/80). Thus a total of 70.51% of GNB showed drug resistance mechanisms with resistance to multiple drugs tested whereas amongst GPC, MRSA was seen in 60% (3/5) isolates of *S. aureus* as shown in the **Chart 80** above.

Overall when different samples were compared, as shown in the **Chart 81** below, for the percentage of resistance mechanisms observed in different samples the isolates obtained from ET samples were the most resistant isolates showing the highest percentage of all the mechanisms i.e. 65% ESBL, 60% MBL and 71% AmpC-beta-lactamase production as compared to the isolates of urine and pus samples.

Chart 81: Percentage Resistance Mechanisms Observed Amongst Isolates of Different Samples



Biomarkers of Sepsis: Procalcitonin (PCT) & C-Reactive Protein (CRP)

PCT:

PCT is considered to be an important biomarker that rises during infection and helps to predict sepsis especially due to bacterial pathogens. Thus PCT was carried out in a total of 288 patients out of 743 patients as shown (in the **Chart 82**). It was carried out by a semi-quantitative test. Ninety seven with tropical sepsis, 47 with non-tropical and 144 with sepsis due to unidentified etiologies were tested making a total of 288 patients. Of the total 288 patients tested, 43 had values of ≤0.5ng/ml, 50 had values of >0.5<2 ng/ml, 168 had ≥2<10ng/ml and 27 had values of ≥10 ng/ml. Thus a larger number of patients showed the values ranging between 2ng/ml and 10ng/ml **(Table 9)**

Chart 82: Distribution of PCT in patients with sepsis due to different microbial etiologies.

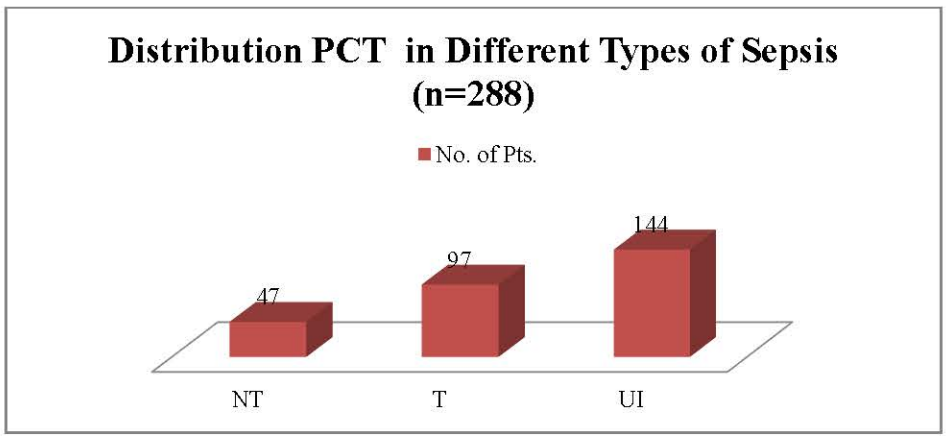
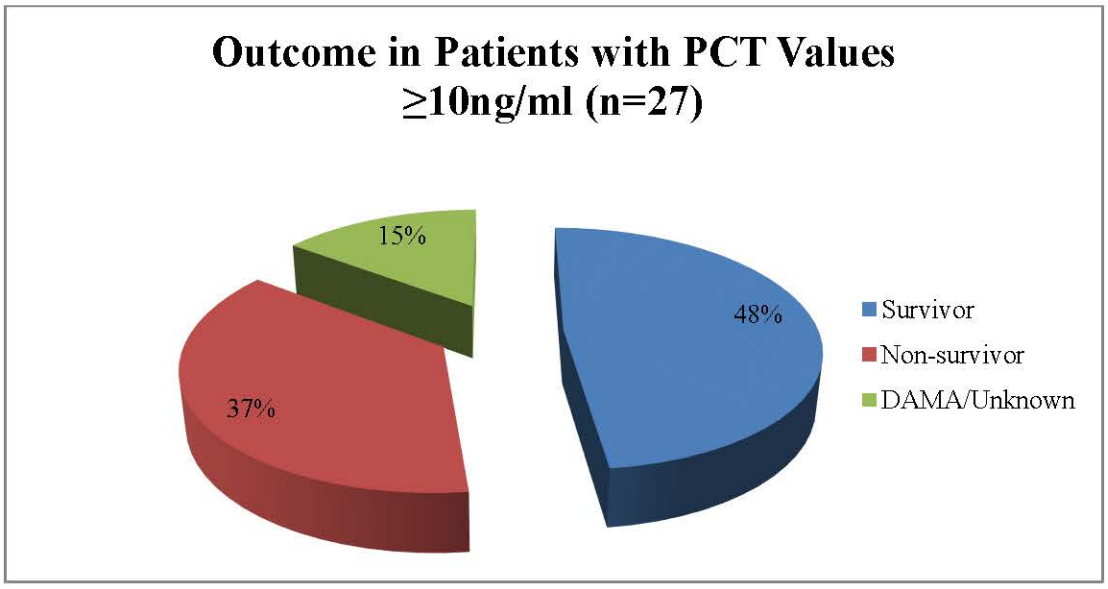


Table 9: Number & Percentage of Patients with different PCT Values

PCT	Frequency	Percentage (%)
≤0.5 ng/ml	43	14.9
>0.5 <2 ng/ml	32	11.1
≥2 <10 ng/ml	186	64.6
≥10 ng/ml	27	9.4
Total	288	100.0

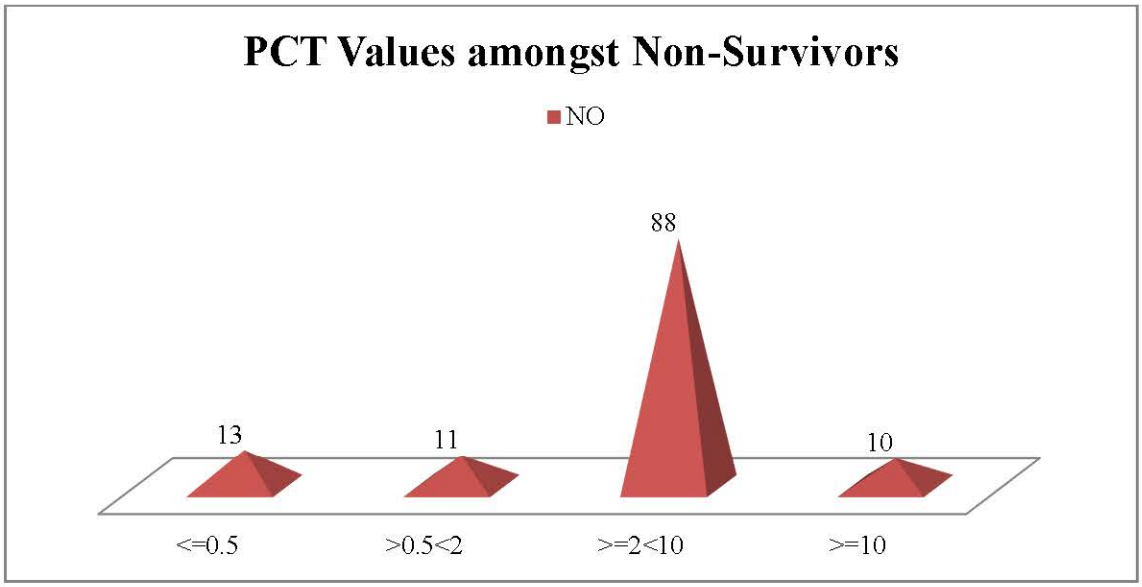
Of the total 288 tested, 27 had values of PCT ≥ 10ng/ml. The outcome amongst these patients is as shown below in the chart. Accordingly 13 survived, 10 did not survive and for 4 patients the outcome was not known. **(Chart 83)**

Chart 83: Outcome in Patients with PCT values ≥10 ng/ml



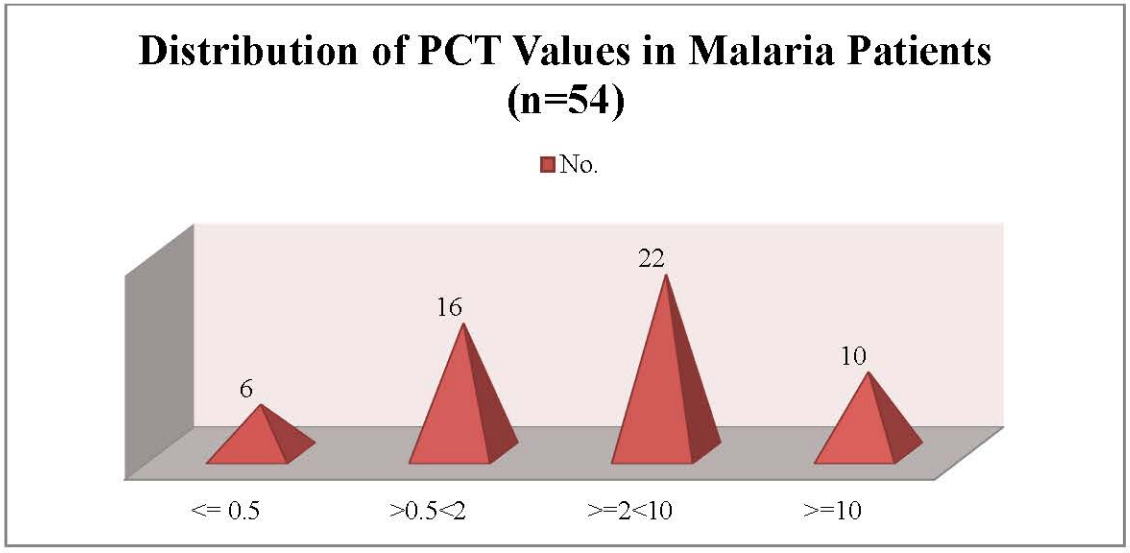
Amongst 122 non-survivors in whom PCT values were determined, the majority i.e. 88 had PCT values of >2<10 ng/ml followed by 13 with values of ≤0.5ng/ml, 11 with >0.5<2 ng/ml and 10 had values of >10ng/ml as shown in the **Chart 84** below.

Chart 84: PCT values amongst Non-survivors



PCT values amongst different groups of microbial etiology i.e. due to malaria, dengue and only bacterial infection were analyzed.

Chart 85: Distribution of PCT Values in Malaria Patients



As shown in the **Chart 85** above, amongst the 54 malaria patients tested for PCT, the maximum number of patients i.e. 22 showed PCT values of ≥2ng/ml <10ng/ml, followed by 16 patients with values of >0.5ng/ml<2ng/ml and 10 patients with ≥10ng/ml and least i.e. 6 patients with ≤0.5ng/ml.

Patients with PCT values ≥10ng/ml were 10. Moreover, 6/10 patients did not survive and again of these 6 non-survivors, 1 had coinfection with *Acinetobacter spp.*, 1 with HAV and 1 patient was HIV positive.

On comparison of PCT values of ≥10 ng/ml with CRP values in these 10 patients, 8 patients had values of >100 mg/L whereas only 2 had values <100 mg/L. This shows that both PCT and CRP are raised with the severity of disease and are associated with a greater mortality. The mean CRP of these patients was 124 mg/L.

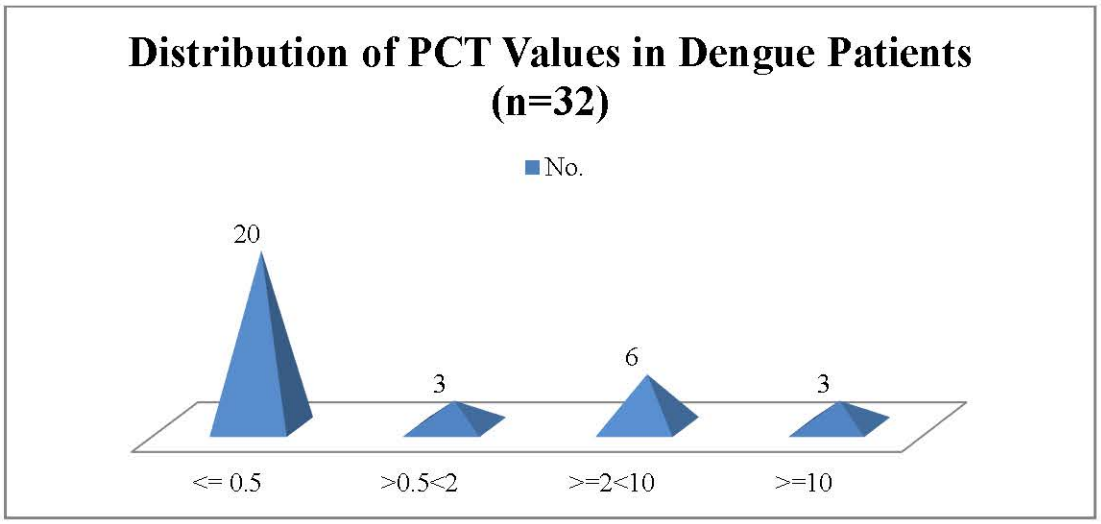
Whereas amongst those with values of ≥2 ng/ml <10ng/ml, 11 patients did not survive. Amongst these 11 patients, 2 had coinfection i.e. 1 with *Staphylcoccus aureus* and 1 with *Pseudomonas spp.* The mean CRP amongst these 11 patients was 93.21 mg/L. Of these 11 patients, 4 patients had CRP values of >100mg/L whereas 6 had CRP values ranging between 50 mg/L and 100mg/L and only patient had 51.21mg/L. Here the CRP values were definitely on rise as compared to PCT indicating the severity of the disease. But according to the PCT interpretation chart, values for PCT of ≥2 ng/ml <10ng/ml indicates an increased risk for progression to severe sepsis and septic shock.

Only 16 patients had values >0.5<2 ng/ml for PCT. Amongst these patients, 6 patients expired. Of these 6 patients, one patient had concomitant enteric fever with *Salmonella paratyphi A* isolate from the blood culture of the patient. The mean CRP values of these 6 patients was 124 mg/L and of which 3 had values >100 mg/L and 2 had >90<100 mg/L and only 1 had values of 27.68 mg/L. Here again the CRP values were on rise in patients with severity and mortality as compared to PCT values. However, values >0.5<2 ng/ml for PCT does not rule out mortality but indicates a moderate risk for progression to severe sepsis.

Overall, the PCT values of ≥10 ng/ml indicate severity of malaria infection in patients in the similar manner as in bacterial infections and CRP values rise with rising severity much more than the PCT values.

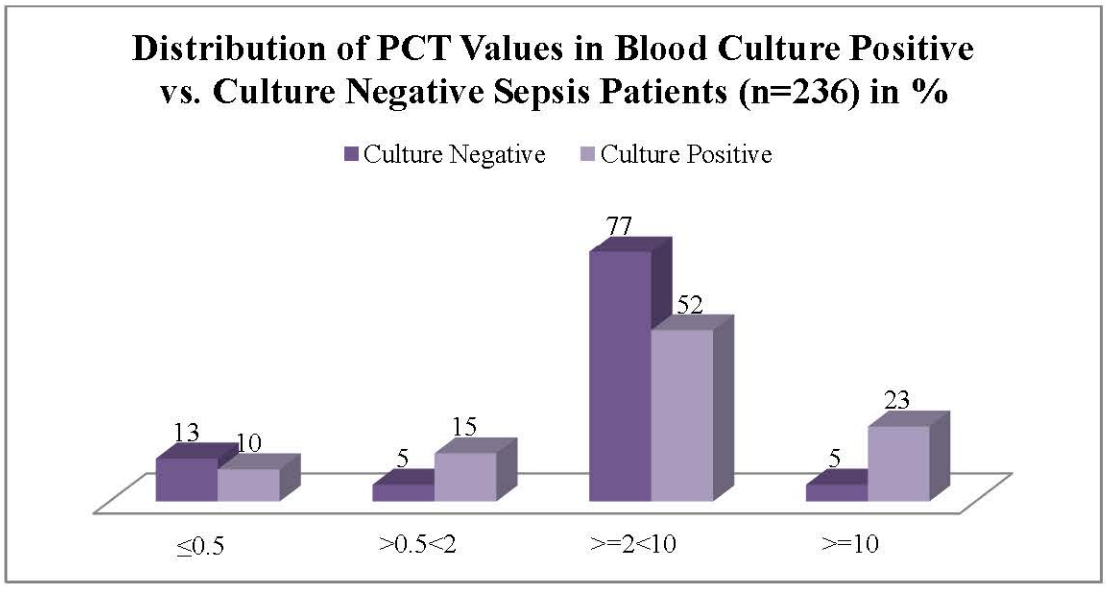
Amongst the 32 dengue patients tested for PCT, majority i.e. 62.5% (20/32) had values of ≤0.5ng/ml followed by 18.75% (6/32) with values ≥2<10 ng/ml and finally, equal numbers of patients i.e. 3 each, had PCT values of >0.5<2 ng/ml and ≥10ng/ml **(Chart 86)**

Chart 86: PCT Values in Dengue Patients tested



Of the total 288 patients in whom PCT was determined blood cultures were performed in 236 patients. Of these 236 patients, 175 patients were culture negative and 61 were culture positive. The following **Chart 87** shows the comparison of the PCT values between culture positive sepsis patients and culture negative sepsis patients.

Chart 87: PCT Values in Blood Culture Positive vs. Culture Negative Sepsis Patients



Thus as shown in the **Chart 87** above, the majority i.e. 77% (134/175) and 52% (32/61), in culture negative and culture positive, had PCT values of ≥2<10 ng/ml respectively. It was also noted that 23% (14/61) and 5% (9/175) amongst culture positive and negative patients, had PCT values of ≥10ng/ml. Though cultures were negative in 77 patients, the PCT values were raised with values of ≥2<10 ng/ml, indicating there was an infection but the microbial etiology could not be established.

C-Reactive Protein (CRP):

CRPis a well known marker of inflammation and has been in use, particularly, higher values indicating infection. Thus CRP was carried out in 183 patients. The mean CRP was 69.23 mg/L. A total of 43 patients showed a CRP values of >100<320mg/L.

Also the mean CRP values were determined for patients in whom PCT was done for each PCT values as shown in the **Table 10**. As shown in the **Table 10**, the CRP values showed a rise in titres with rise in PCT values. Stastically this increase in CRP was significant when compared with rise in PCT values. Also ESR values increased with increasing PCT values, but this was not as significant as CRP values. **(Table 11, 12 and 13)**

Table 10: Comparison of Mean CRP values with PCT values

PCT Values	Mean CRP Values
≤0.5 ng/ml	55.35 mg/L
>0.5<2 ng/ml	78.87 mg/L
≥2 ng/ml <10 ng/ml	63.75 mg/L
≥10 ng/ml	106.20 mg/L

Table 11: One way ANOVA – Comparison of PCT with Mean CRP & Mean ESR Values

	PCT	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
CRP	<=0.5	36	55.3575	40.10434	6.68406	41.7881	68.9269	6.00	140.00
	>0.5 <2	30	78.8703	47.74604	8.71720	61.0417	96.6990	9.81	153.00
	>=2	57	63.7568	47.44723	6.2845	51.167	76.3463	7.30	207.69

	PCT	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
	<10				4	4			
	>=10	22	106.2055	45.82025	9.76891	85.8899	126.5210	6.45	173.00
	Total	145	71.2389	48.12301	3.99640	63.3397	79.1381	6.00	207.69
ESR	<=0.5	18	23.7778	13.61036	3.20799	17.0095	30.5460	12.00	70.00
	>0.5 <2	24	25.7083	6.57055	1.34121	22.9338	28.4828	18.00	39.00
	>=2 <10	44	32.3409	19.25103	2.90220	26.4881	38.1938	10.00	120.00
	>=10	17	35.2941	12.93621	3.13749	28.6429	41.9453	12.00	60.00
	Total	103	29.7864	15.53216	1.53043	26.7508	32.8220	10.00	120.00

Table 12: ANOVA

ANOVA						
		Sum of Squares	df	Mean Square	F	P-value
CRP	Between Groups	40916.489	3	13638.830	6.573	<0.001
	Within Groups	292562.210	141	2074.909		
	Total	333478.699	144			
ESR	Between Groups	1851.816	3	617.272	2.686	.051
	Within Groups	22755.485	99	229.853		
	Total	24607.301	102			

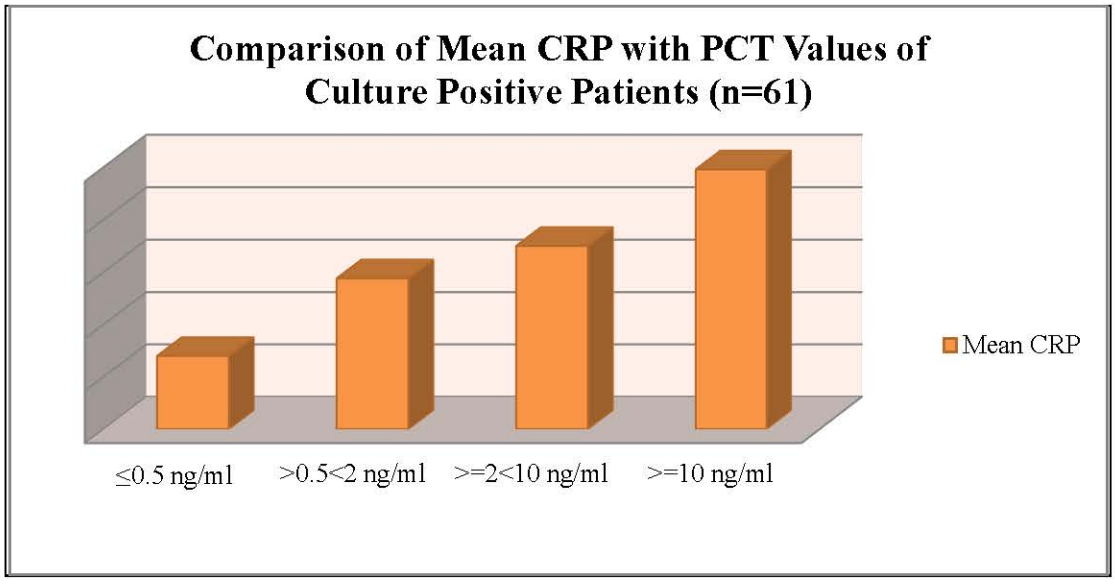
Table 13: Post HOC Test

Dependent Variable	PCT		Mean Difference	Std. Error	P-value	95% Confidence Interval	
						Lower Bound	Upper Bound
CRP	<=0.5	>0.5 <2	-23.51283	11.26055	.162	-52.7895	5.7639
		>=2 <10	-8.39934	9.69733	.822	-33.6118	16.8131
		>=10	-50.84795	12.32682	<0.001	-82.8969	-18.7990
	>0.5 <2	>=2 <10	15.11349	10.27451	.458	-11.5996	41.8265
		>=10	-27.33512	12.78584	.146	-60.5775	5.9072
	>=2 <10	>=10	-42.44861	11.43311	.002	-72.1739	-12.7233

As shown in the tables above, CRP levels were significantly higher in patients with PCT values of ≥10ng/ml as compared to those with PCT values of ≤0.5ng/ml as well as those with ≥2<10 ng/ml PCT values.

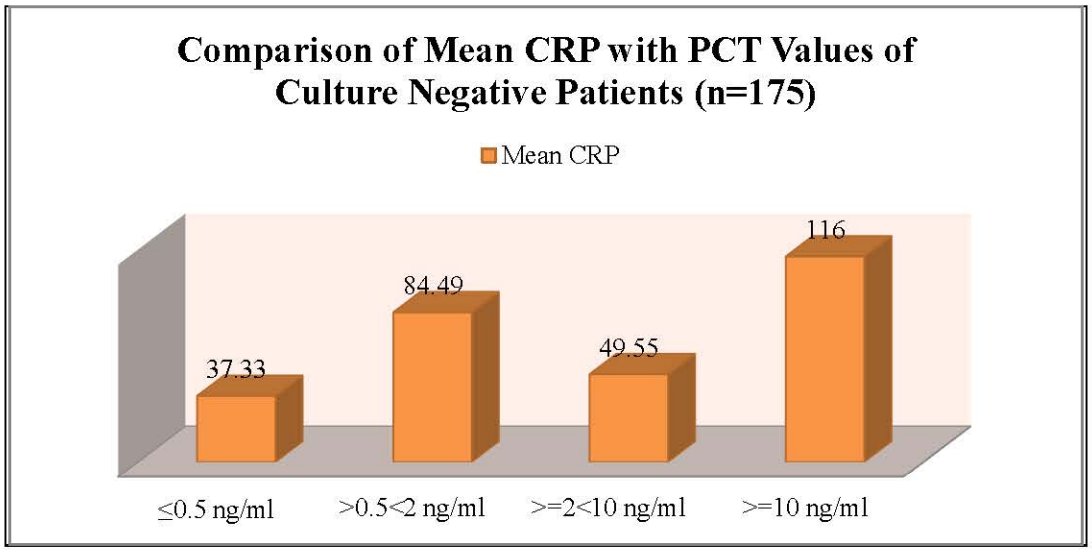
As shown in the **Chart 88** below, the mean CRP values were found to be rising simultaneously with the rise in PCT values among patients with positive cultures.

Chart 88: Comparison of Mean CRP with different PCT values among culture positive patients



However, amongst the culture negative patients as shown in **Chart 89**, the mean CRP values were found to be rising with rising PCT values, but in the category of patients with PCT values ≥2<10 ng/ml, the mean CRP values were found to be low and again a rise in the titres was seen with ≥10ng/ml PCT values.

Chart 89: Comparison of Mean CRP with different PCT values among culture negative patients



Comorbid Conditions, Laboratory Parameters & Organ Involvement Pattern:

Comorbid conditions like hypertension, diabetes mellitus and tuberculosis were noted in all the 743 patients and their association with mortality is as shown below in the **Table 14**.

Table 14: Comorbid conditions and their association with mortality

Comorbid Conditions	In No. Of Pts. (N=743)	No. Of pts. Expired	p value
Hypertension (HTN)	188	88	0.005
Diabetes mellitus (DM)	193	93	0.001
Tuberculosis (TB)	10	7	0.037

Thus as shown in the above **Table 14** comorbid conditions like hypertension, diabetes mellitus and tuberculosis were significantly associated with mortality in these patients as compared to those who did not have these. Other comorbid conditions like COPD (chronic obstructive pulmonary disease), CRF/CKD (chronic renal failure/chronic

kidney disease), liver cirrhosis and sickle cell anemia were observed in 15, 20, 9 and 4 patients respectively.

Table 15: Association of GCS, Mechanical ventilation and dialysis with mortality

Conditions	In No. Of Pts. (N=743)	No. Of pts. Expired	p value
GCS<13	84	47	<0.001
Mechanical Ventilation	196	115	<0.001
Dialysis	53	38	<0.001

Also GCS<13 (Glasgow Coma Scale), mechanical ventilation and dialysis were noted and analyzed for their association with mortality as shown in the table below. Thus as shown in the **Table 15** GCS<13, use of mechanical ventilation and dialysis were significantly associated with mortality in these patients.

Laboratory Parameters:

Laboratory parameters like total leukocyte count (TLC), platelet count, ESR (Erythrocyte sedimentation rate), CRP (C-reactive protein), total bilirubin (TB), urea & creatinine were noted and analyzed for their association with tropical infections (T), bacterial/fungal infections(Non-tropical-NT) and infections with unidentified etiology (UI). To compare average (median) values of these parameters between three groups, Kruskal – Wallis test is applied. Results are given in below **Table 16 & 17**

Table 16: Laboratory parameters & Kruskal-Wallis Test

	T_NT_UI	N	Mean Rank
ESR	T	119	71.23
	NT	28	118.43
	UI	16	98.38
CRP	T	121	104.62
	NT	39	70.74
	UI	20	43.60

	T_NT_UI	N	Mean Rank
TLC	T	250	183.08
	NT	349	489.49
	UI	144	415.24
SGPT	T	132	230.21
	NT	163	158.00
	UI	72	158.13
SGOT	T	129	227.40
	NT	162	155.89
	UI	72	159.41
TB	T	250	453.81
	NT	349	325.96
	UI	144	341.56
DB	T	250	426.43
	NT	349	335.44
	UI	144	366.10
IB	T	250	455.54
	NT	349	328.66
	UI	144	332.01
Urea	T	232	338.00
	NT	348	377.02
	UI	143	364.39
Creatinine	T	232	326.50
	NT	348	377.23
	UI	143	382.53

Table 17: Chi-square test

	ESR	CRP	TLC	SGPT	SGOT	TB	DB	IB	Ur	Creat
Chi-Square Value	24.865	30.701	304.176	39.148	37.541	58.049	28.275	60.081	4.912	9.981
DF	2	2	2	2	2	2	2	2	2	2
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.086*	0.007

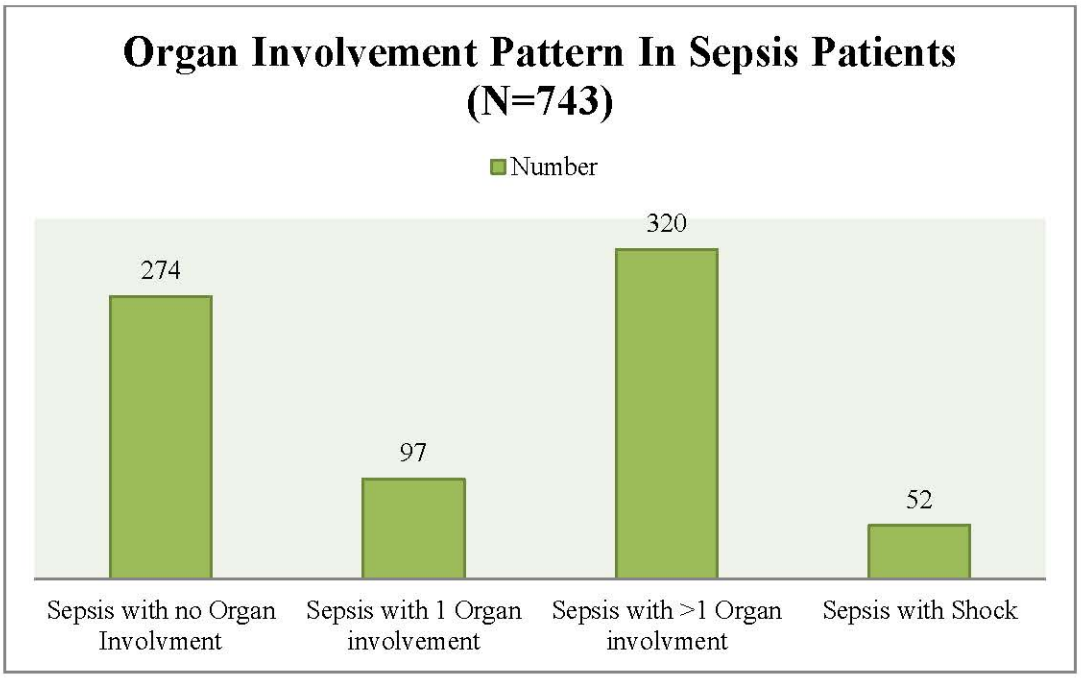
*Borderline significant.

Thus, CRP, TLC and Total bilirubin along with SGOT & SGPT were significantly higher in patients with sepsis due to tropical infections as compared to those with sepsis due to bacterial/fungal infections and unidentified etiology, whereas ESR and Urea were significantly higher in those with sepsis due to bacterial/fungal infections as compared to those with tropical infections and unidentified microbial etiology.

Organ Involvement Pattern:

Of the total 743 patients, 274 patients had sepsis with no organ involvement; 97 with one organ involvement; 320 had sepsis with multiple-organ involvement and 52 with septic shock as shown in **(Chart 90)**. The most common organ involved was kidney with creatinine values >1.2mg/dL in (n=371) followed by liver with bilirubin values of >1.2mg/dL in (n=357) and hemopoetic system with a platelet count of <1, 50,000/µl (n=310). Kidney (n=189/349) and Liver (n=173/250) were the frequently affected organs in sepsis due to bacterial/fungal infections and tropical infections respectively. Thrombocytopenia was most commonly found in those with tropical infections (n=181) followed by those with bacterial/fungal infections (n=88) and least amongst those with unidentified etiology (n=41).

Chart 90: Organ Involvement Pattern In Sepsis Patients (N=743)



Summary of Results:

Overall the results can be summarized as follows:

1. Of 743 patients, 457 were male and 256 were females. The ratio being 1.6, with males being affected more.
2. The mean age of sepsis patients was 48.02 years.
3. Out of 743 patients, 284 patients (38.22%) died (i.e. non-survivors), 411 survived and for 48 patients the outcome was not known as they took discharge against medical advice or referred to higher centre.
4. The average age of non-survivors was 50.86 years and that of survivors was 46.05 years.
5. The above statements 2, 3 and 4 suggest that sepsis occurs in middle age persons and about one-third patients die due to sepsis.
6. Forty-seven percent patients had sepsis due to bacteria and/or fungi, 34% due to tropical infections and 19% sepsis due to unidentified microorganism. This means that in our setup one-third of patients would have sepsis due to tropical infections.
7. Amongst tropical infections present in 250 patients, 48.8% had malaria. Thus half of the patients having tropical sepsis will be of malaria, which means one-sixth of the patients with sepsis in setup like ours will be due to malaria (malaria being totally reversible condition, early diagnosis and management may save life).
8. Dengue is not uncommon as the cause of sepsis, which was present in 76 patients of which in 57 it was dengue alone while 19 patients had dengue with other infections. Hemorrhagic dengue and dengue shock syndrome (DSS) which are described and taught clinically to medical students is part of sepsis which can cause severe epsis according to 1992-2001 definition (haemorrhage can be considered as the one of the signs of the organs involvement) and DSS as part of septic shock.
9. Viral infections, fungal infections and polymicrobial infections, which would have combination of various microbial agents, is not uncommon and should be considered while establishing the microbial etiology of sepsis.
10. A total of 11.44% (85/743) patients had sepsis because of polymicrobial infections. Half of them had predominantly tropical infections (42/85) and other half (43/85) had non-tropical (bacterial & fungal) infections. When

absolute number is considered, polymicrobial infections are more common in tropical group (42/250) than in non-tropical (43/349).

11. Enteric fever due to *Salmonella species* was found in 2.01% (15/743) patients as a cause of sepsis and 6% (15/250) as a cause of tropical sepsis. In two patients it turned out to be fatal as the one had complicated P. falicparum infection and other had dengue.
12. A total of 57.74% (656/1136) samples yielded growth from cultures. A total of 701 isolates were obtained; with 598 bacteria, 100 *Candida species* and 3 other fungi. Thus bacteria were more commonly isolated than fungi.
13. Amongst bacterial isolates, the Gram negative bacilli- 67.22% (402/598) predominated over Gram positive bacteria -32.77% (196/598).
14. Of the gram negative bacilli, *Klebsiella species* was the most common isolate followed by *E. coli*.
15. Amongst Gram negative bacilli, the least resistance was seen against Imipenem (9.71%) and most was seen against Cefuroxime (92%) and Cefotaxime (90%)
16. The most common mechanism of antibiotic resistance observed was ESBL (extended spectrum beta lactamase) production.
17. Of the Gram positive isolates, *Staphylococcus aureus* was the most common isolate followed by *Coagulase negative staphylococci (CoNS)*.
18. Amongst the Gram positive cocci, the least/no resistance was seen against Vancomycin (0%), meaning, all the isolates were susceptible to Vancomycin whereas the highest resistance was seen against Penicillin (97%) followed by 85% against Erythromycin.
19. The methicillin resistance due to mecA gene (by phenotypic detection with cefoxitin disc) was the common form of resistance mechanism observed among Gram positive cocci i.e. 64.55% of MRSA and 40% of MRCoNS.
20. A total of 274 patients had sepsis with no organ involvement; 97 with one organ involvement, 320 with multiple organ involvement and 52 had sepsis with shock (septic shock).
21. Amongst non-survivors (284/743) almost half of the deaths were due to bacterial and/or fungal infections in 44.36% (126/282), 30.28% (86/284) due to tropical infections and 25.35% (72/284) due to unidentified microbial etiology.

22. Amongst those with tropical infections, malaria was the most common cause of death in 14.08% (40/284), dengue in 7.39% (21/284), in 3.16% (9/284) Hepatitis E, in 1.40% (4/284) Hepatitis A, in 1.40% (4/284) Hepatitis B, in 0.35% (1/284) Hepatitis C virus, in 0.35% (1/284) *Leptospira species* infections whereas the rest 6 had mixed infections with malaria, dengue and viral hepatitis.
23. The PCT values were found to be more than ≥0.5ng/ml in most of the patients i.e.245/288 and only 43 showed values ≤0.5 ng/ml.
24. A total of 168 out of 288 tested for PCT, had values ranging from >2<10ng/ml.
25. PCT helped to predict infections due to bacteria, fungi and malaria, with values usually ≥0.5 ng/ml or even >2ng/ml.
26. With viral infections the values remained as low as 0.5 ng/ml or even less than 0.5 ng/ml, thus ruling out other causes as bacterial, fungal or parasitic.

CHAPTER 6
DISCUSSION

6 DISCUSSION

The results of our study are compared and discussed under the following headings:

- **Why this study and the role of Microbiologist?**
- **Gender & Age of Sepsis patients**
- **Outcome in Sepsis patients**
- **Microbial Etiology:**

  Bacterial Isolates and Drug Resistance Mechanisms of bacterial isolates

  Fungal isolates and their antifungal susceptibility

  Tropical Infections: Malaria, Dengue, Viral hepatitis, Leptospira and Enteric fever

  Polymicrobial Etiology
- **Nosocomial Sepsis**
- **Biomarkers: PCT & CRP**
- **Comorbid conditions, Laboratory parameters and Organ involvement pattern**

Why this study and the role of Microbiologist?

Sepsis is interplay of microbial invasion and host response which is overwhelming and life threatening. It damages tissues and body organs which lead to death. Throughout the world, campaigns are in full swing to create awareness among public and society regarding prevention and early management of sepsis. Sepsis is treatable, especially with early recognition and care. This awareness is equally needed amid health care providers and administrators of health care facilities. Awareness and research is given promotion as help can be provided in the community by taking timely action.

All acute infections can culminate to sepsis which is considered a common final pathway. Body's response to an infection leads to tissue damage, shock and multiorgan failure which may result in death. It is one of the deadly and costly diseases, common both in developing and underdeveloped countries. However as mentioned earlier, is treatable and preventable. For treatment and prevention, International Health Authorities had taken active interest in the year 2012 and 2013, when this research work was undertaken. International Guidelines for Management of Severe Sepsis and Septic Shock were updated in 2012 as "Surviving Sepsis Campaign", which is a joint collaboration of the Society of Critical Care Medicine and the European Society of Intensive Care Medicine. This was a landmark conception in Sepsis and focus was "SURVIVAL" with early recognition and treatment [(10)]. Recognition of sepsis early is of great importance as it is found to have link with survival. Global Sepsis Alliance started concept of "World Sepsis Day" in 2013.Their collaborative effort gave momentum to other organizations and individuals for "sepsis awareness and prevention". 13th September is observed as Sepsis day all over the world and September month is observed as Sepsis awareness month [(77)]. Thus all the recent concepts which have emerged as evidences based guidelines are geared around decreasing mortality and prevention of sepsis.

The UK Sepsis trust for educational purposes acknowledges and writes "Sepsis kills more than 44,000 people per year in the UK alone with nearly half of the cases due to *E.coli* and about a third are resistant to antibiotics." For solving this problem, 3 point agenda adopted by them is "SPOT IT", "TREAT IT". "BEAT IT" and further guides "LOOK FOR SIGNS OF SEPSIS IN EVERY PATIENT WHERE INFECTION IS LIKELY."(www.telegraph.co.UK>NewsDec,10, 2016.) [(78&79)]

The agenda "Spot It" and "Treat It" reflect onto diagnosis and treatment of sepsis. Microbiologist's role in diagnosis is important. In treatment of sepsis, the laboratory and "Microbiologist" play an important supporting role to clinicians and intensivists. Third agenda "Beat It" will be prevention – prevention of infection and thus infection control in relation to nosocomial infection, surgical site infection, puerperal sepsis, neonatal sepsis etc. will come in picture. A role of microbiologist in infection control is important at community level and hospital level. Preventing sepsis includes concept of asepsis, infection control, antibiotic stewardship, universal precaution and other concepts where Microbiologist has significant role. Apart from prevention, adaption of these notions results in better patient's outcomes, reduces microbial resistance, and decreases the spread of infections caused by multidrug-resistant organisms. Thus first role of microbiologist in sepsis patient will be in etiological diagnosis of sepsis and to find out microbe responsible, second role will be in treatment where appropriate antimicrobial agent can be recommended and third is in prevention through proper strategic planning of hospital infection control.

Though a lot of research today is done on sepsis, this study, as per our knowledge, is the first one that aimed to identify potential pathogens as causes of sepsis using a predefined set of diagnostic tests covering a wide range of bacteria, viruses, fungi and parasites. We being in a rural setup, our patient mix would be different than urban population as well as from the western world. We encounter cases of sepsis due to typhoid, malaria, puerperal sepsis etc. in which the microbiological research is sparse. Carrying out research in sepsis in a rural based teaching hospital would help us know/improve understanding of the causative organisms of the sepsis, whether, community acquired or hospital acquired, antibiotic sensitivity status, host response, etc. and help us in appropriate antimicrobial selection. This study would also give an insight to various aspects of sepsis like early diagnosis, judicious use of antibiotics, correlating the host factors to mortality, differentiating sepsis from various sepsis-like syndromes. By generating our own data, our aim is to help clinicians know the kind of flora that exists in the hospital setup, their potential as pathogens and developing resistance against the antimicrobials used, thereby allowing clinicians choose the right empiric treatment too.

Gender & Age of Sepsis Patients:

Of the total 743 patients, 61.50% (457/743) were male and 38.49% (286/743) were female patients. The mean age of our patients was 48.02 years. **Table 18** shows comparison of the gender of our study population with that of the other studies which shows that findings of our study are quite similar to the findings of other studies

Table 18: Comparison of Gender with other studies

Gender %	Author
62.6% males/ 37.4% females	Saravu et al [80]
66.1% males/33.9% females	Divatia J et al [81]
61% males/39% females	Abrahamsen et al [82]
60.1% males/39.9% females	Chrispal et al [83]
57% - males/ 43% females	SEAR* study [84]
61.50% - males/38.49% females	Present Study

*SEAR –South East Asia Region conducted by South East Asia Infectious Diseases Clinical Research Network in 3 tropical countries.

Table 19 below shows the comparison of the mean age of our study population with that of the other studies. The difference in the mean age could be because only adult patients were included in the study whereas in other studies, patients of all ages have been included, affecting the mean age of the study population.

Table 19: Comparison of Mean Age with other studies

Age	Author
54.1 years	Divatia J et al [81]
37.5 years	Abrahamsen et al [82]
39.5 years	Chrispal et al [83]
48.02 years	Present Study

In our setup, which is a rural setup catering mainly the rural, underpriveleged population, males, especially, the middle aged, were found to be more commonly affected by sepsis which could be explained as follows: (1) Probably the male patients are brought to hospitals for treatment more than the female patients, as ours is a male dominant society where male is given more importance and in most cases he is

the only person earning the livelihood, considering his survival to be important. (2) The young are brought more to the hospital than the old, as younger generation is the promising generation and future of the family as compared to old and probably for the same reason they formed the main study population.

Outcome in sepsis patients:

Of the total 743 patients, 411 patients survived, 284 did not survive while for 48 patients outcome was not known as they took discharge against medical advice (DAMA). Also it was noted that mortality was higher amongst those patients who had sepsis due to causes other than tropical infections i.e. 44.36% (126/284) followed by those with tropical infections 30.28% (86/284) and in 25.35% (72/284) with unidentified microbial etiology. The comparison of the outcome in our patients with that of the other studies is shown in **Table 20** below.

Table 20: Comparison of the Outcome with the other studies

Author	Outcome %
Saravu et al [80]	Survivors – 63% Non-survivors – 22% Not Known – 15%
Divatia J et al [81]	Survivors – 81.94% Non-survivors – 18.05% Not Known – 0%
Abrahamsen et al [82]	Survivors – 93% Non-survivors – 7% Not Known – 0%
Chrispal et al [83]	Survivors – 89% Non-survivors – 11% Not Known – 0%
SEAR study [84]	Survivors – 85% Non-survivors – 13% Not Known – 1%
Present Study	Survivors – 55.31% Non-survivors – 38.22% Not Known – 6.46%

The average age of non-survivors was 51.03 years (range 18 to 88) and the majority i.e. 22.88% belonged equally to the age group of 51years to 60 years and 61 years to 70 years i.e. 65 each in both the age groups. Of the 284 non-survivors, 59.15% (168/284) were males and 40.85% (116/284) were females with average age of 52.82 years and 48.61 years respectively. The average age of the survivors was 46.00 years. Amongst survivors, 63.74% (262/411) were males and 36.26% (149/411) were females with an average of 47.29 years and 43.69 years. Also the mortality was significantly higher amongst patients with unidentified etiology as compared to non-tropical and tropical group of patients. ($p<0.05$)

The higher mortality rate in our setup could be because of the difference in the patient population, as our patients are either referred, from smaller healthcare settings with inadequate facilities from nearby districts as well as the neighbouring states with incomplete treatment/diagnosis, or may be the patients themselves took discharge without completion of the treatment from elsewhere which lead to a delay in care resulting in an increased risk of complications during transit and eventually they succumbed during treatment in our hospital on the same day or after few days of admission. Also the incomplete treatment reduces the chances of isolation or identification of a microbial etiology, thereby limiting the chances of correct diagnosis and correct treatment leading to greater mortality, stastically significant, in patients with unidentified etiology in our case as compared to those with non-tropical and tropical infections.

Microbial Profile:

Microscopy:

As a part of early diagnosis microscopy plays a critical role. The wet mount preparation of CSF, urine, body fluids and stool revealed not only the information about the absence or presence of the microbe but also the presence and number of pus cells, red blood cells if any, epithelial cells, fungal elements, parasite, motility etc. giving a direction to the identification process. The stained preparations such as gram stain & acid fast stain helped to appreciate the morphology and arrangement of the microbes, a clue leading to identification; as well as the number of pus cells and epithelial cells were important in deciding the appropriateness of the specimen for processing. The microscopic examination done as a first step gave a direction to the

whole process of identification of the microbe. Also the results of microscopy correlated well with the culture outcome and were very much helpful especially, with the interpretation of isolation of commensal microbes from the specimens. The examination of peripheral smear preparations – thick and thin – for malarial parasite, the gold standard, was utilized for diagnosis of malaria mainly by observing the ring forms, gamentocytes and schizonts of *Plasmodium spp.* which could be reported faster thereby helping clinician to initiate the timely treatment. The KOH mount of the swab from a nasal polyp helped identify fungal hyphae in the sample which finally lead to isolation and identification of *Mucor species*. Overall, we found microscopy as a rapid, convenient and cost effective tool in the identification of the most of the microbial etiology from various specimens.

Sepsis due to Bacterial & Fungal Infections:

Culture & Susceptibility:

The microbial etiology could be identified and established in 599/743 (81%) patients and in 144/743 (19%) no pathogen could be established. A total of 47% (349/743) patients had sepsis due to bacteria and/or fungi, followed by 34% (250/743) due to tropical (malaria, dengue, enteric fever, viral hepatitis and leptospira) infections and the rest 19% (144/743) had sepsis due to unidentified microbial aetiology/no pathogen identified. When compard to other studies as shown in **Table 21** our findings are quite similar to results of 2 studies but higher compared to most other studies.

Table 21: Comparison with other studies

Author	Microbial Etiology Proven/Identified (%)
Saravu et al [80]	Identified – 63.8% Unidentified – 36.2%
Divatia J et al [81]	Identified – 35.9% Unidentified – 64.1%
Abrahamsen et al [82]	Identified – 87% Unidentified – 13%
Chrispal et al [83]	Identified – 92% Unidentified – 8%
SEAR study [84]	Identified – 48%

	Unidentified – 52%
Present Study	Identified – 81% Unidentified – 19%

The higher rate of identification of a microbial etiology in our setup could be because of the coordinated efforts of the consultants & the laboratory; timely and appropriate sample collection, processing and reporting.

Out of the selected patients, 47% (349/743) patients had sepsis due to bacteria and/or fungi referred as non-tropical (NT), 34% (250/743) due to tropical (malaria, dengue, enteric fever, viral hepatitis and *Leptospira spp.*) sepsis and the rest 19% (144/743) had sepsis due to unidentified microbial aetiology. The following **Table 22** shows comparison of the microbial profile of sepsis in our patients with that of the others.

Table 22: Comparison of Microbial Profile of Sepsis with other studies

Author	Microbial Profile (% in Patients)
Saravu et al [80]	Reports only Bacterial isolates in 63.8% with Polymicrobial in 13.8% Others Not Reported
Divatia J et al* [81]	Bacteria - 84.8% Malaria - 1.1% Viruses - 1.7% Fungi - 7.5%
Abrahamsen et al [82]	Bacteria - 57% Parasite – 7% Fungi - 4% Viruses - 3%
Chrispal et al [83]	Bacteria – 65.32% Parasite -Malaria – 17.1% Fungi – Not Reported Viruses – 5.51%
SEAR study [84]	Bacteria -27% Viruses -29% Parasite -1% Fungi – Not Reported Polymicrobial Etiology– 3.68%

Author	Microbial Profile (% in Patients)
Present Study	Bacteria – 39.56% Parasite – Malaria – 16.41% Fungi – 3.09% Dengue – 7.80% Viral hepatitis – 2.29% Viruses – 10.09% Polymicrobial Etiology – 11.44%

*Percentage of microbes identified from total number of microbes.

Thus 38.09% (283/743) patients had only bacteria as a cause of sepsis, 16.41% (122/743) had only malaria, 7.80% (58/743) had only dengue, 3.10% (23/743) had only fungal, 2.29% (17/743) had only viral hepatitis, 1.48% (11/743) had only enteric fever whereas the remaining 11.44% (85/743) had polymicrobial etiology. In the above **Table 22** *Salmonella species* have been added to total bacterial count i.e. 283+11=294.

However, from 743 patients, 2183 samples (inclusive of 1136 cultures) were processed and 950 microbes could be identified. Of these 950, 63.05% (599/950) were bacteria (inclusive of one *Leptospira spp.*), 14.73% (140/950) malarial parasite, viruses 11.36% (108/950) [Hepatitis viruses – A, B, C & E - 31; dengue-76 & HIV-1) and 10.84% fungi (103/950).

Culture:

A total of 57.74% (656/1136) of the different samples cultured were positive yielding 701 isolates while 42.25% (480/1136) samples did not yield any bacterial or fungal isolate i.e. they were culture negative. A total of 598 bacteria and 103 fungi were isolated making a total of 701 isolates from 657 culture positive specimens. Of the 598 bacterial isolates, 67.22% (402/598) were Gram negative bacilli and 32.77% (196/598) were Gram positive bacteria.

Table 23: Comparison of Cultue Outcome and Isolates with the findings of the other studies

Author	Culture Outcome (%)	Isolate Type & Percentage
Saravu et al [80]	Culture Positive – 63.8% Culture Negative – 42.17%	Gram negative – 75.3% Gram positive – 24.7

Author	Culture Outcome (%)	Isolate Type & Percentage
Divatia J et al* [81]	Not Reported in relation to culture outcome	Gram negative – 68.9% Gram positive – 15.9%
Abrahamsen et al [82]	Culture Positive – 22.16% Culture Negative – 77.84%	Gram negative – 76.74% Gram positive – 23.25%
Chrispal et al [83]	Not Reported in relation to culture outcome	Not Reported in terms of culture isolates
SEAR study [84]	Not Reported in terms of total culture outcome of samples	Not reported in terms of total culture isolates.
Present Study	Culture Positive – 57.74% Culture Negative – 42.25%	Gram negative – 67.22% Gram positive – 32.77%

Though the percentage of isolation differs, the trend is consistent to the findings of the authors with the predominance of gram negative bacilli in developing countries, especially the tropical. Amongst the gram negative bacilli, *Klebsiella spp.* was the most common isolate followed by *E. coli*, *Acinetobacter spp.*, *Pseudomonas spp.*, *Salmonella species* and also *Proteus spp.*, *Citrobacter freundii* and unidentified GNB. Amongst gram positive cocci, *S. aureus* (n=79) was the major isolate followed by *CoNS* (n=60), *Enterococcus species* (n=49) and *Str. pyogenes* (n=6) and gram positive bacilli, probable contaminants (n=2). However, Lee et al [85] and Kumalo A et al [86] report contradictory findings of gram positive cocci as the predominant isolates.

The overall resistance pattern of the major gram negative bacilli isolated from the total 1136 samples cultured, showed least resistance towards carbapenems as compared to other antibiotics, higher towards 2nd & 3rd generation cephalsporins and no resistance against vancomycin in the gram positive cocci group are comparable to the findings of Saravu et al. [80] However, higher resistance to ciprofloxacin, amoxicillin-clavulanic acid and gentamicin by most of the gram negative & gram positive bacteria is in contrast to other studies [86, 87]. This could be because as these drugs are found to be effective for both gram negative and positive infections, these may have been used in our patients, especially, the referred patients by the consultants and as the treatment is incomplete and infection not cleared, it could have resulted in selection of such bacterial population which has become resistant, which survived and were isolated as well.

Blood Culture & Bacteremia/Blood Stream Infections:

Blood cultures were positive in 49.03% (330/673) samples yielding 339 isolates. The following **Table 24** shows comparison of blood culture outcome and the isolates obtained of our study with that of the others.

Table 24: Comparison of blood culture outcome and isolate types with other studies:

Author	Place	Positive Blood Culture Outcome (%) & Isolate types (%)	Frequent Isolates (%)
Kante Meenakshi et al [87]	Andhra Pradesh, South India	Outcome - 17% Gram negative – 23/34 (67.64%) Gram positive – 11/34 (32.36%)	*Pseudomonas aeruginosa* (6.6%) *S. aureus* (4.5%) *Acinetobacter spp.* (6.6%) *Klebsiella pneumoniae* (2%) *CoNS* (1%)
Abrahamsen et al [82]	Tamil Nadu, South India	Outcome - 7% Gram negative – 42.85% Gram positive –19.04%	*E. coli* (14.3%) *S. typhi/paratyphi* (14.3%) *S. aureus* (9.5%) *Klebsiella spp.* (4.8%) *Enterococcus spp.* (4.8%) *Strep. viridians* (4.8%) Unidentified GNB (4.8%)

			Pseudomonas spp. (0%) *Acinetobacter spp.* (0%) Other (38.1%) Fungi (4.8%)
Sahoo D et al [88]	Odisha, East India	Outcome -26% Gram negative – 69.23% Gram positive – 30.76%	*E. coli* (34.6%) *Klebsiella spp.* (26.9%) *S. aureus* (23.1%) *A. baumanii* (7.7%) *CoNS* (7.7%)
Gupta S et al [89]	New Delhi, North India	Outcome - 16.5% Gram negative – 58.34% Gram positive – 41.65%	*E. coli* (22.4%) *Klebsiella spp.* (19.7%) *S. aurues* (18.3%) *CoNS* (17.4%) *Pseudomonas spp.* (8.40%) *Acinetobacter spp.* (5.20%) *Enterococcus spp.* (4.8%) *Streptococcus spp.* (4.8%) *S. typhi* (1%) *Citrobacter spp.* (0.9%) *Proteus spp.* (0.35%)
Sonawane J et al [90]	Navi Mumbai, West India	Outcome 10.29%	*Klebsiella spp.* (22.38%)

		Gram negative – 83.22% Gram positive – 16.78%	*Pseudomonas spp.* (20.98%) *Acinetobacter spp.* (17.48%) *E. coli* (11.19%) *S. typhi* (7.70%) *Enterococcus spp.* (7.70%) *S. aureus* (6.99%) *Enterobacter spp.* (3.50%) *Strep. spp.* (2.10%)
Oza S et al [91]	Surendranagar, Gujarat	Outcome 18.62% Gram negative – 67.40% Gram positive – 32.60%	*K. pneumoniae* (34.78%) CoNS (15.22%) *Pseudomonas spp.* (10.87%) *E. coli* (8.69%) *S. typhi* (4.35%) *Enterococcus spp.* (4.35%) *Acinetobacter spp.* (4.35%) *Unidentified GNB* (4.35%) *Strep. spp.* (2.17%)
SEA Infectious Diseases Clinical Research Network [84]	South East Asia Region	Outcome 12% (in adults)	*E. coli* (5%) *S. typhi* (0.2%) *Acinetobacter spp.* (1%) *Strep. suis* (1%) *Burkholderia pseudomallei*

			(<1%)
Lee et al [85]	North Carolina, USA	Outcome 73.1% (in first culture)	*S. aureus* (20.70%) *CoNS* (14.10%) *Enterococcus spp.* (4.66%) Strep. spp. (5.46%) *K. pneumoniae* (9.55%) *E. coli* (7.62%) *Pseudomonas spp.* (4.43%) Yeasts (8.64%)
Present Study	Vadodara, Gujarat	Outcome 49.03% Gram negative – 51.03% Gram positive – 38.64%	*Klebsiella spp.* (19.47) *S. aureus* (15.04) *CoNS* (14.45) *E. coli* (14.16) *Candida spp.* (10.03) *Enterococcus spp.* (7.37%) *Acinetobacter spp.* (7.96) *Pseudomonas spp.* (4.13) *Salmonella spp.* (4.42) *Strep. pyogenes* (1.17%) *Citrobacter freundii* (0.58%) Gram positive bacilli (0.58%)

			Proteus mirabilis (0.29%) Unidentified GNB (0.29%) *Candida spp.* (10.32%)

Overall 42.17% (479/1136) of the total samples were culture negative which included 50.97% (343/673) negative blood cultures as well. These findings are much lesser to 88% reported for adult patients in SEAR study [84] but higher compared to 40% reported by Phua J et al (New Ref). However, as suggested in the literature [92], the culture negative outcome could be because of the following reasons:

a. The recent antibiotic treatment which reduces the number of microorganisms in the samples which reduces the chances of isolation.
b. Infection due to anaerobic organisms and if anaerobic cultures are not performed these could have been missed.
c. Infection due to fastidious or slow-growing organisms like *Haemophilus spp.* which do not grow on standard medium.
d. Bacteremia is intermittent and thus blood collected during such period does not yield the growth.

Septicaemia due to Gram negative bacteria predominated over Gram positive and these findings are comparable to those with other studies from India as well as other countries [88, 89, 90, 91, 93, 80, 81, 87] but contrasting findings have been reported by Lee et al [85] and Kumalo A et al [86] who reported Gram positive cocci predominance with 52.33% and 53.3% respectively.

In our study *Klebseilla spp.* was the most frequent isolate from blood culture followed by *Staphyloccoccus aureus*, *CoNS* and *E. coli*. These findings are similar to Gupta S et al [89] and Sonawane J et al [90] Whereas in the other studies, *Klebsiella pneumoniae* [91], *Staphylococcus aureus* [85, 86], *E. coli* [88, 89,] and non-typhoidal *Salmonella* [93] have been reported as the most frequent isolates from blood culture samples.

In our study we found 4.42% (15/339) *Salmonella species* obtained from blood cultures of patients and 1.48% (11/743) patients had only *Salmonella species* as the cause of sepsis. Our findings in regards to isolation of *Salmonella species* are more than 1% and 0.2% reported by Abrahamsen et al [82] and SEAR study [84] respectively

but lesser than 8% reported by Chrispal A et al [83]. Ugas MB [94] reports a case of septic shock with acute respiratory distress syndrome due to *Salmnella Typhi* from Oklahoma, USA, which was found to be susceptible to ceftriaxone, cotrimoxazole and ciprofloxacin. Another case report from New Delhi, India reports [95], *Salmonella paratyphi A*, susceptible to Cefotaxime, Ciprofloxacin, Gentamicin, Amikacin, Cotrimoxazole which is similar to the findings of our study except for Ciprofloxacin in both the reports [94, 95].

CoNS was the third most common blood culture isolate i.e. (n=49) 14.45% as well as the second most common gram positive cocci. Only single blood cultures were performed except for patients who had suspected endocarditis, more than one blood cultures were obtained from different sites. Most of the studies consider it as a contaminant [84] but as these have been isolated from critically ill patients admitted in ICU with clinically diagnosed sepsis these isolates were considered to be pathogenic rather than contamination. S Singh et al [96] used correlation with clinical parameters like temperature >38^{0}C or <36^{0}C, systolic blood pressure of <90mm/Hg, total leukocyte count >12,000/µl or <4000/ µl and presence of >10% immature bands for establishing the significance of *CoNS* isolated from single cultures. In our study these clinical parameters are already fulfilled under sepsis definition. Thus with the same concept these were all considered significant. Also Oza S et al [91] and Kumalo A et al [86] reports 13.3% *CoNS* being the second most common isolates from blood culture. Kumalo A et al suggests that though in past they were considered as non-pathogenic but currently due to increased use of indwelling medical devices they are on the rise. [86]

Least resistance was observed against cabapenems with *Acinetobacter spp.* showing the highest resistance i.e. 29.63%. A total of 89.39%, 55.56% and 45.83% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance to amoxycilin/clavulanic acid. A total of 57.58%, 25.93% and 22.92% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance against Amikacin. Moreover, 93.94%, 96.30% and 87.50% of *Klebsiella spp.*, *Acinetobacter spp.* and *E. coli* showed resistance to Cefuroxime and almost a similar percentage of resistance against Cefotaxime. Thus higher percentage of resistance against amoxicillin/clavulanic acid and 3rd generation cephalosporins is comparable to the findings of Oza S et al [91] from Surendranagar and Kante M et al [87] from AP, India.

No resistance was observed against vancomycin and least towards linezolid (3.88%) and doxycycline (23.26%) whereas 97.42% against Pencillin and 85.57% against Erythromycin with *Enterococcus spp.* showing the maximum resistance. These findings are again comparable to the findings of Oza S et al [91] who report no resistance against vancomycin, linezolid and teicoplanin and also higher resistance by *Enterococcus spp.* towards penicillin. However, findings in regards to Penicillin & Erythromycin are in striking contrast to 100% susceptibility reported by Kante M et al [87]. Also resistance against ciprofloxacin was noted as 60.47% and 74.36% by GNB and GPC respectively. This is in contrast to the findings of Kumalo A et al [86] who report ciprofloxaxin to be 87.5% susceptible and as an effective antibiotic in their setup.

Of the total 174 Gram negative bacteria 22.41% (39/174) isolates showed only ESBL production as a major form of mechanism resistance to which 56.41% (22/39) was contributed by *Klebsiella spp.*, followed by *E. coli* – 33.33% (13/39) and smaller percentage of each of 5.12% (2/39) *Acinetobacter spp.* and *Pseudomonas spp.* Only AmpC-Beta lactamase production with 9.19% (16/174) was the next in the sequence with *E. coli* been the major contributor followed by *Acinetobacter spp.* and finally only MBL production was seen in 5.74% (10/174) with maximum number of *Acinetobacter spp.* (40%). Amongst gram positive cocci, MRSA was seen in 52.94% and MRCoNS in 34.69%. The percentage of ESBL producers has been reported to be 51.85% by Sweta et al [71] and 82% by Sonawane J et al [90]. The latter also reports highest production of ESBL by 55.66% *Klebsiella spp.* and 29.24% by *E. coli*. Saravu et al [80] reports 66.7% and 70% ESBL production in *E. coli* and *Klebsiella spp.* respectively and 35.2% resistance to carbapenems was also reported. Overall the percentage of resistant isolates as well as total resistance against carbapnems and ESBL producing gram negative isolates were less in our study. The percentage of MRSA and MRCoNS was reported to be 33.33% and 0% by Kumalo A et al. [86]

Urine Culture & Urinary Tract Infections:

A total of 167 urine samples were cultured from 143 patients including 58 samples from 53 catheterized patients. Growth was observed in 134/167 (80.24%) samples yielding 143 isolates and in the remaining 33 (19.76) there was no growth. The predominant growth of gram negative bacilli (60.13%) was obtained followed by 23.07% *Candida species* and 16.78% gram positive cocci. The majority isolates

belonged to Enterobacteriaceae- 50.34% (72/143) with *E. coli* 34.96% (50/143) been the most common isolate followed by *Candida species*, *Enterococcus species*, *Klebsiella* spp. and *Acinetobacter spp.* in the sequence. Amongst gram positive cocci, *Enterococcus spp.* was the major isolate showing higher resistance to most of the antibiotics followed by *S. aureus* with 75% being MRSA and 50% of *CoNS* being MRCoNS. Of the 33 *Candida species*, the major species was *C. albicans* (n=20), 12 were *C.non-albicans* and 1 was *C. tropicalis*.

However, of the 167 samples 58 (34.73%) samples were catheterized samples obtained from 53 patients (49 with single specimens, 3 with 2 and 1 with 3 specimens) thus representing hospital acquired infections. Amongst the catheterized samples, growth was obtained in 52 (89.65%) samples yielding 58 isolates with 6specimens yielding 2 isolates from same sample. Here *Candida species* (14/58) were the major isolate followed by *E.coli* (13/58) and equal numbers (7/58) of *Acinetobacter spp.* and *Enterococcus species* and then *Klebsiella spp.* (6/58) *S. aurues* (2/58) and *CoNS* (1/58) in the order.

Amongst the total *E. coli*, 44% (22/50) were ESBL producers. Higher resistance was seen against Cefuroxime, Cefotaxime and Ceftazidime (97.47%, 92.41% and 77.22% respectively) and also 83.54% against ciprofloxacin. As found and discussed under blood culture, the ciprofloxacin resistance in our study is higher compared to 12.5% and 14.3% report by Kumalo A et al [(86)] for GNB and GPC respectively. Amongst gram positive cocci 16.66% inducible clindamycin resistance was seen. There was no resistance observed against vancomycin but 100% against Penicillin and Erythromycin. MRSA were found to be 75% (3/4) and MRCoNS 50% (1/2). Inducible Clindamycin Resistance (ICR) was observed in 16.66% (4/24) gram positive cocci. Overall in 19.24% (143/743) patients, urinary tract infections were suspected as source of in infection leading to sepsis and 18.03% (134/743) patients had culture proven urinary tract infections.

Saravu et al [(80)] from Karnataka report urosepsis in 21.8% patients with Enterobacteriaceae members contributing to 36% and 26% of *E. coli* as the most common isolate with two-thirds being ESBL producers. Kumalo A et al [(86)] report urinary tract infections in 13.7% of patients. The most frequent isolate reported was *S. aureus* 22.07% followed by 19.48% *Pseudomonas spp.*, 16.88% *E. coli* and 15.58% *Klebsiella spp.*

Pus Culture & Soft tissue/Skin and Bone Infections:

Growth was obtained in 87.5% (70/80) samples from 60 patients (8.07%) yielding 77 isolates. Of the 77 isolates, majority were gram negative bacilli 62.33%, 35.06% gram positive cocci and 2.59% *Candida species*. Kumalo A et al [(86)] report skin/soft tissue & bone infections in 4.2% patients. The most common isolate was *S. aureus* (22.07%) followed by *Pseudomonas spp.* (19.48%). *S.aureus* has been commonly reported in skin/soft tissue infections, both community and hospital acquired whereas *Pseudomnas spp.* has been commonly associated with hospital acquired infections. These findings are comparable to Ghanshani et al [(97)] from BHU who report *S. aureus* (23.33%) as the most common isolate followed by *Pseudomonas spp.* (18.88%) as the second most common isolate from skin/soft tissue infections in ICU. Of the 80 samples, 11 samples were obtained from 11 patients with post-operative infections. These have been discussed under hospital acquired sepsis. The antibiotic resistance pattern of gram negative bacilli as well as the gram positive cocci exhibited the same trend as seen with the isolates from other samples. The resistance pattern of *Pseudomonas spp.* revealed 26.67% and 33.33% resistance to Imipenem and Meropenem/Ertapenem. This is in striking contrast to the findings of Ghanshani et al [(97)] from BHU who report 72.8% and 73.6% resistance to both the antibiotics respectively of the total number of *Pseudomonas spp.* isolates obtained from various samples.

Sputum, Endotracheal Specimens & Respiratory Infections:

A total of 148 samples for suspected respiratory infections were processed which included a total of 75 ET specimens from 73 patients and 73 sputum samples from 68 samples making a total of 141 patients. From 7 patients out of these 141 patients both ET & sputum samples were collected together. [Thus 134 patients with either of the sample making a total of 134 samples and 7 patients with both the samples making a total 14 samples made a final total of 134+14=148 samples].

Of the total 148 samples, 102 samples (68.91%) were positive for culture whereas the rest were negative. These 102 samples included 46 out of 75 ET samples & 56 out of 73 sputum samples.

The positive findings of ET samples are suggestive of infection due to mechanical ventilation which in turn is ventilator associated pneumonia and have been discussed under the heading of nosocomial sepsis.

Of the 56 positive sputum samples 61 isolates were obtained from 52 patients, with *Candida spp.* (31%) as the most frequent isolate, followed by *Klebsiella spp.* (26%), *Acinetobacer spp.* (16%), *S. aureus* (7%), *Pseudomonas spp.* (6%), *Enterococcus spp.* (3%), 2% each of *CoNS*, unidentified GNB and *Aspergillus niger*. Here all the *Candida spp.* were considered significant as they were isolated from purulent sputum with plenty of pus cells on microscopy and $>10^5$ CFU/ml colony count.

Klebsiella spp. showed a higher resistance against Amoxycillin/clavulanic acid, Amikacin, Gentamicin, Ciprofloxacin, Cotrimoxazole and Ceftazidime whereas *Acinetobacter spp.* showed a higher resistance towards Imipenem, Ertapenem, Cefuroxime and Cefotaxime. Also ESBL production was the major form of resistance mechanism followed by MBL production.

Amongst gram positive cocci, the higher resistance was exhibited by *Enterococcus* spp. towards most of the antibiotics tested as compared to *S. aureus* which exhibited higher resistance towards doxycycline and cefoxitin. MRSA was found to be 75%.

Thus overall bacteriologically proven respiratory infections were seen in 52 patients (76.47%) out of total 68 patients in whom sputum cultures were performed whereas of the total study population 6.99% (52/743) patients had respiratory infections with *Candida spp.* and highly resistant *Klebsiella spp.* been the frequent causative agents of respiratory infections. The percentage of respiratory infections in the total study population is slightly lower than that reported by Abrahamsen et al [82] in their study population i.e. 11%. The study from 3 tropical countries of SEAR [84] reports respiratory infections in 53.49% (i.e. 436/815 adults; including all those clinically diagnosed but no pathogen was identified) and as the most common infection amongst sepsis & severe sepsis patients.

CSF Culture:

A total of 38 CSF samples were cultured from 38 patients with suspected meningitis. Thus CNS infections were suspected in 5.11% (38/743) of the total patients. It was notable that 95% samples were culture negative and only 5% (2/38) were positive. Both these samples yielded *Klebsiella spp.*, whose antibiotic susceptibility pattern

revealed 100% susceptibility to most of the antibiotics tested and 50% to Amikacin, Cefuroxime, Ceftazidime and 0% to Gentamicin and Ciprofloxacin. None of them were ESBL producers and neither did they exhibit any resistance mechanism. Such a low outcome could be because either these patients had aseptic meningitis i.e. due to viral infections which could be related to the absence of pus cells in majority of samples on wet mount preparation as well as stained smears with Gram and Acid fast and the other possibility could be that the bacteria/fungi did not grow due to the reasons explained above under the blood culture. The SEAR study reports 9.81% (80/815) patients with suspected as well as etiology proven CNS infections. [84]

Body fluids:

A total of 17 different body fluids were cultured from 17 patients and ascitic fluid was the frequent sample. However, pleural fluid and BAL represent patients with suspected respiratory infections. A total of 41% samples showed growth with *E. coli* been the common isolate followed by *Klebsiella spp.*, *Acinetobacter spp.* and one each of *CoNS* & *Candida spp.*

Fungal Culture & Antifungal Susceptibility:

A total 103 fungal isolates were obtained of which 100 *Candida species*, 2 *Mucor spp.* and 1 *Aspergillus niger* were obtained. A total of 14.26% isolates of *Candida species* were obtained from 1136 different samples cultured from sepsis patients yielding 701 bacterial & fungal isolates altogether and 10.60% (35/330) were obtained from blood cultures with 7.90% (26/330) as single isolate and 2.92% (9/330) in combination with another bacterial isolate contributing as the 5th most common blood culture isolate. Of the total 100 *Candida species* isolated, the most common isolate was *C. albicans* (53%) followed by *C.non-albicans* (37%), *C. glabrata* (6%) and *C. tropicalis* (4%). Our findings are consistent with the findings of Dewan E et al [98] who report 10% candidemia in patients with hematological malignancies. In a multicentric one year study conducted in 27 ICUs of India for determining incidence of candidemia by Chakraborti A et al [99] found an incidence of 6.51 cases/1000 ICU admissions with the highest burden from ICUs of North India contributing 8.95 cases/1000 ICU admissions. Tak V et al [100] report candidemia incidence of 7.76 cases/1000 ICU admissions. Delaloye J & Calandra T [101] in their review article mention *Candida species* as the fourth most common blood stream isolate accounting for 10% to 15%

of hospital acquired fungal sepsis and 5% of all cases of severe sepsis and septic shock. It accounts for 8 to 10% of blood stream infections in the United States and about 2-3% in Europe. Sonawane J et al [90] from Mumbai and Gupta S et al [89] from New Delhi, North India report 7.14% and 3.31% isolates of *Candida spp.* from blood cultures. Of the total 35 isolates obtained from blood, *C. albicans* was the most common isolate with 42.86% (15/35) followed by *C. non-albicans*, 40% (14/35), *C. glabrata*, 11.43% (4/35) and *C. tropicalis*, 5.71% (2/35). Our findings are similar to the findings reported in a laboratory based surveillance study carried out across Asia by Tan BH et al [102] who report *C. albicans* as the most common cause of candidemia with the similar percentage of prevalence.

The following tables 25 & 26 shows the comparison of different studies in relation to types of *Candida species* and antifungal susceptibility pattern of *Candida species*.

Table 25: Comparison with other studies in relation to *Candida species* from blood culture

Reference	**Place of Study**	***Candida species* (%)**
Guzman AJ et al, 2011 [103]	USA	*Candida non-albicans* (74%) *C. albicans* (26%)
Giri S et al 2013 [104]	Chennai, Tamil Nadu	*C. tropicalis* (74.35%) *C. albicans* (10.26%) *C. parapsilosis* (7.69%) *C. krusei* (5.13%) *C. glabrata* (2.56%)
Tak V et al, 2014 [100]	New Delhi	*C. tropicalis* (38.7%) *C. parapsilosis* (20.3%) *C. albicans* (13.7%) *C. glabrata* (11.4%) *C. rugosa* (9.4%) *C. hemulonii* (2.8%) *C. guilliermondi* (1.8%) *C. famata* (1.4%) *C. lusitaniae* (0.47%)
Chakraborti A, 2014 [99]	27 ICUs across India	*C. tropicalis* (41.6%) *C. albicans* (20.9%)

		C. parapsilosis (10.9%) *C. glabrata* (7.08%) *C. auris* (5.66%) *C. rugosa* (3.15%) *C. krusei* (1.74%) *C. guilliermondi* (1.74%)
Dewan E et al, 2015 (98)	Uttarakhand, India	*C. tropicalis* (46.67%) *C. ablicans* (26.7%) *C. glabrata* (6.7%) *C. parapsilosis* (6.7%) *C. krusei* (6.7%) *C. dublinensis* (6.7%)
Tan BH et al, 2015 (102)	25 hospitals across Asia	*C. albicans* (41.3%) *C. tropicalis* (25.45%) *C. glabrata* (13.9%) *C. parapsilosis* (12.1%)
Present Study, 2017	Vadodara, Gujarat	*C.albicans* (42.86%) C. non-albicans (40%) C. glabrata (11.43%) C. tropicalis (5.71%)

Table 26: Comparison with other studies in relation to antifungal susceptibility & resistance pattern of *Candida species*

Reference	**Place of Study**	**Resistance Pattern (%)**	**Sensitive Pattern (%)**
Guzman AJ et al, 2011 (103)	USA	Not Reported	Not Reported
Giri S et al, 2013 (104)	Chennai, Tamil Nadu	Fluconazole (30.8%) Ketoconazole (12.8%) Amphotericin B	Fluconazole (69.2%) Ketoconazole (87.2%) Amphotericin B (100%)

		(0%)	
Tak V et al, 2014 [100]	New Delhi	Fluconazole (3.3%) Amphotericin B (3.3%) Flucytosine (0%) Voriconazole (0%)	Fluconazole (93.9%) Amphotericin B (93.9%) Flucytosine (0%) Voriconazole (0%)
Chakraborti A, 2014 [99]	27 ICUs across India	Fluconazole (6.2%) Itraconazole (1.2%) Voriconazole (5.6%) Amphotericin B (2.1%) Anidulafungin (1.7%) Caspofungin (5.6%) Micafungin (1.7%)	Fluconazole (82.8%) Itraconazole (89.5%) Voriconazole (71.5%) Amphotericin B (97.9%) Anidulafungin (96.7%) Caspofungin (84.3%) Micafungin (96.1%)
Dewan E et al, 2015 [98]	Uttarakhand, India	Fluconazole (20%) Clotrimazole (20%) Voriconazole (13.4%) Flucytosine (100%) Nystatin	Fluconazole (80%) Clotrimazole (80%) Voriconazole (86.66%) Flucytosine (0%) Nystatin (33.33%) AmphotericinB(73.33%)

		(66.67%) AmphotericinB (26.67%)	
Tan BH et al, 2015 [102]	25 hospitals across Asia	Not Reported	Not Reported
Present Study, 2017* *Note: it represents overall percentage of all 100 isolates; and also SDD percentage is not mentioned here)	Gujarat, India	Fluconazole (63%) Itraconazole (62%) Ketoconazole (77%) Nystatin (14%) Amphotericin B (20%) Clotrimazole (85%)	Fluconazole (33%) Itraconazole (12%) Ketoconazole (5%) Nystatin (6%) Amphotericin B (80%) Clotrimazole (15%)

In our study a total of 34% of *Candida species* were isolated from urine samples which may be the cause of candidemia. Of these total 34 isolates, 20 (58.82%) were obtained from urine samples of catheterized patients. Giri S et al [104] report urinary catheters in 53.90% patients as a predisposing factor for candidemia while Xess et al [105] report it in 55.6% patients. Moreover in a review article, Giri S [106] quotes that as many as 10% of candiduria cases are significantly associated with development of candidemia. Mahmoudabadi AZ et al [61] report 62.3%, 26.8% and 4.3% *C. albicans*, *C. glabrata* and *C. tropicalis* from urine of 92 patients with candiduria. Besides 35% from blood and 34% from urine culture *Candida species* were also isolated from sputum (19%), non-catheterized urine (14%) and ET tips/secretions (7%), pleural fluid, suction material and throat swab suggest that these sites would have been the source of *Candida* infection leading to sepsis in these patients. Guzman AJ et al [103] report 27% & 51% rate of isolation of *Candida species* from urine, sputum and catheter-related samples of septic shock and no-shock group of patients respectively.

Overall, the difference in the prevalence of infections due to *Candida species*, the types of *Candida species* and the antifungal susceptibility & resistance patterns, may

be due to the difference in the geographical locations i.e. USA & India; the clinical settings i.e. only ICU or hospital; patient demographics i.e. only patients with certain conditions, adult or pediatric; number of & types of samples processed; different media/methods used for isolation & identification like automated vs. conventional/semi-automated; different numbers of *Candida spp.* identified and tested for antifungal agents as well as different types of antifungal agents tested in different studies. Most of the studies have taken into account only those *Candida species* which cause candidemia, but in our study all the *Candida species* isolated from all different samples of sepsis patients to identify the source of infection in them have been considered. Also most of the studies from developed countries and those from premier institutes in India are equipped with automated systems for culture which are useful in increasing the yield of *Candida species*, especially, from blood samples, identification to the species level and determination of susceptibility pattern through MIC values of antifungal agents tested.

Sepsis due to Tropical Infections:

A total of 250 patients i.e. 34% had sepsis due to tropical infections i.e. malaria, dengue, viral hepatitis, enteric fever and leptospira. Of these total tropical patients 65.2% (163/250) were males and 34.8% (87/250) were females. Of the total 250 patients who were grouped in tropical infections 122 (48.8%) had only malaria, 58 (23.2%) had only dengue, 17 (6.8%) had only viral hepatitis, 11 (4.4%) had only enteric fever, and the rest 43 (17.2%) had polymicrobial infections like malaria, dengue, viral hepatitis, enteric fever associated with other mixed infections which includes bacterial, fungal, parasitic and viral in various combinations of two or three microbial agents; including one patient (0.4%) with *Leptospira* spp. infection. In our study malaria was the major cause of tropical sepsis followed by dengue, viral hepatitis and enteric fever.

The percentage of tropical sepsis in our study is comparable to findings of Abrahamsen et al. [82] from South Indian hospital, who reported 35% of the total causes of fever in tropics due to malaria, typhoid fever, scrub typhus, hepatitis viruses but less compared to 84.67% reported by Chrispal A [83] from the same hospital in South India during a different period. However, it was higher to 29% reported as a cause of tropical sepsis in the SEAR study [84] which actually included the patients with sepsis and severe sepsis in the study group. There are only few studies in our

country where malaria, dengue, viral hepatitis, enteric fever and leptospira have been studied as cause of sepsis, most of the studies report these infections as separate entity or as a cause of fever in tropics or pyrexia of unknown origin. [82, 83]

Malaria:

In our study malaria was found to be cause of sepsis in 18.84% (140/743) of the total study population and 56% (140/250) of the tropical sepsis patients. *Plasmodium vivax* was found to be the most common cause in 59.29% (83/140) patients followed by *P. falciparum* in 28.57% (40/140) and mixed infections due to both the species of *Plasmodium* in 12.14% (17/140) patients. Also 18 patients out of 140 had concurrent infections with other microbes i.e. 10 had bacterial, 5 had dengue and 3 had hepatitis A infections and additional one of was also HIV seropositive. Amongst the bacterial isolates obtained from blood cultures of these patients, gram positive bacteria predominated over gram negative. Amongst non-survivors i.e. 31.42% (44/140), *Plasmodium falciparum* was the most common cause of mortality in 43.18% patients and equally important was the mixed infections in 22.73% patients. Interestingly of the 44 non-survivors, 3 had concurrent infections with HAV, 3 with bacterial infections and one with Dengue and also the one with HIV positive result did not survive. Thus *P. falciparum* infection, mixed infections and concurrent polymicrobial etiologies were an important cause of mortality. The identification of mixed infections with species of *Plasmodium* and polymicrobial infections highlights the role of a microbiologist in management of sepsis, as treating only one condition in these cases would have lead to higher mortality than that was noted. Though the rapid malarial antigen test as well as the microscopic examination of the peripheral blood smears helped diagnose malaria early but the delay in the identification of the concurrent bacterial isolates by conventional methods may be costing the lives of patients. PCT & CRP were also performed in 54 patients. Higher values of PCT & CRP were found associated with severity and mortality.

Studies from tropical countries like Sub-Saharan Africa [107] 57%, Nigeria [23] 33.3% and 1% SEAR countries [84] report malaria to be an important cause of sepsis in adults as well as neonates & children. Abrahamsen et al [82] and Chrispal A [83] from India reported 5% and 17.1% malaria as a cause of acute febrile illness, respectively. Authors in study from Nigeria [23], Kenya [108] and Kolkata, India [77/109] also report 4.8%, 6.2% and 6.7% patients with concomitant bacterial septicaemia. Also Mensah DD in the thesis on malaria & bacteraemia in febrile patients found 2.8% patients

with malaria and concomitant bacteraemia [110]. In all the above mentioned studies [23, 108, 109, 110] the concomitant septicaemia was largely due to Gram negative bacteria with *Salmonella species* being the common isolate. This is strikingly different from our finding of Gram positive septicaemia i.e. 60% which predominated over gram negative septicaemia. In our study we found concomitant growth of *Salmonella paratyphi A* in only one of the 49 cases of malaria in whom blood cultures were performed.

Dengue:

Dengue was found to be cause of tropical sepsis in 10.22% (76/743) of the total study population and 30.4% (76/250) of the patients with tropical sepsis. Dengue a single cause of sepsis was seen in 7.80% (58/743) patients. Of the total 76, 18 patients had concomitant infections with bacteria in 9, malaria in 4, *S. typhi* in 2 and 1 each with HAV, HEV+*CoNS* and malaria+*CoNS*. Of the 76 patients, 60.52% (46/76) patients survived, 30.26% (23/76) did not survive and outcome was not known in 9.21% (7/76); of the 23 non-survivors, 3 patients had concomitant bacterial infections, 1 patient with GB syndrome had malaria due to *P. falciparum* and 1 paatient had both *CoNS* and HEV together. Here again the percentage mortality is almost similar to sepsis with malaria as well as the concomitant infections seem to be associated with severity and mortality. Dengue is considered a separate entity but the dengue hemorrhagic fever (DHF) and Dengue Shock Syndrome (DSS) fulfill the criteria of definition of severe sepsis and septic shock. Thus considering them as a cause of sepsis they were included in our study. Though dengue does not need a particular drug/treatment, yet the presence of concomitant infections make it difficult to treat such patients with only supportive therapy thereby increasing the chances of fatality in such patients. Here too, the use of rapid diagnostic kit for detection of NS1 antigen and IgM & IgG antibodies proved to be helpful in early diagnosis of these cases but again, the delayed identification of bacterial isolates probably poses the same challenges in treatment.

Our findings of total dengue 76/743 i.e. 10.22% as cause of sepsis is slightly more compared to 8%, 7% and 1% reported in the SEAR study [84], by Chrispal et al [83] and Abrahamsen et al [82] respectively, as an important cause of sepsis in adults and children. However, these findings very less when compared to a cross sectional study conducted in Brazilian Amazon [111] which reported 37% patients with dengue

infection alone besides patients with concomitant infections. In our study dengue alone was found in 58/743 i.e. 7.80%. Five out of total 76 i.e. 6.57% dengue patients had concurrent malaria whereas 1/23 of the non-survivors had concurrent malaria. About 2.8% patients had concurrent malaria in Brazilian Amazon study. They found that those with coinfection had more severe disease [111]. There are similar individual reports from other parts of India like Srinagar [112] and Patna [113]. In our study we found 11.84% (9/76) of the total dengue patients and 4 out of 23 non-survivors had concurrent culture positive bacterial infection. Similar reports have also been found in literature from tropical countries like Singapore – 22.7% culture positive bacterial infections in dengue patients. [114]. Authors in the SEAR study also found rapid test kits useful in diagnosis of dengue [84].

Viral Hepatitis:

Hepatitis either due to viruses or alcoholism or any other pathological conditions, is an important cause of sepsis as well as sepsis-related MODS. Viral hepatitis as a cause of sepsis was found in 4.17% (31/743) of the total study population whereas 12.44% (31/250) of the tropical sepsis patients. Of the total patients with hepatitis, HAV (13/31) was the most common cause followed by HEV in 10, HBV in 4 and least by HCV in 2 patients. Infection with more than one type of hepatitis viruses was seen in two patients i.e. one with HAV+HEV and one with HAV+HBV with the latter having an *E. coli* isolate also. Thus only viral hepatitis was seen in 17 patients. Concurrent infections with other microbes were observed in 14 (45.16%) out of total 31 patients i.e. 5 with bacterial, 3 with malaria , 2 with dengue, 1 with HAV+ *Candida spp.*, 1 with HCV+*Candida spp.*+ *CoNS* and 1 with HAV+*Candida spp.*+*E.coli* and HAV+HBV+*E. coli*. Of the total 31 patients with viral hepatitis, only 25.81% (8/31) patients survived whereas 74.19% (23/31) did not survive the episode of sepsis due to viral hepatitis. Amongst the non-survivors the fatality was 100% (5/5) in patients with hepatitis B followed by 91% in HEV, 60% (9/15) in HAV patients and 50% (1/2) in patients with hepatitis C virus infection. Eleven of the non-survivors had concomitant infections of which 5 had bacteria, 3 had malaria and 1 had dengue + bacteria, 1 with bacteria & *Candida spp.* together and the last one had HAV+HEV together. Surprisingly the mortality was much higher in this group as compared to malaria and dengue group of patients. This could be because almost half of the non-survivors had concomitant infections, of which malaria, dengue and bacterial

infections could have worsened the condition leading to death in these patients, especially, in pregnant/post-partum females it took a fulminant course resulting in fatality.

These findings are comparable to results of 4% reported by Abrahamsen et al [82] which mainly included HBV (3/100) and HCV (1/100). HAV and HEV were not included in predefined diagnostic set in all the 3 studies from tropical region by Abrahamsen et al [82], Chrispal A et al [83] and SEAR [84] and hence not been reported. Moreover, the studies in India as well as from the other countries have not included viral hepatitis as a cause of sepsis.

Leptospira infections:

Only one patient (0.13%) tested positive for IgM antibodies, against Leptospira, of the total study population and 0.4% of the tropical sepsis patients diagnosed as having septic shock and succumbed during treatment. All the laboratory findings correlated well with the diagnosis. PCT was 10ng/mL and CRP was 110mg/dL, suggesting severity as well as a higher risk for developing shock as well as mortality. The platelet counts were low at 22,000/cumm and while the serum SGOT/SGPT, bilirubin, lipase and amylase were all raised. Importantly, the blood culture yielded *CoNS* and *C. non-albicans* together.

Though *Leptospira* infections are common in Surat, a city located in the Southern part of our state i.e. Gujarat, we did not find much of this infection as it is probably not so prevalent in our district as well as the nearby regions. Abrahamsen et al [82], in a study from South India, did not find any leptospira infection out of 15 tested and total 100 patients in the study group. Our finding is strikingly lesser to 6% reported in the SEAR study carried out in 3 countries [84] and 3% from a tertiary hospital in South India [83]

Polymicrobial Infections:

An important finding of our study was sepsis due to polymicrobial etiology in 11.44% (85/743) patients; it was not only restricted to finding 2 bacterial isolates together or bacterial & fungal isolates together but the microbes belonging to entirely different categories/classes were found together; not only in combination of 2 but also in 3. These resulted in diverse causes of sepsis as well as clinical presentations that were not typical of one infectious condition. Thus multipronged, multidisciplinary approaches towards the diagnosis, utilizing all the available resources and technology

in microbiology, biochemistry, pathology and imaging, lead to these findings and the mortality could be reduced in such patients. These findings are important as treating these patients, focusing onto only one infectious condition would have not lead to recovery of the patient unless the other causes were treated too. Also as noted and discussed with findings of other infections, severity & mortality were higher amongst this group of patients. Moreover, these polymicrobial infections were common amongst those with tropical infections in whom concomitant bacterial/fungal infections were found. Polymicrobial etiology, as a cause of sepsis, severe sepsis, [84, 80] as well as other conditions like VAP [115], has been reported but with lower percentages.

Sepsis due to Hospital Acquired/Nosocomial Infections:

It would be difficult to put up a single value in terms of number or percentage to indicate the sepsis acquired during ICU or hospital stay, as we have included patients presenting with sepsis on admission, with or without the history of previous hospitalization, surgery or treatment for any condition as well as those who developed it later during the stay. Moreover, infections like malaria, dengue and leptospira are mainly community acquired infections. However, indwelling devices like peripheral lines were placed in almost all the 743 patients at any one given point of time during ICU stay for parenteral nutrition or administration of drugs, urinary catheters in 58 patients, 196 patients on mechanical ventilation; 53 patients who underwent hemodialysis and post-operative infections in patients having undergone surgery were found in 20 patients. Thus these can be considered as potential sources of nosocomial infections leading to sepsis in our setup.

Hence patients with urinary catheters in 7.13% (53/743) and those on mechanical ventilation 26.37% (196/743) as well as those who developed infections post-operatively 2.69% (20/743) in our set up have been considered to have acquired nosocomial infections leading to sepsis.

A total of 58 (34.73% i.e. 58/167) samples were obtained from 53 catheterized patients. Of the 58 samples, growth was obtained in 89.65% samples yielding 58 isolates. Here *Candida species* (20/58) were the major isolate followed by *E.coli* (13/58) and equal numbers (7/58) of *Acinetobacter spp.* and *Enterococcus species* and then *Klebsiella spp.* (6/58), *Pseudomonas spp.* (2/58), *S. aurues* (2/58), and *CoNS* (1/58) in the sequence.

Similarly from 73 patients, out of 196 on ventilation, with clinically suspected VAP, 75 ET tips were cultured yielding 54 isolates from 46 samples of 46 patients. Thus 6.19% (46/743) can be labeled to have developed bacteriologically proven VAP whereas 3.63% (27/743) had VAP with an unproven microbial etiology. Eight samples showed polymicrobial growth. Of the total isolates, 45 were gram negative bacilli, 8 were fungi with 7 *Candida species* and 1 *Mucor spp.* and only one isolate of *S. aureus* (2%-1/54)). The most common isolate was *Klebsiella spp.* 35% (19/54), followed by *Acinetobacter* spp. 31% (17/54), *Candida spp.* 13% (7/54), *Pseudomonas spp.* 11% (6/54) and *E. coli* 4% (2/54), 2% each of unidentified GNB (1/8) and *Mucor spp.* (1/54).

Of the total 80 pus samples, 11 pus samples were obtained from patients who underwent surgery and developed infection at the site. The cultures were positive in 8 samples yielding 9 isolates; one sample having 2 isolates together. *E. coli* (3/9) was the most common isolate followed by *S. aureus* & *Enterococcus spp.* in equal numbers i.e. 2 each, *Klebsiella spp.* (1/9) and unidentified GNB (1/9).

Thus overall, the microbial profile of 144 different samples (11+75+58=144) from 137 patients (53+73+11=137) as representatives of nosocomial infections yielding 121 isolates showed that *Candida spp.* with 22.3% (27/121) was most common isolate followed by *Klebseilla spp.* - 21.5% (26/121), *Acinetobacter spp.* 19.8% (24/121), *E. coli* – 14.9% (18/121), *Enterococcus spp.* – 7.4% (9/121), *Pseudomonas spp.* - 6.6% (8/121), *S. aureus* – 4.1% (5/121) and a smaller percentage of unidentified GNB & *CoNS*. Though *Mucor spp.* has been mentioned above as an isolate from ET tube, it was not considered as hospital acquired as it was a part of patient's own condition i.e. nasal polyp. However, *Klebsiella spp.* isolated from the sample along with *Mucor spp.* was considered as hospital acquired. In addition, of the total 78 GNB, ESBL production was seen in 39 (31/80) isolates, MBL in 13% (10/80), AmpC in 18% (14/80). Thus a total of 70.51% of GNB showed drug resistance mechanisms with resistance to multiple drugs tested whereas amongst GPC, MRSA was seen in 60% (3/5) isolates.

These findings of our are in concordance with the findings of Saravu et al [(80)] in terms of predominant GNB infections 64.46% (78/121) as compared to GPC, also majority i.e. 36.36% belonging to Enterobacteriaceae and also the isolate types reported are similar to our study. However, in contrast to ours, they report *Pseudomonas spp.* with 24.62% as the most common isolate and 2.98% of *Candida spp.* as the least

common isolate, but *Klebsiella spp.* is reported to be 20.14%, *Acinetobacter spp.* – 22.38% and *Enterococcus spp.* – 5.22%, which are quite similar and consistent with our findings. ESBL production was reported in 28.57% (30/105) and MDR was seen in 32.38% of the total gram negative isolates reported in the study, which is lower compared to ours. The VAP findings are also comparable to the findings of previous study carried out in our setup on VAP by Patel A et al [115]

Biomarkers of Sepsis: Procalcitonin (PCT) & C - reactive protein (CRP)

The last decade saw many research studies and published articles on utility of various markers – immunological & biochemical, like PCT, IL-6, CD 64, s-TREM, lactate levels, etc. as many as 170, for predicting sepsis with more sensitivity and specificity through these tests, so that the treatment, especially, an antibiotic, can be initiated early during "the golden hour" thereby reducing mortality due to sepsis. However, none of these have been proved to be 100% specific. [116].

PCT was carried out in 288 patients, of which majority i.e. 186 patients had values of ≥2<10 ng/ml, suggesting a moderate risk of progressing to sepsis/severe sepsis, as well as, a most likely infection with bacteria. But interestingly, these values were found even in those patients who had malaria and dengue. Moreover, in those with an unproven microbial etiology, these values helped suspect a microbial etiology as a cause of sepsis, but could not be established due to reasons stated above under blood culture. Patients with PCT values of ≥10 ng/ml were found in 27 patients, of which 10 patients did not survive and other 17 survived despite the higher values and again of which 8 patients had sepsis due to tropical infections and 2 with bacteria/fungi. Amongst the total 66 patients with bacterial infections (inclusive of concurrent infections with other microbes), 21% (14/66) had ≥10ng/ml values and 89.39% (59/66) had values of >0.5ng/ml. PCT values of ≥10ng/ml were found in 10 patients with malaria, of which 60% (6/10) did not survive especially, those with *P. falciparum* malaria or malaria due to mixed infections. In dengue patients, the majority i.e. 62.5% had values of ≤0.5 ng/ml, suggesting a very low risk of progression of sepsis/severe sepsis and probably ruling out the bacterial infections as a cause of sepsis. The data in our study is not sufficient enough to comment onto the levels of PCT in regards to fungal infections. Overall, the PCT values of >0.5ng/ml and ≥2 ng/ml indicated infections due to bacteria and the higher values indicated severity and high risk of progression to severe sepsis/septic shock both in bacterial

infections as well as malaria and values ≤0.5ng/ml indicated either an infection with virus or no infection.

Different studies use different threshold/cut off values of PCT, ranging from <0.1 mcg/L to <0.25mcg/L and even >0.5mcg/L, for deciding whether inflammatory process is infectious or non-infectious. Thus the interpretations differ for e.g. PCT values of <0.1mcg/L means there is no bacteremia or bacterial infection. In a review article by Neslon G et al [117] in 2014, on utilization of biomarkers in sepsis with special attention to India, suggest that there is need for more evidence to make definitive recommendations regarding use of PCT in India whereas Camacho CH and Losa J, in 2014 [118] in their review article suggest that is there enough evidence through various studies regarding the decision making in terms of withholding antibiotic prescription with PCT values of <0.25 ng/mL. Gaini S et al, [119] from Denmark in 2014, carried out work comparing various biomarkers in predicting suspected community-acquired mild infections as well as sepsis. The author suggests (1) that PCT is a severity marker of sepsis and a marker of presence of bacteremia and should be introduced for routine testing; (2) also that more studies are required towards finding new biomarkers with greater specificity and sensitivity. Interestingly all these studies published in 2014, after the introduction of PCT & CRP in 2001ACCP Consensus Definition, indicate a lack of evidence in recommending its use as a sole marker for predicting sepsis as well as the cost-effectiveness in its implementation. Hesselink DA et al [120] from Netherlands, in their study on utility of semi-quantitative test in travelers with imported severe *P. falciparum* malaria found that higher values of PCT like 10ng/ml, helped to predict the severity but the lower values had poor positive predictive value. Chiwakata BC et al [121] from Germany suggest that repeated measurements of PCT may be helpful in determining the prognosis of complicated *P. falciparum* malaria, as they found values of >25ng/mL associated with death and the values did not decrease in these patients.

CRP is more sensitive but less specific marker. [116] CRP has been known since long as a maker of inflammation, tissue injury and infection. It has been used as an adjunctive test in sepsis diagnosis and also the newer biomarkers have been compared with it for their utility & superiority [117]. CRP test was carried out in 183 patients. The mean CRP was 69.23 mg/L. A total of 43 patients showed a CRP values of >100mg/L but <320mg/L. Of these 43 patients, 27 patients were culture positive and 16 were culture negative; also 15 patients expired who had MODS and septic shock.

Moreover, only 2 with unidentified etiology, 37 patients with tropical infections and 4 with bacterial/fungal infections had >100mg/L of CRP values. Also 3 patients with CRP values of >320mg/L had only bacterial/fungal infections; of which 1 expired and two were discharged. The mean CRP was 74.56 mg/L in patients with sepsis due to gram negative bacteria, with majority i.e. 97.29% (36/37) having values above 20 mg/L whereas those with gram positive bacteria had 65.93mg/L mean CRP, of which 75% (18/24) had values of more than 20mg/L. The mean CRP value was 124 mg/L amongst ten malaria patients having PCT of ≥10ng/ml. The mean CRP values when compared to the different groups of PCT values showed a rise in titre with rising PCT values. In our study we found that the rise in both the markers indicated severity and high risk of mortality. However, CRP does not much differentiate between the microbial etiologies of infection/sepsis. Thus higher values of both these markers may help to predict severity and risk of progression of disease.

Nelson G et al [117] in their review article mention that though CRP has been used successfully in diagnosis of sepsis in initial stages but lacks specificity during the course of the disease as its levels don't go down. Camacho et al [118] suggests that low specificity of CRP makes it a poor marker for sepsis in adults but due to the increased levels noted in neonatal sepsis, especially the early onset, it is frequently used as a screening test. Nelson G et al [117] mentions that rise in the levels of CRP were found to be significantly associated with sepsis due to gram negative bacteria compared to that of gram positive. Luzzani A et al [122] suggest that PCT is a better marker of sepsis than CRP and the course of PCT shows closer correlation than that of CRP with severity of infection and organ dysfunction. However, Nelson G et al [117] suggest utilization of combination of biomarkers for diagnosis of sepsis forming an algorithm with better sensitivity and specificity.

Comorbid Conditions, Laboratory Parameters & Organ Involvement Pattern:

Comorbid conditions like hypertension (HTN), diabetes mellitus (DM) and tuberculosis (TB) were recorded and were found significantly ($p<0.05$) associated with mortality. Thus 25.30% (188/743) had hypertension of which 88 expired, 25.97% (193/743) had DM and 93 expired whereas 1.34% (10/743) had TB and 7 expired. Other comorbid conditions like COPD (chronic obstructive pulmonary disease), CRF/CKD (chronic renal failure/chronic kidney disease), liver cirrhosis and sickle cell anemia were observed in 15, 20, 9 and 4 patients respectively. Thus

40.91% (304/743) did not have any comorbidity. Also GCS<13 (Glasgow Coma Scale), mechanical ventilation and dialysis were noted and their association with mortality was found to be significant ($p<0.05$) in patients in whom these were found. Laboratory parameters like total leukocyte count (TLC), platelet count, ESR (Erythrocyte sedimentation rate), CRP (C-reactive protein), total bilirubin (TB), urea & creatinine were noted and analyzed for their association with types of infections. Thus, CRP, TLC and Total bilirubin along with SGOT & SGPT were significantly ($p<0.05$) higher in patients with sepsis due to tropical infections as compared to those with sepsis due to bacterial/fungal infections and unidentified etiology, whereas ESR and Urea were significantly higher in those with sepsis due to bacterial/fungal infections; and Creatinine was higher in unidentified etiology group.

The organ involvement pattern showed majority having multiple-organ involvement 43.06% (320/743) followed by 36.87% (274/743) with no organ involvement, 13.05% with (97/743) single organ involvement and 6.99% (52/743) with septic shock. The most common organ involved was kidney with creatinine values >1.2mg/dL in (n=371) followed by liver with bilirubin values of >1.2mg/dL in (n=357) and hemopoetic system with a platelet count of <1, 50,000/µl (n=310). Kidney (n=189/349) and Liver (n=173/250) were the frequently affected organs due to sepsis with bacterial/fungal infections and tropical infections respectively. Thrombocytopenia was most commonly found in those with tropical infections (n=181).

Desai SR and Lakhani JD [123] report 82% patients having MODS in sepsis patients of rural setup from Gujarat with lungs involved in 80%, kidneys in 78% followed by liver and CNS in equal proportions of 68% and haematological in 62%. Divatia J et al [81] report 62.8% patients without comorbitidies whereas those on mechanical ventilation, inotropes and renal replacement therapy were associated with significantly higher mortality. They also report diabetes in 13.7% patients and other comorbid conditions like COPD, cancer, chronic renal failure and cirrhosis of liver significantly associated with mortality. Abrahamsen et al [82] report diabetes and increasing age as significant risk factors for a fatal outcome but not the gender and HIV infection amongst patients with fever in tropics i.e. South India. Saravu et al [80] report DM, CKD, chronic liver disease, chronic lung disease and HIV in 26.4%, 8.6%, 10.3%, 13.2% and 2.3% patients respectively whereas they report hemodialysis, mechanical ventilation, inotropes and blood product use in 18%, 58%,

67% and 26% patients respectively. They also report 22% mortality amongst their study population.

Kumalo A et al [86] from Ethiopia report comorbidities like HIV/AIDS, heart failure, chronic renal disease, hematological malignancy, DM and stroke in 21%, 16.8%, 9.5%, 7.4%, 6.3% and 5.3% patients and also 1% each of chronic liver disease, solid malignancy and COPD.

Divatia J et al [81] from a multicentric study carried out across 124 ICUs in India report 53.6% patients with no organ failure and 28.3% with severe sepsis or septic shock. They also report 34% mortality amongst patients with severe sepsis. Todi S et al [124] (in a multicentre study carried out across 12 ICUs in India from June 2006 to November 2006) report SIRS in 51.6% patients with no organ dysfunction and 17.1% (n=230) with organ dysfunction and of these 17.1%, 76.5% (176) had organ dysfunction due to sepsis. They also report incidence of severe sepsis as 13.1% and mortality amongst severe sepsis patients as 54.1%.

The overall difference seen while comparing all different aspects of the study could be because of: (1) different study populations like ours is a rural based single setup whereas others are multicentre studies, (2) developing country vs. developed countries, (3) number of patients included is smaller as compared to the larger number of patients in multicentre studies. (4) Different parameters studied in different studies and in addition to these, the reasons discussed under fungal culture & susceptibility explains differences in our study.

Limitations of the study:

There are several limitations of our study yet all efforts have been made, with available resources, to make the findings of the study beneficial to all i.e. patients, clinicians, institution, hospital and me as well.

All the tests/investigations could not be uniformly carried out in all the patients as the tests/investigations were chosen according to the clinicians' request which in turn was dependent upon the chief clinical features and complaints of the condition suspected in patients. Thus the microbial etiology could have been probably missed in patients requiring other investigations.

Each patient could not be followed during the entire period from admission to discharge/death.

Anaerobic cultures were not performed and hence anaerobic microorganisms must have been missed as a cause of sepsis alongwith the limiations discussed under blood culture and susceptibility explains the culture negative sepsis or sepsis due to unidentified etiology.

CHAPTER 7
CONCLUSION

7 CONCLUSION

Our hypothesis that the microbial profile of sepsis in our setup is different from that of microbial profile of sepsis elsewhere, proved to be correct to a greater extent, especially, being in a tropical region infections like malaria, dengue, viral hepatitis, enteric fever and leptospira, not just only fulfill the criteria of sepsis definition, but were found to be a cause of sepsis in almost one-third of our patients which is not the same in regions other than tropical. However at the same time sepsis due to common bacteria and fungi echoes the findings of other researchers from various regions of our country as well as other countries. The study also highlights polymicrobial etiology as a cause of sepsis which warrants treatment with different groups of drugs, emphasizing on the role of microbiologist in management of sepsis patients.

Sepsis is caused due to bacteria, fungi, viruses as well as parasites. Amongst tropical infections, malaria is the most common cause of sepsis followed by dengue and amongst bacteria, *Klebsiella spp.* is the most common cause followed by *E.coli* among gram negative and *Stapylococcus aureus* followed by *Coagulase negative staphylococci* among gram positive bacteria; higher susceptibility towards Imipenem and Vancomycin render these as choice of empirical treatment for aerobic gram negative bacilli and gram positive cocci respectively; *Candida albicans* amongst *Candida spp.* and other fungi is the frequent cause of invasive fungal infections leading to sepsis and susceptibility to amphotericin B makes it an effective treatment choice; biomarkers like CRP and procalcitonin rise during infections and higher values indicate severity, which means if they are carried out at the time of admission can help clinician decide the course of action which in turn can reduce mortality. Overall, our findings in relation to microbial etiology, mortality and comorbid conditions in sepsis patients represent the findings in a tropical country.

Thus establishing microbial etiology through microscopy, culture or rapid tests, antibiotic and antifungal susceptibility pattern, detection of antibiotic resistance mechanisms expressed by bacteria; procalcitonin and CRP as markers of sepsis facilitate in early diagnosis and timely treatment of sepsis patients resulting in better outcome.

CHAPTER 8
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8 REFERENCES

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CHAPTER 9
ANNEXURE

ANNEXURE I

PATIENT PROFORMA

Name: Age: Gender: M/F

Residential Address:

Urban/Rural:

IPD/OPD No.: Marital Status: Married/Single

Socioeconomic Class: Upper/Middle/Lower

Date of Admission: Date of Discharge:

Diagnosis on Admission:

Co-morbid conditions/Past history:

1. DM: Yes/No. If yes, since how long?
2. HIV status: HIV positive/HIV negative/HIV status not determined
3. Hypertension
4. Immunocompetent/Immunocompromised (HIVpositive/Steroids/ Immunosuppressant)
5. Others: Sickle cell anaemia/Pregnancy/_____.

Clinical History:

Chief complaints:

ODP (Origin, Duration & Progress):

Inclusion Criteria: 1. Fever (oral temperature >38^{0}C) or hypothermia (<36^{0}C). ____

2. Tachypnea (>20 breaths/minute). _____

3. Tachycardia (heart rate>90 beats/minute). _____

4. Leucocytosis (> 12,000/mm3)/Leucopenia (< 4,000 mm3) ___

Clinical Examination Findings:

1. Pulse: Tachycardia (P>100)/Bradycardia (P<60)/Normal (P=70-72) _____
2. BP: Hypotension (<100mmHg)/Normal (100-140)/Hypertension (>140) ___
3. Respiratory Rate: Decreased (<12)/Normal (12-16)/Increased (>16) _____
4. Patient Conscious/Unconscious. If unconscious: Glasgow Coma Scale: ____.
5. Organ Involvement (Based on clinical features & investigations)
   a. Liver: Serum bilirubin (total)≥1.2 _____
   b. Kidney: Serum creatinine ≥1.2 _____
   c. Spleen: Clinical examination _____
   d. Hemopoetic: Platelet count < 1.5 lakh _____
   e. Brain: GCS<13 _____
   f. Heart: Mean arterial pressure <70mmHg _____
   g. Lungs: ARDS/PaO2/F1O2<400 _____

Septic Shock = Sepsis with hypotension (BP<90mmHg or 40mmHg less than patient's normal BP despite adequate fluid resuscitation) and Mean Arterial Pressure <70mmHg requiring vasopressive agent.

Supportive Investigations:

1. Hemoglobin
2. TC/DC
3. ESR
4. RBS/FBS/PP2BS
5. S. Bilirubin
6. SGOT/SGPT
7. S. Creatinine
8. S. Urea/BUN
9. X-ray chest
10. ECG
11. Echocardiograph
12. ABG Analysis
13. CT Scan/MRI
14. Others

Drug Treatment:

Antibiotics started: Initial

Later

Empiric/Culture Report

Supportive Treatment:

a. Use of ventilator: Yes/No
b. Use of Dialysis: Yes/No
c. Use of Inotropes: Yes/No
d. Use of plasma exchange: Yes/No
e. Use of blood/blood products: Yes/No

Follow up details:

Probable Diagnosis:

Final Diagnosis on Discharge:

Outcome:

Length of hospital stay:

<u>Microbiological Profile:</u>

1. Blood Culture (BACTEC followed by conventional method):
   Organism isolated:
   AST Report:

2. Additional Culture: CSF/Sputum/ETsecretions/TTA/Bronchoscopic aspirate/Urine/Stool/Catheter tips/others.
   Organism Isolated:
   AST Report

3. Specimen Gram Stain:

4. PSMP: for malarial parasite detection

5. Malarial Antigen: Positive/Negative

6. Serological Tests:
   a. CRP: Quantitative/Qualitative. Value: _____.
   b. PCT: Semi-quatitative. Value: _____ & Interpretation: _____
   c. Others: (e.g. Salmonella typhi, Dengue, Leptospira)

Annexure II **Sumandeep Vidyapeeth University**

Piparia, Ta. Waghodia, Dist. Vadodara

Pin 391760

Informed Consent Form (ICF) for Participants in Research Programmes involving studies on human beings

Study title

Study Number: SVU/SBKS/ /2012-____

Participants Initials: _____ Participant's Name _____

Date of Birth / Age _____ (Years)

1. I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]
2. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]
3. I understand that the investigator of this study, others working on the investigator's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records, both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I agree to this access. However, I understand that my identity will not be revealed in any information related to third party or published. [ ]
4. I agree not to restrict the use of any data or results that arise
5. from this study provided such a use is only for scientific purpose(s). [ ]
6. I agree to take part in the above study. [ ]

Signature (or thumb impression) of the participants /

Legally acceptable representative _____

Signatory's Name _____ Date _____

Signature of the investigator _____ Date _____

Study Investigator's Name _____

Signature of the impartial witness _____ Date _____

Name of the witness _____

સુમનદીપ વિદ્યાપીઠ યુનીવર્સિટી

પીપરીયા, તા.વાઘોડીયા, જી.વડોદરા

માનવજાતને સંબંધી અભ્યાસમાં ભાગ લેવા માટે, સમજી-વિચારીને આપેલી પરવાનગી માટેનું સંમતિ પત્રક

અભ્યાસનું નામ : માઇક્રોબાયોલોજીકલ પ્રોફાઈલ ઓફ સૈપ્રીસ વિથ સ્પેશ્યલ રેફરેનસ ટુ ટ્રોપિકલ સેપ્સીસ

અભ્યાસનો નંબર : એસ.વી.યુ./એસ.બી.કે.એસ. /2011

અભ્યાસમાં ભાગ લેનારનું ટુંકુ નામ :

અભ્યાસમાં ભાગ લેનારનું પુરું નામ :

અભ્યાસમાં ભાગ લેનારની જન્મતારીખ : ઉંમર :

(1) હું ખાત્રી આપું છું કે મે ઉપરોક્ત અભ્યાસ માટેની (તારીખ : / /2012) માહિતી વાંચી છે અને સમજી છે અને તે અંગેના મુંઝવતા પ્રશ્નો પુછવાની મને તક આપવામાં આવી છે.

(2) હું જાણું છું કે આ અભ્યાસમાં ભાગ લેવો મારા માટે મરજિયાત છે અને કોઈપણ જાતનું કારણ આપ્યા વગર, તેમાંથી ગમે ત્યારે ખસી જવાની મને છૂટ છે, અને આમ કરવાથી મારી તબીબી સારવારને અથવા કાયદેસરના હક્કોને કોઈ અસર નહીં થાય.

(3) હું જાણું છું કે આ અભ્યાસના તપાસકર્તા, તેમના મદદનીશો, એથિકલ ટીમ અને તેના ઉપર દેખરેખ રાખતા અધિકારીઓને મારા સ્વાસ્થ્યની કોઈપણ જાતની માહિતી, સદર અભ્યાસને લગતી કે તે સિવાયની, મેળવવા માટે મારી પરવાનગીની જરૂર રહેશે નહિ. ભલે ને પછી હું સદર અભ્યાસમાંથી ખસી જાઉં. હું જાણું છું કે મારી આ પ્રમાણેની કોઈપણ જાતની માહિતીની અન્ય કોઈને જાણ કરવામાં, કે કોઈપણ રીતે પ્રસિદ્ધ કરવામાં નહી આવે.

(4) આ અભ્યાસ દરમિયાન, અથવા તેના અંતે પ્રાપ્ત થતી માહિતી, કોઈપણ જાતની વૈજ્ઞાનિક શોધ માટે હું સ્વૈચ્છિક રીતે છૂટ આપું છું.

(5) હું અભ્યાસમાં / અભિયાનમાં ભાગ લેવા માટે / જોડાવા માટે સહમતિ આપું છું.

અભ્યાસમાં ભાગ લેનારની સહી આ અથવા અંગુઠાનું નિશાન.....

કાયદેસરના સ્વિકૃત તપાસકર્તાની સહી .....તારીખ.....

તપાસકર્તાનું નામ .....તારીખ.....

તટસ્થ સાહેદ/સાક્ષીનું નામ .....

તટસ્થ સાક્ષીની સહી.....

Scanned by CamScanner

Annexure IV

Participant Information Sheet

Title of the study: "Microbiological Profile of Sepsis With Special Reference to Tropical Sepsis"

Study No. _____ Date _____

Invitation to participant:

You are invited to participate in a study titled "Microbiological Profile of Sepsis With Special Reference to Tropical Sepsis"

1. Purpose & nature of the study: To study the microbiological profile of sepsis with special reference to tropical sepsis. There will be very little, if any, direct involvement of yours in this study. You are requested simply to agree to allow us to process sample (s) belonging to you and to agree to cooperate in the study if need arises.
2. Voluntary nature of the participation: Your participation in this study programme will be absolutely voluntary.
3. Study methods: When your sample arrives from Dhiraj General Hospital to microbiology department for culture & antibiotic susceptibility testing for bacteria or for detection of malarial antigen/CRP/Widal/PCT etc. for fungal/ parasitological examination will be processed as per the standard protocol.
4. Participant's responsibilities: After agreeing to participate in the study, you are expected to extend full support. You should provide real facts when inquired into, make yourself available wherever and whenever required.
5. The benefits of participation: Study is likely to benefit both to you and community. Carrying out research in sepsis in a rural based teaching hospital would help us know the causative organism of the sepsis, whether, community acquired or hospital acquired, antibiotic sensitivity status, host response, etc. This study would also give an insight to various aspects of sepsis like early diagnosis, judicious use of antibiotics, differentiating sepsis from various sepsis-like syndromes. By generating our own data, our aim is to help clinicians know the kind of flora that exists in the hospital setup, their potential as pathogens and developing resistance against the antimicrobials used thereby allowing clinicians choose the right empiric treatment too.

6. Confidentiality of the record: Information regarding your health and other personal facts, if any, will be kept confidential.
7. If any problem develops, you can contact (person's name & address with Tel. No.):

Name: Ms. Radhika Rana,

Address: A-48, Bhartinagar, Chhani Jakat Naka, Baroda.

Mobile No.: 9898778632.

8. Financial Considerations: If you are requested to be called for repeat sample or your travel cost for additional visit will be compensated by the investigator. In short, any extra cost, purely for the purpose of this study, will not have to be borne by you. If need arises, you may be contacted to inquire about past, personal, and family history. Also religious background, social customs, believes etc. can be inquired into.
9. Protection for patient and security: Since, no new drug or procedure is being tested, there is no additional risk anticipated, for which may need any protection or security. However, after getting this information, if you decide not to participate in this study or for some reason at a later date, you want to withdraw from the study; your subsequent medical care will not be affected and will continue as per the standard treatment guidelines.
10. Obtaining additional information: If need be additional information would be obtained.

ETHICAL ISSUES (and means of tackling)

1. Patients, whose samples show any signs of positive culture (for culture & sensitivity) or positive while detecting malarial antigen/CRP/Widal/PCT etc. will be taken into confidence, will be adequately informed of the aims, methods, anticipated benefits and potential hazards, if any, of the study.
2. Total care will be taken not to divulge name, OPD or IPD number of the case, facts of ill-health and other information of the patient to anybody without patients' or their guardian's permission.
3. Care will be taken that the patient is not hurt mentally or otherwise due to processing his/her sample for the studies.
4. Prior consent of the patient (or relative or guardian, as the case may be) will be taken on a consent form, printed in patient's vernacular language and contents of the consent form clearly explained to the patient.
5. Patient will be informed of his right to refuse his/her sample for processing for the project.
6. Basic human rights will not be violated.

(If any ethical issue arises even after full precautions, an attempt will be made to solve or diffuse on humanitarian and/or legal grounds)

Annexure V

સુમનદીપ વિદ્યાપીઠ

પીપરિયા, તા.:-વાઘોડિયા, જી.:- વડોદરા

ભાગ લેનારને માટે માહીતી પત્રક

અભ્યાસ નું શીર્ષક:- માઇક્રોબાયોલોજીકલ પ્રોફાઇલ ઓફ સેપ્સિસ વિથ સ્પેશિયલ રેફરન્સ ટુ ટ્રોપીકલ સેપ્સિસ.

અભ્યાસ નો ક્રમાંક:- _____ /2011

ભાગ લેનાર ને નિમંત્રણ:-

પ્રિય ભાગીદાર,

હું તમને મારા અભ્યાસ માં કે જે ધીરજ જનરલ હોસ્પિટલ, પીપળીયા , વડોદરા માં કરવામાં આવે છે, તેમાં ભાગ લેવાનું નિમંત્રણ આપું છુ. આ અભ્યાસ ડો.એસ. જે. લાખાણી, પ્રોફેસર. માઇક્રોબાયોલોજી વિભાગ, એસ. બી. કે. એસ. મેડિકલ ઇન્સ્ટીટ્યુટ એન્ડ રીસર્ચ સેન્ટર, સુમનદીપ વિદ્યાપીઠ ના માર્ગદર્શન હેઠળ કરવામાં આવશે.

(1) અભ્યાસ નો હેતુ અને પ્રકાર:-

હેતુ:- પીએચ.ડી. કક્ષા એ મારો અભ્યાસ નિબંધ બનાવવા માટે.

પ્રકાર:- વૈજ્ઞાનિક રીતે સ્વીકૃત ઉપકરણ અને પધ્ધતિઓ કે જેમાં જોખમી રસાયણો નો કે શરીર માં અંદર ઊંડે કોઈ પ્રવેશ ન કરાવતી પ્રણાલીકતા નો પ્રયોગ કરાય છે, તેવી સલામત અને માન્ય ઉપકરણો કે પધ્ધતી ઓ વડે જ અભ્યાસ થશે.

(2) ભાગ લેવા બાબતે સ્વેછિકતા:-

આ અભ્યાસ માં સંપૂર્ણ સ્વેચ્છા થી ભાગ લેવાનો છે. અને ભાગ લેનાર તેની ઇચ્છા મુજબ ગમે ત્યારે, અભ્યાસ ના કોઈ પણ તબક્કે અભ્યાસ થી અલગ થઈ શકે છે.

(3) અભ્યાસ ની પધ્ધતિઓ:-

જ્યારે આપના લોહી/પેશાબ/પરુ વગેરે ના નમૂના , ધીરજ જનરલ હોસ્પિટલ દ્વારા, જુદી જુદી તપાસ જેવી કે કલ્ચર અને એન્ટીબાયોટીક સસેપ્ટિબિલિટી ટેસ્ટિંગ (બેક્ટેરિયા માટે) અથવા મલેરિયાનાં એંટીજેન/ સી. આર. પિ/ પિ.સી. ટી/અથવા ફૂગ/પરોપજીવી જીવો ની તપાસ માટે માઈક્રોબાયોલોજી વિભાગ માં મોકલવામાં આવશે ત્યારે એ દરેક ની તપાસ નિર્ધારિત યોક્કસ પદ્ધતિ દ્વારા કરવામાં આવશે.

(4) ભાગ લેનારની જવાબદારીઓ:

- સહકાર આપવો અને સાચી પૂર્વ વિગત આપવી.
- જો કોઈ સમસ્યા ઉત્પન્ન થાઈ તો તુરંત જાણ કરવી.

(5) અપેક્ષિત પ્રતિકૂડ ઘટના અને નિવારણ કરવાના ઉપાયો:

સામાન્ય સંજોગોમાં ઉપરોક્ત (3) માં દર્શાવેલ કોઈ પણ પ્રકાર નાં ટેસ્ટ માં કોઈ પ્રતિકૂડ અસર કે જોખમ અપેક્ષિત નથી.

(6) ભાગ લેવાના ફાયદા:

આ અભ્યાસ દરમિયાન જે ટેસ્ટ કરવામાં આવશે તે ભાગ લેનાર નાં રોગનું નિદાન કરવા માટે જ થશે જેથી કે ભાગ લેનારનાં રોગોની વહેલી તકે નિદાન થવાની શક્યતાઓ વધશે.

(7) રેકોર્ડનું ખાનગી રહવું:

આ અભ્યાસનાં રેકોર્ડ અન્ય કોઈપણ વૈગનાનીક સંશોધનનાં રેકોર્ડનાં ધોરણે ખાનગી રખાશે.

(8) આ અભ્યાસ દરમિયાન કોઈ સમસ્યા ઉત્પન્ન થતાં નીચે દર્શાવેલનો સંપર્ક સાધી શકાશે:

વ્યક્તિનું નામ : રાધિકા રાણા (આસિસ્ટન્ટ પ્રોફેસર – માઈક્રોબાયોલોજી)

સરનામું : માઈક્રોબાયોલોજી વિભાગ, એસ. બી. કે એસ. મેડિકલ કોલેજ અને રિસર્ચ સેંટર, પો. પીપરિયા, તા. વાઘોડિયા, જી. વડોદરા.

મોબાઇલ: 9898778632

(9) આર્થિક પાસાઓ:

ભાગ લેનાર પાસેથી કોઈપણ પ્રકાર ની રોકડ કે ભેટ સોગાદ કે અન્ય કોઈપણ સ્વરૂપે કે ફી રૂપે આ અભ્યાસ માટે કાઇ લેવામાં આવશે નહીં એટ્લે કે સંપૂર્ણપણે મફત છે. આ અભ્યાસ માટે કોઈપણ પ્રકાર નો ખર્ચ કરવો પડે તો એ ભાગ લેનારે આપવાનો નહીં રહે.

(10) દર્દીનાં રક્ષણ અને સલામતી બાબત પગલાઓ:

આ અભ્યાસમાં કોઈપણ પ્રકારની નવી દવા અથવા તો પદ્ધતિનો ઉપયોગ કરવામાં આવશે નહીં કે જેના થકી કોઈ નુકશાન થવાની શક્યતા નથી.

(11) વધારાની માહિતી મેળવવી હોય તો માર્ગદર્શક ગાઈડ પ્રોફેસર એસ. જે. લખાણિ, માઈક્રોબાયોલોજી વિભાગ, એસ. બી. કે. એસ. પીપરિયા ની સરનામે સંપર્ક સાધી શકશે.

CHAPTER 10
PUBLICATIONS

10 PUBLICATION

1. Detection of ESBL Producing *Escherichia coli* isolates from blood culture and its effect on the outcome of Sepsis patients at a rural based tertiary care and teaching hospital in Vadodara district, Gujarat. International Archives of Integrated Medicine, 2017; 4(5):111-116.
2. Isolation, Identification and Antifungal Susceptibility Testing of Candida species from Sepsis patients from rural based tertiary care and teaching hospital in Vadodara district, Gujarat. International Archives of Integrated Medicine, 2017; 4(7):151-160.

Original Research Article

Detection of ESBL Producing *Escherichia coli* isolates from blood cultures and its effect on outcome of Sepsis Patients at a rural based tertiary care and teaching hospital in Vadodara district, Gujarat

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Abstract

Background: Sepsis is one of the most common clinical conditions that cause substantial morbidity and mortality all over the world. Blood culture is considered to be the gold standard for the identification of bacteria as a cause of sepsis along with the pattern of antibiotic susceptibility that helps clinicians to choose the appropriate empirical antibiotic. *E. coli*, belonging to the family Enterobacteriaceae, is a very important pathogen causing infections in humans. It causes a number of important clinical conditions like urinary tract infections, diarrhea, peritonitis, visceral abscesses, endovascular infections, septicemia pneumonia, meningitis, osteomyelitis, wound and soft tissue infections. Due to increased resistance to drugs and ability to produce variety of beta-lactamase enzymes (extended-beta lactamases) poses a difficulty in treating infections caused due to *E. coli.*

Objectives: Objectives of this study were (a) to detect ESBL production amongst the *E.coli* isolated from the blood cultures of patients with sepsis and (b) to determine its effect on the outcome of sepsis patients from a rural based tertiary hospital in Vadodara district of Gujarat.

Materials and methods: A total of 48 *E. coli* isolates were obtained from the blood culture of 46 patients with clinically diagnosed sepsis. These 48 *E. coli* isolates were tested for detection of ESBL production according to the CLSI guidelines using phenotypic screening & confirmatory methods.

Results: From a total of 48 *E. coli* isolates obtained from blood culture of 46 clinically diagnosed sepsis, 24 were female patients (as 2 patients had 2 blood samples cultured) and 22 were males. Of the total 48 *E. coli* isolates tested for ESBL production, 23 (47.91%) isolates from 23 (50%) patients were found to be producing ESBL and 25 (52.08%) isolates from other 23 (50%) patients did not produce ESBL. Of the 23 patients with ESBL producing *E. coli*, 14 (60.86%) patients did not survive the episode of sepsis, whereas 7 (30.43%) survived and for 2 (8.69%) patients the outcome was not known as they took discharge against the medical advice. Amongst the 23 patients with 25 blood samples yielding non-ESBL producing *E. coli*, 8 (34.78%) did not survive, 14 (60.86%) survived and for 1(4.34%) patient the outcome was not known. Thus the mortality was more in patients with sepsis due to ESBL producing *E. coli* as compared to patients with non-ESBL producing *E.coli*. Also the urinary tract/kidneys were the common source of infection and kidneys were the organ affected. The ESBL producing *E. coli* showed a higher resistance to most of the antibiotics used but a higher susceptibility to Imipenem and Ertapenem.

Conclusion: The findings of our study suggests a higher prevalence of ESBL producing *E. coli*, which exhibit a higher resistance to most of the antibiotics, are associated with greater mortality and pose a real challenge in the management of patients with sepsis.

Key words

E.coli, ESBL- producers, Sepsis.

Introduction

Sepsis is an important clinical condition which is a result of the exaggerated inflammatory response against the microbe beginning first at the primary site and then spreading throughout the blood stream ultimately resulting in multiorgan failure and death [1].

E. coli, a member of the family Enterobacteriaceae, equipped with flagella, capsule and ability to form various toxins like enterotoxins and hemolysins, is an important cause of most of the human infections. It is the normal commensal of the GIT of the humans and animals. *E. coli* causes a number of clinical conditions in humans, both, community acquired as well as hospital acquired like urinary tract infections, diarrhea, peritonitis, visceral abscesses, endovascular infections, septicemia pneumonia, meningitis, osteomyelitis, wound and soft tissue infections [2]. Moreover these infections have been on rise due to patients with primary immunodeficiency or acquired immunodeficiency as well those on immunosuppressants like cancer patients or those having neutropenia [3].

There is an increase in the incidence of ESBL producing Enterobacteriaceae members, especially, *E. coli* which is frequently resistant to the most of the antibiotics thereby limiting the options for treating infections with these microorganisms. Also these are associated with higher mortality leading to an undesirable clinical outcome in such patients [4].

The objectives of the present study were (a) to detect ESBL production amongst the *E.coli* isolated from the blood cultures of patients with sepsis and (b) to determine its effect on the outcome of sepsis patients from a rural based tertiary hospital in Vadodara district of Gujarat.

Materials and methods

This study was carried out in Clinical Microbiology Laboratory under Dept. of Microbiology at Dhiraj General Hospital after ethical approval from institutional ethical

committee. A total of 48 *E. coli* isolates obtained from blood cultures of 46 adult patients with sepsis diagnosed clinically with the following inclusion criteria were included.

Inclusion Criteria [1]

Adult patients (age >18 years) and having 2 or more of the following:

a. Body temperature: >38^{0}C or <36^{0}C
b. Tachypnea: >20 breaths/minute
c. Tachycardia: Heart rate>90 beats/minute
d. Leuckocyte count: >12,000/µl or <4, 000/ µl

These 48 isolates of *E. coli* obtained on Mac Conkey's agar and blood agar after 24 hours incubation at 37^{0}C, were identified using the standard biochemical tests [5]. The antimicrobial susceptibility testing of these 48 isolates was carried out in the following manner:

Antimicrobial Susceptibility Testing [6]

Anti-microbial susceptibility test was performed for all 48 isolates on Mueller Hinton agar, by modified Kirby-Bauer method according to CLSI guidelines, against Imipenem (10µg), Amikacin (30µg), Gentamicin (10µg), Cefepime (30µg), Cefuroxime (30µg), Ceftazidime (30µg), Cefotaxime (30µg) Ciprofloxacin (5µg), Amoxycillin+Clavulanic Acid (30µg i.e 20/10 µg) and Cotrimoxazole (25µg i.e. 1.25/23.75) and observed after 18-24 hours incubation at 37^{0}C.

Detection of ESBL Production [7]

All the isolates of *E. coli* obtained from blood culture were tested for ESBL production by phenotypic screening and confirmatory tests. For this a Ceftazidime disk (30 µg) and Ceftazidime-clavulanic acid disk (30 µg /10 µg) were placed on surface of MHA plate and incubated at 35±2^{0}C in ambient air for 16-18 hours. A ≥5mm increase in zone diameter of cetazidime-clavulanic acid as compared to ceftazidime alone was considered positive for ESBL production. For confirmatory testing both cefotaxime (30 µg) and ceftazidime (30 µg) were placed on the surface of MHA along with their clavulanic combinations (30 µg /10µg) and incubated at 35±2^{0}C for 16-18 hours. A ≥5mm increase in zone diameter of either of the antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone was considered ESBL producer **(Figure - 1)**.

Figure - 1: ESBL detection.

Control Strains used: When performing the ESBL confirmatory tests, *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were tested. *E. coli* ATCC 25922: ≤ 2-mm increase in zone diameter for antimicrobial agent tested alone vs. its zone when tested in combination with clavulanic acid. *K. pneumoniae* ATCC 700603: ≥ 5-mm increase in ceftazidime-clavulanic acid zone diameter; ≥ 3-mm increase in cefotaxime-clavulanic acid zone diameter.

The data that was obtained was recorded in Microsoft Excel (2007 version) and analyzed. The results were expressed in frequency (number) and in percentage.

Results

A total of 48 *E.coli* isolates were obtained from 46 patients with clinically diagnosed sepsis. Of these, 24 were obtained from female patients and 22 from males. Of the total 48 *E. coli* isolates tested for ESBL production, 23 (47.91%) isolates from blood cultures of 23 (50%) sepsis patients were found to be ESBL producers and the 25 (52.08%) isolates from the remaining 23 (50%) patients were non-ESBL producers.

The antibiotic resistance pattern of both the types of *E.coli* isolates is shown in the **(Chart - 1)**.

Thus, the ESBL producing *E. coli* showed 13.04% resistance to Imipenem & Meropenem as compared to 4% in non-ESBL producers. Also 95.65% and 80% respectively against Ciprofloxacin by ESBL and non-ESBL producing E.coli. Also a higher resistance against amoxicillin-clavulanic acid (73.91%), Gentamicin (69.57%), Cefepime (52.17%), Cotrimoxazole (47.83%), Amikacin (39.13%) and 86.9% against Cefuroxime and Cefotaxime both was observed. All the ESBL producing isolates were resistant to Ceftazidime whereas non-ESBL producers showed 68% resistance. Overall the antibiotic resistance pattern of ESBL producers showed a higher percentage of resistance to most of the antibiotics used.

Chart - 1: Comparison Chart of Antibiotic Resistance Pattern of ESBL vs. Non-ESBL Producing *E. coli*.

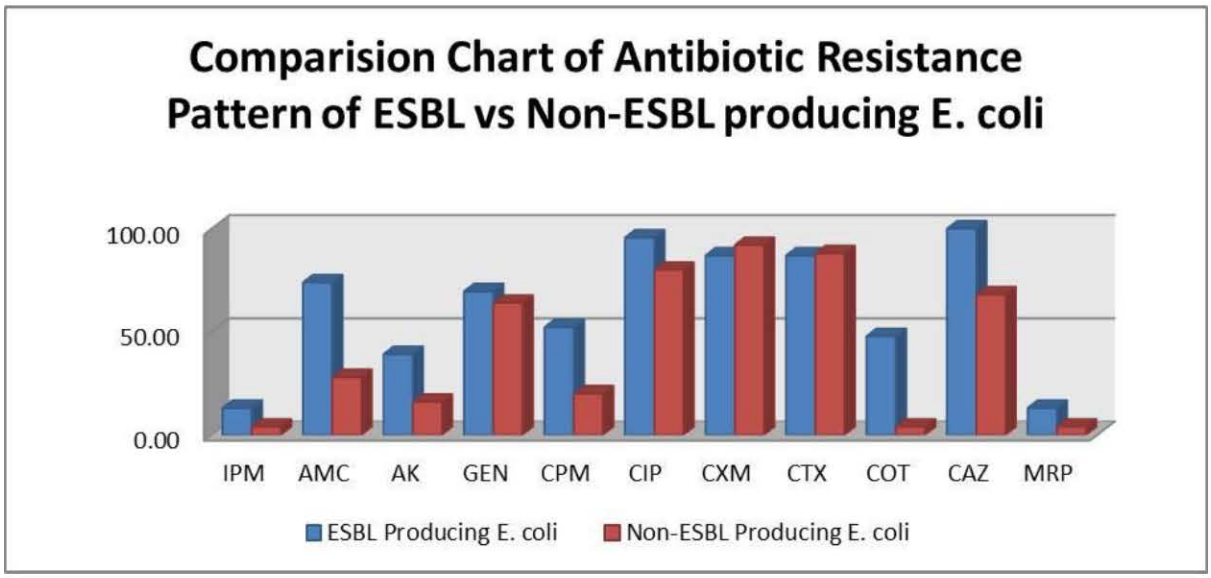
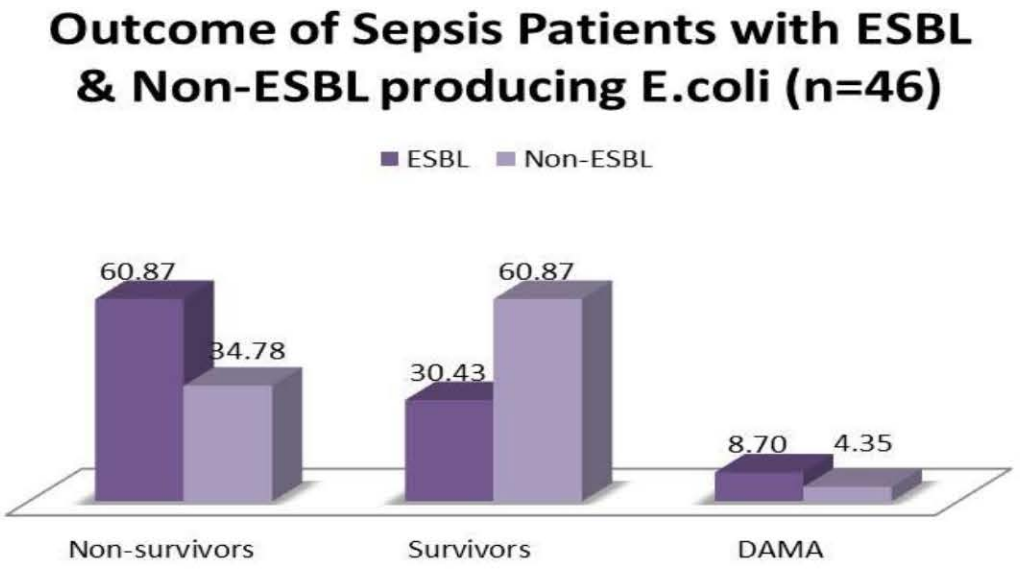


Chart - 2: Outcome among patients with ESBL and Non-ESBL producing *E. coli* Infection.



Of the 23 patients with ESBL producing *E. coli*, 14 (60.86%) patients did not survive the episode of sepsis, whereas 7 (30.43%) survived and for 2 (8.69%) patients the outcome was not known as they took discharge against the medical advice (DAMA). Amongst the 23 patients with 25 blood

samples yielding non-ESBL producing *E. coli*, 8 (34.78%) did not survive, 14 (60.86%) survived and for 1(4.34%) patient the outcome was not known **(Chart - 2)**. Thus the mortality was more among patients with sepsis due to ESBL producing *E. coli* as compared to patients with non-ESBL producing *E.coli.*

Moreover, 17 (36.95%) patients out of 46 had either urinary tract (UTI) or kidneys (Chronic Kidney Disease, Acute Renal Failure, Renal parenchymal disease or pyelonpehritis) as the source of infection or involvement of kidneys in the form Acute Kidney Injury, as a part of organ dysfunction. These were more common with patients having infections with ESBL producing *E. coli* i.e. in 11 (64.70%) patients out of 17 patients.

Discussion

We found 47.91% (23/48) of *E. coli* that produced ESBL. These findings are higher than that reported by Sonawane J, et al. [8] from Mumbai who reported 29.24% of ESBL production in their study; Taneja, et al. [9] from New Delhi who report 38.5%; 14.28% reported by Sweta, et al. [10] from Surendranagar district of Gujarat and 7.7% reported by Kang, et al. [4] from Seoul . When compared to 63.6% reported by Jain A, et al. [11] and Goyal A, et al. [12] from two different institutes of Lucknow, our findings are less. Moreover a higher percentage of resistance against cephalosporins (in **Chart - 2**) and other non-β-lactam antibiotics amongst ESBL producers are comparable to the findings of Jain A, et al. [11] and Sweta, et al. [10]. ESBL producing *E. coli* isolates showed least resistance to Imipenem and Ertapenem i.e. 13.04%. These findings are comparable to that of Sweta, et al. [10] and Taneja, et al. [9].

Amongst the patients with sepsis due to ESBL producing *E. coli*, 60.86% person did not survive the episode of sepsis whereas amongst those with non-ESBL producers 34.87% expired. Thus the mortality was higher amongst patients with sepsis due to ESBL producing *E. coli*. These findings are comparable to the findings of Jain A, et al. [11] who reports 60% and 35.7% mortality due to ESBL-producers and non-ESBL producers respectively. However, our findings are quite higher to 7.8% reported by Taneja, et al. [9] and 19.4% by Kang, et al. [4].

The urinary tract or involvement of kidneys was the most common source of infection site as well as the most common organ affected due to sepsis in 36.95% (17/46) patients of which 64.70% (11/17) had infections due to ESBL producing *E. coli*. Kang, et al. [4] reported solid tumors as the most common underlying disease in 33.8% and most common primary site of infection as pancreatobiliary tract in 43.6% and UTI in 14.3% patients, an overall percentage in patients with sepsis.

Conclusion

Sepsis being an important clinical condition leading to mortality and the information regarding the kind of bacterial flora prevalent in a setup and the antibiotic susceptibility pattern of those isolates helps the clinician in choosing the right empirical treatment, framing the policy for antibiotic use and measures to control infection in any healthcare setting. From our study we found that ESBL producing *E.coli* are an important cause of sepsis, exhibit higher resistance to most of the antibiotics and are associated with higher mortality posing a real challenge in the management of such patients.

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Original Research Article

Isolation, Identification and Antifungal Susceptibility Testing of *Candida species* from Sepsis Patients from a rural based tertiary care and teaching hospital in Vadodara district, Gujarat

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Abstract

Background: Sepsis is a global problem causing substantial morbidity and mortality to the patients afflicted with it. Moreover sepsis due to fungal infections, especially, the *Candida* infections has increased in the recent times due to increase in patients with immunocompromised conditions. They are the normal commensal of the oral cavity, GIT and the mucosal surfaces in the body as well as the pathogens leading to colonization and also infection. Though *Candida* albicans is the most commonly isolated fungal pathogen from clinical samples, gradually *non-albicans Candida species* are becoming predominant pathogens. The increased use of anti-fungal agents for treatment and also for prophylaxis especially in ICU patients has lead to development of resistance against commonly used anti-fungal agents in the treatment like various azoles. Thus this study was carried out to identify different *Candida species* from specimens of clinically diagnosed sepsis patients and their antifungal susceptibility pattern which can be utilized for better management of sepsis patients.

Objectives: The objectives of this study were to isolate and identify the species of *Candida* from different samples of clinically diagnosed sepsis patients and to determine the susceptibility pattern of the *Candida* species isolates against the commonly used anti-fungal agents from the clinical samples of sepsis patients from a rural based tertiary care and teaching hospital.

Materials and methods: A total of 100 *Candida species* were isolated from different samples of clinically diagnosed sepsis patients. These were identified on the basis of gram stain of the samples, colony morphology on Saboraud's Dextrose agar and HiCrome also germ tube and chlamydospore formation. The antifungal susceptibility testing was done according to CLSI M44-A2 for yeasts.

Results: A total of 100 (14.26%) *Candida species* were isolated out of the total 701 isolates from 1136 different samples cultured from clinically diagnosed sepsis patients. Of these, 53% were *C. albicans, 37% C. non-albicans, 6% C. glabrata* and *4% C. tropicalis*. Also 35% were obtained from blood, 20% from catheterized urine, 19% from sputum, 14% from non-catheterized urine, 7% from ET (Endotracheal) tips/secretions and the smaller percentage from other specimens. The antifungal testing showed a higher resistance to most of the antifungal agents tested with 80% towards clotrimazole, 77% to ketoconazole and 63% to fluconazole and 62% towards itraconazole. However, 80% of *Candida species* were susceptible to amphotericin B followed by 33% to fluconazole and 80% were susceptible-dose-dependent to nystatin.

Conclusion: The findings of our study suggest that *Candida species* are an important pathogen causing various infections in our patients leading to sepsis as well as a higher resistance to most of the antifungal agents tested poses a real challenge in the management of patients with sepsis due to *Candida*. Thus routine identification using HiCrome Media and antifungal susceptibility testing by disc diffusion method for yeasts will help in better management of sepsis due to *Candida* infections.

Key words

Candida species, HiCrome, Antifungal susceptibility, Sepsis.

Introduction

Sepsis, a serious clinical condition causes substantial morbidity and mortality amongst the patients globally [1]. *Candida species* are the normal commensal of the oral cavity, GIT and the mucosal surfaces in the body as well as the pathogens leading to colonization and infection. In the recent times incidence of fungal infections has increased with the increased incidence of immunocompromised patients [2]. *Candida* species are frequently isolated from such patients as well as those who are diabetic [3], on immune-suppressants or neutropenic [4] with malignancy undergoing chemotherapy/ radiotherapy [5], long term steroid therapy, long term antibiotic therapy etc. [6]. It has been implicated as a cause of UTI, respiratory infections, septicaemia as well as cutaneous and mucocutaneous infections. The invasive fungal infections often lead to sepsis, severe sepsis and septic shock in critically ill patients in ICU with *Candida species* being the most common cause of fungal sepsis, especially in the hospital acquired infections [7]. More than 17 different *Candida species* are known to be aetiological agents of human infections. Though *Candida albicans* is the most commonly isolated fungal pathogen from clinical samples, gradually *non-albicans Candida species* are becoming predominant pathogens [6]. Moreover, the increased use of anti-fungal agents for treatment and also for prophylaxis especially in ICU patients has lead to development of resistance against commonly used anti-fungal agents in the treatment like various azoles [2, 4, 6]. However the *Candida species* have variable resistance towards various antifungal agents. Thus this study was carried out to identify different *Candida species* from specimens of clinically diagnosed sepsis patients and their antifungal susceptibility pattern which can be utilized for better management of sepsis patients in our set up.

The objectives of this study were to isolate and identify the species of *Candida* from different samples of clinically diagnosed sepsis patients and to determine the susceptibility pattern of the *Candida species* isolates against the commonly

used anti-fungal agents from the clinical samples of sepsis patients from our setup in a rural area.

Materials and methods

A total of 100 *Candida* isolates were obtained from 1136 different samples cultured from clinically diagnosed sepsis patients with the following inclusion criteria:

Inclusion Criteria [1]

Adult patients (age >18 years) and having 2 or more of the following:

a. Body temperature: >38^{0}C or <36^{0}C
b. Tachypnea: >20 breaths/minute
c. Tachycardia: Heart rate>90 beats/minute
d. Leuckocyte count: >12,000/µl or <4, 000/ µl

Thus the specimens whose Gram stained smears showed presence of any yeast cells or yeast-like cells with budding and with or without pseudohyphae were processed for fungal culture and inoculated on Sabourad's Dextrose Agar (SDA). Plates were incubated aerobically at 37^{0}C for 24 hours. The colonies of *Candida species* were obtained after overnight incubation. The colonies were identified by colony morphology on SDA, colony colour on HiCrome Media, germ tube test and chlamydospore formation as follows [8]:

Thus colonies of white, creamy white or yellow-white with smooth, pasty consistency on SDA after overnight incubation were considered as suggestive of yeast or yeast-like fungi. In addition the presence of pseudohyphae in yeasts from direct specimen or colony was suggestive of infection with *Candida species*. The gram stain of the colonies was also performed. Well isolated colonies suggestive of *Candida species* from SDA were picked up with the sterile inoculating loop and streaked onto HiCrome medium (from HiMedia) for species identification. Different species of *Candida* grow with different coloured colonies on this medium. The colonies were identified according to colour and interpreted as shown in the **Table - 1** and **Figure - 1, 2**.

Table - 1: Interpretation of the colour of the colonies [9, 10, 11].

Colour of the colony	Species Identified
Light green	*Candida albicans*
Cream to White	*Candida glabrata*
Purple, fuzzy	*Candida krusei*
Blue purple and Bluish green	*Candida tropicalis*

Figure - 1: Colonies of *Candida* species on HiCrome.

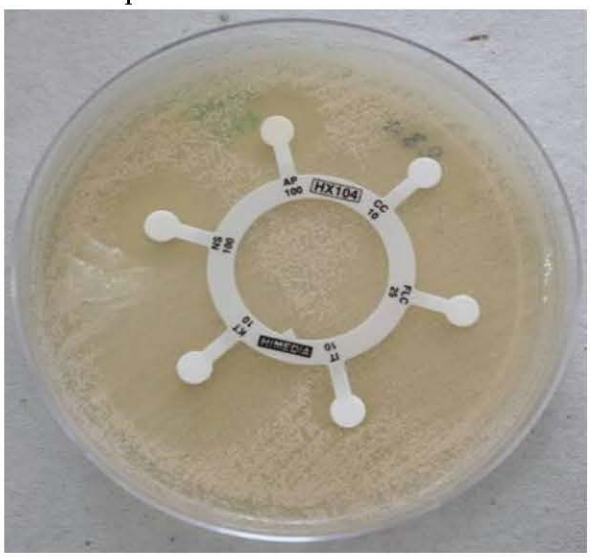
Figure - 2: Colonies of *Candida* species on SDA.

Those which could not be identified as one of the species as mentioned in the **Table - 1**, were labelled as *Candida non-albicans*.

In addition to the colour of the colonies on HiCrome, a germ tube test and observation of chlamydospore formation on cornmeal agar were carried out for identification of *Candida albicans*. For germ tube test, a well isolated colony from SDA was emulsified in 0.5 ml of human serum using sterile straight wire. The test tubes were incubated at 35^{0}C and no longer than 2 hours. A drop of serum sample was placed on a clean, grease free slide and a coverslip was placed over it. This slide was then observed first under 10X and then under 40X objective lens of microscope for the presence of germ tubes. Germ tube is a filamentous extension from yeast cell without constriction at the neck (true germ tube) and is seen in *C. albicans*. For inoculation of corn meal agar, 3 parallel cuts about 1 cm apart were made on the surface of the agar holding the sterile straight wire at about 45^0 angle. A cover slip was placed over the inoculated areas. The plates were then incubated for 24-48 hours at 30^0 C. The cover slips were then observed under the microscope for chlamydospore [8].

Antifungal Susceptibility Testing [12, 13, 14]
Antifungal Susceptibility test was carried out for *Candida species* according to CLSI guidelines for testing anti-fungal agents for yeasts [12]. In order to carry out antifungal susceptibility testing, an inoculum of 0.5McFarland was prepared from a well isolated colony from SDA. A lawn culture was made on Mueller-Hinton Agar + Glucose-Methylene-Blue (GMB) medium from the above inoculum using a sterile swab. The GMB was prepared according to the method described in CLSI-M44-A2. Thus MHA was prepared first (according to the manufacturer's instructions) and to this 2% of glucose and 0.5 µg/ml methylene blue were added [12]. A hexadisc (Hexa-Antimyco-01/HX104 from HiMedia) containing Amphotericin B (100 units), Clotrimazole (10 µg), Fluconazole (25 µg), Itraconazole (10 µg), Ketoconazole (10 µg) and Nystatin (100 units) was used. The plates were then incubated at 37^{0}C for 24 hours. Zones of inhibition for antifungal agents used for *Candida species* were measured (**Figure - 3**) and interpreted according to the Mahmoudabadi AZ, et al. [14]. For Amphotericin B and Clotrimazole, the zones were interpreted according to the manufacturer's manual [13].

Figure - 3: Antifungal Susceptibility Testing of *Candida* species.



Results

A total of 14.26% isolates of *Candida* species were obtained from 1136 different samples cultured from clinically diagnosed sepsis patients yielding 701 bacterial and fungal isolates altogether. The distribution of different fungal isolates is as shown in the **Chart - A**. Thus the most frequent isolate was *C. albicans* (53%), followed by *C. non-albicans* (37%), *C.glabrata* (6%) and *C. tropicalis* (4%).

The **Chart - B** shows the number & types of specimens from which these *Candida* species were obtained. Thus most frequently these were isolated from blood (35%) followed by catheterized urine (20%), sputum (19%), non-catheterized urine (14%), ET tips/secretions (7%) and also in small percentages from other samples as shown in the chart. The overall susceptibility pattern of the antifungal agents tested is as shown in the **Chart - C**.

Chart - A: Type & Percentage of *Candida* species isolated and identified.

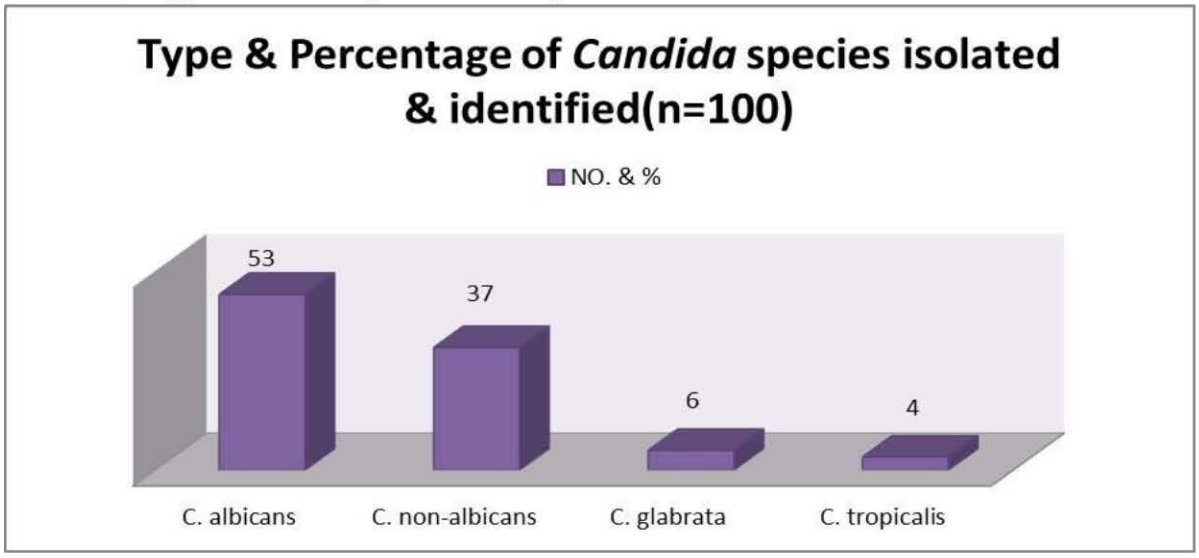


Chart - B: Distribution of *Candida species* amongst different samples (n=100).

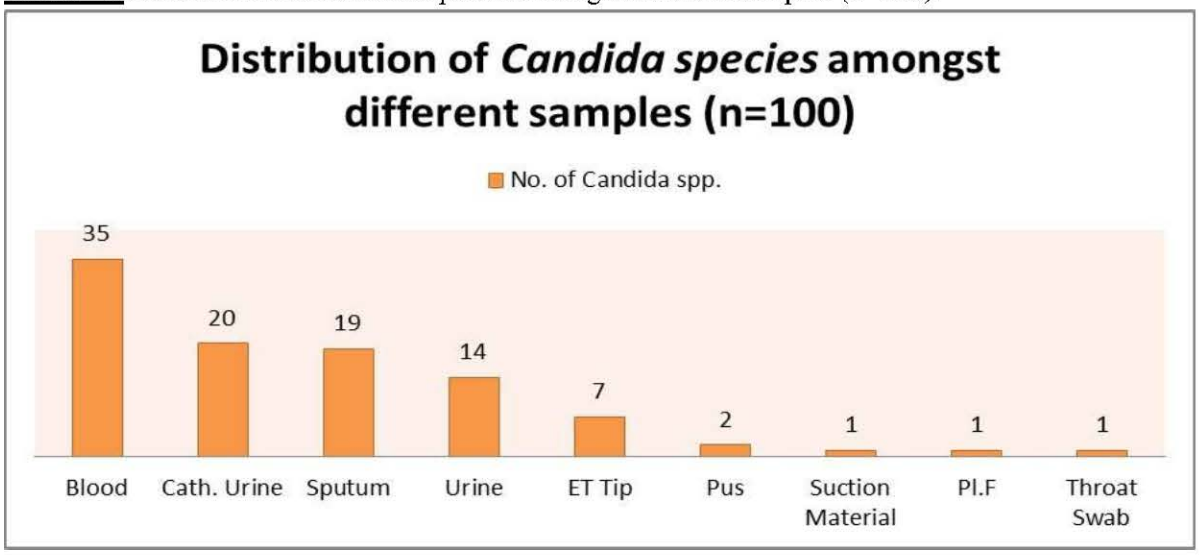
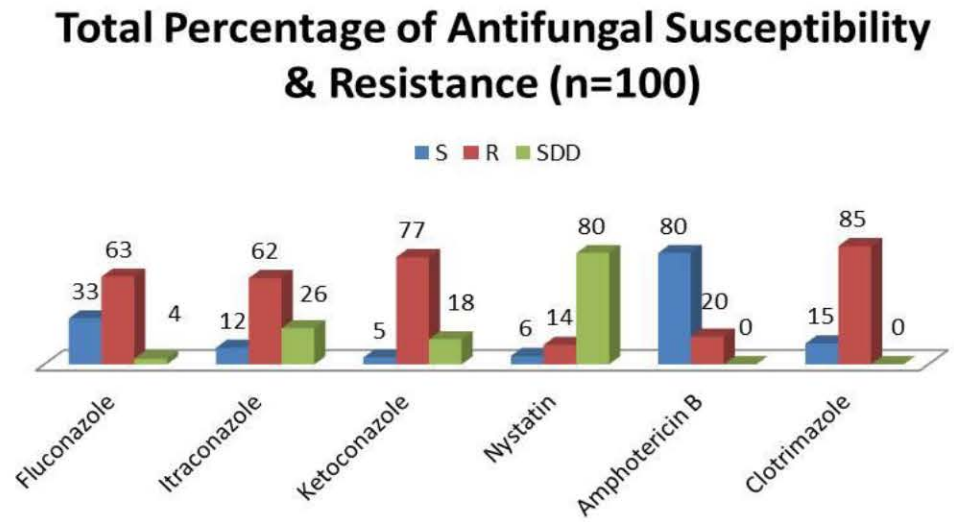


Chart - C: Total Percentage of Antifungal Susceptibility and Resistance (n=100).



Susceptibility to Fluconazole:
Of the isolates tested 33%, 63% and 4% were susceptible, resistant and dose dependent respectively. The maximum resistance was exhibited by *C. albicans* followed by *C. non-albicans, C. tropicalis* and least by *C.glabrata.*

Susceptibility to Itraconazole:
As shown in the **Chart - C** 62%, 26% and 12% were resistant, dose dependent and susceptible to Itraconazole respectively. The maximum resistance was shown by *C. albicans* followed by *C. tropicalis* and least but almost equally by *C.non-albicans* and *C.glabrata*.

Susceptibility to Ketoconazole:
It showed that 77%, 18% and 5% were resistant, dose dependent and susceptible to Ketoconazole respectively. The maximum yet equally the dose dependence was shown by *C. glabrata* and *C. tropicalis* followed by *C. non-albicans* and least by *C. albicans*, which also showed a maximum resistance.

Susceptibility to Nystatin:
Of the total isolates 80%, 14% and 6% were dose dependent, resistant and susceptible to Nystatin respectively. The maximum dose dependence was shown by *C. albicans*, *C. glabrata*, *C. non-albicans* and finally *C. tropicalis*.

Susceptibility to Amphotericin B:
It shows that 80% and 20% were susceptible and resistant respectively. The SDD could not be reported for this category as the interpretation was not available for this category from either the guidelines or the literature. The maximum resistance was shown by *C. tropicalis* followed *C. glabrata*, *C. non-albicans* and least by *C. albicans*.

Susceptibility to Clotrimazole:
Here also no SDD could be reported but 85% and 15% resistance and susceptibility were seen against Clotrimazole respectively. The maximum resistance was shown by *C. albicans* followed equally by *C. non-albicans* and *C. glabrata* and least by *C. tropicalis*.

Discussion

A total of 14.26% isolates of *Candida species* were obtained from 1136 different samples cultured from sepsis patients yielding 701 bacterial and fungal isolates altogether. All these were obtained from patients who fulfilled the inclusion criteria for defining sepsis and thus all the isolates were considered to be significant here rather than contamination. Of the total 100 *Candida species* isolated, the most common isolate was C. albicans (53%) followed by C.non-albicans (37%), C. glabrata (6%) and C. tropicalis (4%). In a multicentric one year study conducted in 27 ICUs of India for determining incidence of candidemia by Chakraborti A, et al. [15] found an incidence of 6.51 cases/1000 ICU admissions with the highest burden from ICUs of North India contributing 8.95 cases/1000 ICU admissions. Tak V, et al. [16] report candidemia incidence of 7.76 cases/1000 ICU admissions whereas Dewan E, et al. [17] report 10% candidemia in patients with hematological malignancies. Delaloye J and Calandra T [7] in their review article mention *Candida species* as the fourth most common blood stream isolate accounting for 10% to 15% of hospital acquired fungal sepsis and 5% of all cases of severe sepsis and septic shock. It accounts for 8 to 10% of blood stream infections in the United States and about 2-3% in Europe. Sonawane J, et al. [18] from Mumbai and Gupta S [19] from New Delhi, North India report 7.14% and 3.31% isolates of *Candida spp*. from blood cultures. Of the total 35 isolates obtained from blood, *C. albicans* was the most common isolate 42.86% (15/35) followed by *C. non-albicans*, 40% (14/35), *C. glabrata*, 11.43% (4/35) and *C. tropicalis*, 5.71% (2/35). Our findings are similar to the findings reported in a laboratory based surveillance study carried out across Asia by Tan BH, et al. [20] which reports *C. albicans* as the most common cause of candidemia with the similar percentage of prevalence.

The **Table - 2** shows the comparison of different studies in relation to *Candida* species and **Table - 3** shows comparison in relation to antifungal susceptibility.

Table - 2: Comparison of studies in relation to *Candida* species.

Reference	Place of Study	*Candida* species (%)
Guzman AJ, et al. 2011 [21]	USA	*Candida non-albicans* (74%) *C. albicans* (26%)
Giri S, et al. 2013 (JPGM) [22]	Chennai, Tamil Nadu	*C. tropicalis* (74.35%) *C. albicans* (10.26%) *C. parapsilosis* (7.69%) *C. krusei* (5.13%) *C. glabrata* (2.56%)
Tak V, et al. 2014 [16]	New Delhi	*C. tropicalis* (38.7%) *C. parapsilosis* (20.3%) *C. albicans* (13.7%) *C. glabrata* (11.4%) *C. rugosa* (9.4%) *C. hemulonii* (2.8%) *C. guilliermondi* (1.8%) *C. famata* (1.4%) *C. lusitaniae* (0.47%)
Chakraborti A, 2014 [15]	27 ICUs across India	*C. tropicalis* (41.6%) *C. albicans* (20.9%) *C. parapsilosis* (10.9%) *C. glabrata* (7.08%) *C. auris* (5.66%) *C. rugosa* (3.15%) *C. krusei* (1.74%) *C. guilliermondi* (1.74%)
Dewan E, et al. 2015 [17]	Uttarakhand, India	*C. tropicalis* (46.67%) *C. ablicans* (26.7%) *C. glabrata* (6.7%) *C. parapsilosis* (6.7%) *C. krusei* (6.7%) *C. dublinensis* (6.7%)
Tan BH, et al. 2015 [20]	25 hospitals across Asia	*C. albicans* (41.3%) *C. tropicalis* (25.45) *C. glabrata* (13.9%) *C. parapsilosis* (12.1%)

In our study a total of 34% of *Candida* species were isolated from urine samples which may have lead to candidemia in these patients. Of these total 34 isolates, 20 (58.82%) were obtained from urine samples of catheterized patients. Giri S, et al. [22] report urinary catheters in 53.90% patients as a predisposing factor for candidemia while Xess, et al. [23] report it in 55.6% patients. Moreover in a review article, Giri S [24] quotes that as many as 10% of candiduria cases are significantly associated with development of candidemia. Mahmoudabadi AZ, et al. [14] report 62.3%, 26.8% and 4.3% *C. albicans*, *C. glabrata* and *C. tropicalis* from urine of 92 patients with candiduria. Besides 35% from blood and 34% from urine culture *Candida species* were also isolated from sputum (19%), non-catheterized urine (14%) and ET

tips/secretions (7%), pleural fluid, suction material and throat swab suggesting that these sites would have been the source of *Candida* infection leading to sepsis in these patients. Guzman AJ, et al. [21] report 27% and 51% rate of isolation of *Candida species* from urine, sputum and catheter-related samples of septic shock and no-shock group of patients respectively.

Table - 3: Comparison of studies in relation to antifungal susceptibility and resistance pattern.

Reference	Place of Study	Resistance Pattern (%)	Susceptible Pattern (%)
Guzman AJ, et al. 2011 [21]	USA	Not Reported	Not Reported
Giri S, et al. 2013 (JPGM) [22]	Chennai, Tamil Nadu	Fluconazole (30.8) Ketoconazole (12.8) Amphotericin B (0)	Fluconazole (69.2) Ketoconazole (87.2) Amphotericin B (100)
Tak V, et al. 2014 [16]	New Delhi	Fluconazole (3.3) Amphotericin B (3.3) Flucytosine (0) Voriconazole (0)	Fluconazole (93.9) Amphotericin B (93.9) Flucytosine (0) Voriconazole (0)
Chakraborti A, 2014 [15]	27 ICUs across India	Fluconazole (6.2) Itraconazole (1.2) Voriconazole (5.6) Amphotericin B (2.1) Anidulafungin (1.7) Caspofungin (5.6) Micafungin (1.7)	Fluconazole (82.8) Itraconazole (89.5) Voriconazole (71.5) Amphotericin B (97.9) Anidulafungin (96.7) Caspofungin (84.3) Micafungin (96.1)
Dewan E, et al. 2015 [17]	Uttarakhand, India	Fluconazole (20) Clotrimazole (20) Voriconazole (13.4) Flucytosine (100) Nystatin (66.67) Amphotericin B (26.67)	Fluconazole (80) Clotrimazole (80) Voriconazole (86.66) Flucytosine (0) Nystatin (33.33) Amphotericin B (73.33)
Tan BH, et al. 2015 [20]	25 hospitals across Asia	Not Reported	Not Reported
Present Study, 2017*	Gujarat, India	Fluconazole (63) Itraconazole (62) Ketoconazole (77) Nystatin (14) Amphotericin B (20) Clotrimazole (85)	Fluconazole (33) Itraconazole (12) Ketoconazole (05) Nystatin (06) Amphotericin B (80) Clotrimazole (15)

*It represents overall percentage of all 100 isolates; and also SDD percentage is not compared here for any of the studies.

Also we found HiCrome media and disc diffusion method for antifungal susceptibility testing to be cost effective, convenient and useful methods for identification of *Candida species* and determining susceptibility and resistance to antifungal agents respectively on daily basis in our laboratory. Other authors too have found HiCrome to be equally good when compared to CHROME agar, Nested PCR and standard

biochemical methods for identification of *Candida species* [9, 10, 11].

Overall, the difference in the prevalence, of *Candida species*, the types of *Candida species* and the antifungal susceptibility and resistance patterns, may be due to the difference in the geographical locations; the clinical settings i.e. only ICU or hospital; patient demographics i.e. only patients with certain conditions, adult or pediatric; number of and types of samples processed; different media/methods used for isolation and identification like automated vs. conventional/semi-automated; different numbers of *Candida spp.* identified and tested for antifungal agents as well as different types of antifungal agents tested in different studies. Most of the studies have taken into account only those *Candida species* which cause candidemia, but in our study we have studied all the *Candida* species isolated from all different samples of sepsis patients to identify the source of infection in them. Also most of the studies from developed countries and those from premier institutes in India are equipped with automated systems for culture which are useful in increasing the yield of *Candida species*, especially, from blood samples, identification to the species level and determination of susceptibility pattern through MIC values of antifungal agents tested.

Conclusion

Candida species are important pathogens causing various infections leading to sepsis in our patients. Also we found HiCrome media and disc diffusion method for antifungal susceptibility testing to be cost effective, convenient and useful methods for identification of *Candida species* and for determining susceptibility/resistance to antifungal agents respectively and using these routinely in our laboratory will help determine the prevalence of *Candida* infections along with their susceptibility pattern in our setup which in turn will help in better management of sepsis patients as well as all other patients with *Candida* infections.

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