Original Article

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Prevalence and Factors Associated with Wound Colonisation by *Staphylococcus* Species at Tertiary Care Hospital: A Cross-sectional Study

Microbiology Section

J LAKHANI SUCHETA[1], HATKAR SUNIL[2], J LAKHANI SOM[3]

ABSTRACT

Introduction: The *Staphylococcus* species do not directly cause infection but invade an already existing wound and causing extensive tissue necrosis and enlarging an already existing wound. The human host factor plays a major role in the pathogenesis by making a suitable environment for pathogens, like diabetic mellitus, hypertension, and so on. The emergence of methicillin resistant strains worsens the clinical output and very few therapeutic alternatives are left to treat such infections.

Aim: The present study was attempted to see the relation of *Staphylococcus* species with host factors among wound infections.

Materials and Methods: The specimens taken from various types of wound infection were screened for *Staphylococcus* species as per standard microbiological guidelines. The clinical history of the patients were taken from the 'patient's case record form' and Medical Record Department (MRD) section. The data was statistically analysed, p-value ≤0.05 was considered significant.

Results: A total of 421 *Staphylococcus* species were isolated from various clinical samples, out of which 159 (37.7%) were isolated from wound infections. Of which 142 (89.3%) were *S.aureus* and 17 (10.7%) were *S.epidermidis*. Diabetic mellitus 29 (18.2%) and hypertension 11 (7%) were found to be a major host factor facilitating the infection. Methicillin Resistant *Staphylococcus aureus* (MRSA) was 100 (62.9%) while Methicillin Resistant *Staphylococcus epidermidis* (MRSE) was not found in the single strain. Inducible clindamycin resistant strains among wound infection were found to be 58 (36.5%).

Conclusion: Staphylococcal wound infections should be carefully treated to prevent microbial spread especially in immune-compromised patients, better patient recovery, and reducing healthcare costs.

Keywords: Chronic wounds, Co-morbidities, Methicillin resistant, *Staphylococcus aureus*

INTRODUCTION

The *Staphylococcus aureus* is the normal flora and it presents primarily in the anterior nares of human which accounts for 30-40%, and it became pathogenic under immune impairment and followed by migration from the usual site to another anatomical site, although they don't invade the host tissue unless gaining access into a traumatic site/abrasion [1,2]. The diagnosis and clinical management of *Saphylococcus* infections solely depend on the microbiological investigation to rule out the specific antimicrobial agent for fast recovery and prevent the serious form of uncomplicated infection [3,4]. The Community Acquired- MRSA (CA-MRSA) causing Skin and Soft Tissue Infection (SSTI) are increasing day by day and if proper treatment is not started in time may pose deep tissue infections and subsequent involvement of vital organs [5,6]. The *Staphylococcus* species do not directly cause infection but invade an already existing wound causing extensive tissue necrosis and enlarging an already existing wound. The human host factor plays a major role in pathogenesis by making a suitable environment for pathogens, like diabetic mellitus, hypertension, and so on. The emergence of methicillin resistant strains makes worsens the clinical output and is left with very few therapeutic alternatives to treat such infection [7-9].

The wounds of hospitalised patients may result from surgery, pressure ulcers, and diabetic ulcers, etc. The wound can be classified as clean wounds or contaminated based on their degree of contamination [10]. The clean wounds have minimal risk of infection, i.e., lipoma excision, thyroidectomy, simple herniorrhaphy, and lumpectomy limited to 1-2% risk of infection. The contaminated wounds have a high rate of infection (15-20% infection rate), and it occurs when there is a major breach in aseptic conditions even without entry into the abscess cavity. Dirty or Infected wounds have a very high risk of infection (>40% infection rate). Dirty wounds appear as grossly infected with pus with entry into an abscess cavity such as in gangrenous bowel [11]. In hospital environments, wounds are likely to get colonised with multidrug-resistant organisms, especially MRSA. Bacterial colonisation of wounds can increase wound severity and interfere with healing. Surveillance of the pattern of Staphylococcal infections and their antibiogram in a hospital environment is therefore warranted, especially in postoperative surgical wounds, which may be a source of cross-contamination by MRSA and Coagulase-Negative *Staphylococcus* species (CoNS) [12].

Staphylococcus aureus is the most common cause of skin infections. Its capacity to adapt has led to the selection of MRSA [13]. The emergence of multidrug resistance among *Staphylococcus* species is still challenging, especially methicillin resistant strains. In the recent past, several studies are carried out in the world show that methicillin resistant strains of *Staphylococcus* species are not limited to hospital-acquired infection but significantly associated with community-acquired infection [4,9,12,14], hence the clinician facing the challenge while selecting the antimicrobial agents for a better outcome. The antimicrobial susceptibilities are varying with different geographical areas and even hospital to hospital, hence the evaluation of antimicrobial profile helps to tackle severe *Staphylococcus* infections. Therefore, the present study was an attempt to see the relation of *Staphylococcus* species with host factors among wound infections.

MATERIALS AND METHODS

The present cross-sectional study was carried out after the approval from Sumandeep Vidyapeeth Institutional Ethics Committee (Ref. No.- SVIEC/ON/Medi/PhD/17007) in August 2017 and data collection was done for two years (from August 2017 to July 2019). The clinical history of the patient having staphylococcal infection was taken from the MRD section of the study place. As in the present study, there was no direct involvement of human, animal, or their body parts, hence consent from the patients were not required.

Inclusion criteria: The pus samples collected from the infected chronic wounds by aspiration in sterile syringes or by sterile swab received in the Department of Microbiology were included in the present study.

Exclusion criteria: Repeat sample from the same patients were excluded from the study.

Study procedure: The specimens were inoculated on 5% sheep blood agar and MacConkey Agar and incubated at 37°C for 24 hours. Subsequently, a smear made from the direct specimen and stained with gram stain and examined under oil immersion lens, and the primary report was sent to the clinician for initial treatment. After 24 hours of incubation of previously inoculated clinical specimens, isolated colonies were taken to make a smear and stained with gram stain to rule out Gram-positive cocci arranged in clusters. Confirmed gram-positive cocci were further subjected to the Catalase test to differentiate staphylococci from streptococci. The catalase-positive isolated colonies were tested for coagulase production to categorise *Staphylococcus aureus* and CoNS species. The tube coagulase test was done, the tube was incubated at 37°C for 4 hours if the clot was not observed at the end of four hours; the tube was further incubated at room temperature and read after 18-24 hours. The Catalase-positive and Coagulase-negative isolates were further subjected to antimicrobial susceptibility testing by using Novobiocin (5µg), Polymyxin B (300U) disc, and Urease activity as per the standard procedure for the speciation of CoNS species [15,16].

Furthermore, a well-isolated colony was taken and suspended in peptone water and incubated at 37°C for four hours, the bacterial suspension was compared with 0.5 McFarland turbidity standard, a comparison was corrected by using the addition of peptone water or further incubation. The 0.5 bacterial suspensions were used for antimicrobial susceptibility testing and biochemical test as per standard microbiological procedure and Clinical Laboratory Standards Institute (CLSI) guidelines [17].

The erythromycin-resistant and clindamycin sensitive isolates were further subjected to D-test to rule out inducible clindamycin resistant strains of *Staphylococcus* species.

D-test: (disc diffusion test/disc approximation test): In D-test, erythromycin (15 µg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2 µg) disc on Muller Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspensions and incubated at 37^{0}C, flattening "D shaped" zone of inhibition around clindamycin in the area between two disc, indicated inducible clindamycin resistance [17,18].

STATISTICAL ANALYSIS

It was done by using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) (20 version) software. Frequencies and percentages were calculated for all the parameters. Non-parametric test was run by selecting one sample, which automatically compares observed data to hypothesised using the Chi-square test to calculate the p-value (CI-95%) and Kolmogorov-Smirnov was used to calculate average age means and Standard Deviation (SD).

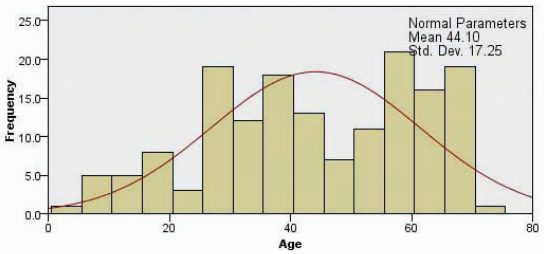
RESULTS

The wound infection was separately evaluated from the SSTIs because of the criteria considering the wound infection like open wounds, closed wounds, contusion/bruise, laceration, avulsions, punctures, perforating and penetrating wounds. A total of 159/421 (37.7%) pus samples were received from wound infections which were further evaluated for Staphylococcal species. Of which, 142/159 (89.3%) were *Staphylococcus aureus* and 17/159 (10.7%) were *Staphylococcus epidermidis*.

Demographic data of Staphylococcal wound infection is presented in [Table/Fig-1]. The majority of the patients were male 87/159 (54.7%) and female were 72/159 (45.3%). All the infected wound specimens were procured from hospitalised patients 159 (100%) those who are admitted for diabetic foot (n=29), postoperative surgical site infection (n=08), and mechanical trauma leads to contusion (closed wound, n=07) and laceration (open wound, n=115). The patients were from the age group of 1 to 80 years and the average mean age of the patients was 44.10±17.25 years (p-value-0.013) [Table/Fig-2].

Demographic data	N	Percent	Bootstrap for Percent			
			Bias	Std. Error	95% CI	
					Lower	Upper
Male	87	54.7	0.1	4.3	45.3	64.2
Female	72	45.3	-0.1	4.3	35.8	54.7
In-patient	159	100.0	0.0	0.0	100.0	100.0
Out-patient	0	0	0	0	0	0
Statistical test- One sample Chi-square test (p-value <0.005, 95% CI)						
Age groups of the Patients						
Age group (Years)		**Frequency**		**Percent**		
01-10		06		3.77		
11-20		13		8.17		
21-30		22		13.84		
31-40		30		18.87		
41-50		20		12.58		
51-60		32		20.13		
61-70		35		22.01		
71-80		01		0.63		
Total		159		100		
Statistical test- Kolmogorov-Smirnov (p-value <0.013, 95% CI)						

[Table/Fig-1]: Demographic data of staphylococcal wound infection.



Total N		159
Most Extreme Differences	Absolute	.126
	Positive	.073
	Negative	-.126
Test Statistic		1.591
Asymptotic Sig. (2-sided test)		.013

[Table/Fig-2]: Average mean age of the patients with wound infection.

Clinical data of Staphylococcal wound infection is presented in [Table/Fig-3]. All the clinical specimens were from surgical Departments i.e., Orthopaedic 98/159 (61.6%) and Surgery 61/159 (38.4%). The majority of the patients were of diabetes 29/159(18.2%) followed by hypertension 11/159 (7%). A prior history of antibiotic therapy relevant to wound infection was also taken [Table/Fig-3].

Results of Antibiotic Sensitivity Testing (AST) of *Staphylococcus* spp. of wound infection is depicted in [Table/Fig-4]. The antimicrobial susceptibility results are always under the influence of prior antimicrobial therapy, it is noted that patients are having exposure to baseline drugs and it might be due to the cost of the regimen, hence the staphylococcal species remain susceptible to second-line drugs (expensive). A total of 100/159 (62.9%) MRSA strains were

Clinical data of the patients		Frequency	Percent
Clinical Departments	Orthopaedics	98	61.6
	Surgery	61	38.4
Clinical specimen	Pus swab/aspiration	159	100
Co-morbid conditions	Diabetic mellitus	29	18.2
	Hypertension	11	7.0
	None	119	74.8
History of prior antimicrobial therapy	Cefuroxime	22	13.8
	Amoxyclav	18	11.3
	Cefotaxime	13	8.2
	Piperacillin-Tazobactam	8	5.0
	Levofloxacin	7	4.4
	Ofloxacin	2	1.3
	Not given	89	56.0

[Table/Fig-3]: Clinical data of the patients of Staphylococcal wound infection.

multidrug-resistant [Table/Fig-4], all the isolates of *Staphylococcus epidermidis* were sensitive to methicillin (MSSE) [Table/Fig-5].

Antimicrobial agents	Sensitive		Resistant	
	Frequency	Percent	Frequency	Percent
Penicillin	0	0	159	100
Erythromycin	17	10.7	142	89.3
Gentamicin	27	17	132	83
Ofloxacin	28	17.6	131	82.4
Chloramphenicol	37	23.3	122	76.7
Trimethoprim/ Sulfamethoxazole	45	28.3	114	71.7
Cefoxitin (MIC)	59	37.1	100	62.9
Tetracycline	58	36.5	101	63.5
Clindamycin	108	67.9	51	32.1
Rifampin	159	100	0	0
Linezolid	159	100	0	0
Vancomycin (MIC)	159	100	0	0
Ceftaroline (MIC)	159	100	0	0

[Table/Fig-4]: Antimicrobial susceptibility of *Staphylococcus* species of wound infection.

Methicillin resistance	Frequency	Percent	Bootstrap for Percent			
			Bias	Std. Error	95% CI	
					Lower	Upper
MRSA	100	62.9	0	3.4	57.2	69.2
MSSA	42	26.4	-0.1	3.4	20.1	33.3
MRSE	0	0	0	0	0	0
MSSE	17	10.7	0.1	2.3	6.9	15.1
Total	159	100	0	0	100	100

[Table/Fig-5]: Methicillin resistant strains of *Staphylococcus* species of wound infection.
MRSA: Methicillin resistant *Staphylococcus aureus*; MSSA: Methicillin sensitive *Staphylococcus aureus*; MRSE: Methicillin resistant *Staphylococcus epidermidis*; MSSE: Methicillin sensitive *staphylococcus epidermidis*; Statistical test: One sample Chi-square test (p-value <0.005, 95% CI)

Inducible clindamycin resistance in *Staphylococcus* species of wound infection is shown in [Table/Fig-6]. Out of 159 *Staphylococcus* species isolated from wound infections, 142/159 (89.3%) were erythromycin-resistant; these were further subjected to D-test to rule out inducible clindamycin resistant strains of *Staphylococcus* species. Inducible clindamycin resistant strains among wound infection were 58/159 (36.5%).

DISCUSSION

It is defined as the injury to the living tissue caused by different agents with various degrees of severity from a minor wound to a severe wound that can damage the whole organ, tissue, or

Resistant phenotypes	Frequency	Percent	Bootstrap for percent			
			Bias	Std. Error	95% CI	
					Lower	Upper
Erythromycin (S)	17	10.7	0	2.4	6.3	17.0
cMLSb (E-R,CD-R)	51	32.1	-0.3	3.7	24.5	39.0
iMLSb(E-R, CD-S)	58	36.5	0.3	3.6	29.6	45.3
MSb(E-R, CD-S)	33	20.8	0	3.3	14.5	27.7
Total	159	100	0	0	100	100

[Table/Fig-6]: Inducible clindamycin resistance in *Staphylococcus* species of wound infection.
cMLSb: Constitutive MLSb phenotype, iMLSb: Inducible MLSb phenotype; MSb: macrolide Streptogramin phenotype; E-R: Erythromycin resistant; CD-S: Clindamycin sensitive CD-R: Clindamycin resistant; Statistical test: One sample Chi-square test (p-value <0.005, 95% CI)

cell. There are different agents to cause tissue injury/wound like Mechanical agents, Chemical agents, Radiant agents, Biological agents (pathogenic organisms) [19].

Infections by *Staphylococcus* species are often associated with wounds, especially in hospitalised patients. The wounds of hospitalised patients may result from community-acquired injuries, after surgery, pressure ulcers, and diabetic ulcers. Wound infections can result in recurrent hospitalisation [12,20]. The wound serves as a major factor for colonisation with methicillin resistant strains of *Staphylococcus aureus*, and it increases the severity of the infection [7,12].

In the present study, 159/421 (37.7%) Staphylococcal species were isolated from wound infection; out of which 142/421 (89.3%) were *Staphylococcus aureus* and 17/421 (10.7%) *Staphylococcus epidermidis*, these were further evaluated for the significant association of co-morbid conditions with wound infections. The average age of the patients was 44.10±17.25 years.

All the infected wound samples were taken from hospitalised patients admitted for diabetic foot, mechanical trauma (contusion), open wound (laceration), and postoperative surgical site infection.

Staphylococcus species are normally present on the skin, anterior nares, axilla, and groin areas that invade already existing wound that leads to infection and related clinical implications. Prevalence of Staphylococcal wound infections varies from the different geographical areas, as it is influenced by occupation, socio-economic background, and host immune response. Comparison of prevalence of *Staphylococcus* species in wound infections is compared with other studies which is represented in [Table/Fig-7] [21-26].

Different studies (Year)	*S.aureus*		CoNS	
	N	%	N	%
Sewunet T et al., (2013) [21]	-	-	15/50	30
Mama M et al., (2014) [22]	-	-	21/145	14.48
Al Tayyar IA et al., (2015) [23]	-	-	14/223	6.3
Harshan KH et al., (2015) [24]	205/620	30	-	-
Nanthini Devi P et al., (2017) [25]	63/196	32.14	-	-
Bora P et al., (2018) [26]	-	-	52/120	43.3
Present study (2020)	142/421	33.72	17/421	4.0

[Table/Fig-7]: Comparison of prevalence of *Staphylococcus* species in wound infections [21-26].

The *Staphylococcus* species were further evaluated for antimicrobial susceptibility testing which shows that all the isolates were resistant to penicillin; however, all the isolates were 100% sensitive to rifampin, linezolid, vancomycin, and ceftaroline. These results are indicating that the patients had low exposure to second-line antimicrobial agents hence the infection can easily be treatable in this region.

Methicillin resistant strains of *Staphylococcus* species among wound infections were 62.9% while all the isolates of CoNS

(*S. epidermidis*) were 10.7% susceptible to methicillin. This is per a study carried out by Šiširak M et al., antimicrobial susceptibility testing showed that 73% of MRSA isolates were with the same antibiotic sensitivity pattern and these were sensitive only to vancomycin [14].

In this study, inducible clindamycin resistant strains among wound infection were also observed in 36.5% strains, indicating a high prevalence of such strains in wound infection that should be ruled out on a routine basis to avoid treatment failure. In a study by Hatkar SS et al., the inducible clindamycin-resistant strains of *Staphylococcus aureus* were 46 (26.13%) and among them, 42 (91.3%) were MRSA stains [18].

Wound infection because of *Staphylococcus* can be an important cause of sepsis and its consequences. Of 55 patients studied of SSTIs leading to sepsis by Lakhani Som J et al., 29 had wound infection as a cause of sepsis, and *Staphylococcus aureus* was the commonest organism. Diabetes mellitus, yellow pus discharge unhealthy granulation tissue with slough, depth, and size of the wound was important risk factors in this study [27]. In the present study, diabetes was found to be the most significant host factor/co-morbid condition. The antimicrobial susceptibility of the isolated strain is the only key to recover the wound and reduce the healthcare cost burden.

Limitation(s)

As total eight patients was of postoperative surgical site infections, the screening of source of these infections was (hospital-acquired) not done.

CONCLUSION(S)

The patients suffering from diabetes are more prone to get infected with *Staphylococcus* species which leads to uncomplicated infection and the emergence of MRSA strains has left very few therapeutic alternatives to treat wound infection colonised with Staphylococcal species. Staphylococcal wound infections should be carefully treated to prevent microbial spread especially in immunecompromised patients, better patient recovery, and reducing healthcare costs.

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PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Microbiology, SBKSMI & RC, Sumandeep Vidyapeeth Deemed University, Piparia, Vadodara, Gujarat, India.
2. Associate Professor, Department of Microbiology, SMBT Medical College, Nashik, Maharashtra, India.
3. Associate Professor, Department of Dermatology, SBKSMI & RC, Sumandeep Vidyapeeth Deemed University, Piparia, Vadodara, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sunil Hatkar,
Associate Professor, Department of Microbiology, SMBT Medical College,
Nashik-422403, Maharashtra, India.
Email address: sunilhatkar25@gmail.com

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