

# 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.

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**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

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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:

- Body temperature: >38°C or <36°C
- Tachypnea: >20 breaths/minute
- Tachycardia: Heart rate >90 beats/minute
- Leucocyte count: >12,000/ $\mu$ l or <4,000/ $\mu$ l

These 48 isolates of *E. coli* obtained on MacConkey's agar and blood agar after 24 hours incubation at 37°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 (10 $\mu$ g), Amikacin (30 $\mu$ g), Gentamicin (10 $\mu$ g), Cefepime (30 $\mu$ g), Cefuroxime (30 $\mu$ g), Ceftazidime (30 $\mu$ g), Cefotaxime (30 $\mu$ g), Ciprofloxacin (5 $\mu$ g), Amoxicillin+Clavulanic Acid (30 $\mu$ g i.e. 20/10  $\mu$ g) and Cotrimoxazole (25 $\mu$ g i.e. 1.25/23.75) and observed after 18-24 hours incubation at 37°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  $\mu$ g) and Ceftazidime-clavulanic acid disk (30  $\mu$ g /10  $\mu$ g) were placed on surface of MHA plate and incubated at 35 $\pm$ 2°C in ambient air for 16-18 hours. A  $\geq$ 5mm increase in zone diameter of ceftazidime-clavulanic acid as compared to ceftazidime alone was considered positive for ESBL production. For confirmatory testing both cefotaxime (30  $\mu$ g) and ceftazidime (30  $\mu$ g) were placed on the surface of MHA along with their clavulanic

combinations (30  $\mu$ g /10 $\mu$ g) and incubated at 35 $\pm$ 2°C for 16-18 hours. A  $\geq$ 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:  $\leq$  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:  $\geq$  5-mm increase in ceftazidime-clavulanic acid zone diameter;  $\geq$  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.

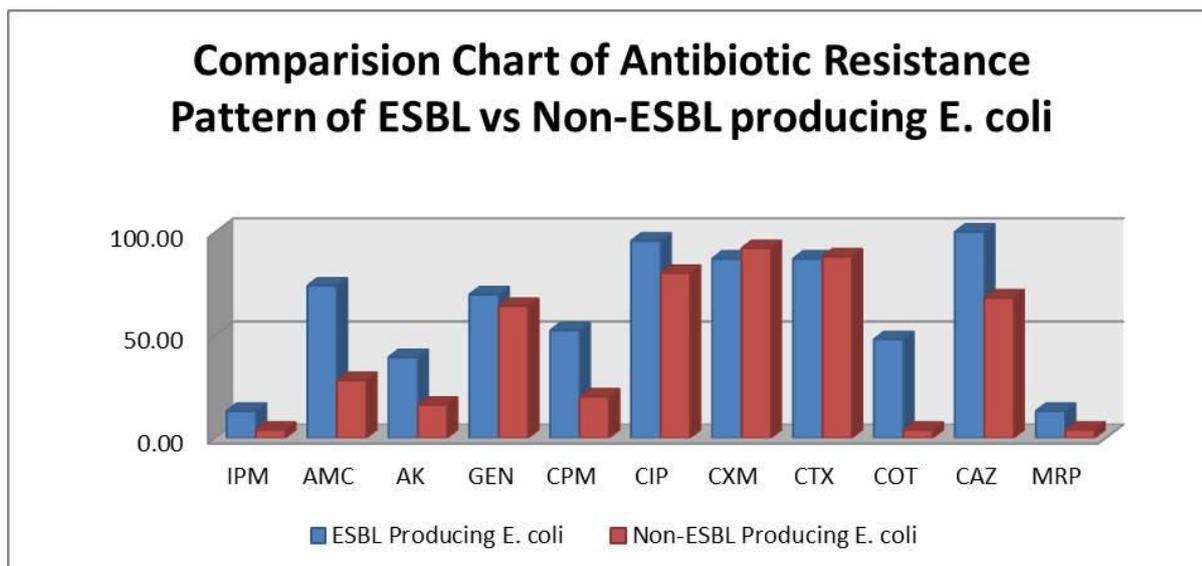
The antibiotic resistance pattern of both the types of *E. coli* isolates is shown in the (**Chart - 1**).

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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%),

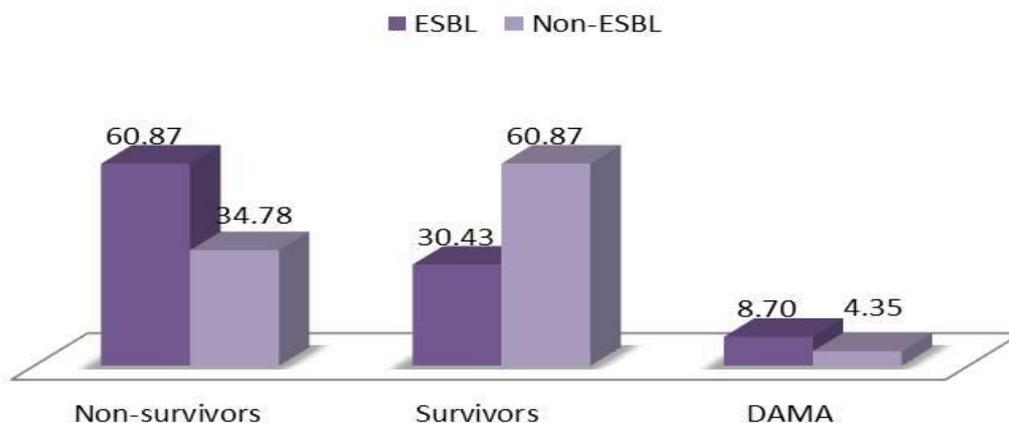
Cotrimoxazole (47.83%), Amikacin (39.13%) and 86.9% against Cefuroxime and Cefotaxime both was observed. All the ESBL producing isolates were resistant to Ceftazidime whereas non-ESBL producers showed 68% resistance. Overall the antibiotic resistance pattern of ESBL producers showed a higher percentage 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.

### Outcome of Sepsis Patients with ESBL & Non-ESBL producing *E.coli* (n=46)



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

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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 pyelonephritis) 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- $\beta$ -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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