

# Prevalence and antimicrobial susceptibility pattern of ESBL producing Gram Negative Bacilli

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## ABSTRACT

The production of extended-spectrum- $\beta$  lactamases (ESBLs) is an important mechanism for resistance to the third-generation cephalosporins. Awareness and the detection of these enzymes are necessary for optimal patient care.

To determine the prevalence and the antibiotic sensitivity pattern of ESBL producing gram negative bacilli. A prospective study was conducted at a tertiary care teaching hospital

A total of 213 isolates which were recovered between February 2008 and January 2009 from various samples were tested for ESBL production by using both the double-disk approximation and the combination disk methods.

Among the 132 *Escherichia coli*, 54 *Klebsiella pneumoniae* and 27 *Pseudomonas* isolates which were tested, 81%, 74%, and 14%, respectively were found to be ESBL producers. The ESBL producing *E. coli* showed maximum susceptibility to imipenem (100%), followed by piperacillin-tazobactam (84%), amikacin (68%), gentamicin (9%), ciprofloxacin (9%) and amoxicillin-clavulanic acid (7%). Similarly, the ESBL producing *K. pneumoniae* showed very good susceptibility to imipenem (98%), followed by piperacillin-tazobactam (68%), amikacin (40%), gentamicin (15%), ciprofloxacin (15%) and amoxicillin-clavulanic acid (5%).

About 87% and 88% of the ESBL producing *E. coli* and *K. pneumoniae* respectively showed multi-drug resistance to amoxicillin-clavulanic acid, gentamicin and ciprofloxacin.

It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinician in prescribing the proper antibiotics. Piperacillin-tazobactam and imipenem are the most active and reliable agents for the treatment of infections which are caused by ESBL producing organisms.

## KEY MESSAGES

ESBL producing gram negative bacilli are the important emerging nosocomial pathogens.

ESBL production should be tested by the conventional methods and should be reported along with routine antibiotic susceptibility testing in every microbiology lab, to help the physicians choose the appropriate antibiotics.

Occurrence of multi-drug resistance to the third generation cephalosporins, aminoglycosides and fluoroquinolones is common among ESBL producers.

Carbapenem is an effective drug for the treatment of infections which are caused by ESBL producers.

**Key Word:** extended-spectrum- $\beta$  lactamases, double-disk approximation test, combination disk method, multi-drug resistance, prevalence

## INTRODUCTION

Antimicrobial resistance is a growing threat worldwide. Resistance mechanisms have been found for every class of antibiotic agents. The predominant mechanism for resistance to the  $\beta$ -lactam antibiotics in gram-negative bacteria is the production of  $\beta$ -lactamase. The production of extended-spectrum  $\beta$ -lactamases (ESBLs) is an important mechanism which is responsible for the resistance to the third-generation cephalosporins. During the last 2 decades, ESBL producing gram-negative bacilli have emerged as a major problem in many settings [1].

The ESBLs mediate resistance to broad-spectrum cephalosporins (e.g., ceftazidime, ceftriaxone and cefotaxime) and aztreonam. The genes for the ESBL enzymes are plasmid borne and have evolved from point mutations, thus altering the configuration of the active site of the original and long known  $\beta$ -lactamases [2].

The problems which are associated with ESBLs include multidrug resistance, difficulty in detection and treatment, and increased mortality. Awareness and the detection of these enzymes are necessary for optimal patient care. The judicious use of antimicrobial agents and improved infection control methods must become health care priorities.

The objective of the present study was to determine the prevalence and antibiotic sensitivity pattern of ESBL producing gram nega-

tive bacilli which were isolated from various samples from both in-patients and out-patients who attended a tertiary care hospital in Pondicherry, India.

## MATERIALS AND METHODS

A prospective study was conducted over a period of one year (February 2008 to January 2009) at a tertiary care teaching hospital. The gram negative bacilli which were isolated from both the in-patients and the out-patients, which showed resistance to the third generation cephalosporins as per the Clinical Laboratory Standards Institute (CLSI) guidelines, were included in this study [3].

They were isolated from various specimens such as pus, sputum, tracheal aspirate, cerebrospinal fluid, ascitic fluid, pleural fluid, blood and urine which were received in our lab during the study period. The isolates were identified, based on the standard bacteriological techniques and were tested for ESBL production by using the double-disk approximation test which was described by Jarlier et al and the combination disk method which was recommended by CLSI [3-5].

## DOUBLE-DISK APPROXIMATION TEST

An overnight culture suspension of the test isolate which was adjusted to 0.5 McFarland's standard was inoculated by using a ster-

ile cotton swab on the surface of a Mueller Hinton Agar plate. A disc of amoxycylav (20 µg amoxycillin and 10 µg clavulanic acid) and a 30-µg disc of ceftazidime were placed 15 mm apart. After incubating overnight at 37°C, the presence of synergy between the two discs was interpreted as positive for ESBL production [4].

**COMBINATION DISK METHOD**

The combination-disk test using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, was performed for the detection of ESBL according to the CLSI guidelines [3].

In this test, an overnight culture suspension of the test isolate which was adjusted to 0.5 McFarland's standard was inoculated by using a sterile cotton swab on the surface of a Mueller Hinton Agar plate. The Cefotaxime (30 µg) and cefotaxime-clavulanic acid (30 µg/ 10 µg) disks were placed 20 mm apart on the agar. Similarly, the ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg/ 10 µg) disks were placed 20 mm apart. After incubating overnight at 37°C, a ≥ 5-mm increase in the zone diameter for either antimicrobial agent which were tested in combination with clavulanic acid vs. its zone when tested alone, was interpreted as positive for ESBL production [3].

**ANTIBIOTIC SENSITIVITY TESTING**

The susceptibility of the ESBL producing bacteria to amikacin, piperacillin- tazobactam, amoxicillin/ clavulanic acid, gentamicin, ciprofloxacin and imepenem was determined by the Kirby-Bauer disk diffusion method according to the Clinical Laboratory Standards Institute guidelines [3].

**QUALITY CONTROL**

Klebsiella pneumoniae ATCC 700603 and Escherichia coli ATCC 25922 were used for the quality control of the ESBL testing methods. Escherichia coli ATCC 25922 was used for the quality control of the Kirby-Bauer disk diffusion method [3].

**RESULTS**

A total of 213 isolates were tested for ESBL production during a period of one year (February 2008 to January 2009) in this study. A majority of the isolates were from various exudates, followed by urine specimens. Overall, 69% percent of the isolates which were resistant to the third-generation cephalosporins were detected to be ESBL producers. [Table/Fig 1] shows the ESBL positivity in the isolates from various types of samples which were obtained from the out-patients and in-patients. [Table/Fig 1]

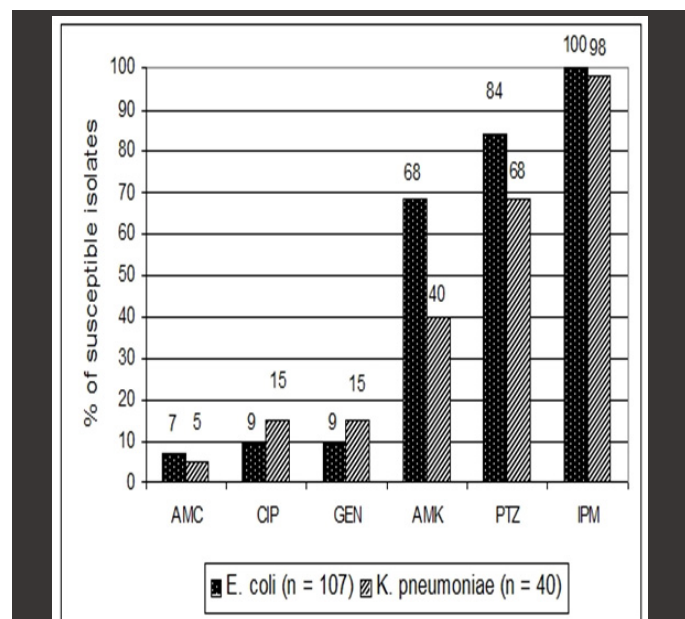
Organism	Urine		Exudates		Blood	
	In patients	Out patients	In patients	Out patients	In patients	Out patients
<b>Escherichia coli (n = 132)</b>						
No. of ESBL producers (%)	44 (75.9)	14 (82.4)	36 (87.8)	12 (80.0)	1 (100)	-
No. of isolates tested	58	17	41	15	1	-
<b>Klebsiella pneumoniae (n = 54)</b>						
No. of ESBL producers (%)	11 (68.8)	-	22 (78.6)	5 (71.4)	2 (66.7)	-
No. of isolates tested	16	-	28	7	3	-
<b>Pseudomonas aeruginosa (n = 27)</b>						
No. of ESBL producers (%)	-	0 (0)	3 (14.3)	0 (0)	-	-
No. of isolates tested	-	1	21	5	-	-

[Table/Fig 1]: Rate of ESBL positivity in different samples obtained from in-patients and out-patients

A total of 117 isolates from the exudates were tested for ESBL production and it was found that 78 (66.7%) were positive. Out of the 92 isolates from the urine samples, 69 (75%) were positive. Of the 4 isolates from the blood samples, which were resistant to the third generation cephalosporins, 3 were positive for ESBL production.

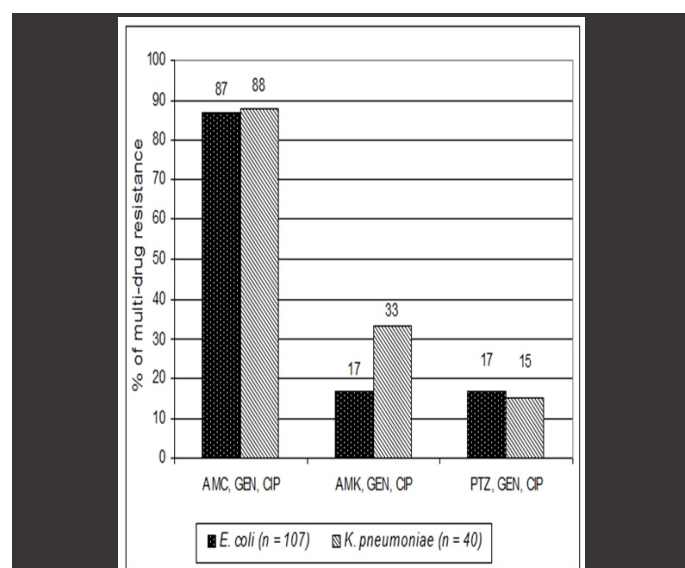
E. coli was the most common isolate which was obtained from the samples and tested in our study. Among the 132 E. coli isolates which were tested, 107 (81.06%) were ESBL positive by the combination disk method, but only 58 (43.9%) were positive by the double disk approximation test. In the same way, K. pneumoniae also showed high (74.07%) ESBL positivity by the combination disk method as compared to the double disk approximation test (40.7%). By both the methods, ESBL positivity in the Pseudomonas spp. was 14.3%.

The susceptibility of the ESBL producing E. coli and K. pneumoniae to various antibiotics is depicted in [Table/Fig 2].



[Table/Fig 2]: Susceptibility of the ESBL producing E. coli and K. pneumoniae to various antibiotics. AMC -amoxicillin-clavulanic acid, CIP – ciprofloxacin, GEN – gentamicin, AMK – amikacin, PTZ - piperacillin-tazobactam, IPM – Imipenem.

The multi-drug resistance which was observed among the ESBL producing E. coli and K. pneumoniae is shown in [Table/Fig 3].



[Table/Fig 3]: Multi-drug resistance observed among the ESBL producing E. coli and K. pneumoniae. AMC -amoxicillin-clavulanic acid, CIP – ciprofloxacin, GEN – gentamicin, AMK – amikacin, PTZ - piperacillin-tazobactam

## DISCUSSION

The resistance to extended spectrum cephalosporins is mainly mediated by the production of ESBLs (2). A number of nosocomial outbreaks which were caused by ESBL-producing organisms, have been reported in the United States [6-8].

Although most of the outbreaks were limited to high-risk patient-care areas such as ICUs, oncology units, etc., the first report of an outbreak in nursing homes appeared in the literature in the year 1999 [9].

Therefore, the threat of these resistant organisms is not limited to intensive care units or tertiary hospitals.

Recent studies on ESBL production among the members of Enterobacteriaceae which were isolated from clinical specimens, showed an increase in the occurrence of ESBL producers [10].

A study from North India on uropathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter*, *Proteus* and *Citrobacter* spp., showed that 26.6% of the isolates were ESBL producers [11]. A study from Nagpur showed that 48.3% of their cefotaxime resistant gram negative bacilli were ESBL producers [11].

A report from Coimbatore (India) showed that ESBL production was 41% in *E. coli* and 40% in *K. pneumoniae* [12].

In a similar study by Mathur et al, 62% of the *E. coli* and 73% of the *K. pneumoniae* isolates were reported to be ESBL producers [13].

In the present study, we also observed that 81% of the *E. coli* and that 74 % of the *K. pneumoniae* isolates were ESBL producers. Although *K. pneumoniae* were more often reported as ESBL producers in other studies, we observed that ESBL production was more common among the *E. coli* isolates as compared to the *K. pneumoniae* isolates [4],[11],[12].

In *Pseudomonas* spp. ESBL production is less as compared to Enterobacteriaceae, because their resistance is mediated by various other mechanisms such as the production of metallo-beta-lactamases, lack of drug penetration due to mutations in the porins and the loss of certain outer membrane proteins and efflux pumps [13][14],[15].

We observed that ESBL production among *E. coli* and *K. pneumoniae* isolates was more frequently detected by the combination disk method than the double disk approximation test. The Clinical Laboratory Standards Institute therefore also recommended the use of the combination disk method for the phenotypic confirmation of ESBL production among Enterobacteriaceae [3].

Although we could not document any significant differences between the ESBL detection rates of the two methods in *Pseudomonas aeruginosa*, the CLSI recommends the double disk approximation method for testing ESBL production among the *Pseudomonas aeruginosa* isolates [3].

Our failure to detect the better performance of the double disk approximation test as compared to the combination disk method for the detection of ESBL production among the *Pseudomonas aeruginosa* isolates could be due to the relatively small number of isolates which were tested in our study.

In our study, we observed that a majority of the isolates were susceptible to imipenem and piperacillin-tazobactam. Similarly, in a study from Coimbatore, all the members of Enterobacteriaceae were found to be susceptible to piperacillin-tazobactam and imipenem [16].

In both the studies, amikacin also showed good activity against gram-negative bacteria. We studied the occurrence of multi-drug

resistance among the *E. coli* and *K. pneumoniae* isolates and found that co-resistance to amoxicillin-clavulanate, gentamicin and ciprofloxacin was very common.

ESBL producing organisms, being the commonest nosocomial pathogens, it is essential to detect and treat them as early as possible. Since ESBL production is more common among the nosocomial pathogens, early detection will definitely help in controlling hospital infections which are caused by this group of organisms. Enterobacteriaceae are the common isolates in most of the laboratories. Now-a-days, a majority of these isolates are multi-drug resistant. The control of these multidrug resistant organisms is a therapeutic challenge. This difficulty is enhanced further by the co-existence of the resistance to  $\beta$ -lactams, aminoglycosides and fluoroquinolones, as observed in our study. Of all the available antimicrobial agents, carbapenems are the most active and reliable treatment options for infections which are caused by the ESBL producing isolates [10].

However, the overuse of carbapenems may lead to resistance in gram-negative organisms. The regular detection of ESBLs by conventional methods should be carried out in every lab where molecular methods cannot be performed, as genotyping is not more informative for the treatment.

In conclusion, the ESBL-producing organisms are a breed of multi-drug-resistant pathogens that are increasing rapidly and becoming a major problem in the area of infectious diseases. It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinicians in prescribing proper antibiotics. Piperacillin-tazobactam and imipenem are the most active and reliable agents for the treatment of infections which are caused by ESBL producing organisms.

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