

Original Research Article

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Prevalence of ESBL Producing *Escherichia coli* and *Klebsiella* Species among Clinical Isolates and their *in vitro* Antimicrobial Susceptibility Pattern in a Tertiary Care Hospital

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A B S T R A C T

E. coli and *Klebsiella* spp. are the main opportunistic pathogens under family Enterobacteriaceae. Cephalosporins are the first line of drugs against these organisms, but the inappropriate use of beta-lactam antibiotics render these organisms more vulnerable for the production of enzymes. Prevalence of ESBLs has resulted in widespread development of drug resistance. The present study was done to detect the prevalence of ESBLs producing *E. coli* and *Klebsiella* spp with their antibiotic resistance patterns at TMMC Tertiary care hospital. A total no of 200 isolates were obtained from clinical samples from March to February 2015. Samples were inoculated on MacConkey and sheep blood agar. The organisms were identified based on standard microbiological techniques. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method, as per CLSI guidelines. Following screening with Cefotaxime (30 Åµg) disc, resistance was then confirmed for ESBL production by phenotypic confirmatory disc diffusion test using Ceftazidime (30 Åµg) and Ceftazidime + clavulanic acid (30 Åµg) disc. Result: Out of 120 *Escherichia coli* isolates 80 isolates of *Klebsiella* species, 31(25.83%) and 19 (23.75%) were ESBL producers respectively. These ESBL producers showed high-degree of resistance to ampicillin (100%) followed by ceftazidime, piperacillin and cefotaxime for both *E. coli* and *Klebsiella* species. The most sensitive antibiotics were found to be imipenem followed by Amikacin and meropenem. The present study highlights a relatively higher prevalence ESBL among *E. coli* and *Klebsiella* spp In view of these findings, we recommend that ESBL testing should be done routinely to reduce resistance to antibiotics and to strengthen the enforcement of infection control measures. It is pertinent for the treating clinicians so that they can make an informed choice as far as appropriate antibiotics and precautions are concerned.

Keywords

ESBL, *E. coli*,
Klebsiella spp.,
Ceftazidime,
Ceftazidime+
clavulanic acid,
Multidrug
resistance.

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Introduction

Beta-lactamases act as a major defense of Gram negative bacilli (GNB) against beta-lactam antibiotics. Treatment of Extended Spectrum Beta-Lactamase (ESBLs) producing Gram negative bacilli has posed a major challenge in hospitalized patients. ESBLs are β-lactamases conferring resistance to

oxymino-Cephalosporins (3rd Generation Cephalosporins) and Aztreonam but not Cephamycins or Carbapenems.

Their numbers have increased significantly since 1989 and represent one of the largest groups of novel enzymes ¹.

Ever since its discovery in 1983, pathogens producing Extended-spectrum- β lactamases (ESBLs) have become an increasing cause of clinical concern for the clinicians for several reasons². First, systemic infections due to ESBL producing pathogens are associated with adverse clinical outcomes. Second, initially ESBLs producing pathogens was restricted to certain geographical areas which subsequently spread across the globe and their prevalence varies throughout the world. Third, primarily ESBLs was characterized among certain members of family Enterobacteriaceae such as *E. coli* and *Klebsiella* spp but then it spread among the other genera such as *Citrobacter*, *Enterobacter* and *Proteus* Spp. Finally, ESBL production is associated with more complex phenotypes due to multiple enzyme production which include: inhibitor-resistant ESBL variants, plasmid-borne AmpC, production of ESBLs in AmpC beta lactamases – producing bacteria, production of ESBLs in KPC (Carbapenamases)-producing bacteria, enzyme hyperproduction and porin loss³.

Genus *Klebsiella* and *Escherichia coli* among family Enterobacteriaceae are frequently encountered opportunistic pathogens in clinical settings. Infection due to ESBL producers range from uncomplicated Urinary tract infection to life threatening sepsis. Cephalosporins are considered as first line drugs against *Escherichia coli* and *Klebsiella* species. The mechanism behind Cephalosporin resistant *Klebsiella pneumoniae* was the production of Extended Spectrum β -Lactamase (ESBLs).

In India, the prevalence of ESBL production varies from 10% to 84%. ESBLs are more prevalent in *Klebsiella* spp. (pneumonia and oxytoca) followed by *Escherichia coli*⁴. With the spread of ESBL producing Gram negative bacilli (GNB) in hospitals worldwide, the aim

of the present study was to find out the prevalence of ESBL producing *Klebsiella* species and *Escherichia coli* among clinical isolates and their antibiotic susceptibility patterns.

Materials and Methods

The present prospective study was carried out from March 2014 to February 2015 in the Department of Microbiology, in Teerthankar Mahaveer Medical College and Research Center Moradabad. During this period, a total of 200 isolates were obtained after processing of the clinical samples.

Sample processing

All clinical samples were inoculated on Blood agar, MacConkey's agar and the *Klebsiella* Species and *Escherichia coli* isolates that were identified based on colony morphology and biochemical reactions as per standard microbiological procedures⁵ were only considered for the study.

Antimicrobial susceptibility testing

The isolates were tested for antimicrobial susceptibility by modified Kirby-Bauer disk diffusion method on Muller Hinton agar plate as per clinical and laboratory standards institute guidelines (CLSI 2017)⁶.

The antibiotic discs used were Amikacin (30 μ g), gentamycin (10 μ g), ampicillin (10 μ g), ampicillin/sulbactam (10/10 μ g), ciprofloxacin(5 μ g), levofloxacin (5 μ g), chloramphenicol (30 μ g), co-trimoxazole (1.25/23.75 μ g), ceftriaxone (30 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), cefoxitin (30 μ g), amoxicillin clavulanic acid (20/10 μ g), tigecycline (30 μ g) piperacillin-tazobactam (100/10 μ g), imipenem (10 μ g) and meropenem (10 μ g), ertapenem (10 μ g). Norfloxacin (10 μ g) and nitrofurantoin (300

µg) were tested against isolates from urine samples only. Dehydrated media and antibiotic discs were obtained from Hi-Media, Mumbai.

Klebsiella pneumoniae ATCC 700603 and *Escherichia coli* ATCC 25922 strains were used as controls during this study.

Screening for ESBL Producers for *Escherichia* and *Klebsiella* species

According to CLSI guidelines, strains showing zone of inhibition less than 27mm for Cefotaxime and less than 22 mm for Ceftazidime were taken as indicators for possible ESBL production. Phenotypic confirmatory Disc Diffusion test was used for confirmation amongst the potential ESBL-producing isolates. The discs of Ceftazidime (30mcg) and Ceftazidime plus Clavulanic + (30/10mcg) were placed on Mueller Hinton agar plates within a distance of 20 mm between two discs. Overnight incubation was done at 37° C. An increase of zone diameter > 5 mm of Ceftazidime plus clavulanic acid in comparison to the zone diameter of Ceftazidime alone confirmed for ESBL production.

Results and Discussion

A total of 120 isolates of *E. coli* and 80 isolates of *Klebsiella* species isolated in the bacteriology laboratory of Department of Microbiology of Teerthankar Mahaveer Medical College and Research Center Moradabad were taken up during the study.

Among the 120 isolates of *E. coli*, 31 were identified as ESBL producers by phenotypic confirmatory disc diffusion test (PCDDT). Ceftazidime Clavulanic acid (93.54%), Imipenem (83.87%) and Meropenem (80.64%) were the most effective antibiotics against ESBL producing *E. coli*. Imipenem

(88.76%), Meropenem (86.51%) and Ceftazidime Clavulanic acid (86.51%) were the most effective antibiotics against non-ESBL producing *E. coli* (Table 2).

Among the 80 isolates of *Klebsiella*, 19 were identified as ESBL producers by phenotypic confirmatory disc diffusion test (PCDDT). Imipenem (89.47%), Meropenem (84.21%) and Ceftazidime Clavulanic acid (84.21%) were the most effective antibiotics against ESBL producing *Klebsiella*.

Imipenem (93.44%), Meropenem (88.52%), and Ceftazidime Clavulanic acid (85.24%) were the most effective antibiotics against non-ESBL producing *Klebsiella* (Table 3).

In the present study, the percentage of ESBL producers among *E. coli* isolates was 25.83% and *Klebsiella* isolates was 23.75% (Table 4).

For the *E. coli* isolates, the most effective antibiotics were Imipenem (75.83%) followed by Amikacin (74.16%) and Meropenem (73.33). Highest resistance was seen against Ampicillin (80.83%), Cotrimoxazole (59.16%) and Ceftazidime (57.5%) (Table 1, Figure 1).

For the *Klebsiella* isolates, the most effective antibiotics were Imipenem (81.25%) followed by Meropenem (78.75%) and Ceftazidime Clavulanic acid (78.75%).

Highest resistance was seen against Ampicillin (87.5%), Cotrimoxazole (60.0%) and cefepime (56.25%) (Table 1, Figure 2).

In the present study, the prevalence of ESBL producing *E. coli* is 25.83% (31 ESBL producers out of total 120 *E. coli* isolates and the prevalence of ESBL producing *Klebsiella* spp. is 23.75% (19 ESBL producers out of total 80 *Klebsiella* spp. Isolates. The studies which are listed in Table 5 conducted in wide

geographical areas of India by various authors, from the past 12 years reported a widely varied prevalence ranging from 10.10% to 87%.⁷⁻¹⁹

Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, residence in an institution with high rates of Ceftazidime and other 3-GC use and

instrumentation or catherization.⁴ Though ESBLs are quite prevalent in Indian hospitals and community, they are not routinely tested by diagnostic Microbiology laboratories.

The possible factor could be different geographical locations, different levels of proficiency of Microbiology trained technical staffs, different antibiotics cut offs used, different guidelines followed and varied techniques used for E detection of ESBLs.

Table.1 Comparative studies in different regions of India

Author	Place	Year	Prevalence (%)
Kumar <i>et al.</i> , (7)	Hyderabad	2006	10.10%
Agrawal <i>et al.</i> , (8)	Pune	2008	16 %
Vemula <i>et al.</i> , (9)	Kadappa	2011	17 %
Variaya <i>et al.</i> , (10)	Mumbai	2008	20 %
Tankhiwale <i>et al.</i> , (11)	Nagpur	2004	25%
Shukala <i>et al.</i> , (12)	Aligarh	2004	30.18 %
Babypadmini et a (13)	Coimbatore	2004	40 %
Metri <i>et al.</i> , (14)	Bijapur	2012	44.90%
Taneja <i>et al.</i> , (15)	Chandigarh	2008	51.20 %
Hawser <i>et al.</i> , SMART study (16)	7 hospitals India	2010	55 %
Rao <i>et al.</i> , (17)	Davangere	2008	62.20 %
Rudresh <i>et al.</i> , (18)	Bangalore	2011	63.30 %
Sharma <i>et al.</i> , (19)	Jaipur	2013	67.04 %

Table.2 Antibiotic sensitivity patterns of *E. coli* and *Klebsiella* species

Antibiotics	<i>E. coli</i> n= (45)		<i>Klebsiella</i> n=(15)	
	S (n%)	R (n%)	S (n%)	R (n%)
Amikacin	89 (74.16)	31 (25.83)	62 (77.5)	18 (22.5)
Ampicillin	23 (19.16)	97 (80.83)	10 (8.33)	70 (87.5)
Ceftazidime	51(42.5)	69 (57.5)	36 (45)	44 (55)
Ceftazidime +Clavulanic acid	77 (64.16)	43 (35.83)	63 (78.75)	17 (21.25)
Cefepime	52 (43.33)	68 (56.66)	35 (43.75)	45 (56.25)
Ciprofloxacin	71 (54.16)	49 (40.83)	50 (62.5)	30 (37.5)
Levofloxacin	73 (60.83)	47 (39.16)	49 (61.25)	31 (38.75)
Pipracillin	56 (46.66)	64 (53.33)	39 (48.75)	41 (51.25)
Pipracillin+Tazobactam	84 (70)	26 (21.66)	36 (30.66)	18 (22.5)
Gentamicin	78 (65.33)	42 (35)	55 (68.75)	25 (31.25)
Co-trimoxazole	49 (40.83)	71 (59.16)	32 (40)	48 (60)
Nitrofruntine	82 (68.33)	38 (31.66)	54 (67.5)	26 (32.5)
Imipenem	91 (75.83)	29 (24.16)	65 (81.25)	15 (18.75)
Meropenem	88 (73.33)	32 (26.66)	3(78.75)	17 (21.25)

Table.3 Antibiotic sensitivity pattern of ESBL and non-ESBL producing *E. coli*

E. coli n= (120)	ESBL producers (31)		Non-ESBL producers (89)	
	S (n%)	R (n%)	S (n%)	R (n%)
Amikacin	19(61.29)	12 (38.70)	59 (66.29)	30(34.88)
Ampicillin	00	31(100)	23 (25.84)	56 (49.43)
Ceftazidime	6(19.35)	25 (80.64)	45 (50.6)	44 (50.56)
Ceftazidime +Clavulanic acid	29 (93.54)	2 (6.45)	77 (86.51)	12 (86.51)
Cefepime	52 (22.58)	24 (77.41)	43 (48.31)	46 (51.68)
Ciprofloxacin	15(48.38)	16 (51.61)	28 (31.46)	61 (68.53)
Levofloxacin	17(54.83)	14 (45.16)	34 (38.20)	55 (61.79)
Pipracillin	10 (32.25)	21 (67.74)	56 (62.92)	33 (62.92)
Pipracillin+Tazobactam	15 (48.38)	16 (51.61)	61 (68.53)	28 (31.64)
Gentamicin	13 (41.93)	18 (58.06)	53 (59.55)	36 (40.44)
Co-trimoxazole	9 (29.03)	22 (70.96)	29 (32.58)	66 (74.15)
Nitrofruntine	14 (45.16)	17(54.83)	43 (48.31)	46 (51.68)
Imipenem	26 83.87)	5(16.12)	79 (88.76)	10 (11.23)
Meropenem	25 (80.64)	6 (19.35)	77 (86.51)	12 (13.43)

Table.4 Antibiotic sensitivity pattern of ESBL and non-ESBL producing *Klebsiella*

<i>Klebsiella</i> n= (80)	ESBL producers (19)		Non-ESBL producers (61)	
	S (n%)	R (n%)	S (n%)	R (n%)
Amikacin	9(47.36)	10 (52.63)	33 (54.09)	28 (45.90)
Ampicillin	00	19 (100)	24 (39.34)	27 (44.26)
Ceftazidime	4 (21.05)	15 (78.94)	28 (45.90)	33 (54.09)
Ceftazidime +Clavulanic acid	16 (84.21)	3 (15.78)	52 (85.24)	9 (14.75)
Cefepime	5 (26.31)	14 (73.68)	31 (50.81)	30 (49.18)
Ciprofloxacin	8 (42.10)	11 (57.89)	29 (47.54)	32 (52.45)
Levofloxacin	9 (47.36)	10 (52.63)	32 (52.45)	29 (47.54)
Pipracillin	7 (36.84)	12 (63.15)	27 (44.26)	34 (55.73)
Pipracillin+Tazobactam	11 (57.89)	8 (42.10)	37 (60.65)	24 (39.34)
Gentamicin	9 (47.36)	10 (52.63)	29 (47.54)	32 (52.45)
Co-trimoxazole	7 (36.84)	12 (63.15)	22 (36.06)	39 (63.93)
Nitrofruntine	10 (52.63)	9 (47.36)	33 (54.09)	28 (45.90)
Imipenem	17 (89.47)	2 (10.52)	57 (93.44)	4 (6.55)
Meropenem	16 (84.21)	3 (15.78)	54 (88.54)	7 (11.47)

Table.5 Percentage of ESBL and non-ESBL producing *Klebsiella* and *Escherichia coli*

Total samples	200	ESBL producers (50=25%)	Non-ESBL producers (150=75%)
<i>Klebsiella Species</i>	80 =40%	19 =23.75%	61 =76.25%
<i>Escherichia coli</i>	120 =60%	31 =25.83%	89 =74.16%

Fig.1 Antibiotic sensitivity pattern of *Escherichia coli*

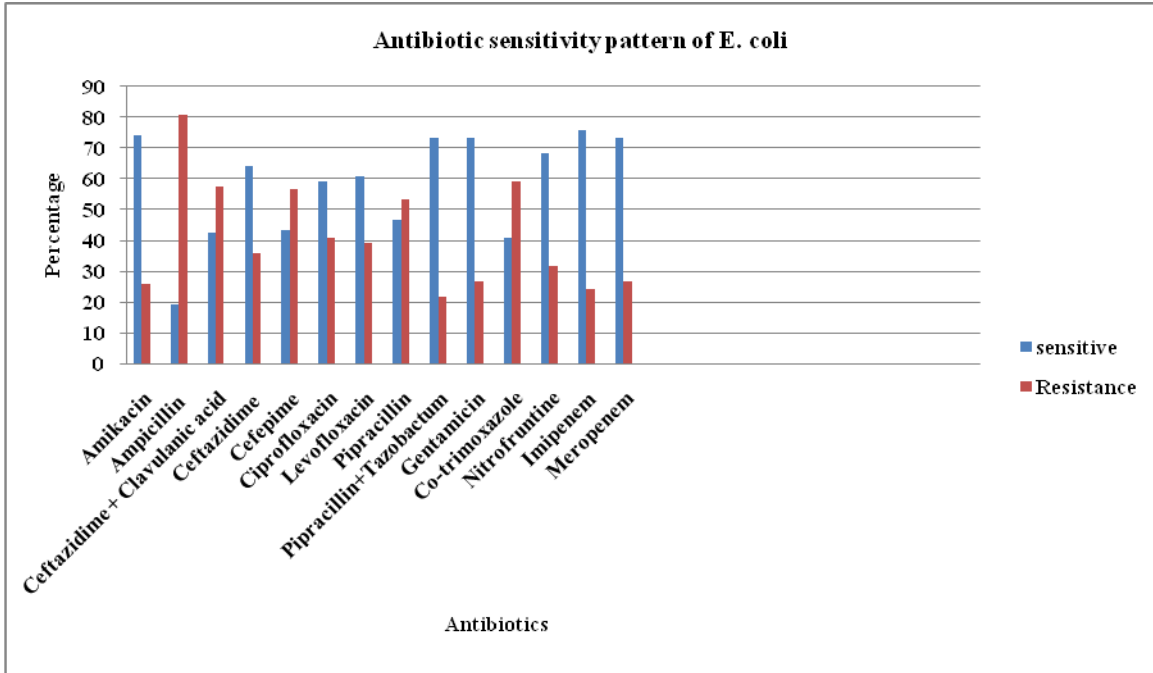
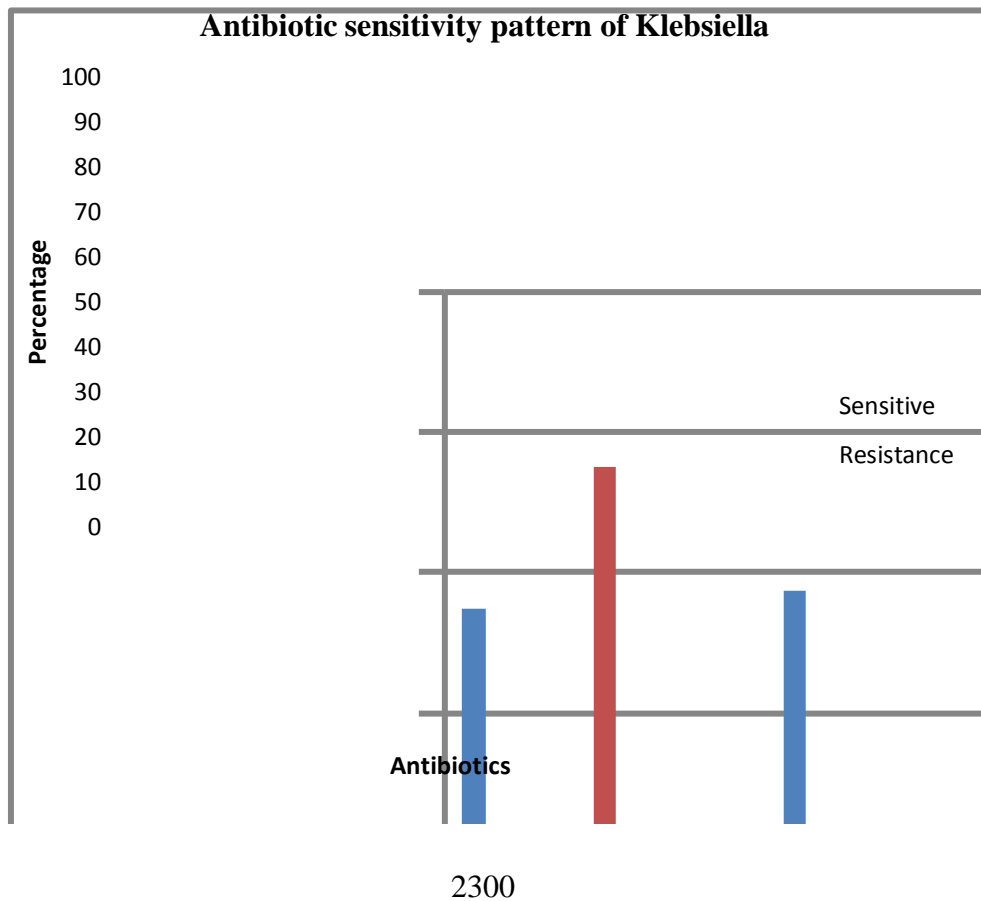


Fig.2 Antibiotic sensitivity pattern of *Klebsiella* species



In developing country like India, ESBL detection and its antimicrobial susceptibility testing should be routinely undertaken to enable the clinician to select appropriate drug regimen

Klebsiella species and *Escherichia coli* remain the major ESBL producing organisms isolated worldwide which are recommended to be routinely tested and reported by the Clinical and Laboratory Standards Institute.⁶

ESBLs are more prevalent in *Klebsiella* spp. (pneumonia and oxytoca) followed by *Escherichia coli*⁴. In the present study, *Escherichia coli* (60%) was the most frequent and statistically significant ESBL producing organisms ($p < 0.05$) followed by *Klebsiella* spp. (40%). Predominant prevalence of ESBL among *Escherichia coli* isolates was also reported in a study by Ananthakrishnan *et al.*,²⁰ and Kumar *et al.*,²¹. A similar study from central India reported the incidence of 69% and 41% ESBL producing isolates of *E. coli* and *Klebsiella pneumoniae* respectively.²²

In the present study, ESBL detection was carried out by the Phenotypic Confirmatory Disc Diffusion test (PCDDT) on 120 isolates of *E. coli* and 80 isolates of *Klebsiella* species. Imipenem was found to be the most effective (75.83%) antibiotic followed by Amikacin (74.16%) and Meropenem (73.33%). Our findings correlate with a similar study done by Chika *et al.*,²³ and Sharma *et al.*,²⁴ where imipenem sensitivity was reported to be 84.6% and 96.2% among the isolates. The isolates of *E. coli* in our study were found to be most resistance to Ampicillin, Cotrimoxazole and Ceftazidime which coincides well with studies done by Chika *et al.*, and Sharma *et al.*, and Sarojamma *et al.*,²⁵

Amongst the *Klebsiella* isolates, Imipenem was found to be the most effective (81.25%)

followed by Amikacin (77.5%) and Piperacillin + Tazobactam (77.5%). These findings correlate with a study done by Chander *et al.*, in which 100% sensitivity of *Klebsiella* to Imipenem was reported. However, resistance to Amikacin and Piperacillin + Tazobactam was only 22.5%. In our study, 31 isolates (25.83%) of *E. coli* and 19 isolates (23.75%) of *Klebsiella* spp. were found to be ESBL producers.

Ceftazidime + Clavulanic acid (93.5%) was the most effective antibiotic against ESBL producing *E. coli* followed by Imipenem (83.87%) and Meropenem (80.64%). Against ESBL producing *Klebsiella*, Imipenem was most effective (89.47%) followed by Ceftazidime + Clavulanic acid (84.21%) and Piperacillin + Tazobactam (84.21%). These findings are marginally better than studies conducted by Chika *et al.*,⁷ where ESBL producing *Klebsiella* were 26%. However, Chander *et al.*,¹⁰ had lower ESBL rates for *E. coli* (13.5%) and *Klebsiella* (16.5%).

In a study done by Mumtaz S *et al.*,¹¹ antibiotic sensitivity correlates with our study where most of the isolates are sensitive to Imipenem (95.7%) followed by Amikacin (76%).

As observed in our study, Imipenem was effective among both ESBL and non-ESBL producing *E. coli* and *Klebsiella* species.

It should be kept in mind that Carbapenems should only be used in emergency and life threatening situations and not as a part of routine use so that the therapeutic value of these drugs is preserved and available when absolutely necessary¹².

A well designed antibiotic policy, effective implementation of the same, awareness on ESBLs in hospitals are important points to be taken into account while handling resistant

organisms such as ESBL producers. The present study highlights a relatively higher prevalence ESBL among *E. coli* and *Klebsiella* spp. In view of these findings, we recommend that ESBL testing should be done routinely to reduce resistance to antibiotics and to strengthen the enforcement of infection control measures. It is pertinent for the treating clinicians so that they can make an informed choice as far as appropriate antibiotics and precautions are concerned.

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