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Prevalence of Extended Spectrum β-Lactamase and AmpC β-Lactamase among Enterobacteriaceae and Pseudomonadaceae Isolated at Tertiary Care Set up in Tripura, India.

Tapan Majumdar*, Shibabrata Bhattacharya, and Raunak Bir.

Department of Microbiology, Agartala Government Medical College, Kunjavan, Agartala, Tripura, India.

Research Article

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***For Correspondence**

Dept. of Microbiology, Agartala Government Medical College, Kunjavan, Agartala, Tripura, India.

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ABSTRACT

β-lactam antibiotics are the most widely used chemotherapeutic agents. Commonest cause of resistance towards β-lactam antibiotics is the production of β-lactamases. Isolation, identification and antibiogram of the members of *Enterobacteriaceae* and *Pseudomonaceae* from the clinical samples. Detection of Extended spectrum β-Lactamase and AmpC β-Lactamase producing *Enterobacteriaceae* and *Pseudomonadaceae* by phenotypic method. Culture isolation, identification and antibiogram of the isolates were performed followed by detection of Extended spectrum β-Lactamase by Double Disc Synergy Test and Phenotypic disc confirmatory test, whereas AmpC β-Lactamase was detected by Disc Antagonism Test and Double Disc Synergy Test. Out of 200 isolates, 54% are Extended spectrum β-Lactamase producers, 48.5% are AmpC β-Lactamase producers. Predominant ESBLs producers are *Klebsiella pneumonia* and *Escherichia coli.* In case of *Pseudomonadaceae*, 88% of *Pseudomonas aeruginosa* and 100% of *Pseudomonas fluorescens* has shown presence of AmpC β-Lactamase and 66% of *Providencia alcalifaciens* produced combined Extended spectrum β-Lactamase and AmpC β-Lactamase. Out of the 97AmpC β-Lactamase producers, 45.4% and 54.6% are inducible and plasmid mediated AmpC β-Lactamase producers respectively. This study shows high rate of circulating extended spectrum β-Lactamase and AmpC β-Lactamase producers in our hospital setup. Development of antimicrobial stewardship program based on the local epidemiological data and national guidelines is the need of the hour.

INTRODUCTION

β-lactam antibiotics are the most widely used chemotherapeutic agents. Commonest cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamases. Many second and third generation cephalosporins and extended spectrum penicillins were specifically designed to resist the hydrolytic action of major β-lactamases. However, new β-lactamases emerged against each of these new classes of β-lactams that were introduced and caused resistance, most important among them being the Extended spectrum β-Lactamase (ESBLs) and AmpC β-Lactamase(AmpC) [1].

ESBLs are enzymes that hydrolyse oxyimino-cephalosporins conferring resistance to third generation cephalosporins such as cefotaxime, ceftazidime and ceftriaxone and to monobactams such as aztreonam. They are not active against α-methoxy-β-lactams or cephamycins and the carbapenems, but are susceptible to β -lactamase inhibitors like clavulanic acid. Being plasmid mediated, it facilitates the dissemination of resistance not only to β-lactams but also to other commonly used antibiotics such as fluroquinolones and aminoglycosides [2].Whereas, AmpC apart from being resistant to all generations of cephalosporins, cephamycins and monobactam except cefepime and cefpirome are not inhibited by clavulanic acid.They are not active against carbapenems and are inhibited by cloxacillin and boronic acid [3].

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The prevalence of bacteria producing ESBLs varies from 20-71% in India and 8-45% worldwide [4,5].In case of AmpC, Moland *et al* 1998 and Sanguinetti *et al* found the prevalence of the AmpC production to be 10.67% and 15.1% respectively whereas in India, prevalence of AmpC ranges from 3.3-47.3% [6,7,8,9].As injudicious use of antibiotics not only expose people to the danger of acquiring infection from ESBLs and AmpC producing organisms, but also with β-lactams being the most frequently prescribed antimicrobials, the emergence of ESBLs and AmpC producing organisms in clinical infections can result in treatment failure which constitutes a serious threat to current β-lactam therapy.

Though non-response to therapy by β- lactam groups of antibiotics is being reported by the clinicians there is no such data currently available for the state of Tripura. Therefore this study was undertaken to evaluate the prevalence of β-lactamase producing organisms isolated in the Department of Microbiology from different clinical samples with special reference to ESBLs and AmpC with an objective:

- To isolate and identify the common members of *Enterobacteriaceae* and *Pseudomonaceae* in the clinical samples.
- To evaluate their antibiotic susceptibility pattern.
- To detect the presence of ESBLs and AmpC producing *Enterobacteriaceae* and *Pseudomonadaceae*.

MATERIALS AND METHODS

This is a hospital based cross sectional study conducted in Department of Microbiology at tertiary care hospital in Tripura over a period of six calendar months (May-Oct, 2012).The study was conducted following clearance by the institutional ethical committee.

Sample size

A total of 200 non-repeating bacterial isolates belonging to members of *Enterobacteriaceae* and *Pseudomonadaceae* from different clinical samples received in the department of microbiology were evaluated in the study.

Methods

The received samples for culture and sensitivity test were inoculated in Blood agar and MacConkey agar respectively and kept overnight at 37°C in the incubator. Next day isolated colony was identified by gram staining and conventional biochemical tests without any automated instruments as per standard laboratory protocol [10]. Antibiotic susceptibility testing of the isolated organisms were performed in Mueller Hinton agar(MHA) by Kirby-Bauer disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines with amoxycillin 30µg , cefoxitin 30µg, cefuroxime 30µg, cefotaxime 30µg, ceftazidime 30µg, ceftriaxone 30µg, cefepime 30µg, aztreonam 30µg, imipenam 10µg, ofloxacin 5µg, norfloxacin 10µg, amikacin 30µg, gentamycin 10µg, tetracycline 30µg, chloramphenicol 30µg and piperacillin/tazobactam 100/10µg (Himedia, Mumbai, India) [10, 11].β-lactamase negative *Escherichia coli* ATCC 25922 was used as the negative control and ESBL-producing *Klebsiella pneumoniae* ATCC 700603 was used as the positive control throughout the study. In case of *Pseudomonas*, *Pseudomonas aeruginosa* ATCC 27853 was used as control strain in this study.

An isolate was suspected to be an ESBL producer if it had the zone sizes for the cephalosporins like cefotaxime (30µg) ≤ 27 mm, ceftazidime (30µg) ≤ 22 mm, ceftriaxone (30µg) ≤ 25 mm and aztreonam (30µg) ≤ 27 mm [11]. In case of AmpC, cefoxitin resistance i.e. zone sizes for the cefoxitin (30µg) ≤ 17 mm was treated as AmpC suspected strains [8].

Double Disc Synergy Test (DDST) for ESBLs

The isolates were then subjected to double disc synergy test(DDST) where a disc of augmentin (20 µg amoxycillin + 10 µg clavulanic acid) was placed on the surface of the MHA; then, discs of cefotaxime (30 µg) and ceftazidime (30 µg) were kept 20 mm apart from the augmentin disc (centre to centre). The plates were incubated at 37°Covernight.The enhancement of the zone of inhibition of the cephalosporin disc towards the clavulanic acid disc was taken as evidence of ESBLs production [4].

Phenotypic Disc Confirmatory Test (PDCT) for ESBLs

This was done on MHA. Two discs, containing cefepime (30 µg), cefepime+clavulanic acid (30 µg+10 µg), were used. A ≥ 5 mm increase in zone diameter for cefepime tested in combination with clavulanic acid versus its zone when tested alone confirmed ESBLs production [4].

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Disc Antagonism Test (DAT) for inducible AmpC

The inducible AmpC were detected on primary antibiogram plates by the presence of D-shaped zone of inhibition of ceftriaxone (30 µg) disc kept at 20mm (center to center) adjacent to the imipenem (10 µg) disc [12].

Double Disc Synergy Test (DDSTa) for plasmid mediated AmpC

The AmpC were tested by a double-disc synergy test based on the utilisation of cloxacillin as inhibitor of AmpC. Each isolate was inoculated on a Mueller Hinton agar plate, according to CLSI guidelines. Discs of cefotaxime (30 µg) and cefoxitin (30µg) were placed 10 mm (edge to edge) from cloxacillin (500 µg) disc. After incubation at 37 °C overnight, an enhanced zone of inhibition between cefotaxime disc and cloxacillin disc was interpreted as evidence of AmpC production. Plasmid mediated AmpC producers were found out by subtracting the number of inducible AmpC producers by DAT from the total number of AmpC producers by DDSTa [13].

Statistical tools

Compiled data was analysed and compared using Chi-square test.

RESULTS AND DISCUSSION

During the study period total 200 consecutive non-repetitive isolates were tested for production of ESBLs and AmpC. The source of the isolates were from urine, pus, blood, sputum, stool, CSF and others includes amniotic fluid, peritoneal fluid, pleural fluid, bile, etc (Fig-1). Clinical spectrum representing the isolates shows 40% were from urinary tract infection (UTI) , 20% from surgical site infection (SSI) ,15% from septicaemia, 8% from lower respiratory tract infection and 6% each from gastro-intestinal tract infection and local abscess (Fig-2).

Figure 1

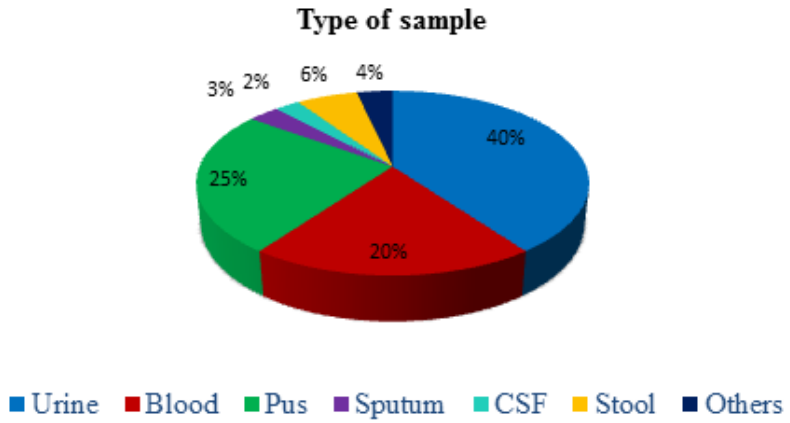
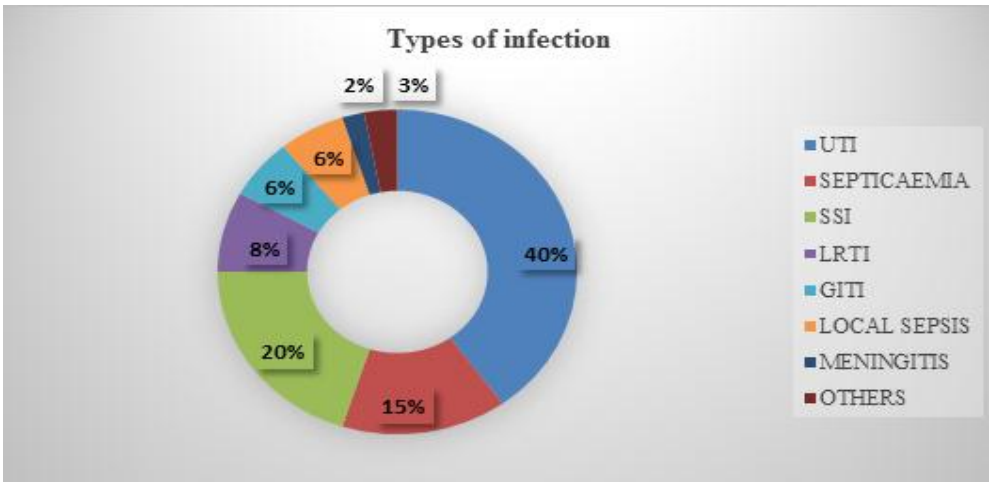


Figure 2: Types of infection



UTI- urinary tract infection, SSI- surgical site infection, LRTI- lower respiratory tract infection, GITI- gastro-intestinal tract infection.

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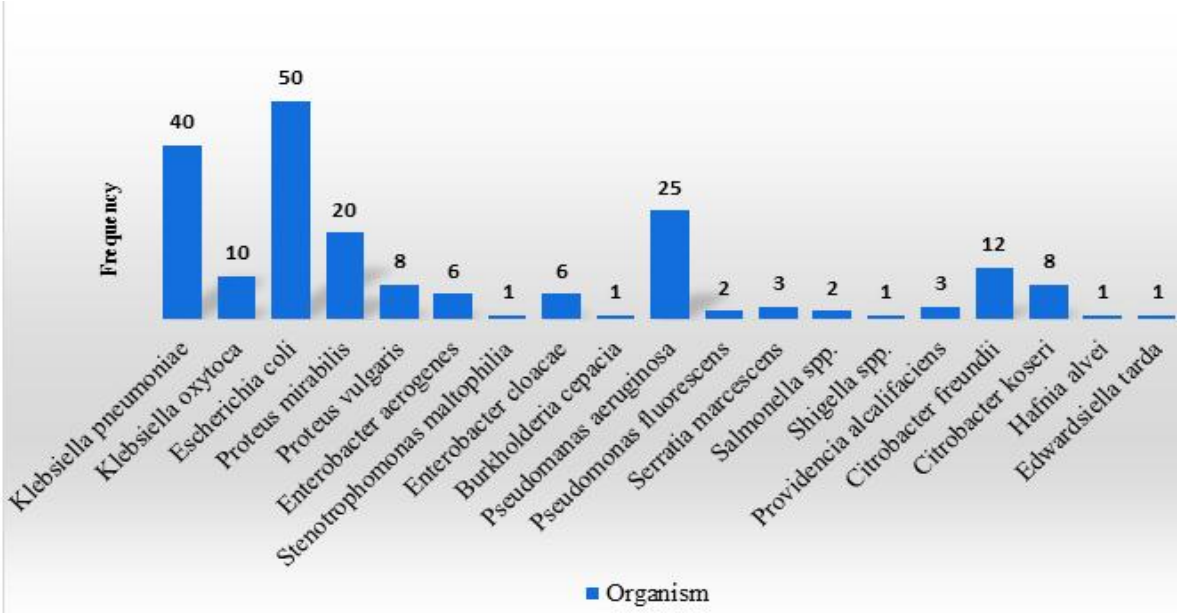
Distribution pattern of isolated organisms shows that *Escherichia coli* was the most commonly isolated organism followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis* (Fig-3).

Figure 3: Distribution pattern of isolated organism.



This study showed high rate of circulating ESBLs producers in our hospital setup. Among the 200 tested strains of *Enterobacteriaceae* and *Pseudomonadaceae* ,54%(108/200) are ESBLs producers which is similar to others studies like Rodrigues C. *et al*., Sridhar Rao P.N. *et al*. and Mathur P. *et al.*,where rate of ESBLs occurrence was found to be 53.3%, 61% and 68% respectively[14, 15, 16].Factors which might have led to the high prevalence of the ESBL producers could be indwelling catheters, invasive procedures, severity of the illness and excessive use of cephalosporins[17].Regarding distribution of the ESBLs producers similar results were shown by Jain A. *et al*., Umadevi S. *et al*.,Mathur P. *et al.* [17, 18, 16]. Species wise distribution of ESBLs and AmpC producing organisms shows predominant ESBLs producers were *Escherichia coli* (56%) followed by *Klebsiella pneumonae* and *Proteus mirabilis* (Table-1). In case of *Pseudomonadaceae*, 88% of *Pseudomonas aeruginosa* and 100% of *Pseudomonas fluorescens* has shown presence of AmpC. Predominant Non-ESBLs, Non-AmpC producers were *Burkholderia cepacia*, *Edwardsiella tarda* and *Shigella spp* whereas 66% of *Providencia alcalifaciens* produced combined ESBLs and AmpC. A study done by Chaudhuri B.N. *et al*. showed that 79% of *E. coli* and 70% of *Klebsiella*spp. were ESBL producers, which is also in line with our results[19]. In contrast, studies done outside India like Tsering D.C. *et al*. and Nijssen S. *et al*. showed lower rates of ESBLs in these places which may be due to rational use of antibiotics and active surveillance [20, 21].In case of *Pseudomonadaceae*, studies like Umadevi S. *et al*. 2011 and Laghawe A. *et al*. found prevalence of ESBLs to be 14% and 11.5% which is similar to our results [18, 8]. Our study showed 48.5 %(97/200) isolates were producers of AmpC similar to the results shown by Tan T.Y. *et al*.,Hemalatha V.*et al*. andNagdeo N.V. *et al*. where rate of Amp C occurrence was found to be 49.8%, 47.3% and 47.8% respectively[22, 9, 23].

Species wise distribution of inducible and plasmid-mediated AmpC shows, out of 97AmpC producers 45.4% were inducible AmpC and 54.6% were plasmid –mediated. *Escherichia coli* expressed highest number of plasmid mediated AmpC isolates i.e. 83.3% (10/12) whereas 81.8% (18/22) *Pseudomonas aeruginosa* expressed inducible AmpC (Table-2).Regarding pattern of inducible and plasmid mediated AmpC producers, results shown in other studies like Shobha K. L. *et al*., Parveen, M. *et al*.2010 and Laghawe A. R. *et al*. 2012 are reflecting similar trends [24, 25, 26]. In *Pseudomonas spp*. ESBLs production is less as compared to *Enterobacteriaceae*, because their resistance is mediated by various other mechanisms such as the production of high degree of AmpC, metallo β-lactamases, lack of drug penetration due to mutations in the porins and the loss of certain outer membrane proteins and efflux pumps [18, 27].

In our study, we also observed 5% (10/200) resistance shown towards imipenem which may be due to production of carbapenamase and metallo β-lactamasesis similar to the assertions made by Chaudhuri B.N. *et al*. and Umadevi S. *et al*. 2011[19, 18]. Along with it we also suspected presence of K1 β- lactamase in 2% (4/200) and Inhibitor-resistant β-lactamase in 1% (2/200) of the isolates, which needs further investigation and characterization for their confirmation.

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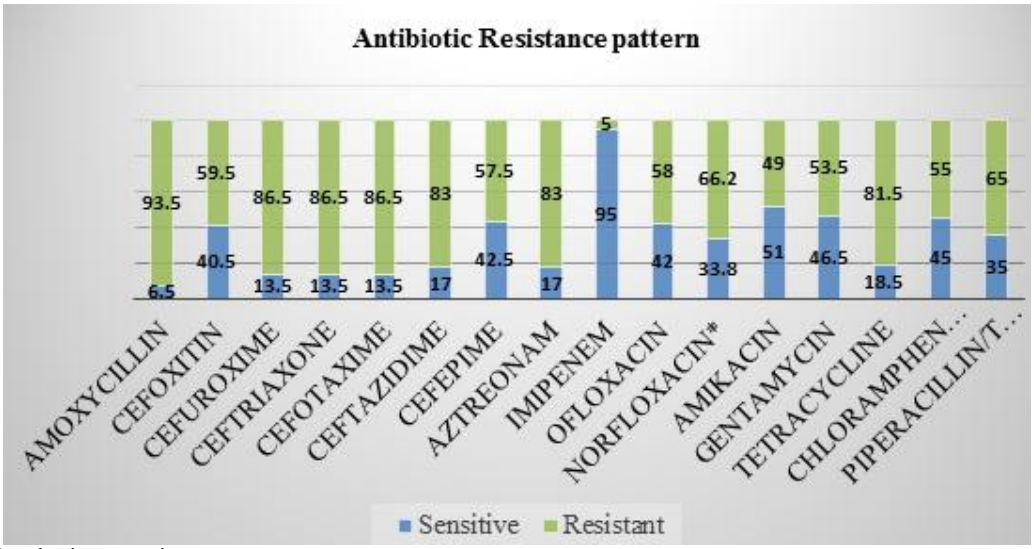
Overall antibiotic resistance pattern of the isolates showed high degree of resistance toward amoxycillin, 3rd generation cephalosporin and tetracycline whereas cefepime imipenam, aminoglycosides were showing less resistance (Fig-4).

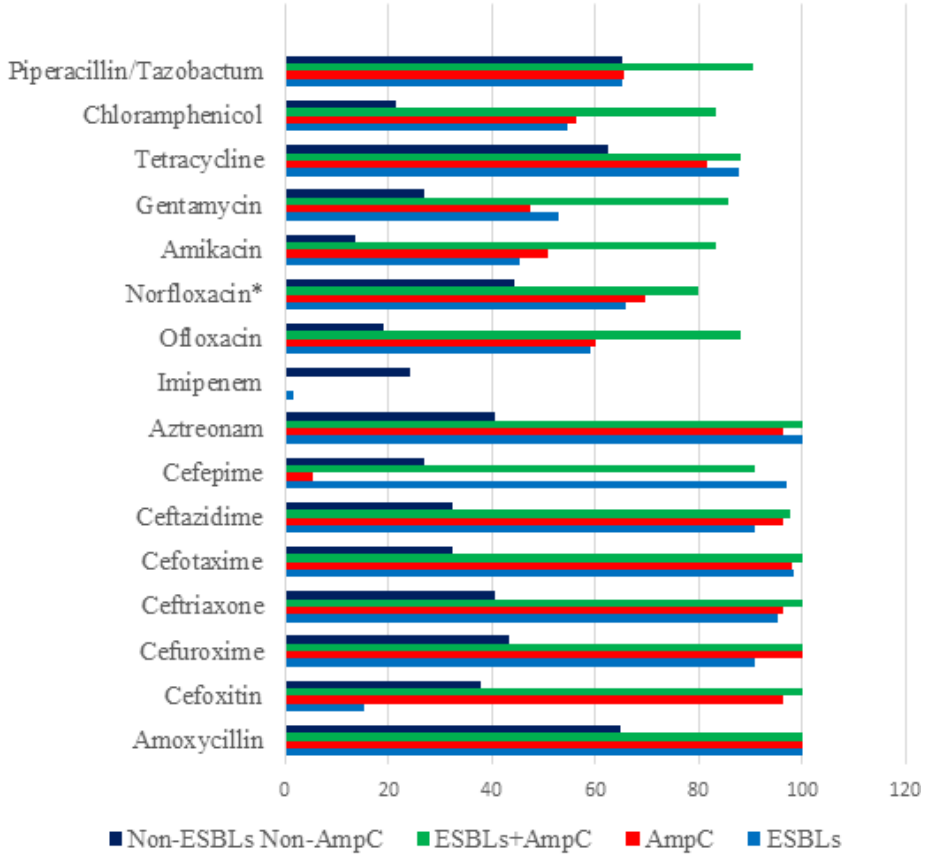
Figure 4: Antibiotic sensitivity pattern isolated organism



*Used in urine isolates only.

Further analysis of resistance pattern of the isolates shows co-expression of ESBLs and AmpC was also associated with higher degree of resistance towards non β-lactam antibiotics, whereas 5% resistance shown to imipenem were produced mostly by non-ESBLs non-AmpC expressors (Fig-5), similar to studies done by Mshana E.S. *et al*. and Tsering D.C. *et al*. [28, 20].

Figure 5: Antibiotic resistance pattern



*Used in urine isolates only.

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Table 1: Species wise distribution of ESBL and AmpC producing organisms

Organisms(n)	ESBLs(%)	AmpC (%)	ESBLs+AmpC (%)	Non-ESBLs NonAmpC(%)
200	33(66/200)	27.5(55/200)	21(42/200)	18.5(37/200)
	Distribution of ESBL and AmpC producing organism			
Klebsiella pneumonia(40)	52.5(21/40)	15(6/40)	20(8/40)	12.5(5/40)
Klebsiellaoxytoca (10)	10(1/10)	20(2/10)	20(2/10)	50(5/10)
Escherichia coli(50)	56(28/50)	10(5/50)	14(7/50)	20(10/50)
Proteus mirabilis(20)	40(8/20)	10(2/20)	25(5/20)	20(4/20)
Proteus vulgaris(8)	37.5(3/8)	12.5(1/8)	25(2/8)	25(2/8)
Enterobacter aerogenes(6)	16.7(1/6)	50(3/6)	33.3(2/6)	0(0/6)
Enterobacter cloacae(6)	16.7(1/6)	33.3(2/6)	33.3(2/6)	16.7(1/6)
Citrobacter freundii (12)	16.7(2/12)	33.3(4/12)	33.3(4/12)	16.7(2/12)
Citrobacter koseri(8)	0(0/8)	37.5(3/8)	37.5(3/8)	25(2/8)
Serratia marcescens(3)	0(0/3)	66.7(2/3)	33.3(1/3)	0(0/3)
Salmonella spp.(2)	0(0/2)	50(1/2)	50(1/2)	0(0/2)
Shigella spp.(1)	0(0/1)	0(0/1)	0(0/1)	100(1/1)
Providencia alcalifaciens(3)	0(0/1)	33.3(1/3)	66.7(2/3)	0(0/1)
Hafnia alvei(1)	0(0/1)	100(1/1)	0(0/1)	0(0/1)
Edwardsiella tarda(1)	0(0/1)	0(0/1)	0(0/1)	100(1/1)
Pseudomanas aeruginosa(25)	0(0/25)	76(19/25)	12(3/25)	12(3/25)
Pseudomonas fluorescens(2)	0(0/2)	100(2/2)	0(0/2)	0(0/2)
Stenotrophomonas maltophilia(1)	100(1/1)	0(0/1)	0(0/1)	0(0/1)
Burkholderia cepacia(1)	0(0/1)	0(0/1)	0(0/1)	100(1/1)

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Table 2: Species wise distribution of inducible and plasmid-mediated AmpC β lactamases

Organisms(n)	Inducible AmpC- β lactamases (%)	Plasmid-mediated AmpC- β lactamases (%)
Klebsiella pneumonia	28.6(4/14)	71.4(10/14)
Klebsiella oxytoca	75(3/4)	25(1/4)
Escherichia coli	16.7 (2/12)	83.3(10/12)
Proteus mirabilis	25(2/8)	75(6/8)
Proteus vulgaris	0(0/3)	100(3/3)
Enterobacter aerogenes	60(3/5)	40(2/5)
Enterobacter cloacae	50(2/4)	50(2/4)
Citrobacter freundii	50(4/8)	50(4/8)
Citrobacter koseri	33.3(2/6)	66.7(4/6)
Serratia marcescens	33.3(1/3)	66.7(2/3)
Salmonella spp.	0(0/0)	100(2/2)
Shigella spp.	0(0/0)	0(0/0)
Providencia alcalifaciens	0(0/3)	100(3/3)
Hafnia alvei	100(1/1)	0(0/0)
Edwardsiella tarda	0(0/0)	0(0/0)
Pseudomanas aeruginosa	81.8(18/22)	18.2(4/22)
Pseudomonas fluorescens	100(2/2)	0(0/0)
Stenotrophomonas maltophilia	0(0/0)	0(0/0)
Burkholderia cepacia	0(0/0)	0(0/0)
Total(97)	45.4(44/97)	54.6(53/97)

CONCLUSIONS

The study was undertaken to evaluate prevalence of ESBLs and AmpC among Enterobacteriaceae and Pseudomonadaceae isolated at tertiary care set up. High degree of resistance was observed against most of the isolates from different clinical samples which resulted in non-response to therapy by β- lactam groups of antibiotics. Outcome of the study further emphasised the need for development of antimicrobial stewardship program based on local epidemiological data and national guidelines. Preventive measures like a continuous surveillance and a strict implementation of infection control practices can go a long way in containing the menace of drug resistance in our settings. In addition to the trends of prevalence, precise knowledge about the exact ESBL subtypes, status of other β-lactamases (AmpC, carbepenemases, K1β-lactamases, etc.) is essential in relation to institution of necessary interventions and strategies directed at further worsening of the present scenario. This necessitates further study in large scale with molecular characterization like DNA probing, polymerase chain reaction, restriction fragment length polymorphism and isoelectric focusing. Also regular surveillance will highlight the changes in the organism distribution, antibiotic sensitivity patterns and MICs of the commonly used drugs. This will help in formulating a working antibiotic policy for our hospital which will aid the clinicians in prescribing proper antibiotics.

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