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PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASES AMONG GRAM NEGATIVE CLINICAL ISOLATES FROM A TERTIARY CARE HOSPITAL

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ABSTRACT

Beta-lactamases production is the most common mechanism of bacterial resistance in gram negative bacteria. These enzymes are capable of mutation in response to the selective pressure of antibiotic use. This study was undertaken to detect the prevalence of Extended Spectrum β-lactamases (ESBLs) from Gram negative clinical isolates. A total of 500 gram negative isolates were subjected to Kirby Bauer disk diffusion method. Isolates showing resistance or reduced susceptibility to third generation cephalosporins were subjected to ESBL screening tests by Double Disc Synergy test and Phenotypic confirmatory disc diffusion test .The prevalence of ESBL producing Gram negative bacilli in this study was found to be 62%.A total of 310 isolates showed reduced susceptibility to third generation cephalosporins. Out of 310, 232(74.83%) isolates were positive for ESBL screening. All the 310 isolates were tested positive by confirmatory method. Early detection of these β-lactamases producing isolates in a routine laboratory could help to avoid treatment failure.

KEYWORDS: ESBL, Gram negative bacilli, DDST, PCDDT, E-test,

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INTRODUCTION

Among the Gram negative bacteria, a major mechanism of β-lactam resistance is the production of β-lactamases that disrupt the amide bond of the characteristics four member β-lactam ring. Extended Spectrum β-lactamases (ESBLs) are plasmid mediated enzymes that confer resistance to a wide variety of β-lactams, including third generation cephalosporins, penicillins and aztreonam [1,2]. These enzymes are predominantly of class A and class D β-lactamases and are generally inhibited by available β-lactam inhibitors. Being plasmid mediated, these enzymes spread fast amongst various bacteria and poses a major threat to clinical therapeutics. Wide spread use of third generation cephalosporins and aztreonam is believed to be the major cause of mutation in these enzymes [3]. In recent years, there has been an increased incidence and prevalence of extended spectrum β-lactamases (ESBLs). Resistance to multiple drugs are seen in ESBL producing bacteria as the genes with other mechanism of resistance often resides on the same plasmid as the ESBL gene .ESBL are now found in significant percentage of *E.coli* and *K.pneumoniae* strains. The most prevalent types of ESBLs, TEM / SHV enzymes have been reported in members of the Enterobacteriaceae including *E.coli, Klebsiella spp, Enterobacter spp, Citrobacter spp, Serratia marcescens, Shigella dysenteriae, Morganella morgani, Proteus spp, Providencia spp, Salmonella spp* etc as well as in *Burkholderia cepacia, Capnocytophaga ochracea, Aeromonas spp, Pseudomonas aeruginosa, Acinetobacter baumannii* etc [4,5]. Hence this study was undertaken to detect the prevalence of ESBLs (Extended spectrum β-lactamases) producing Gram-negative bacilli in various clinical samples and to determine their antimicrobial resistance pattern.

MATERIALS AND METHODS

Study Period

This study was carried out in the Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College from November 2010 to Feb 2012.

Study Population

Inpatients and outpatients of VMKV Medical College Hospitals, Salem, Tamilnadu

Sample size

500 non-repetitive gram negative bacilli isolates.

Inclusion criteria

A total of 500 Gram-negative bacilli obtained from various clinical specimens such as blood, urine, sputum, body fluids, tracheo-bronchial aspirates, drainage tube tips, pus and wound swab of patients attending the hospital were included in the study.

Exclusion criteria

All the clinical isolates other than gram negative bacilli were excluded from the study.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was done on Mueller Hinton agar plates by Kirby-Bauer's disc diffusion method using commercially available discs (obtained from Hi-media laboratories limited, Mumbai, India) as per Clinical and Laboratory Standard Institute (CLSI) guidelines [3]. The antibiotic discs used namely, Amikacin (30µg), Amoxycillin / Clavulanic acid (20/10µg), Co-trimoxazole (25 µg), Cefotaxime (30µg), Ceftazidime (30µg), Cefoxitin (30µg), Cefepime (30µg), Gentamicin (30µg), Imipenem (10µg), Piperacillin / tazobactam (100/10µg) were tested for quality control by *Escherichia coli* ATCC 25922. Isolates showing resistance or reduced susceptibility to third generation cephalosporins viz Ceftazidime (≤22mm) and Cefotaxime (≤27mm) were subjected to ESBL screening tests [6].

SCREENING FOR ESBL BY DOUBLE DISC SYNERGY TEST (DDST)

A lawn culture of test organism was made on MHA plate and a modified double disc synergy

test (Disc approximation test) was carried out by the following method. Amoxicillin + clavulanic acid (20 µg + 10 µg) disc was placed in the centre and the ceftazidime (30 µg) and cefotaxime (30 µg) discs were placed on either side at a distance of 15 mm centre to centre from the amoxicillin + clavulanic disc. Plates were incubated at 37°C for 18-24 hours and the pattern of zones of inhibition was noted [7, 8].

INTERPRETATION

Isolates that exhibited a distinct shape / size with potentiation towards amoxicillin + clavulanic discs were considered potential ESBL producers and short listed for confirmation of ESBL producers. (Figure-I & Figure-II).

Double Disc Synergy Test (DDST)

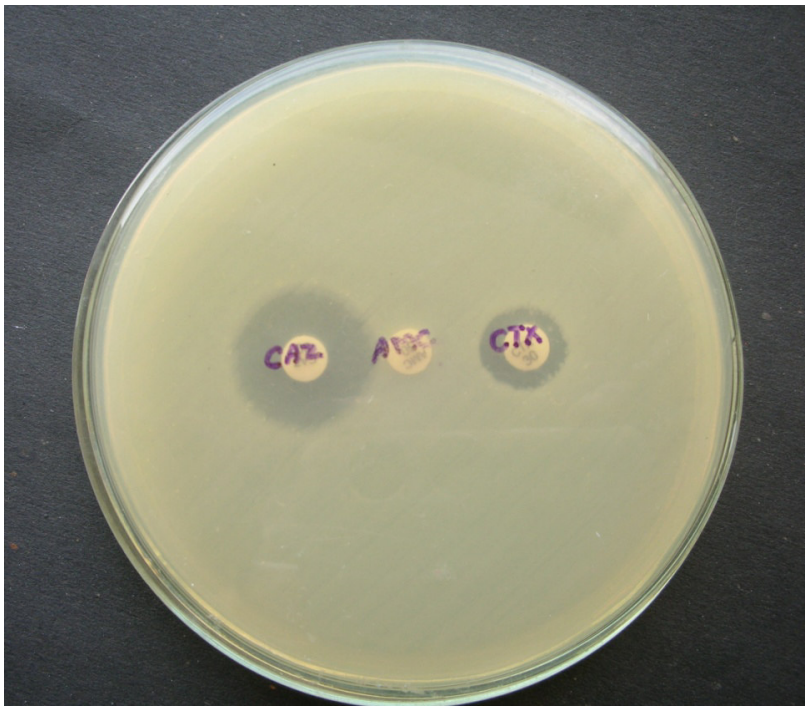


Figure I
ESBL producer by DDST method showing potentiation towards Amoxyclav

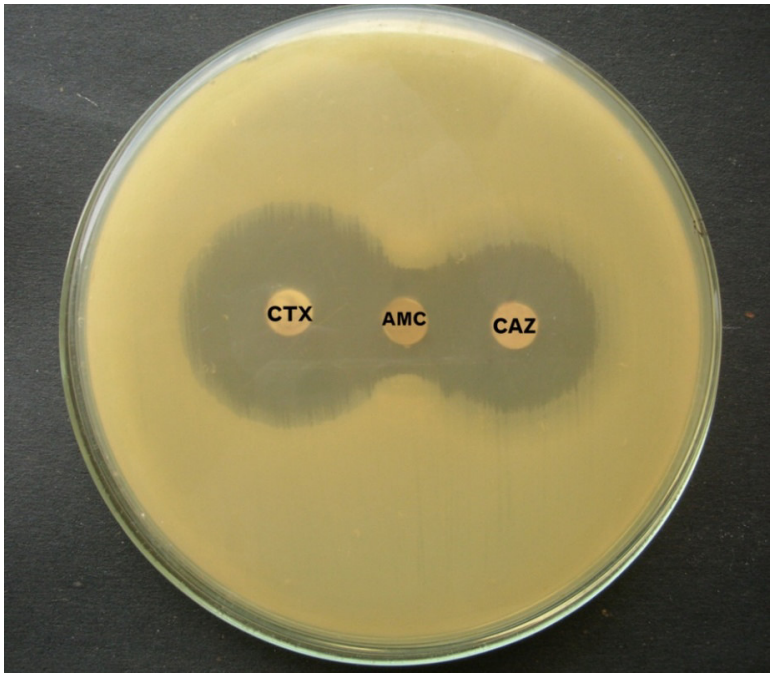


Figure II
ESBL producer by DDST method with presence of Amoxycillin / clavulanic acid (AMC) at the centre with cefotaxime (CTX) and Ceftazidime (CAZ) on either side showing potentiation towards AMC

PHENOTYPIC CONFIRMATION OF ESBLS
PHENOTYPIC CONFIRMATORY DISC DIFFUSION TEST (PCDDT)

A lawn culture of test organism was made on MHA plate with a sterile cotton swab soaked in the broth. Sensitivity discs containing third generation cephalosporins with and without clavulanic acid are used as follows: ceftazidime 30 µg (CA/CAZ), ceftazidime 30 µg + clavulanic acid 10 µg (CAC), cefotaxime 30 µg (CE/CTX), and cefotaxime 30 µg + clavulanic acid 10 µg (CEC). Disc diffusion assay was carried out as per guidelines of CLSI and the plates were then incubated aerobically at 37^{o}C for 18-24 hours. *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* strain ATCC 700603 were used as negative and positive controls[7, 8].

INTERPRETATION

The zone of inhibition of the antibiotic alone (cefotaxime / ceftazidime) was compared with the zone of inhibition in combination with clavulanic acid (CEC / CAC). A difference of ≥5mm increase in zone diameter between the two agents (Zone of CEC / CAC is more than CE / CA) confirms the presence of ESBL. (Figure-III & Figure-IV)

Phenotypic Confirmatory Disc Diffusion Test (PCDDT)

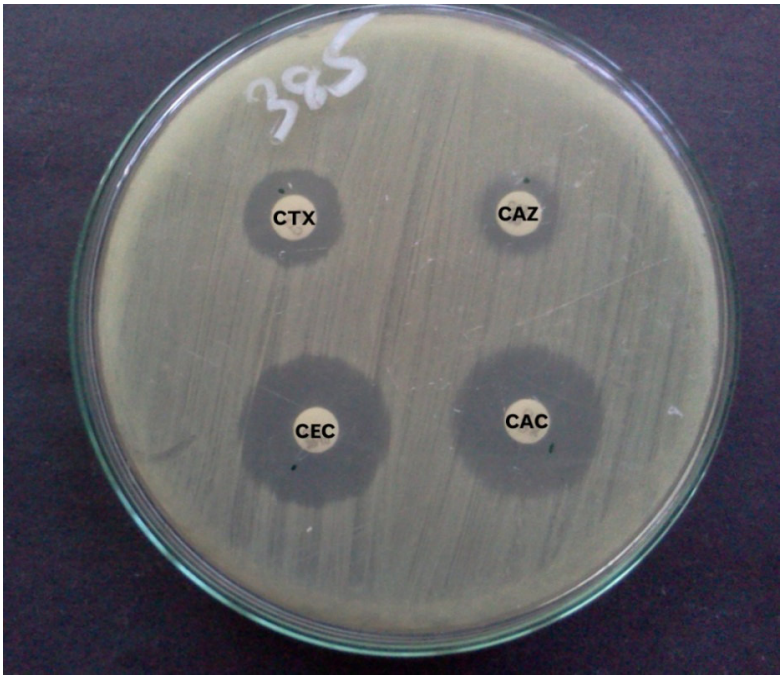


Figure III
ESBL producer by PCDDT method showing CA (ceftazidime) and CAC (Ceftazidime / clavulanic acid), CE (cefotaxime) and CEC (cefotaxime / clavulanic acid) CAC and CEC Shows enhanced zone of inhibition (>5mm)

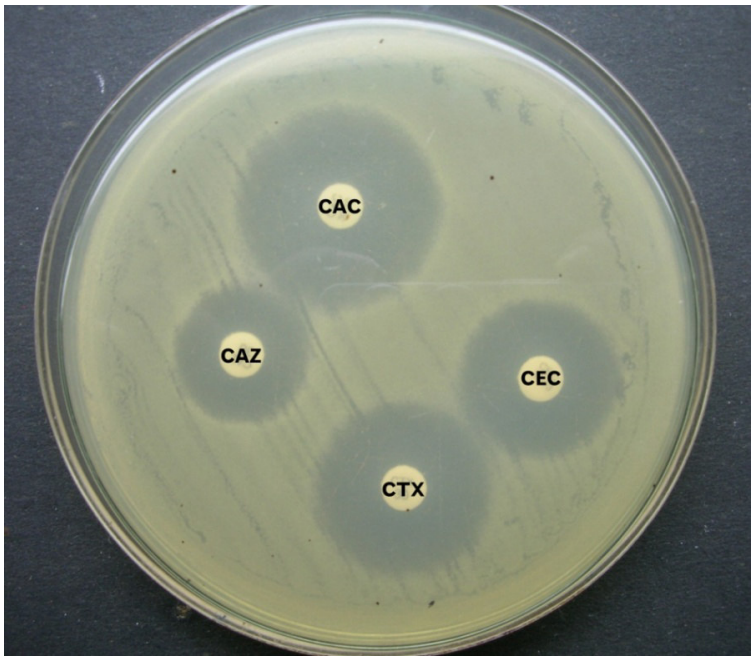


Figure IV
ESBL producer by PCDDT method showing enhanced zone of inhibition of CAC than CAZ (> 5mm)

MINIMUM INHIBITORY CONCENTRATION (MIC) OF ESBL ISOLATES

ESBL E-TEST STRIPS

Double ended strips containing cefotaxime (CT) / cefotaxime + clavulanic acid (CTL) with a concentration gradient of 0.25-16mcg/ml of cefotaxime (CT) and 0.016-1mcg/ml of cefotaxime with 4mcg/ml of clavulanic acid and ceftazidime (TZ) / ceftazidime + clavulanic acid (TZL) with a concentration gradient of 0.5-32mcg/ml of ceftazidime and 0.064-4 mcg/ml of ceftazidime + clavulanic acid (4mcg/ml) were used. The E-test strips are procured from AB BIODISK, Solna, Sweden. The E-test procedure, reading and interpretation were done according to the manufacturer's instructions [9].

RESULTS

Out of 500 clinical isolates included in this study 310 were found to be ESBL producers. Prevalence of ESBL producing organism in this study was found to be 62%. Among 310 isolates, 232 isolates were positive for ESBL screening. All the 310 isolates tested were positive by a confirmatory method for ESBL production. (Table-I)

Table I

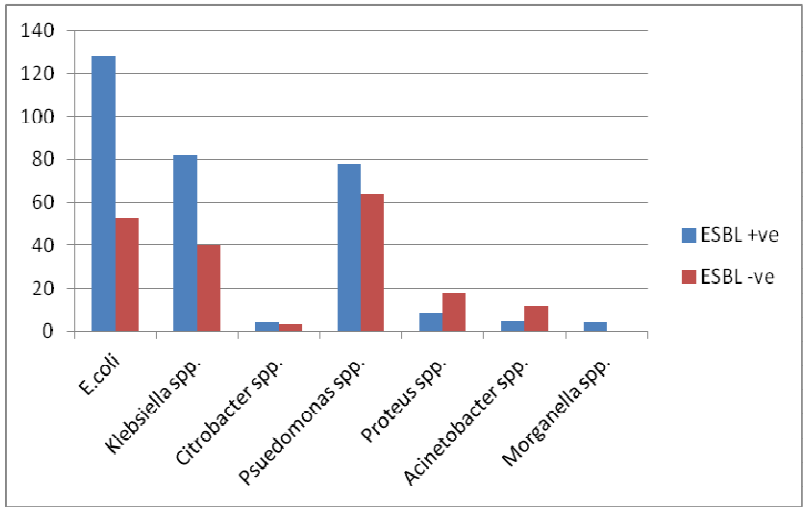
Percentage of ESBL producing GNB by screening and Confirmatory methods

	Screening (DDST)		Confirmatory (PCDDT)	
	Number	**%**	**Number**	**%**
ESBL +ve	232	74.83	310	100
ESBL -ve	78	25.16	0	0
TOTAL	310		310	

ESBL production was found to be highest in *E.coli* (128/181=70.7%),followed by *Klebsiella species* (67.21%) and *Pseudomonas spp*. (54.9%). (Chart-I)

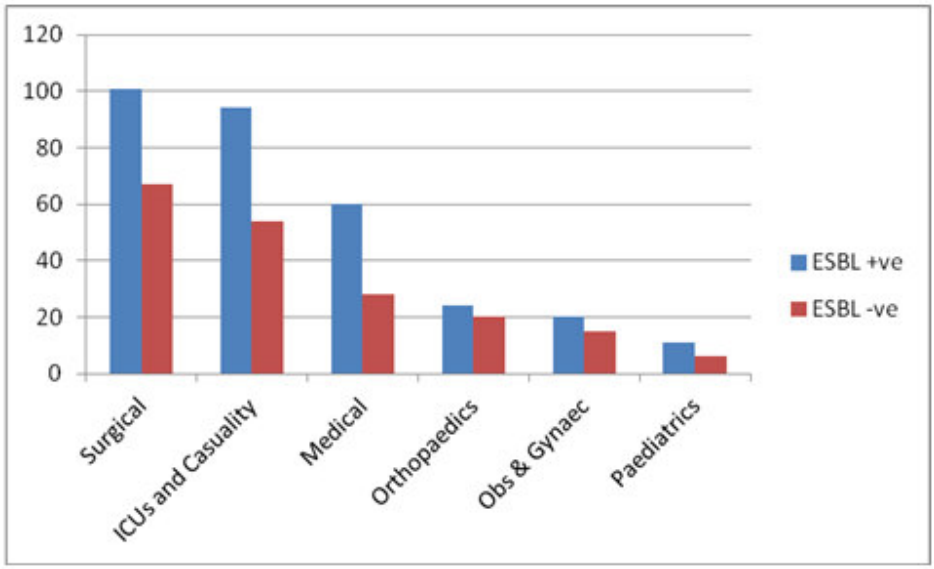
Chart I

Percentage of isolation of various organisms



The prevalence of ESBL producers obtained from patients admitted in Medical, ICU, Surgical wards were found to be 68.2%, 63.5% and 60.1 % respectively. (Chart-II)

Chart II
Ward wise distribution of ESBL positive organisms



Among the ESBL producers 93% exhibited 3GC resistance. Resistance percentage to Gentamicin, Ciprofloxacin and co-trimoxazole was found to be 86, 82, and 94 respectively.

Susceptibility of ESBL producers to Imipenem was found to be 98% followed by PT (92%) and Amikacin (79%). (Table-II)

Table II
Antibiotic susceptibility pattern of ESBL producers

Antimicrobial agent	Symbol	No. of Resistant strains	%	No. of Sensitive strains	%
Amoxyclav (Amoxycillin / Clavulanic acid)	AC / AMC	307	99	3	1
Gentamicin	G / GEN	268	86	42	14
Amikacin	AK	66	21	244	79
Co-trimoxazole	COT / CO	290	94	20	6
Ciprofloxacin	CF / CIP	254	82	56	18
Cefoxitin	CN / CX	308	99	2	1
Cefotaxime	CE / CTX	289	93	21	7
Ceftazidime	CA / CAZ	288	93	22	7
Cefepime	CPM	262	85	48	15
Piperacillin / Tazobactam	PT / PIT	26	8	284	92
Imipenem	I / IPM	6	2	304	98

E-TEST

Among the ESBL producers, 30 isolates were randomly selected and subjected to E-test. All the thirty isolates were confirmed to be ESBL producers by E-test. (Figure-V and Figure-VI)

Epsilometer Test (E-Test)

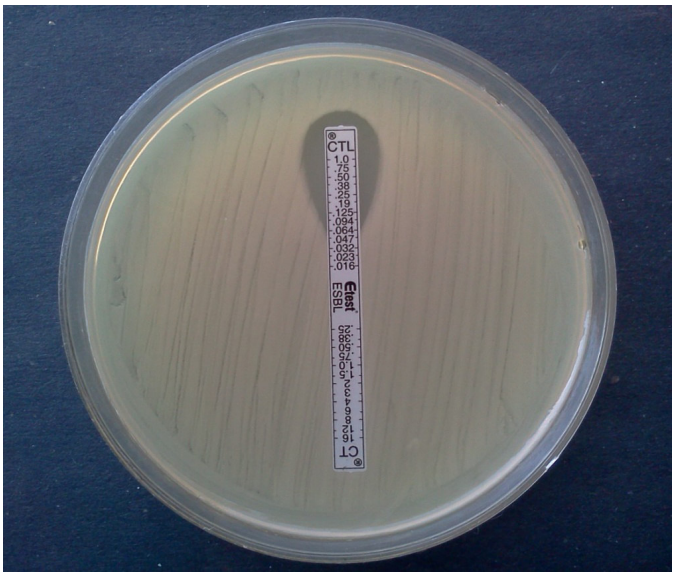


Figure V
E-Test strips showing ESBL producer with a MIC ratio of CT/CTL ≥ 8

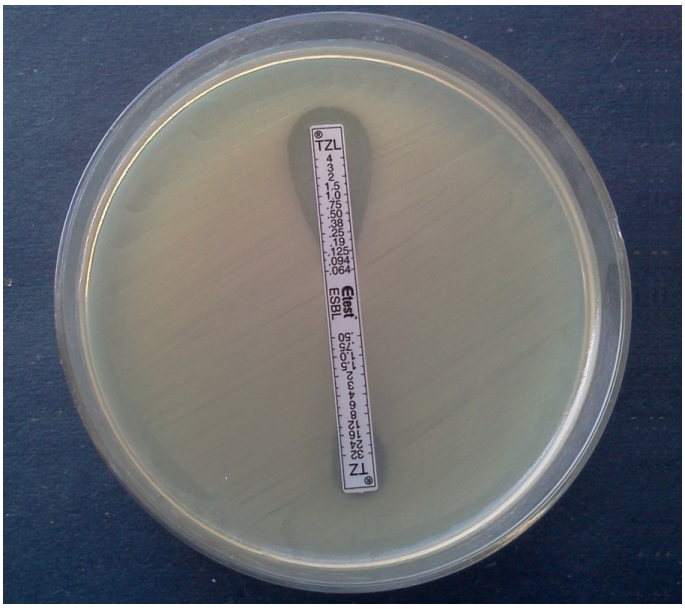


Figure VI
E-Test strips showing ESBL producer with a MIC ratio of TZ/TZL ≥ 8

DISCUSSION

In the present study, the prevalence of ESBL was found to be 62% (310/500).The overall prevalence of ESBL producers was found to vary greatly in different geographical areas and institutes. Various studies from India have reported ESBL production from 6% to 87 %.

Menon et al (Chennai) [10] have reported 20%, Bhattacharjee et al (Varanasi) [11] reported 32%, Khan et al (New Delhi) [8] reported 44% and Rodrigue et al (Mumbai) [12] reported 53%, Singhal et al (New Delhi) [13] reported 64%. The prevalence of ESBL producers obtained from patients admitted in Medical, ICU, Surgical wards were found to be 68.2%, 63.5% and 60.1 % respectively. This is similar to the study conducted by Baby padmini et al [14] among in-patients**.** Admissions in ICU and surgical units were found to be the risk factors in this study. In the present study, regarding inpatients ESBLs producing *E.coli* (70.7%) was found to be most prevalent organism followed by *Klebsiella spp.* (66.39%) and *Pseudomonas spp.* (54.9%). This is similar to the study conducted by Shamim mumtaz et al [15], which showed 51% in *E.coli*, 40 % in *Klebsiella spp.* and 5.8% in *Pseudomonas spp.* A study conducted by Kumar.M.S et al [7] has detected 63.7% ESBL producers among *E.coli* and 14% among *Klebsiella spp.* The present study showed a resistance of 2% among the ESBL producers to Imipenem and 8% to Piperacillin / Tazobactam. A study conducted by Taneja et al [16] showed a percentage resistance of 8.2 and 9.5 to Imipenem and piperacillin tazobactam respectively. Babypadmini et al [14] have reported susceptibility of ESBL producers to Imipenem as 100%. A similar study conducted by Khan et al [8] and Bhattacharjee et al [11] have found 100% sensitivity with Imipenem and Piperacillin Tazobactam. 100 % sensitivity with Imipenem was also reported by, Srujana mohanty et al [17] and Varaiya et al [18].

CONCLUSION

The present study demonstrates a high degree of resistance among gram negative bacilli in clinical isolates. The isolates producing this enzyme often shows a susceptible phenotype in routine susceptibility testing. Accurate Screening or detection of these β-lactamases producing isolates in a routine laboratory could be of help for Hospital Infection Control Committee (HICC) in the formulation of antibiotic policy, to increase the awareness among clinicians regarding the drug resistant pathogens & to control indiscriminate use of antibiotics by strictly adhering to the antibiotic policy. To minimize the emergence of this multiple β-lactamases producing pathogens in hospital, measures are to be taken to limit the indiscriminative use of cephalosporins and carbapenems.

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