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ORIGINAL ARTICLE

Extended Spectrum Beta-Lactamases (ESBL) In Gram Negative Bacilli At A Tertiary Care Hospital

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ABSTRACT

Extended spectrum beta lactamases (ESBL) hydrolyse expanded spectrum cephalosporins like ceftazidime and cephotaxime ,which are used in the treatment of Pseudomonas and other gram negative bacteria. ESBL producing bacteria may not be detectable by the routine disk diffusion susceptibility test, which leads to inappropriate use of antibiotics and treatment failure. Not much information on ESBL producing organisms causing infection is available from the south west coastal region. An effort was therefore made to study the ESBL producing gram negative organisms by the phenotypic confirmatory test and the novel fashion method. Antibiotic susceptibility pattern of ESBL organisms were also analysed.

Methods:160 clinical strains were included in the study. Strains were obtained from adult patients who were either admitted to or attended the outpatient departments of medicine, surgery, obstetrics and gynaecology in a tertiary care hospital .The study was conducted from June 2005 to December 2005. Informed consent was taken from the patients for collecting the samples. The samples were processed for the identification of organisms and were screened for ESBLs. The isolates were also tested for antimicrobial susceptibility by the Kirby-Bauer disc diffusion technique, using Muller Hinton agar. Screening for ESBL was done as per the guidelines recommended by CLSI . Organisms were further tested for confirmation of ESBL production by the phenotypic confirmatory test and by the Novel fashion .

Results And Conclusion:Out of the total 160 strains, the common organisms isolated were Klebsiella pneumoniae with 73 strains (45.62%), followed by Escherichia coli with 63 strains (39.37%) and Pseudomonas spp with 14 strains (8.75%), respectively. ESBL positive strains detected by the screening test for Klebsiella pneumoniae were 20(27.39%), for Escherichia coli were 16 (25.39%) and for Pseudomonas species were 03(21.42%), respectively. ESBL positive organisms were also found to remain positive by the Phenotypic confirmatory test, when combinations of Cefotaxime against amoxicillin /clavulanic acid and Cefipime against piperacillin/tazobactum were used. The novel fashion method showed that ESBL and de-repressed mutants in E.coli were 29(46.03%), only de-repressed mutants strains were 15 (23.80%) and inducible Amp C gene producers were 03(4.76%) . Among 48(65.75%) strains, Klebsiella pneumoniae showed ESBL and de-repressed mutants , de-repressed mutants alone in 08(10.95%) strains and inducible Amp C mutants in 02(2.73%). The antimicrobial susceptibility test showed that ESBL organisms were resistant to gentamicin and trimethoprim / sulphamethoxazole, but all were susceptible to imipenem, We conclude that clinical laboratories should develop quick screening methods to assess the different mechanisms of ESBL production, so that the patients can be treated with appropriate antibiotics .

Key Message: ESBL by phenotypic confirmatory test, novel fashion method

Key Words: ESBL, Gram negative bacilli, novel fashion method

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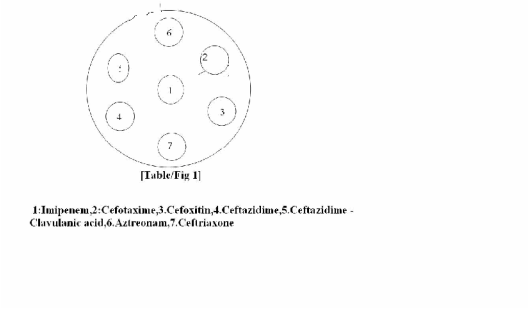
Introduction

The widespread use of antibiotics in hospitals has led to the emergence of multi-drug resistant organisms like Klebsiella spp, Pseudomonas spp , Escherichia coli and Enterobacter spp .Over the last few years, numerous outbreaks of infections with organisms producing extended spectrum beta lactamase (ESBLs), has been observed worldwide. The advent of ESBL producers has posed a great threat to the use of antibiotics like cephalosporins .There are indications that poor outcome occurred when patients with serious infections caused by ESBL producing organisms were treated with antibiotics to which the organisms were resistant .The aim of the present study was to study the prevalence of ESBL production among gram negative organisms by the different mechanisms and to analyse the antibiotic susceptibility of ESBL producing organisms.

MATERIALS AND METHODS

A total number of 160 clinical strains were included in the study .The strains were obtained from patients admitted to or those who attended the outpatient departments of medicine, surgery , obstetrics and gynecology at a tertiary care hospital. Out of the 160 patients, 87 were males and 73 were female patients in the age group of 18 years to 70 years. No specific criteria were followed in the selection of males and females. The study was conducted during the period from June 2005 to December 2005. Informed consent was taken from the patients before collecting the samples, which included urine, pus and sputum. The samples were processed for the identification of organisms according to W.winn et al[1] and were later screened for ESBLs. Screening for ESBL[2] was done according to the guidelines recommended by the Clinical Laboratory Standards Institute (CLSI). Control strains, Escherichia coli ATCC 25922 (Beta – Lactamase negative) and Klebsiella pneumoniae ATCC 700603(ESBL positive) were used for quality control. The screening test for ESBL was considered positive when an inhibition zone of <27 mm for cefotaxime and an inhibition zone of <22 mm for ceftazidime was indicated. ESBL producing organisms were further confirmed by the phenotypic confirmatory test .The method followed for the phenotypic confirmatory test was the antibiotic disc test, An amoxicillin - clavulanic acid (20/10 µg) disc was placed in the centre of the Muller Hinton culture plate, and a disc of cefotaxime was placed at a distance of 20 mm from the amoxicillin - clavulanic acid disc. The third antibiotic disc, aztreonam (30 µg) was placed at a distance of 25 mm from the amoxicillin-clavulanic acid disc and another antibiotic disc ceftazidime (30 µg) was placed at a distance of 30mm from the amoxicillin-clavulanic disc. In the same culture medium, a piperacillin – tazobactum (100 µg/10 µg) disc was placed at a distance of 25 mm from the cefipime disc, and on the opposite side, a tazobactum (110 µg) disc was placed at a distance of 25 mm from the cefipime disc. All the afore said antibiotic discs were

purchased from Hi-Media, ,Mumbai, India . The plates were incubated at 37^0C overnight, and were examined for formation of a synergistic zone or enhancement of zone of inhibition by the cefotaxime and cefipime disc at the side facing the amoxicillin-clavulanic acid (20/10 µg) disc and the piperacillin-tazobactum disc (100/10 µg) . The organisms that showed a clear extension of zone of inhibition towards the amoxicillin-clavulanic acid and the piperacillin-tazobactum discs were considered to be ESBL strains. Apart from the above procedure, a different method called the Novel fashion method for detection of ESBL[3] , was also studied for ESBL strains. The disc placements in the novel fashion method were as shown in the [Table/Fig 1].



[Table/Fig 1]

1:Imipenem,2:Cefotaxime,3.Cefoxitin,4.Ceftazidime,5.Ceftazidime - Clavulanic acid,6.Aztreonam,7.Ceftriaxone

The ceftazidime and ceftazidime-clavulanic acid discs were kept 15-20 mm apart from each other .Imipenem, an inducer, was placed in the centre. On either side of the imipenem discs, at a distance of 15 mm,the ceftazidime and cefotaxime discs were placed (indicators of induction). Another inducer, cefoxitin was placed at 15mm from cefotaxime (indicator). Cefoxitin discs were placed opposite the ceftazidime - clavulanic acid discs to avoid any effect of inducible beta -lactamase on the zone of inhibition of the latter. The remaining discs were placed as showed in [Table/Fig 1]. The criteria used in the Novel fashion method [3] for ESBL producer detection was, the resistance of the strain to 3rd generation cephalosporins, susceptibility of the strains to cefoxitin and the tendency of the strains to show increase of zone size by 5mm with the addition of an inhibitor. Inducible Amp C lactamase production was considered when there was **a** blunting of the zone towards the inducer, when there was no increase in zone size with the addition of an inhibitor and when the strain was susceptible to cefipime. De-repressed mutants were considered when the strain was resistant to cefotaxime and cefoxitin, when it showed blunting of the zone towards the inducer and when it showed no increase in zone size with the addition of an inhibitor. The strain was considered to have multiple mechanisms of resistance to cefoxitin, showed blunting of the zone towards the inducer and showed increase of zone size by 5mm with the addition of an inhibitor. The isolates were also tested for antimicrobial susceptibility by the Kirby-Bauer disc diffusion technique, using Muller Hinton agar [4] . Muller Hinton agar was purchased from Hi-media, Mumbai, India. In the Kirby-Bauer disc diffusion method , test strains were pre-incubated in peptone water at 37^0C at an optical density of 0.5Mc Farland standard .This suspension was used to inoculate the strains on to the Muller Hinton agar plate by swabbing them with a sterile cotton swab. The different antibiotic discs used for the disc diffusion test were amikacin(30µg), gentamicin (10µg), ciprofloxacin (5µg), trimethoprim/sulphamethoxazole(TSX) (1.25/23.75µg), netilmicin((30µg), cefotaxime(30µg) ,ceftazidime (30µg) and imipenem (110µg). The antibiotic discs were purchased from Hi-media, Mumbai, India .The plates were incubated at 37^0C overnight, a zone of inhibition was measured for each antibiotic and it was recorded as sensitive or resistant.

Results

73 strains of Klebsiella pneumoniae , 63 strains of Escherichia coli, 14 strains of Pseudomonas spp , 04 strains of Enterobacter spp and 06 strains of Acinetobacter spp were isolated from 160 strains. ESBL strains which tested positive by the screening test were 20(27.39%) Klebsiella pneumoniae, 16(25.2%)

Escherichia coli, 03(21.42%) Pseudomonas spp, 01(25%) Enterobacter spp and 01(17%) Acinetobacter spp. In the phenotypic confirmatory test, when the combinations of cefotaxime against amoxicillin /clavulanic acid and cefipime against piperacillin/tazobactum was used, the results showed that the strains which were positive by the screening test were also positive by phenotypic confirmatory test . The novel fashion method showed that ESBL and de-repressed mutants in E.coli were 29(46.03%), only de-repressed mutants strains were 15 (23.80%) and inducible Amp C gene producers were 03(4.76%). Klebsiella pneumoniae spp showed 48(65.75%) strains of ESBL and de-repressed mutants 08(10.95%) strains of de-repressed mutants alone and 02(2.73%) of inducible Amp C mutants [Table/Fig 2].

(Table/Fig 2) Percentage of β-Lactamase production by different mechanisms in Novel fashion method

Organism	ESBL+de-repressed	De-repressed	Inducible AmpC	Plain ESBL
E.coli (N *63)	29(46.03%)	15(23.80%)	03(4.76%)	16(25.3%)
Klebsiella (N73)	48(65.75%)	08(10.95%)	02(2.73%)	11(15%)
Pseudomonas (N14)	02(14.28%)	01(7.14%)	10(71.42%)	01(7.14%)
Enterobacter (N04)	02(50%)	02(50%)	0(0%)	01(25%)
Acinetobacter (N06)	03(50%)	02(33.33%)	01(16.66%)	01(16.66%)

Different mechanisms of Beta –Lactamase production were identified by the novel fashion method. Antibacterial susceptibility test to various antibiotics, indicated that 17 (85%) ESBL producing Klebsiella pneumoniae organisms were sensitive to amikacin and all ESBL strains were sensitive to imipenem [Table/Fig 3].

(Table/Fig 3) Antibiotic susceptibility test results of ESBL isolates

Organisms	Antibiotics				
	Amikacin (30µg)	Netilmicin (30µg)	Ciprofloxacin (5µg)	Trimethoprim-sulfamethoxazole (1.25/23.75 µg)	Gentamicin (10µg)
Klebsiella (20/73)	17(85%)	17(85%)	16(80%)	14(70%)	13(65%)
E.coli 16/63(25.39%)	16(100%)	15(93.7%)	15(93.7%)	12(75%)	7(43.7 %)
Pseudomonas 03/14(21.42%)	03(100%)	03(100%)	02(66.6%)	01(33.3%)	01(33.3%)

Discussion

The number of strains which tested positive by the screening test, were also found to be positive by the phenotypic confirmatory test. The percentage of ESBL producers detected by the phenotypic confirmatory method for Klebsiella pneumoniae strains were 20/73 (27.39%), for Escherichia coli were 16/63 (25.39%), for Pseudomonas spp were 3/14 (21.42%) , for Enterobacter spp were 1/4(25%) and for Acinetobacter spp were 1/6 (17%). These results were in concordance with the study conducted by C.Rodriques [3], David L Peterson [7] and S.Babypadmini et al[8] . The novel fashion method of study indicated that ESBL organisms like E.coli, Pseudomonas spp and Klebsiella pneumoniae produced various different mechanisms for the production of multiple β-Lactamases. . Our study was in concordance with the study conducted by C.Rodrigues et al[3]. The percentage of plain ESBL producers among Ecoli was 16(25.3%) and among Klebsiella pneumoniae were less frequent (15%), when compared to studies conducted by A Varaiya et al[9], and Pseudomonas spp had fewer plain ESBL producers when compared to studies conducted by C Rodrigues et al[3].The percentages of acinetobacter spp and enterobacter spp were 01(16.66%) and 01(25%), respectively, and the percentages of those producing plain ESBL was less, when compared to Mahua et al [10] A similar study conducted by Giuseppe Celenza[11] from a Bolivian hospital, showed a higher percentage (34%) of ESBL production. It could be because only a few strains of enterobacter spp and acinetobacter spp were isolated at our centre. De-repressed mutants in our study were 29(46.03%) for Ecoli and 02(14.28%) for Pseudomonas, which was less when compared to studies conducted by Rodrigues et al[3] . However, in our study, 48(65.75%) klebsiella pneumoniae, 03(50%) acinetobacter spp and 02(50%) enterobacter spp were both ESBL producers with

derepressed mutants, which was high when compared to studies conducted at Mumbai [3]. Pseudomonas spp 01(7.14%) and acinetobacter spp 02(33.33%) were detected to be de-repressed mutants, which was less when compared to studies conducted at Mumbai [3]. All the four organisms tested, namely Escherichia coli 3/63(4.76%), Pseudomonas spp 10/14(71.42%) , Klebsiella pneumoniae 2/73(2.75%) and acinetobacter spp 1/6(16.66%), were detected to produce inducible Amp C lactamase, except Enterobacter spp. The percentage of inducible Amp C lactamase production was higher when compared to studies done at Mumbai. At Mumbai, only 26.5% Pseudomonas spp showed inducible Amp C lactamase production. When the phenotypic confirmatory method was compared to the novel fashion method, the latter was found to be better than the former, because it assesses ESBL producers, de-repressed mutants, inducible AmpC lactamase production and multiple mechanisms in a single culture plate and it was also easy to perform. The antimicrobial susceptibility test showed that strains were resistant to gentamicin, netilmicin, ciprofloxacin and trimethoprim/sulphamethoxazole.

Susceptibility to imipenem and amikacin was found to be 14(100%) in Pseudomonas spp , 63(100%) in E.coli 63, 17(85%) in Klebsiella pneumoniae and the susceptibility to netilmicin for Pseudomons spp was 14 (100%) strains, for E.coli it was 15(93.75%) and for Klebsiella pneumoniae it was 17 (85%), but all ESBL producers were found to be susceptible to imipenem and amikacin. However, amikacin and carbapenems are usually used only as the reserve drugs . A similar study conducted by Hanstia JB et al [5] and Abigal S Mathai et al [6] showed 100% susceptibility to amikacin and imipenem. The marked increase in β-Lactamase production, including the high level constitutive ESBL producers (de-repressed mutants), have left us with few alternatives in combating serious infections.

Conclusion

Clinical laboratories must be aware of the importance of ESBL and plasmid mediated AmpC Beta- Lactamase production. Although CLSI recommendations exist, they are limited to ESBL producers of Ecoli and Klebsiella spp . No recommendations exist for ESBL detection and reporting for other organisms, or for the detection of Amp C Beta- Lactamases .Clinical laboratories need to develop quick screening methods to assess the mechanisms of Beta- Lactamase resistance in their isolates, so that appropriate antibiotics can be given to patients. Screening methods of ESBL with recommended zone size should be immediately applied to suggest the presence of an ESBL. From the study, it was apparent that various different mechanisms exist for production of multiple Beta-Lactamases, especially in places where newer Beta-Lactams were being routinely prescribed. The marked increase in Beta-Lactamase production and the high level constitutive producers (de-repressed mutants) with ESBL leave us with few alternatives in combating serious infections. Good infection control practice and careful introspection while prescribing Beta-Lactam drugs, with the background of high risk category in acquiring infections with ESBL producing organisms, have to be considered for good anti-microbial stewardship in hospitals. The battle between the antibiotics and bacteria possessing Beta-Lactamase, is far from over .Unfortunately, as new antibiotics are introduced that are capable of resisting the effects of bacterial Beta Lactamases, bacteria develop novel ways of overcoming the new antibiotics. A small number of new Beta-Lactamase antibiotics and Beta-Lactam inhibitors are undergoing development at the present time[12]. It is unclear whether we will be able to keep pace with bacterial genetic changes and be able to effectively treat gram negative infections in future.

References

[1]. WinnW.Jr, Allen S, Janda W et al.Koneman's color atlas and textbook of diagnostic microbiology.6th edn. 2006 Philadelphia USA.

[2]. Amitha Jain, Indranil Roy, Mahendra KGupta ,Mala Kumar and S.K. Agarwal .Prevalence of ESBL producing gram negative bacteria in septicaemic neonates in tertiary care hospital .J Medl Microbiology 2003; 52: 421-25 .

[3]. C.Rodrigues,P.Joshi ,SH Jain ,M Aiphons ,R Radhakrishna,A Mehata .2004. Detection of ß lactamases in nosocomial gram negative clinical isolates. JCM. 22(4):247-50.

[4]. Clinical and Laboratory Standards Institute, 2006. Performance standards for antimicrobial susceptibility testing; 16th informational supplement. CLSI document M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.

[5]. Hanstia JB ,Agarwal V,Patha KAA,Saoji AM.ESBL mediated resistance to 3rd generation cephalosporins in Klebsiella pneumonia ,Nagpur Central India.Indian Journal of Medical Research.1997;105:158-61.

[6]. Abigal S Mathai ,E Jesudasan MV,Jhon TJ: Ceftazidim resistance among Klebsiella pneumoniae in South India.Indian Journal Of Medical research .1995:102:53-88

[7]. David L Peterson MD. ß lactamase inhibitor and new ß lactamase :JCM.1999; 85-87

[8]. S Babypadmini, B Appalaraju:Extended spectrum –lactamases in urinary isolates of Escherichia coil and Klebsiella pneumoniae-Prevalence and susceptibility pattern in a tertiary care hospital:IJMM. 2004;22:3:172 - 74.

[9]. A Varaiya ,J Dogra ,M Kulkarni ,PBhalekar :Extended spectrum beta lactamase(ESBL) producing Escherichia coli and Klebsiella pneumoniae in diabetic foot infection:IJMM. 2009; 26:3:281- 82.

[10]. Mahua Sinha, H Srinivasa and R Macaden:ESBL-Production of Acinetobacter species :IJMR. 2007;126:63 – 67.

[11]. Giuseppe Celenza, Cristina Pellegrini, Marisa Caccamo ,Bernardella Segatore ,Gianfranco Amicosante and Mariagrazia Perilli :Spread of bla CTX- Mtype and bla PER-2 ß- Lactamase genes in clinical isolates from Bolivian hospitals. Journal of Antimicrobial Chemotherapy.2006;57(5):975 – 78.

[12]. K.Bush:Beta-lactamase inhibitors from laboratory to clinic.Clin Microbiol Rev. 1988;1(1):109 – 23.