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Research Article

ESBL- A CONTINUOUS DIAGNOSTIC CHALLENGE TO CLINICAL MICROBIOLOGY LABORATORIES.

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ABSTRACT: Objectives: Extended spectrum beta lactamase (ESBL) producers have posed a great threat to the use of many classes of antibiotics, particularly cephalosporins. Their detection has proved to be difficult for many laboratories because the resistant ESBL producing organisms appear to be susceptible by in vitro routine testing but result in treatment failure.The present study aims to detect the prevalence of ESBLs in organisms like E.coli and Klebsiella spp. which are responsible for many serious infections. **Method:** Isolates were screened for ESBL production using cefotaxime, ceftazidime and ceftriaxone by disk diffusion method. Isolates showing resistance to one or more than one of these drugs were futher subjected to Phenotypic Confirmatory Test (PCT) using CAZ/CAZ-CAC as per CLSI guidelines. **Results:** Of the 230 isolates, 116 (50.43%) tested positive by initial screening method. But on PCT only 94 tested positive. Out of 94 ESBL producers, 59 (62.76%) were E.coli and 35(37.23%) were Klebsiella spp. Of the various clinical samples urine 90(39%) showed maximum number of ESBL producers (32, 34%), followed by pus (27, 29%). Out of 230, 126 (54.7%) were females and 104 (45.2%) were males with a male to female ratio of 0.82:1 showing female preponderance. This study also showed increasing resistance to fluoroquinolones among ESBL producers. **Conclusion:** The results of our study show that there is an increased prevalence of ESBL producers in our tertiary care centre and also an increased resistance to fluoroquinolones among ESBL producers. Hence infections caused by E.coli and Klebsiella spp. which are prime producers of ESBL have to be considered seriously and proper screening methods and antibiotic policies have to be drawn to confine their spread.
Key words: ESBL, E.coli, Klebsiella spp, Phenotypic Confirmatory Test, Ceftazidime/ Ceftazidime-Clavulanic acid.

INTRODUCTION

Emergence of resistance to antibiotics has put burden on both clinical microbiologists as well as physicians in advocating proper antibiotics in hospitals. Spreading of resistance to commonly used antibiotics in both human and animal populations has posed adverse impact on morbidity and mortality due to diseases caused by resistant bacteria. Gram negative bacteria are the common pathogens causing wide spread infections, both nosocomial and community acquired. In the Gram negative bacteria, one of the important mechanisms of resistance is production of beta lactamases (Kolar et al., 2010). Since ESBL distribution has been shown to differ from region to region, establishing proper screening methods and a proper treatment protocol is the dictum of the day to limit them from spreading globally. There are more than 340 different beta lactamases so far identified and the growth spurt shows no signs of slowing (Koneman et al , 2006). The extended spectrum of beta lactamases belong to group 2be Bush's functional classification and are due to point mutation in original plasmid mediated TEM-1 and SHV-1 beta lactamases (Bush et al., 1995).

The first ESBL-producing organism was isolated in Germany in 1983. The ESBL enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but inactive against cephamycins and imipenem. These organisms exhibit resistance to many other classes of antibiotics resulting in limited therapeutic option (Alipourfard et al., 2010).
The indiscriminate use of antibiotics in hospitals has led to wide spread occurrence of these resistant organisms. Hence their detection calls for measures to be taken to control their further spread and eminent threat they pose on global health scenario. Clinical Laboratory Standards Institute (CLSI) has established ESBL confirmation method which is used worldwide (CLSI M100-S17, 2007).

MATERIALS AND METHODS:

A total of 230 non repetitive isolates of Enterobacteriaceae from various clinical samples were obtained from patients admitted to Bapuji and Chigateri government hospital, Davangere over a period of one year, from November 2010 to October 2011. The samples were processed and isolates were identified by standard laboratory methods. Only Escherichia coli and Klebsiella spp. were included in the study.
Antibiotic susceptibility testing was done on Muller Hinton agar (Hi Media Laboratory-Mumbai, India.) by Kirby Bauer's disk diffusion method as per CLSI guidelines. Antibiotics (in micrograms) used were ampicillin(10), cotrimoxazole(25), ciprofloxacin(5), norfloxacin(10) gentamycin(10), cefoxitin(30), cefotaxime(30), cefuroxime(30), ceftazidime(30), ceftriaxone(30), cefepime(30), imipenem (10),meropenem(10), (Hi Media-Mumbai) .

Screening method:

All isolates which showed resistance pattern to ceftazidime (zone of inhibition ≤ 22mm), cefotaxime(zone of inhibition ≤27mm), were considered to be probable producers of ESBL.

CLSI phenotypic confirmatory method:

According to CLSI, phenotypic confirmation can be performed by either broth microdilution method or by disk diffusion method. In the present study less laborious disk diffusion method has been adopted.
Disk diffusion method - A lawn culture of test organism was prepared on MHA plate. Ceftazidime and cefotaxime disks were placed alone and each in combination with clavulanic acid. Plates were incubated at 35^{0} C overnight. Interpretation of the result was done as follows- a ≥5mm increase in zone diameter for either microbial agent tested in combination with clavulanic acid versus its zone when tested alone confirms an ESBL producing organism.
To ensure quality control a non ESBL producing strain (E.coli ATCC 25922) and ESBL producing strain (Klebsiella pneumoniae ATCC 700603) were tested simultaneously while performing screening and phenotypic confirmatory tests.

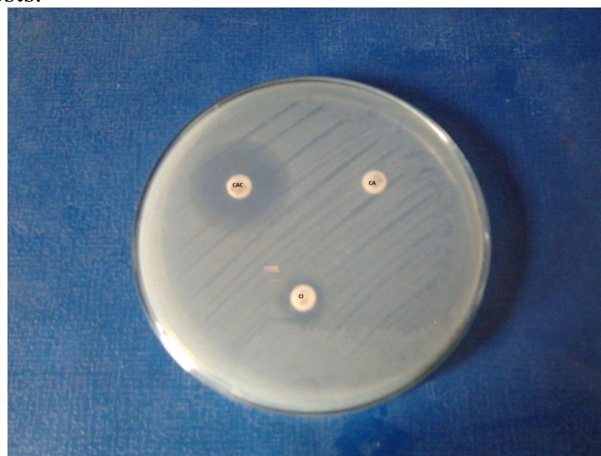

Fig1: Muller – Hinton agar plate showing an isolate resistant to ceftazidime (CA) and ceftriaxone (CI) along with an increase zone of inhibition around ceftazidime clavulanic acid (CAC)

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RESULTS

Of the various clinical samples tested during the study period, a total of 230 isolates were obtained. 104 isolates were from males and 126 were from females with a male to female ratio of 0.82:1. Out of 230 isolates, 90(39.1%) were from urine, 55(23.9%) from pus, 45(19.5%) from sputum, 20(8.69%) from blood and 20(8.69%) were from suction tips. Among 230 isolates, 143(62.17%) were E. coli, 87(37.8%) were Klebsiella spp. and 116 (50.43%) showed resistance to both ceftazidime and cefotaxime . These were suspected to be ESBL producers (screen test positive). By phenotypic confirmatory method 94(81.03%) were found to be true ESBL producers. Out of 94, 59(62.76%) were E. coli and 35 (37.23%) were Klebsiella spp.

The remaining 22 isolates (3-E.coli, 19-Klebsiella spp) which were negative by phenotypic confirmatory method could be co- producers of AmpC hence giving false positive results.

Table I: Isolates from various clinical samples.

Isolate	No.	%
E.coli	143	62.17
Klebsiella spp	87	37.82
Total	230	100

Table II: ESBL producers among the isolates.

Isolate	Screening positive	PCT positive
E.coli(143)	64(55.2%)	59(62.76%)
Klebsiella spp(87)	52(44.8%)	35(37.23%)
Total (230)	116	94

Table III: Specimen wise distribution of ESBL producers

Specimen	ESBL positives- No. (%)
Urine -90	32 , (34%)
Pus-55	27 ,(29%)
Blood-20	8 , (9%)
Sputum-45	13 , (14%)
Suction tips-20	14 , (15%)
Total-230	94 , (81.03%)

DISCUSSION

Infections caused by members of family Enterobacteriaceae are commonly treated using cephalosporins. Their indiscriminate use has resulted in the increase prevalence of ESBLs and AmpC producing organisms. With the discovery of ESBL and AmpC many clinical laboratories are facing problems in detecting these enzymes. There is a lot of confusion about the importance of resistance conferred by these enzymes, their detection methods and appropriate reporting of the same.

Failure in detection of these enzymes has lead to their uncontrolled spread and therapeutic failures (Rattan et al., 2005). Recent studies have revealed that serious infections such as septicemia caused by ESBL producing organisms have high fatality rate (Mehrgan et al ., 2008). The occurrence of ESBL among clinical isolates varies greatly worldwide and is rapidly changing over time (Babypadmini et.al 2001). ESBLs are typically inhibitor susceptible β- lactamases that hydrolyze penicillins, cephalosporins, and aztreonam. They are encoded by mobile genes. Most commonly found ESBLs belong to CTX-M, SHV, and TEM families (Bradford, 2001, Paterson, 2005). Since ESBL genes are transmissible, they are spreading from E.coli , Klebsiella and Proteus to many other organisms as well.
In our study urine was the most common source of ESBL producing isolates (34%). Out of 94 ESBL producing isolates, 62.76% were E.coli and 37.23% were Klebsiella spp which is similar to a study done by Alipourfard et al where urine (70.4%) was the most common source of organisms producing ESBL followed by blood (16.5%) and they found that 60% were E. coli and 40% were K. pneumoniae (Kenneth et al., 2010).Another study done by A Rattan et al., detected ESBL producing Klebsiella spp 73% and E.coli 62% . Our study shows a high rate of E.coli (62.17%) producing ESBL followed by Klebsiella spp (37.82%) which is similar to the findings of study done by Shahina Mumtaz et al where E.coli is the leading producer of ESBL followed by Klebsiella. In one study from Turkey the prevalence rate of ESBLs was 12-47%. This range is consistent with our finding of 40.86% of ESBL producers.

ESBL detection can be done either by doing confirmatory tests of screen positive or by doing confirmatory tests without prior screening as it does not have 100% sensitivity (CLSI M100-S15 2005). The Clinical and Laboratory Standards Institute has published guidelines for ESBL detection in Enterobacteriaceae specifically for E. coli, Klebsiella spp. and Proteus spp. In the UK, the Health Protection Agency (HPA) has also prepared guidelines. Screening with any of cefotaxime, ceftazidime, ceftriaxone, or aztreonam, followed by confirmatory tests using both cefotaxime and ceftazidime in combination with clavulanate or the Etest ESBL strips is also done by various researchers. The use of more than one antibacterial agent for screening improves the sensitivity of detection of ESBLs. If disk diffusion is used by the laboratory, a ≥5mm increase in zone diameter for either cefotaxime or ceftazidime tested with clavulanic acid versus its zone size when tested alone is considered a positive phenotypic ESBL test (Yagi et al ., 2005).

In our study, 116 isolates tested positive (resistance) to cefotaxime, ceftazidime and ceftriaxone. Of these 94(81.03%) showed increase in zone diameter (≥5mm) when tested with ceftazidime-clavulanic acid combined disk and ceftazidime alone kept at a distance of 20 mm center to center.
The remaining 22 (18.96) screen positive isolates could be co-producers of AMP-C β lactamases.
The CLSI recommended phenotypic confirmatory method fails to detect ESBL in the presence of AmpC as the latter is resistant to CA (WuTu et al., 2001). CA may induce high level expression of chromosomal AmpC, masking the synergy arising from the inhibition of an ESBL. Loss of outer membrane protein with co existing TEM-1 and SHV-1 β lactamases has been reported to give false positive results (Helene garrec et al., 2010). Of the 22 PCT negative isolates, 18 (81.81%) showed resistance to cefoxitin. This finding correlates with that of the study done by Nagarathnamma T et al.
The high sensitivity of the disk diffusion method by using three or more third generation cephalosporins has been reported by others, despite different settings and ESBL tested (HPA , 2008). HPA recommends testing cefpodoxime or both cefotaxime and ceftazidime as first screening test (Linscott et al., 2005). The ability of the combined disk method to detect ESBL is very satisfactory, and sensitivity can reach 100% when testing both cefotaxime and cefepime against group1-2 Enterobacteriaceae (CLSI M100-S20, 2010). Hence the feasible recommended approach for ESBL detection is to use a screening test, usually the routine susceptibility method used in the clinical laboratory, and to apply a confirmatory method dedicated to ESBL detection on all strains selected by the screening test (Khan et al ., 2008).

Many researchers have found that among the disk method, PCT has more sensitivity and specificity as compared to DDST (Shukla et al., 2004, Merela et al., 2011, Rashid et al., 2010). Confirmation should ideally be done using molecular methods like PCR or isoelectric focusing. Because of the skill and expertise involved in these advanced methods, E-Test can be used as next option. Studies have shown that the double disc method and E-test have almost same sensitivity and specificity (Merla et al., 2011). E-test though easier to do, it still remains expensive for routine use and interpretation is difficult when the enzymes are underexpressed.

The antibiogram pattern though have been studied by various other researchers, none of the patterns are identical to ours. In our study we found that the ESBL producing isolates showed increased resistance to quinolones (ciprofloxacin-89%,norfloxacin82%),cotrimoxazole(90%), ampicillin(100%) . 96.2% of our isolates showed sensitivity to Imipenem and meropenem. Our findings are similar to study done by Iraj Alipourfard , Rashid Ramazanzadeh and Morosini et al. Hence Carbapenems appear to be the drug of choice for ESBL producers. However, we need to keep in mind that the carbapenems are antimicrobials that are usually kept in reserve. In the case of non-life threatening infections and in non outbreak situations, it is not necessary to administer carbapenems. This approach intends to preserve the therapeutic value of these precious drugs. This could be due to the reason that ESBL is located on a plasmid that can be transferred from one organism to another rather easily and can incorporate genetic material coding for resistance to other antimicrobial classes.

CONCLUSION

All members of the Enterobacteriaceae are known to be potential ESBL producers, especially Escherichia coli and Klebsiella spp. High prevalence of ESBL producers in our hospital (81%) calls for strict policies regarding antibiotic usage and their screening methods. Combinations of a β-lactam with a β-lactamase inhibitor, e.g. piperacillin/tazobactam, are still good choices for therapy in the treatment of infections with these pathogens.

When the combination of a β-lactam with a β-lactamase inhibitor fails, third generation cephalosporins are still good options for therapy. However, local surveillance should be taken into account. For treatment of patients with an isolate showing lower susceptibility rates to these agents or an ESBL producing isolate, cefepime, a fourth-generation cephalosporin and

ultimately the carbapenems could be used (Sirot et al ., 2002). ESBL screening and confirmation can be done by using disk diffusion test and CLSI advised PCT to get a more reliable estimation of true ESBL-phenotypes as compared to more laborious broth microdilution method , expensive E-Test or molecular methods when the sensitivity and specificity is found to be almost equal. ESBLs occurrence and spread need to be controlled. Appropriate antimicrobial selection, surveillance systems and effective infection control procedures are required for the containment of their spread worldwide.

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RFERENCES

Alipourfard Iraj, Nilufar Yeasmin Nili. (2010). Antibiogram of Extended Spectrum Beta-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae isolated from Hospital Samples Bangladesh J Med Microbiol, 04 (01) : 32-36

Babypadmini S, Appalaraju B. (2004). Extended spectrum lactamase in urinary isolates o f E.coli and Klebsiella pneumoniae – prevalence and susceptibility pattern in a tertiary care hospital. Indian J. Med. Microbiol; 22:172-174

Bradford,P.A (2001). Extended spectrum beta lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev.; 14:933-951.

Bush k, Jacoby GA, Mederios AA.(1995). A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother ;39:1211-33

Clinical and Laboratory Standards Institute.(2005). Performance Standards for Antimicrobial Susceptibility testing; 15th Informational Supplement M100-S15 Clinical and Laboratory S tandards Institute,Wayne, PA, USA,

Clinical and Laboratory Standards Institute. (2007). Performance standards for antimicrobial susceptibility testing; 17thinformal supplement. CLSI document M100-S17. Wayne , PA, USA

Clinical and Laboratory Standards Institute. (2010). Performance standards for antimicrobial susceptibility testing. M100-S20. CLSI, Wayne, PA, USA

Hélène Garrec , Laurence Drieux-Rouzet , Jean-Louis Golmard ,Vincent Jarlier , and Jérôme Robert(2011). Comparison of Nine Phenotypic Methods for Detection of Extended-Spectrum ß-Lactamases (ESBL) Production by Enterobacteriaceae J. Clin. Microbiol. doi:10.1128/JCM.02130-10

Health Protection Agency.(2008). Laboratory detection and reporting of bacteria with extended spectrum β-lactamases - National Standard Method QSOP 51 Issue 2.2.
Kenneth S. (2010). Thomson Extended –Specrum-Beta-Lactamase, AmpC, and Carbapenemase Issues J Clin. Microbiol.; 48(4): 1019-1025

Khan MKR, Thukral SS, Gaind R. (2008). Evaluation of a modified doubledisc synergy test for detection of extended-spectrum β-lactamases in AMPC β-lactamase-producing Proteus mirabilis Indian J Med Microbiol ;26(1): 58-61.

Kolar, J.Bardon, M.Chroma, K.Hricova, T.Stosova et al.,(2010). ESBL and AmpC beta- lactamase producing Enterobacteriaceae in poultry in the Czech Republic Veterinarni Medicinia, 55 , (3): 119-124.

Linscott, A. J., and W. J. Brown.(2005) . Evaluation of four commercially available extended-spectrum beta-lactamase phenotypic confirmation tests. J Clin Microbio .; l43:1081-1085.

Mehrgan H, Rahbar M.(2008). Prevalence of extended-spectrumbeta-lactamase producing Escherichia coli in a tertiarycare hospital in Tehran, Iran. Int. J. Antimicrob. Agents; 31: 147-51.

Mirela Flonta, Ariana Almas. (2011). Methods for detection of beta-lactamase mediated resistance in E. coli and Klebsiella , Therapeutics, Pharmacology and Clinical Toxicology ; XV, 15(1).

Morosini, M.I., García-Castillo, M., Coque, T.M. et al. (2006). Antibiotic coresistance in Extended Spectrum- β lactamase-producing Enterobacteriaceae and invitro activity of tygicyclin Antimicrob AgentsChemother ; 50: 2695-2699.

Nagarathnamma T, R M. Shoorashetty , Prathibha J. (2011). Comparison of the Boronic acid disc potentiation test and Cefepime – clavulanic acid method for the detection of ESBL and AmpC producing Enterbacteriaceae. Indian J Med Microbiol, 29(3);297-301

Paterson, D.L., and R.A. Bonomo (2005). Extended-spectrum-beta lactamases: a clinical update. Clin. Microbiol. Rev.; 18: 657-686

Rashid Ramazanzadeh (2010). Prevalence and characterization of extended-spectrum -beta-lactamase production in clinical isolates of Klebsiella spp. African Journal of Microbiology Research ; 4 (13), 1359-1362,

Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Rattan.A. (2005). Evaluation of methods for AMPC-β-lactamase in gram negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol 23 (2) : 120-124

Shukla I, Tiwari R, (2004). Agarwal M Prevalence of Extended spectrum β-lactamase producing Klebsiella pneumoniae in a tertiary carehospital. Indian J Med Microbiol ;22 (2): 87-91.

Sirot J, Nicolas-Chanoine MH, Chardon H (2003). Susceptibility of Enterobacteriaceae to β-lactam agents and fluoroquinolones: a 3-year survey in France. Clin Microbiol Infect :207-213.

Washington C Winn, Stephen D Allen, William M Janda, Eimer W Koneman, Gary W Procop, C Schreckenberger, (2006). Gail LWoods - Koneman'S Color Atlas and Textbook of Diagnostic Microbiology.,6th ed Philadelphia, USA: Lippincott Williams & Wilkins; ;pp959-960.

Wu TL, Siu LK, Su LH,(2001). Outer membrane protein change combined with co-existing TEM-1 and SHV-1β-lactamases lead to false indentificationof ESBL-producing Klebsiella pneumonia. Antimcrob Agents Chemother ; 47: 755-61.

Yagi T,Wachino J , Kurokawa H, Suzuki S, Yamamone K, Doi Y. (2005). Practical methods using Boronic acid compounds to identification of class C β lactamases producing Klebsiella pneumonia and Escherichia coli J. Clin. Microbiol ;43:2551-8