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Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae

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A B S T R A C T

Antimicrobial resistance is a growing threat worldwide. Increasing resistance to third generation cephalosporins has become a cause for concern among Enterobacteriaceae. The prevalence of extended spectrum β-lactamases (ESBLs) and metallobetalactamases (MBL) among members of Enterobacteriaceae constitutes a serious threat to current β-lactam therapy leading to treatment failure. 100 Enterobacteriaceae isolated from various clinical samples were included in the study. ESBL was detected by phenotypic confirmatory disc diffusion test (PCDDT) using ceftazidime alone and in combination with clavulanic acid. MBL detection was done by Imipenem EDTA combined disc diffusion test. Out of 100 Enterobacteriaceae isolates, 43(43%) were ESBL producers and 18(18%) MBL producers. None of the isolates showed the coexistence of ESBL and MBL in the same isolate. ESBL and MBL production was observed in *E.coli*, *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp. isolated from various clinical samples. The study underlines problem of ESBL and MBL mediated resistance, which has created a therapeutic challenge for the clinicians and microbiologists. Simple disk method can be routinely employed to detect these common resistance mechanisms which will reduce the mortality and also spread of such resistant strains.

Introduction

The rapid and irrepressible increase in antimicrobial resistance of pathogenic bacteria is widely accepted as a major problem that has been observed over the last decade (Fam *et al*., 2006). Enterobacteriaceae have become one of the most important causes of nosocomial and community acquired infections. Beta-

lactams (mainly extended-spectrum cephalosporins and carbapenems) and fluoroquinolones constitute the main therapeutic choices to treat infections caused by these microorganisms (Coque *et al*., 2008). The enzymes, namely ß-lactamases, which are responsible for the wide-spread of ß-lactam resistance. These ß -lactamases hydrolyse the amide bond of the four-membered characteristic ß-lactam ring, thus rendering the antimicrobial ineffective (Prashant Durwas Peshattiwar and Basavaraj Virupaksappa Peerapur, 2011).

The introduction of the third generation cephalosporins was very much helpful in fighting against the beta-lactamases in clinical practice (Rajesh Kondian Rangachari *et al*., 2010). Resistance to third generation oxyimino-cephalosporins is mediated by extended spectrum beta-lactamase enzymes (Sridhar Rao *et al*., 2008). ESBL producing isolates, in addition to being resistant to ß-lactam antibiotics, often exhibit resistance to other classes of drugs such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones (Dinesh *et al*., 2011). ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species (Mark E. Rupp and Paul D. Fey, 2003).

Carbapenems represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant bacteria (Hodiwala *et al*., 2013). Due to their broad spectrum of activity and stability to hydrolysis by most β-lactamase, the carbapenems have been the drugs of choice for treatment of infections caused by penicillin or cephalosporin resistant gram negative bacilli (Nirav P. Pandya *et al*., 2011). But extensive and sometime unnecessary use of the carbapenems, poor sanitation and large population has facilitated the emergence of carbapenem resistant bacteria (Debasrita Chakraborty *et al*., 2010). Resistance to carbapenem is predominantly mediated by metallo-beta-lactamases, a class B type of betalactamases that recognize bivalent metal ions (Anil Rajput *et al*., 2012). The multidrug resistant isolates that are present in the ICU and in the hospital environment pose not only therapeutic problems but also serious concerns for infection control management (Varun Goel *et al*., 2013). Early detection of MBL and ESBL producing organisms is crucial to establish appropriate antimicrobial therapy and to prevent their interhospital and intrahospital dissemination (Nirav P. Pandya *et al*., 2011). So the present study was undertaken to detect ESBL and MBL in clinical isolates of Enterobacteriaceae.

Materials and Methods

A total of 100 clinical isolates of Enterobacteriaceae, which were isolated from various samples (blood, urine, sputum, exudates) were identified by standard procedures (Collee *et al*., 2007). The susceptibility of isolates to antibiotics was determined by Kirby- Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) guidelines (2011). Antibiotics included were aztreonam (30 µg), cefipime (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), imipenem (10 µg). Isolates resistant to the third generation cephalosporins were tested for ESBL production and isolates showing resistance to imipenem were tested for MBL production.

Detection of ESBL

This was performed by phenotypic confirmatory test as per the

recommendations of CLSI. The ceftazidime (30 μg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 μg discs) were used. An increase of ≥ 5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer.

Detection of MBL

This was performed by Imipenem EDTA combined disc test. Two (10 μg) imipenem discs were placed on a plate inoculated with the test organism, and 10 μl of 0.5 M EDTA solution was added to one disc. A zone diameter difference between the imipenem and imipenem + EDTA of ≥ 7 mm was interpreted as a positive result for MBL production.

Result and Discussion

Out of 100 Enterobacteriaceae isolates, 43 were ESBL producers and 18 MBL producers.

E. coli showed maximum ESBL production (50%). Maximum MBL production was seen in *Klebsiella* spp. (33.3%) and *Enterobacter* spp. (16.6%).

Majority of ESBL and MBL producers were from blood followed by sputum, urine and exudates.

The worldwide emergence of multi-drug resistant bacterial strains is a growing concern which are usually found in those hospitals where antibiotic use is frequent and the patients are in critical condition (Shahanara Begum *et al*., 2013). Broad resistance spectrum is a cause for concern and necessitates the restricted use of extended-spectrum cephalosporins, and a trial of other suitable alternatives (Amita Jain *et al*., 2003). Therapeutic options for the infections which are caused by the ESBL producers have also become increasingly limited (Metri Basavaraj *et al*., 2011). A study has found ciprofloxacin to be highly effective in treating multiresistant Gram-negative infections (Amita Jain *et al*., 2003). Recent studies on ESBL production among the members of Enterobacteriaceae which were isolated from clinical specimens, showed an increase in the occurrence of ESBL producers (Umadevi *et al*., 2011).

In our study of the 100 Enterobacteriaceae isolates, 43 were ESBL producers. ESBLs were predominantly present among *E. coli* 26(50%) compared to *Klebsiella* spp. 9(37.5%), *Enterobacter* spp. 6(33.3%) and *Citrobacter* spp. 2(33.3%). Majority of ESBL producers were from blood (50%). Our findings are similar to that of Nachimuthu Ramesh *et al.*, (2008) and Kumar *et al*., (2006) who reported a high prevalence of ESBLs among *E. coli*.

Correct identification of ESBL positive Enterobacteriaceae in due time is mandatory not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organisms (Irith Wiegand *et al*., 2007). Early detection will definitely help in controlling hospital infections which are caused by this group of organisms (Umadevi *et al*., 2011). The double disc synergy test (DDST) lacks sensitivity because of the problem of optimal disc space and the correct storage of the clavulanic acid containing discs. Assuming that a laboratory is currently testing the sensitivity for ceftazidime by using the disc diffusion test and it required only one disc

Table.1 ESBL and MBL producers among different isolates

Organism	No. of isolates	ESBL producers No. (%)	MBL producers No. (%)
E. coli	52	26 (50.0)	07 (13.4)
Klebsiella spp.	24	09 (37.5)	08 (33.3)
*Enterobacte*r spp.	18	06 (33.3)	03 (16.6)
Citrobacter spp.	06	02 (33.3)	00 (0)
Total	**100**	**43 (43.0)**	**18 (18.0)**

Table.2 Distribution of ESBL and MBL producers in various clinical specimens

Sample	No. of isolates	ESBL producers No. (%)	MBL producers No. (%)
Urine	26	11 (42.3)	03 (11.5)
Exudate	37	14 (37.8)	04 (10.8)
Blood	30	15 (50.0)	10 (33.3)
Sputum	07	03 (42.8)	01 (14.2)
Total	**100**	**43 (43.0)**	**18 (18.0)**

Figure.1 ESBL positive

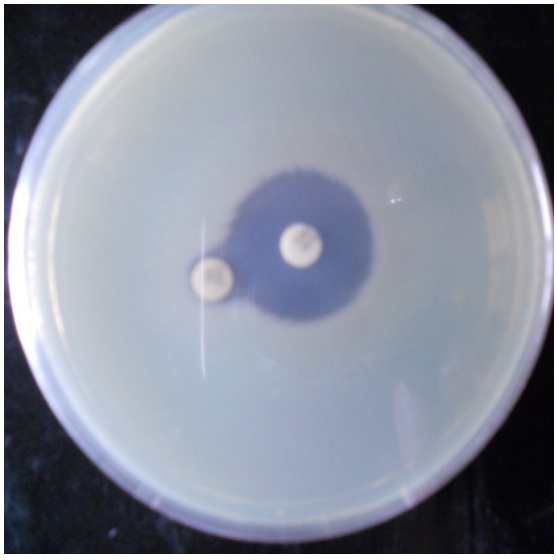
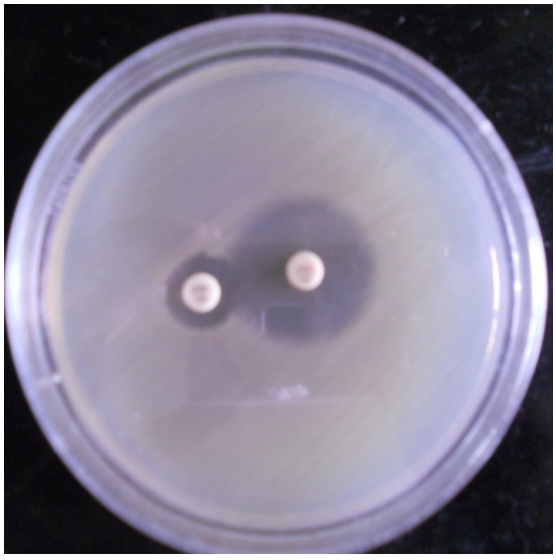


Figure.2 MBL positive



to be added to the sensitivity plate by phenotypic confirmatory disc diffusion test and would screen all gram negative bacteria in the diagnostic laboratory for ESBL production. This method is technically simple and inexpensive (Gaurav Dalela, 2012). The only β-lactam active against co-AmpC and ESBL producers are carbapenems; however, recently resistance to carbapenems has been increasing, which is mostly due to the production of metallo-β-lactamases (Supriya Upadhyay *et al*., 2010).

Metallo- β-lactamases (MBLs) are enzymes belonging to Ambler's class B that can hydrolyze a wide variety of β-lactams, including penicillins, cephems, and carbapenems except aztreonam (Hisaaki Nishio *et al*., 2004; Krishna,2010). Although, PCR method is simple to use in detecting MBL producing isolates, it has become more difficult with the increased number and types of MBL (Uma Chaudhary *et al*., 2008). Combined disc test is simple to perform and highly sensitive in differentiating MBL-producing isolates (Irene Galani *et al*., 2008).

Thus, implementation of simple method using imipenem- EDTA disk for MBL detection is quick, specific, sensitive and reproducible (Uma Chaudhary *et al*., 2008). Our study showed MBL production in 18 isolates with maximum production in *Klebsiella* spp. (33.3%), *Enterobacter* spp. (16.6%) and *E.coli* (13.4%) which is consistent with studies by Varun goel *et al*., (2013) and Pandya *et al*., (2011). Production of MBL has tremendous therapeutic consequences since these organisms also carry multidrug resistance genes and the only viable option remains the potentially toxic polymyxin B and colistin (Veenu gupta *et al*., 2013).

The early detection of beta lactamase producing isolates would be important for the reduction of mortality rates for patients and also to avoid the intra hospital dissemination of such strains. Simple phenotypic screening tests are proved to be rapid and convenient for their detection in the clinical laboratory. To overcome the problem of emergence and the spread of multidrug resistant organisms, a combined interaction and cooperation between the microbiologists, clinicians and the infection control team is needed.

References

Amita Jain, Indranil Roy, Mahendra K. Gupta, Mala Kumar and S. K. Agarwal. 2003. Prevalence of extended spectrum β lactamase producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. J. Med. Microbiol. 52: 421–425.

Anil Rajput, Bhavin Prajapati,.Bimal Chauhan, Atit Shah, Toral Trivedi, and Mina Kadam. 2012. Prevalence of Metallo-beta-lactamases (MBL) producing Pseudomonas aeruginosa in a Tertiary care Hospital. Indian. J. Basic.Appl. Med. Res. 1(4): 304-308.

Clinical Laboratory Standards Institutes. 2011. Performance Standards for antimicrobial susceptibility testing, XXI International Supplement (M100-S21). Wayne, Pennsylvania, US A: National Committee for C linical Laboratory Standards.

Collee, J.G., Barrie P. Marmion, AG Fraser, and A. S immons. 2007. Mackie and McCartney Practical Medical Microbiology, 14th ed. Edinburgh: Churchill Livingstone.

Coque, T.M., F baquero, and R canton. 2008. Increasing prevalence of ESBL producing Enterobacteriaceae in Europe. Eurosurveillance. 13, issue 47.

Debasrita Chakraborty, Saikat Basu and Satadal Das. 2010. A Study on Infections Caused By Metallo Beta Lactamase Producing Gram Negative Bacteria in Intensive Care Unit Patients. American. J. Infect. Dis. 6 (2): 34-39.

Dinesh, S. Chandel, Judith A. Johnson, Rama Chaudhry *et al*. 2011. Extended spectrum beta-lactamase-producing Gram-negative bacteria causing neonatal sepsis in India in rural and urban settings. J. Med. Microbiol.60: 500–507.

Fam,N., M. Diab, H. Helmi and I. El-Defrawy. 2006. Phenotypic Detection of Metallo-β-Lactamases and Extended Spectrum β-Lactamases among Gram Negative Bacterial Clinical Isolates. Egyptian. J. Med. Microbiol. 15 (4): 719-30.

Gaurav Dalela. 2012. Prevalence of Extended Spectrum Beta-Lactamase (ESBL) Producers among Gram Negative Bacilli from Various Clinical Isolates in a Tertiary Care Hospital at Jhalawar, Rajasthan, Indian. J. Clini.Diagnostic Res.6(2): 182-187.

Hisaaki Nishio, Masaru Komatsu, Naohiro Shibata *et al*. 2004. Metallo β Lactamase Producing Gram-Negative Bacilli: Laboratory-Based Surveillance in Cooperation with 13 Clinical Laboratories in the Kinki Region of Japan. J. Clini. Microbiol. 42(11): 5256–5263.

Hodiwala (bhesania), A., R. Dhoke, and A.D. Urhekar. 2013. Incidence of metallo-beta-lactamase producing Pseudomonas, Acinetobacter & enterobacterial isolates in hospitalised patients. Inter. J. Pharm. Biol. Sci. 3(1): 79-83.

Irene Galani, Panagiota Danai Rekatsina, Despina Hatzaki *et al*. 2008. Evaluation of different laboratory tests for the detection of metallo- β -lactamase production in Enterobacteriaceae. J. Antimicro. Chemothera. 61: 548–553.

Irith Wiegand, Heinrich K. Geiss, Dietrich Mack, Enno Stu¨renburg, and Harald Seifert. 2007. Detection of Extended Spectrum Beta-Lactamases among Enterobacteriaceae by Use of Semiautomated Microbiology Systems and Manual Detection Procedures. J. Clini. Microbiol. 45(4): 1167–1174.

Krishna, B.V.S., 2010. New Delhi metallo-beta-lactamases: A wake up call for microbiologists. Indian. J. Clini. Microbiol. 28(3): 265-66.

Mark E. Rupp and Paul D. Fey. 2003. Extended spectrum beta-lactamase (ESBL)-Producing Enterobacteriaceae. Drugs. 63(4): 353-365.

Metri Basavaraj C., Jyothi P., and Peerapur Basavaraj V. 2011. The Prevalence of ESBL among Enterobacteriaceae in a Tertiary Care Hospital of North Karnataka, India. J. Clini. Diagnostic Res. 5(3): 470-475.

MS Kumar, V Lakshmi, and R Rajagopalan. 2006. Occurance of extended spectrum β-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. Indian J. Med. Microbiol. 24(3): 208-11.

Nachimuthu Ramesh, Chettipalayam Samiappan Sumathi *et al*. 2008. Urinary tract infection and antimicrobial susceptibility pattern of extended spectrum β-lactamase producing clinical isolates. Adv. Biol. Res. 2(5-6): 78-82.

Nirav P. Pandya, Sweta B. Prajapati, Sanjay J. Mehta, Kunjan M. Kikani and Pratima J. Joshi. 2011. Evaluation of various methods for detection of metallo-β-lactamase (MBL) production in gram negative bacilli. Int. J. Biol. Med.Res. 2(3): 775-777.

Prashant Durwas Peshattiwar and Basavaraj Virupaksappa Peerapur. 2011. ESBL

and MBL Mediated Resistance in Pseudomonas aeruginosa. J. Clini. Diagnostic Res.5(8): 1552-1554.

Rajesh Kondian Rangachari, Mathavi Suresh Kumar and Indra Priyadharsini. 2010. Detection of extended spectrum beta-lactamase producing gram negative bacilli in urinary isolates. Int. J. Biol. Med. Res. 1(4): 130-132.

Shahanara Begum, Md Abdus Salam, Kh Faisal Alam, Nurjahan Begum, Pervez Hassan and Jalaluddin Ashraful Haq. 2013. Detection of extended spectrum β-lactamase in Pseudomonas spp. isolated from two tertiary care hospitals in Bangladesh. Biomedcentral Res. Notes. 6:7.

Sridhar Rao,P.N., K.G. Basavarajappa, and G. Leela Krishna. Detection of extended spectrum beta-lactamase from clinical isolates in Davangere. Indian. J. Pathol. Microbiol. 51(4): 497-99.

Supriya Upadhyay, Malay Ranjan Sen, and Amitabha Bhattacharjee. 2010. Presence of different beta-lactamase classes among clinical isolates of Pseudomonas aeruginosa expressing AmpC beta lactamase enzyme. J. Infect. Dev. Ctries. 4(4): 239-242.

Uma Chaudhary, Hemlata Bhaskar and Madhu Sharma. 2008. Imipenem-EDTA disk method for rapid identification of metallo- β -lactamase producing Gram-negative bacteria. Indian. J. Med. Res. 406-407.

Umadevi S, Kandhakumari G, Joseph NM, Kumar S, Easow JM, Stephen S and Singh UK. 2011. Prevalence and antimicrobial susceptibility pattern of ESBL producing Gram Negative Bacilli. J. Clini. Diagnostic Res. 5(2): 236-239.

Varun Goel, Sumati A. Hogade, and SG Karadesai. 2013. Prevalence of extended spectrum beta-lactamases, AmpC beta-lactamase, and metallo-beta-lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in an intensive care unit in a tertiary care hospital. J. Scientific Soc.40(1): 28-31.

Veenu gupta, Deepinder chhina and Amarjeet kaur. 2013. Incidence of metallo- β -lactamase (MBL) producing nonfermenters isolated from respiratory samples in ICU patients. Int. J .Pharm. Bio. Sci. 4(2): (B) 580 – 585.