

## Extended Spectrum Beta-lactamases, AmpC and Metallo Beta-lactamases in Emerging Multi-drug Resistant Gram-negative Bacteria in Intensive Care Units

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### Abstract

**Introduction:** Emerging multidrug resistance is a problem worldwide, particularly in the Intensive Care Units (ICUs). Here we present a cross-sectional surveillance study of resistance patterns of most seen Multidrug Resistant (MDR) Gram negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

**Methods:** The study was held in a tertiary care training and research hospital. MDR *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* strains were collected between 15th June 2011 and 15th June 2012, mainly from ICUs. Ceftazidime and ceftazidime-clavulanate; cefoxitin and cefoxitin-boronic acid; imipenem and imipenem- EDTA discs were used to detect extended spectrum beta-lactamase (ESBL), AmpC and metallo beta-lactamase (MBL), respectively.

**Results:** Totally 78 multi-drug resistant strains were isolated from the clinical specimens of the patients from ICUs: 12 *E. coli*, 15 *Klebsiella spp*, 7 *P. aeruginosa* and 44 *A. baumannii*. Amp-C beta-lactamase was present in 4 (33%), 7 (46.7%) and 41 (93%) of *E. coli*, *Klebsiella spp* and *A. baumannii* respectively. All of the *P. aeruginosa* strains showed Amp-C type beta-lactamase. All *E. coli* strains were susceptible to carbapenems and none showed MBL. None of 6 carbapenemase producing *Klebsiella* showed MBL as well. Only three (42.8%) *Pseudomonas* strains and 31 (70%) of *A. baumannii* strains were found to be MBL positive. ESBL was positive in 8 (66.7), 11 (73.3%), 1 (4.2%) and 2 (4%) of *E. coli*, *Klebsiella*, *P. aeruginosa* and *A. baumannii* strains respectively.

**Conclusion:** Amp-C, ESBL and MBL were the main resistance patterns of the strains evaluated in this study. Further phenotypic and genotypic studies based on these results are suggested.

**Keywords:** Multi-drug resistance; *Acinetobacter spp*, *Pseudomonas spp*, *E. coli*, *Klebsiella spp*, Amp-c; Metallo beta lactamase; Extended spectrum beta lactamase

### Introduction

The rapid global dissemination of *Enterobacteriaceae* harboring plasmid-borne extended-spectrum  $\beta$ -lactamases (ESBLs) and plasmid-mediated AmpC  $\beta$ -lactamases represents a significant clinical threat [1,2]. Beta-lactamases are the most important mechanism of drug resistance among Gram-negative bacteria. Extended spectrum  $\beta$ -lactamases (ESBLs) belong to Group 2be of Bush's functional classification [3]. AmpC beta-lactamases are well defined enzymes with broad substrate specificity and classified as class C according to Ambler and group 1 by Bush-Jacoby-Medeiros [4]. These enzymes, both chromosomal and plasmid mediated show an action spectrum similar to ESBLs [5]. Carbapenems are often considered as the last resort antibiotics in the treatment of infections due to clinical multidrug-resistant *Enterobacteriaceae* isolates, since they are stable even in response to extended-spectrum beta-lactamases (ESBLs) and AmpC enzymes. However, during the last decade carbapenem resistance has been increasingly reported among *Enterobacteriaceae* and is largely attributed to the production of Ambler class B acquired metallo-beta-lactamases (MBLs) [6].

Extended spectrum  $\beta$ -lactamase producing organisms confer resistance to penicillin, cephalosporins, and monobactams. They cannot hydrolyze cephamycins and are inhibited by Clavulanic Acid (CA) [7]. Like ESBLs, plasmid-mediated AmpC  $\beta$ -lactamases have a broad substrate profile that includes penicillin, cephalosporins, and monobactams. In contrast to ESBLs, they hydrolyze cephamycins and

are not inhibited by commercially available  $\beta$ -lactamase inhibitors [8,9].

Carbapenemases are  $\beta$ -lactamases, which include serine- $\beta$ -lactamases (KPC, OXA, GES, etc.) and metallo- $\beta$ -lactamases (MBLs). The latter require metal ion zinc for their activity, which is inhibited by metal chelators like EDTA and thiol-based compounds but not by sulbactam, tazobactam and clavulanic acid. MBL production is typically associated with resistance to aminoglycosides and fluoroquinolones, further compromising therapeutic options. Among the seven types of MBL genes described throughout the world, *bla*-IMP and *bla*-VIM are the most common [10,11]. The genes responsible for MBL production may be chromosomal or plasmid mediated and poses a threat of horizontal transfer among other Gram-negative bacteria [12].

### Materials and Methods

#### Bacterial isolates

A total of 78 consecutive non-repetitive clinical isolates of ESBL

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(+) *E. coli* [12], ESBL (+) *Klebsiella* spp., [13], MDR *P. aeruginosa* [7] and *A. baumannii* [14] were isolated from various clinical samples such as urine (n=26), deep tracheal aspirat (n=19), skin-mucosa (n=17), catheter (n=9), blood (n=4) cerebrospinal fluid (n=1), pleura (n=1) over a period of one year from 15th June 2011 to 15th June 2012, mainly from ICUs.

### Antimicrobial susceptibility testing

Bacterial identification was performed by Vitek 2 compact system (bioMerieux, France) with the GN cards, according to the manufacturer's instructions. Susceptibility of the isolates to antimicrobial agents was tested with AST-N266 cards for urine isolates, AST-N261 for the isolates other than urine, AST-N174 for non-fermenter isolates and gram-negative identification cards (GNID) in Vitek 2 compact system (bioMerieux, France). Additionally antibiotic susceptibilities were determined by Kirby-Bauer disk diffusion method and the results were interpreted according to the guidelines of the Clinical Laboratory Standard Institute [15]. The antibiotic discs used were ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), gentamicin (10 µg), imipenem(10 µg), meropenem (10 µg), piperacillin-tazobactam (100/10 µg), cefoxitin (30 µg), cefuroxime (30 µg), amoxicillin/ clavulanic acid (20/10 µg), aztreonam (30 µg) for *E. coli* and *K. pneumoniae*. Ceftazidime (30 µg), ceftriaxone (30 µg), cephoperazon-sulbactam (75/30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), gentamicin (10 µg), imipenem(10 µg), meropenem(10 µg), piperacillin-tazobactam (100/10 µg), cefoxitin (30 µg), cefuroxime (30 µg), amoxicillin/ clavulanic acid (20/10 µg), aztreonam (30 µg) and colistin(10 µg) were used for *P. aeruginosa*. Ceftazidime (30 µg), cephoperazon sulbactam (75/30 µg), ciprofloxacin (5 µg), netilmicin (10 µg), imipenem (10 µg), meropenem (10 µg), piperacillin-tazobactam (100/10 µg), cefoxitin (30 µg), ampicillin-sulbactam (10/10 µg), tigecycline (15 µg) and colistin (10 µg) were used for *A. baumannii*.

All of the 78 isolates were screened for ESBL production by CLSI phenotypic confirmatory test of double-disk diffusion method [15]. One disc of ceftazidim (30 µg, Bioanalyze) alone and one in combination with clavulanic acid (30 µg/10 µg, Bioanalyze) were placed at a distance of 20mm on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards, and incubated overnight at 37°C. The ESBL-producing strains showed at least 5mm differentiation between the inhibition zones around cefotaxime or ceftazidime discs alone in comparison with the inhibition zone around cefotaxime+clavulanic acid or ceftazidime+clavulanic acid discs. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative control strains respectively.

Totally 78 isolates were screened for AmpC production as described by Coudron [14]. Disks containing boronic acid were prepared as follows: Phenylboronic acid (120mg) (benzeneboronic acid; Sigma-Aldrich, Australia) was dissolved in 3ml of dimethyl sulfoxide. Three milliliters of sterile distilled water was added to this solution. Twenty microliters of the stock solution was dispensed onto disks containing 30 µg of cefoxitin. Disks were allowed to dry for 30 min and used immediately or stored in airtight vials with desiccant at 4°C. The boronic acid disc test was performed by inoculating Mueller-Hinton agar by the standard disc diffusion method and placing a disc containing 30 µg of cefoxitin and a disc containing 30 µg of cefoxitin and 400 µg of boronic acid onto the agar. Inoculated plates were incubated overnight at 35°C. An organism that demonstrated a zone diameter around the disk containing cefoxitin and boronic acid that was 5 mm or greater than the zone diameter around the disk containing cefoxitin

was considered as an AmpC producer. *K. pneumoniae* ATCC 700603 was used as a negative control strain.

All 78 isolates were screened for metallo-beta-lactamase production as described by Yong et al. [15]. A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA•2H<sub>2</sub>O (Sigmachemicals, Germany) in 1,000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. The mixture was sterilized by autoclaving. One disc of imipenem (10 µg) alone and one with imipenem (10 µg) in combination with EDTA were placed at a distance of 20 mm, from center to center, on a Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight at 35°C. The MBL producing strains showed a variation greater than 7 mm between the inhibition zone around imipenem discs alone and the inhibition zone around imipenem+ EDTA discs, and they showed a variation greater than 5mm between the inhibition zone around imipenem+EDTA discs and EDTA discs alone. *P. aeruginosa* ATCC 27853 was used as a negative control strain.

### Results

Of the total 78 strains 12 were *E. coli*, 15 were *Klebsiella* spp., 7 were *P. aeruginosa*, and 44 were *A. baumannii*. Among the 12 isolates of *E. coli* and 15 of *K. pneumoniae*, 8 (67%) and 11 (73%) isolates were found to be ESBL (+) by CAZ/CZC combined disc method, respectively. In contrast to this result, ESBL was present only two (4%) and one (14%) of *A. baumannii*, *P. aeruginosa*, respectively.

Among the 12 isolates of *E. coli* and 15 *K. pneumoniae*, 4 (33%) and 7 (47%) isolates were producing AmpC by cefoxitin-cefoxitin/boronic acid disc method, respectively. All of the isolates of *P. aeruginosa* (100%) and 41 (93%) of *A. baumannii* were AmpC (+) by the same method.

All *A. baumannii* isolates were carbapenem resistant whereas 5 (71%) of *P. aeruginosa* were resistant to carbapenems. MBL was present in 31 (70%) and 4 (57%) of *A. baumannii* and *P. aeruginosa* respectively by imipenem-EDTA disc method. Among 12 *E. coli* and 15 *K. pneumoniae* isolates, 2 *E. coli* and one *K. pneumoniae* showed resistance against carbapenem by disc diffusion method and Vitek 2 compact system. Carbapenem resistant *E. coli* isolates did not show MBL activity.

### Discussion

The infections which are caused by multidrug-resistant gram negative bacteria that produce various β-lactamase enzymes have been reported with an increasing frequency in the intensive-care units and they are associated with a significant morbidity and mortality [16]. The numerous β-lactamases are encoded either by the chromosomal genes or by the transferable genes which are located on the plasmids or the transposons [17]. These enzymes were commonly found in *Klebsiella* and *E. coli* species. However, recently these enzymes are reported to be produced by all members of *Enterobacteriaceae* and other gram negative bacilli [18,19].

In the study of Baykal et al. [20] ESBL was confirmed by combined disc method in each of 26 *E. coli* and 70 *K. pneumoniae* strains that were determined by initial screening test. In our study ESBL was confirmed in 19 of 27 (70%) isolates (12 *E. coli* and 19 *K. pneumoniae*), ESBL was detected by Vitek 2 compact system. Microorganisms that are producing enough AmpC beta-lactamase are typically positive according to ESBL screening criteria. However, sensitivity tests based on the increase in the presence of clavulanic acid are negative in AmpC [21]. In our study, among eight strains that were not represented ESBL

on the confirmation method, AmpC was detected in one whereas not in remaining seven isolates. On the other hand, in the laboratories using CLSI 2010 as a reference, the ESBL positive results of Vitek 2 compact system should be considered with suspicion and phenotypic confirmatory tests should be done. However, only susceptibility patterns of antibiotics will be given in the laboratories taking CLSI 2012 as a reference, therefore false positive results are insignificant. The increasing prevalence of AmpC  $\beta$ -lactamase resistance among *E. coli* and *K. pneumoniae*, which are the most commonly isolated species of *Enterobacteriaceae* in the clinical laboratory, is becoming a serious problem worldwide. High-level AmpC production is typically associated with in-vitro resistance to third-generation cephalosporins and cephamycins. In connection with this, high clinical treatment failures with broad-spectrum cephalosporins have been documented [22,23]. While the number of isolates of *E. coli* and *K. pneumoniae* is low in our study, AmpC was present in a substantial proportion. Therefore, these parameters should be considered in the initiating of treatment. The most immediate problem is detection of plasmid-encoded AmpC-mediated resistance in Gram-negative organisms but there is no exact guideline on this issue [24]. In a study by Coudron [14], only 55 of 271 screen-positive clinical isolates were AmpC-PCR-positive, and the boronic acid disk test detected 54 of the isolates in this group. This study demonstrated that the boronic acid inhibition method is very sensitive for detecting the presence of AmpC beta-lactamases [14]. In our study this method was used and a further PCR test was planned to perform for comparing the sensitivity.

*P. aeruginosa* may be intrinsically resistant or have acquired resistance to antibiotics due to permeability barrier of the cell surface, multidrug efflux pumps and production of  $\beta$ -lactamases (AmpC  $\beta$ -lactamase, extended spectrum  $\beta$ -lactamases and metallo- $\beta$ -lactamases) [25]. Multiple beta-lactamase producing *P. aeruginosa* can cause major therapeutic failure, and poses a significant clinical challenge if remain undetected. Therefore, early identification of the infections due to these microorganisms is necessary as the appropriate treatment might reduce the spread of these resistant strains as well as the mortality in hospitalized patients. This emphasizes the need for the detection of isolates that produce these enzymes to avoid therapeutic failures and nosocomial outbreaks [26,27]. All of our MDR *P. aeruginosa* strains were AmpC  $\beta$ -lactamase producers. Carbapenems were the effective antibiotics for MDR gram-negative bacteria infections, especially in high-risk hospital settings [28]. In a study from India, Vahdani et al. [29] reported 18% ESBL and 38% MBL in *Pseudomonas aeruginosa* strains. Our prevalence of ESBL and MBL in *Pseudomonas aeruginosa* does not correlate with the Vahdani's study (14% for ESBL positive and 57% for MBL positive strains). But the number of the isolates evaluated in our study is low for comparison. Besides, the proportion of AmpC was high and the production of MBL was 30% in isolates of this study. Therefore, when administering empirical treatment in patients with hospital-acquired infections due to *Pseudomonas* spp, if patients do not responding carbapenem therapy, MBL should be considered.

Reports on carbapenemase-producing *Acinetobacter* isolates are on rise globally due to increased carbapenem usage and selection of resistant bacteria under antibiotic pressure [30-32]. The current CLSI document has no guidelines for detecting MBLs, however, it has recommended modified Hodge test for detection of carbapenemases but in members of *Enterobacteriaceae* only [33]. Lee et al. [34] reported that the sensitivity of the detection of MBL by IMP / IMP-EDTA double-disk synergy method for *Acinetobacter* spp. is 100%. Jesudason et al. [17] used double-disk synergy method and found 72% MBL positive isolates among nonfermentative imipenem resistant gram-

negative bacilli. They also used a modified Hodge test however, double-disk synergy method was found to be more sensitive to detect MBL [17]. In our study, 31 of 44 (70%) *A. baumannii* strains were found to be producing MBL by IMP/IMP-EDTA method. However, as genotypic resistance genes are mainly includes blaVIM and blaIMP and we did not study these in our strains definitive conclusions are unlikely. The level of MBL production among MDR strains are in substantial rates in our study.

As conclusion, Amp-C, ESBL and MBL were the main resistance patterns of the strains evaluated in this study. Further phenotypic and genotypic studies based on these results are suggested.

## References

1. Bradford PA (2001) Extended-spectrum  $\beta$ -lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 14: 933-951.
2. Livermore DM (2003) Bacterial resistance: origins, epidemiology, and impact. *Clin Infect Dis* 36: S11-S23.
3. Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 39: 1211-33.
4. Babic M, Hujer AM, Bonomo RA (2006) What's new in antibiotic resistance? Focus on beta-lactamases. *Drug Resist Updat* 9: 142-156.
5. Barlow M, Hall BG (2002) Origin and Evolution of the AmpC  $\beta$ -Lactamases of *Citrobacter freundii*. *Antimicrob Agents Chemother* 46: 1190-1198.
6. Queenan AM, Bush K (2007) Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev* 20: 440-458.
7. Paterson DL, Bonomo RA (2005) Extended spectrum  $\beta$ -lactamases: A clinical update. *Clin Microbiol Rev* 18: 657-686.
8. Philippon A, Arlet G, Jacoby GA (2002) Plasmid-determined AmpC-type beta-lactamases. *Antimicrob Agents Chemother* 46: 1-11.
9. Rodriguez-Martinez JM, Pascual A, Garcia I, Martinez-Martinez L (2003) Detection of the plasmid-mediated quinolone resistance determinant qnr among clinical isolates of *Klebsiella pneumoniae* producing AmpC-type beta-lactamase. *J Antimicrob Chemother* 52: 703-706.
10. Uma Karthika R, Srinivasa Rao R, Sahoo S, Shashikala P, Kanungo R, et al. (2009) Phenotypic and genotypic assays for detecting the prevalence of metallo-beta-lactamases in clinical isolates of *Acinetobacter baumannii* from a South Indian tertiary care hospital. *J Med Microbiol* 58: 430-435.
11. Amudhan SM, Sekar U, Arunagiri K, Sekar B (2011) OXA beta-lactamase-mediated carbapenem resistance in *Acinetobacter baumannii*. *Indian J Med Microbiol* 29: 269-274.
12. Varaiya A, Kulkarni N, Kulkarni M, Bhalekar P, Dogra J (2008) Incidence of metallo beta lactamase producing *Pseudomonas aeruginosa* in ICU patients. *Indian J Med Res* 127: 398-402.
13. Clinical and Laboratory Standards (2008) Performance standards for antimicrobial susceptibility testing: Eighteenth Informational Supplement. USA.
14. Coudron PE (2005) Inhibitor-based methods for detection of plasmid-mediated AmpC beta-lactamases in *Klebsiella* spp., *Escherichia coli*, and *Proteus mirabilis*. 43: 4163-4167.
15. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, et al. (2002) Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 40: 3798-3801
16. Itokazu G, Quinn JP, Bell-Dixon C, Kahan FM, Weinstein RA (1996) Antimicrobial resistance rates among aerobic gram-negative bacilli recovered from patients in intensive care units: evaluation of a national postmarketing surveillance program. *Clin Infect Dis* 23: 779-784.
17. Jesudason MV, Kandathi AJ, Balaji V (2005) Comparison of the methods for the detection of the carbapenemase and the metallo-beta- lactamases production in the clinical isolates. *Indian J Med Res* 121: 780-783.
18. Mathur P, Kapil A, Das B, Dhawan B (2002) Prevalence of extended spectrum

- beta lactamase producing gram negative bacteria in a tertiary care hospital. *Indian J Med Res* 115: 153-157.
19. Kumar MS, Lakshmi V, Rajagopalan R (2006) The occurrence of extended spectrum  $\beta$  lactamases among the *Enterobacteriaceae* spp. isolated at a tertiary care institute. *Indian J Med Microbiol* 24: 208-211.
  20. Baykal A, Çöplü N, Şimşek H, Esen B, Gür D (2012) The presence of extended spectrum beta-lactamase, KPC-type carbapenemase and plasmid-mediated AmpC beta-lactamase in *E.coli* and *K.pneumoniae* strains isolated from blood cultures. *Mikrobiyol Bul* 46: 159-169.
  21. McFee RB (2009) Nosocomial or hospital-acquired infections: an overview. *Dis Mon* 55: 422-438.
  22. Klerens RM, Edwards JR, Richards CL Jr, Horan TC, Gaynes RP, et al. (2007) Estimating health care-associated infections and deaths in U.S. hospitals, 2002. *Pub Health Rep* 122: 160-166.
  23. Doi Y, Paterson DL (2007) Detection of plasmid-mediated class C beta-lactamases. *Int J Infect Dis* 11: 191-197.
  24. Hanson ND (2003) AmpC beta-lactamases: what do we need to know for the future? *J Antimicrob Chemother* 52: 2-4.
  25. Pai H, Kang CI, Byeon JH, Lee KD, Park WB, et al. (2004) Epidemiology and clinical features of bloodstream infections caused by AmpC-type-beta-lactamase-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 48: 3720-3728.
  26. Livermore DM (2002) Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? *Clin Infect Dis* 34: 634-640.
  27. Kumar V, Sen MR, Nigam C, Gahlot R, Kumari S (2012) Burden of different beta-lactamase classes among clinical isolates of AmpC-producing *Pseudomonas aeruginosa* in burn patients: A prospective study. *Indian J Crit Care Med* 16:136-140.
  28. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, et al. (2010) New Delhi Metallo-beta lactamase (NDM-1) in *Enterobacteriaceae*: treatment options with carbapenems compromised. *J Assoc Physicians India* 58: 147-149.
  29. Vahdani M, Azimi L, Asghari B, Bazmi F, Rastegar Lari A (2012) Phenotypic screening of extended-spectrum  $\beta$ -lactamase and metallo- $\beta$ -lactamase in multidrug-resistant *Pseudomonas aeruginosa* from infected burns. *Ann Burns Fire Disasters* 25: 78-81.
  30. Esterly J, Richardson CL, Eltoukhy NS, Qi C, Scheetz MH (2011) Genetic mechanisms of antimicrobial resistance of *Acinetobacter baumannii*. *Ann Pharmacother*.
  31. Taneja N, Singh G, Singh M, Sharma M (2011) Emergence of tigecycline and colistin resistant *Acinetobacter baumannii* in patients with complicated urinary tract infections in north India. *Indian J Med Res* 133: 681-684.
  32. Goel N, Wattal C, Oberoi JK, Raveendran R, Datta S, et al. (2011) Trend analysis of antimicrobial consumption and development of resistance in non-fermenters in a tertiary care hospital in Delhi, India. *J Antimicrob Chemother* 66: 1625-1630.
  33. Clinical Laboratory Standards Institute (2009) Performance standards for antimicrobial disk susceptibility testing. (9th edn) M2-A9, Wayne, PA.
  34. Lee K, Chong Y, Shin HB, Kim YA, Yong D, et al. (2001) Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect* 7: 88-91.