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 β-lactamases (ESBLs) and plasmid-mediated AmpC β-lactamases represents a significant clinical threat [1,2]. Beta-lactamases are the most important mechanism of drug resistance among Gram-negative bacteria. Extended spectrum β-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-betalactamases (MBLs) [6].

Extended spectrum β-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 β-lactamases have a broad substrate profile that includes penicillin, cephalosporins, and monobactams. In contrast to ESBLs, they hydrolyze cephamycins and

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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 uid (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 identi cation 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 identi cation cards (GNID) in Vitek 2 compact system (bioMerieux, France). Additionally antibiotic susceptibilities were determined by Kirby- Bauer disk di usion method and the results were interpreted according to the guidelines of the Clinical Laboratory Standard Institute [15]. e antibiotic discs used were ce azidime (30 g), ce riaxone (30 g), cipro oxacin (5 g), levo oxacin (5 g), gentamicin (10 g), imipenem(10 g), meropenem (10 g), piperacillin-tazobactam (100/10 g), cefoxitin (30 g), cefuroxime (30 g), amoxicillin/ clavulanic acid (20/10 g), aztreonam (30 g) for E. coli and K. pneumoniae Ce azidime (30 g), ce riaxone (30 g), cephoperazon-sulbactam (75/30 g), cipro oxacin (5 g), levo oxacin (5 g), gentamicin (10 g), imipenem(10 g), meropenem(10 g), piperacillin-tazobactam (100/10 g), cefoxitin (30 g), cefuroxime (30 g), amoxicillin/ clavulanic acid (20/10 g), aztreonam (30 g) and colistin(10 g) were used for P. aeruginosa Ce azidime (30 g), cephoperazon sulbactam (75/30 g), cipro oxacin (5 g), netilmicin (10 g), imipenem (10 g), meropenem (10 g), piperacillin-tazobactam (100/10 g), cefoxitin (30 g), ampicillin-sulbactam (10/10 g), tigecycline (15 g) and colistin (10 g) were used for A. baumannii

All of the 78 isolates were screened for ESBL production by CLSI phenotypic con rmatory test of double-disk di usion method [15]. One disc of ce azidim (30 g, Bioanalyze) alone and one in combination with clavulanic acid (30 g/10 g, 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. e ESBL-producing strains showed at least 5mm di erentiation between the inhibition zones around cefotaxime or ce azidime discs alone in comparison with the inhibition zone around cefotaxime+clavulanic acid or ce azidime+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. ree milliliters of sterile distilled water was added to this solution. Twenty microliters of the stock solution was dispensed onto disks containing 30 g of cefoxitin. Disks were allowed to dry for 30 min and used immediately or stored in airtight vials with desiccant at 4°C. e boronic acid disc test was performed by inoculating Mueller-Hinton agar by the standard disc di usion method and placing a disc containing 30 g of cefoxitin and a disc containing 30 g of cefoxitin and 400 g 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-betalactamase production as described by Yong et al. [15]. A 0.5 M EDTA solution was prepared C Z E J T T P M W J O H H P G E J T P E J V N & % Germany) in 1,000 ml of distilled water and adjusting it to pH 8.0 by using NaOH. e mixture was sterilized by autoclaving. One disc of imipenem (10 g) alone and one with imipenem (10 g) 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. e 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

on the con rmation 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 Vi4>sk 2

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