RESEARCH ARTICLE

Carbapenemases among *Acinetobacter* species isolated from NICU of a tertairy care hospital in Karachi

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Abstract

Objective: To determine the carbapenemases in carbapenem-resistant *Acinetobacter* species.

Methods: This descriptive, cross-sectional study was carried out at the Jinnah Postgraduate Medical Centre, Karachi, from March to December 2014, and comprised *Acinetobacter* species isolated from the clinical specimen collected from hospitalised neonates. The screening for carbapenem resistance was performed by meropenem and imipenem discs, and minimum inhibitory concentrations. SPSS 16 was used for data analysis.

Results: A total of 100 *Acinetobacter* isolates were included. The patients' age ranged from 1-28 days. The main species 95(95%) was *Acinetobacter* calcoaceticus-baumannii complex, followed by *Acinetobacter lwoffii* 5(5%). The overall resistance to carbapenem was 95(95%); it was higher 100 (100%) in *Acinetobacter lwoffii* in comparison to *Acinetobacter* calcoaceticus-baumannii complex 90 (94.7%). Phenotypic characterisation revealed that 89 (93.6%) of both the species were class D carbapenemase producers, 2 (2.1%) were metallo-β-lactamases and 4 (4.2%) were non-producers.

Conclusion: Among carbapenem-resistant *Acinetobacter* species, the class D carbapenemases were the main mode of resistance to carbapenems.

Keywords: *Acinetobacter calcoaceticus baumannii*, *Acinetobacter lwoffii*, Carbapenem-resistant, Carbapenemase.
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Introduction

Acinetobacter species is found in a variety of environments such as soil, water, animals and human skin. The classification of the organism has remained under revision and currently based on deoxyribonucleic acid (DNA)-DNA hybridisation studies; four species are found closely related to each other that cannot be distinguished phenotypically. The species are collectively known as *Acinetobacter calcoaceticus-baumannii* complex (ABC) accounting for more than 80% of infections caused by any *Acinetobacter* species.[1] The organism can survive for long period in different environments[2,3] including hospitals and has been isolated from hospitalised patients.[4] Therefore, many reports describe cross-infection between patients, particularly in case of longer stay in intensive care units (ICUs). Historically, *Acinetobacter* spp. have been considered health care-related pathogens, accounting for 1-3% of hospital-acquired infections, out of which 2-10% of infections occur in ICUs.[5] The situation becomes grave when there is an involvement of patients with prolonged antimicrobial therapy as it results in colonisation with multidrug-resistant (MDR) strains. Indeed, *Acinetobacter* sp. is also included in most common and serious MDR pathogens group, ESKAPE, i.e. *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* sp.[5,6]

Until recently, the carbapenems were considered as the last resort against the infections by *Acinetobacter* and were regarded as the most powerful antibiotics due to potent antibacterial activity and low toxicity. However, various mechanisms conferring to carbapenem-resistant Enterobacteriaceae (CRE) have been reported, notably through the production of carbapenemase enzymes. This recent emergence of carbapenems resistance in *A. baumannii* has become a global concern and has limited the choice of therapeutic agents. Non-existence of therapeutic agents with patient safety and predictable activity against *A. baumannii*[3] is a challenge for global healthcare in general and for countries likes Pakistan in particular. Owing to poor infection control practices and illegitimate use of antibiotics, *A. baumannii* has now emerged as a pandrug-resistant (PDR) pathogen in Pakistani hospitals.[6,7] Among carbapenemases, class D oxacillinases (OXAs) are the most common[8] and less frequent are metallo-β-lactamases (MBLs).[9,10]

Rapid detection of carbapenemase producers provides critical information for antibiotic stewardship and swift implementation of outbreak control measures.[11] In

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Pakistan, a limited work has been done on *Acinetobacter*, in general, and on carbapenemases production and carbapenem resistance, in particular. The current study was designed to determine the prevalence of carbapenemases in acinetobacter species in Pakistani hospitals. We used the RAPIDEC® CARBA NP (Biomeriux, France) first time in Pakistan for the rapid detection of carbapenemases in *Acinetobacter*.

Materials and Methods

This descriptive, cross-sectional study was carried out at the Department of Microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre (JPMC), Karachi, from March to December 2014, and comprised *Acinetobacter* species isolated from the clinical specimen collected from hospitalised neonates. The carbapenem-resistant *Acinetobacter* strains were collected from neonatal intensive care unit (NICU) of the hospital.

Approval for the study was obtained from the institutional review board and informed consent was taken from each guardian.

The sample size was calculated by OpenEpi calculator, and confidence level was set at 95% in the light of an earlier study.[9]

Isolates were initially identified by Gram-staining, followed by growth characterisation on blood and MacConkey agar, and analysis by standard biochemical methods including analytical profile index (API) 20NE (BioMerieux, France).

Using the Kirby-Bauer disc diffusion method, the isolates were tested for antimicrobial susceptibilities to antibiotics: piperacillin-tazobactam, ampicillin-sulbactam, cefepime, ceftazidime, ceftriaxone, meropenem, imipenem, amikacin, colistin, trimethoprim-sulfamethoxazole, tetracycline and gentamicin. The strains were classified as susceptible, intermediate or resistant according to Clinical Laboratory Standards Institute (CLSI) guidelines.[12] The minimum inhibitory concentration (MIC) for all isolates to imipenem and meropenem was also determined by Etest (BioMerieux, France). *Escherichia coli* (ATCC 25922) was used as a quality control strain.

Carbapenemase production was confirmed (Figure-1) by the modified Hodge test[13] (MHT) and Rapidec Carba NP (BioMerieux, France; Figure-2). Two different β-lactamase inhibitors (ethylenediaminetetraacetic acid [EDTA] and boronic acid) were used for the evaluation of class A and B production. A double-disc synergy test protocol recommended by Song et al.[14] was followed. The discs of 10μg meropenem and 400μg of phenylboronic acid (PBA) (Sigma-Aldrich, St. Louis, Missouri, United States) were placed on the inoculated plate, 15mm apart centre to centre, and incubated for 24 hours (Figure-3). In combination with β-lactamase inhibitors phenotypic double disc diffusion method, temocillin 30μg disc was also used for the OXA carbapenemase detection. Interpretation of carbapenemase by phenotypic methods was modified from the interpretation of Dijk et al.[15] *E. coli* ATCC 25922 and NCTC 13476 were used as negative and positive control, respectively.

The data was initially made on Microsoft Excel. SPSS 16 was used for data analysis. The frequencies were analysed and represented in percentages. Hypothesised percentage frequency of outcome of carbapenemases in the organism was 94±5% and the margin of error was taken as 5% at confidence level of 95%.

Results

A total of 100 *Acinetobacter* isolates were included. Of them, 35(35%) were from blood samples and 65(65%) from tracheal aspirates samples. Patients' age ranged from 1 to 28 days, with 47(47%) being 1-5 days old. Moreover, 71(71%) patients had fever and 57(57%) had respiratory distress syndrome. Interestingly, 26(26%) of the patients had normal white blood cell levels but 84(84%) had elevated levels of neutrophils (>70%) and 88(88%) had elevated levels of C-reactive protein (CRP >8 mg/dl). The gestational age of 61(61%) subjects were <38 weeks (Table-1).

The main species was ABC 95(95%), followed by *A. lwoffii* 5(5%). The isolates were tested for antibiotic sensitivity

Table-1: Demographic characteristics of subject in NICU (n=100).

Characteristics	No. of Pt (%)
Age (days)	
1-5	47
6-10	23
11-15	13
16-20	11
21-28	6
Male	59
Fever	71
WBC count >10×109/L	49
WBC count <3.5×109/L	25
Neutrophils >70%	84
Elevated CRP>8	88
GA at birth (weeks) <38	61
Respiratory distress syndrome	57

GA: Gestational age
CRP: C-reactive protein
NICU: Neonatal intensive care unit
WBC: White blood cells.

Table-2: Antibiotics resistance pattern in *Acinetobacter* species isolated in this study.

Antibiotics	Resistant Acinetobacter sp. (%)	
	***A. calcoaceticus baumannii* complex (n=95)**	***A. lwoffii* (n=05)**
Amikacin	95.78	100
Gentamicin	100	100
Ceftazidime	100	100
Cefepime	100	100
Piperacillin-tazobactam	100	100
Ampicillin-sulbactam	100	100
Imipenem	94.7	100
Meropenem	94.7	100
Trimethoprim- sulfamethoxazole	95.78	100
Tetracycline	83.15	80
Colistin	00	00

Table-3: Detection of cabapenemases (and their classes) in carbapenem resistant *A. calcoaceticus baumannii* complex and *A. lwoffii* by different phenotypic methods (n=95).

Phenotypic test	**No.** A. calcoaceticus ***baumannii* complex Positive (n=90)**	**No.A. lwoffii Positive (n=5)**	**No. of total positive (%)**
Modified Hodge Test	10	02	12 (12.6)
RAPIDEC CARBA NP	86	05	91(95.78)
Class B	00	02	02(2.1)
Class D	86	03	89(93.6)
Non-producers	04	00	04(4.2)

Figure-1: A Positive Control strain of *Pseudomonas aeruginosa* for Modified Hodge Test.

and it was found that the resistance to cefoperazone/sulbactam in *Acinetobacter* sp. was 100(100%), whereas the resistance to aminoglycosides, quinolones, and cephalosporins were 95(95%)-100(100%). The resistance to tetracycline was, however, 80(80%)-83(83%) (Table-2).

The overall resistance to carbapenem was 95(95%) but higher 05(100%) in *A. lwoffii* in comparison to ABC which was 90(94.7%). Phenotypic characterisation of carbapenemase revealed that 89(93.6%) of both the

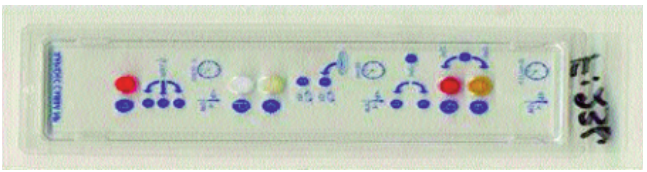

Figure-2: RAPIDEC® CARBA NP Positive for carbapenemase: Yellow color Shows positive and Red color indicate negative.

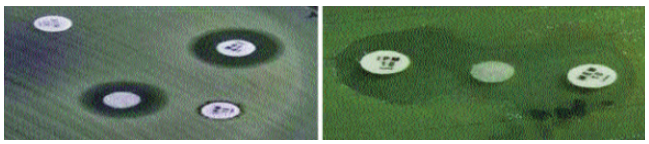

Figure-3: Sample no. F#78 is Negative (Left) *Acinetobacter* sp. and F#288 *Acinetobacter* sp. is Positive (Right) for EDTA synergy test.

species produced D carbapenemase (Table-3).

Discussion

Acinetobacter has emerged as a significant pathogen, posing a continuous threat and challenge to the healthcare system.[6,7] In addition to the severity of infections, the emergence of extensively drug-resistant (XDR) and PDR *Acinetobacter* species is aggravating the situation into a number of complications and deaths in hospitals. But limited data is available about crabapenem-resistant *A. baumannii* from Pakistan. In an earlier study, a high prevalence of MDR *A. baumannii* was found in healthcare facilities in Pakistan, with carbapenem resistance overriding.[16] In this study, the prevalence of *A. calcoaceticus baumannii* was found to be higher, which is consistent with a study conducted by Kaur et al (2014).

Surveillance by the ChiNet project in China revealed that the rate of resistance of *A. baumannii* to the carbapenem, such as imipenem, has been doubled from 30.1% in 2006 to 62.8% in 2013.[17] In Pakistan, the resistance to carbapenems in gram-negative bacteria was found to be increased by 11% from 2002 to 2012.[18] In this study, 95% of *Acinetobacter* species were found to be PDR, which indicates resistance to all classes of antibiotics except the colistin. This resistance pattern is much higher than 75.2% in Algeria and 60.9% in China,[17] whereas similar results to this study (higher resistance) was reported from Pakistan Institute of Medical Sciences (PIMS) Islamabad.[3] Interestingly, in this study, all strains were found sensitive to colistin, whereas up to 50% heteroresistance to colistin has been reported globally.[16]

The MHT showed low sensitivity (12.6%) to detect the carbapenemases, whereas sensitivity and specificity of Rapidec Carba NP were 100%. The Rapidec Carba NP is

more reliable and easy to perform test that does not need highly skilled personnel like in MHT because there is clear change in colour from red to yellow. The results of Rapidec Carba NP were confirmed by the polymerase chain reaction (PCR) using specific primers for carbapenemases at the Centre of Clinical Microbiology, Royal Free Campus, University College London, United Kingdom (UK) (data not shown). These results are in accordance with results of an earlier study.[19] The MHT have poor sensitivity and specificity to detect the carbapenemases in A.sp. because the OXA-type producer shows weak synergetic images when there is low production of carbapenemases. These findings are in accordance with those of Bonnin et al.[20] Out of the 95 carbapenem-resistant *Acinetobacter* .spp. only two strains were positive for MBL production; both strains were *A. lwoffii*, whereas all carbapenem-resistant strains of *A. calcoaceticus baumannii* complex were negative for MBLs. The MBLs in *A. baumannii* have been reported globally by several studies.[21-24] The common mode of resistance mechanism in the *Acinetobacter* .sp. was oxacillinases (Class D; 93.6%), which is in accordance with studies from Pakistan[9] and other countries. The present observations showed that only 2.1% carbapenem resistance in *Acinetobacter* .sp. was due to MBL production, which is in contradiction of a study[6] where all the *Acinetobacter* species were found to be MBL producers. This variation may be due to the presence of the same clone in that environment. The finding of the present study that the major mode of resistance are oxacillinases is in accordance with the findings from Pakistan.[9] This emphasises the necessity and urgency to deal with the dissemination of these superbugs. We recommend that those clinical laboratories without molecular set-up can use the Rapidec Carba NP test for routine screening of carbapenemases in imipenem/meropenem-resistant *Acinetobacter* sp.

Previous reports have shown that carbapenem resistance among *Acinetobacter* species ranged from 6% to 52% in Western countries, 2% to 26% in Asian/Pacific countries[25] and 74% in Egyptian intensive care units.[26] Our results indicate high prevalence of carbapenems resistance (95%) in comparison to developing countries which may result from poor hygiene and ineffective infection control practices in the hospital setting. This also represents a deficiency or failure of the strict measures to control hospital-acquired infections and the potential spread of emerging resistant pathogens. The study revealed that amongst carbapenem-resistant *Acinetobacter* .sp. the class D carbapenemases are the main mode of resistance to carbapenems.

Conclusion

The study revealed that amongst Carbapenem-resistant *Acinetobacter* sp. the class D carbapenemases are the main mode of resistance to carbapenems and RAPIDEC CARBA NP is reliable and sensitive tool for rapid detection of carbapenemases.

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