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Genotypic Studies of Carbapenem Resistant *Acinetobacter baumanii* (CRAB) with Special Reference to Presence of *OXA bla* Genes

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A B S T R A C T

Acinetobacter baumannii is a significant pathogen in health care settings, where it causes a multitude of infections that include bacteremia, pneumonia, meningitis and urinary tract and wound infections. The increasing importance of infections caused by *Acinetobacter* spp., and the multi-drug resistance of the strains render studies of epidemiology and antibiotic resistance necessary for the prevention of further infections with this organism. Hence the study was carried out with the following aim and objective - to screen the Carbapenem Resistant *Acinetobacter baumanii* (CRAB) isolates for the presence of *bla*OXA-23, *bla*OXA-24, *bla*OXA-51, *bla*OXA-58-like gene by PCR. Polymerase chain reaction (PCR) was performed for the detection of OXA (*bla*OXA 23 like, *bla*OXA 24 like, *bla*OXA-51 like and *bla*OXA-58 like genes). Out of 190, 63% (120/190) were CRAB strains of which (63/120) i.e. 53 % were found to be MBL producers by MHT. Among 120 *A. baumannii*, OXA genes were detected in 102 isolates. *bla*OXA 51 like (n = 100) and *bla*OXA -23 like (n = 98) were the most common and they coexisted in 96 isolates. *bla*OXA 24 and *bla*OXA 58 were not detected in any of the isolates. This study concludes that there is high incidence of Multi drug resistance seen in *Acinetobacer baumanii* (AB) isolates acquired from samples of hospitalized patients. *bla*OXA-23 like and *bla*OXA 51 like genes are the most common types of OXA carbapenamases contributing to carbapenem resistance in clinical isolates of *A. baumannii*. The co-production of OXA and metallo- betalactamases is not an uncommon phenomenon in *A. baumannii*. This will go a long way in early detection and also help control infections due to this highly resistant organism.

Introduction

Acinetobacter baumannii has emerged as an important nosocomial pathogen in outbreaks of hospital infections and is ranked second after *Pseudomonas aeruginosa* among nosocomial pathogens. *A. baumannii* infections are often difficult to eradicate due to high-level resistance to many antibiotics as a result of both intrinsic and acquired mechanisms. Lactamase production is the most important mechanism of acquired lactam resistance in gram-negative pathogens (Jeon *et al.*, 2005).

A. baumannii is often resistant to a wide variety of antimicrobial agents, including carbapenems (e.g., imipenem and

meropenem). Carbapenem resistance in *A. baumannii* is due to a variety of combined mechanisms such as hydrolysis by beta-lactamases, alterations in the outer membrane protein and penicillin-binding proteins and increased activity of efflux pumps.

Acquired resistance to carbapenems, mediated by the Ambler class D beta-lactamases or OXA type carbapenamases and Ambler class B metallo-betalactamases are of greatest concern as they are encoded by genes which are transmissible and account for most of the resistance to carbapenems (Peleg, *et al.*, 2008; Poirel, *et al.*, 2006).

Carbapenem-hydrolyzing *bla*OXA-23 was first reported in *A. baumannii* in 1985. Since then several of them have been reported worldwide. Four families of OXA genes have been identified in *A. baumannii*: *bla*OXA-23 like (*bla*OXA-23, *bla*OXA-27 and *bla* OXA-49); *bla* OXA-24 like (*bla*OXA-24, *bla*OXA-25, *bla*OXA-26 and *bla*OXA-40); *bla*OXA-58 and *bla*OXA-51 like.

The last group constitutes a family of chromosomal enzymes typically present in *A. baumannii* (Peleg, *et al.,* 2008; Poirel, *et al.*, 2006). Though the presence of *bla* OXA genes in *A. baumannii* is widely known, there is a paucity of information on the distribution of different types of OXA carbapenamases in isolates from the Indian subcontinent (Karunasagar, *et al.*, 2011).

Hence, this study was undertaken to detect the prevalence of different *bla*OXA-type carbapenamases among nosocomial isolates of *A. baumannii.*

Materials and Methods

This study was carried out at the Department of Microbiology, MGM Medical College and Hospital Navi Mumbai between January 2014 and November 2015.

A total of 120 non repetitive Carbapenem resistant *A. baumannii (CRAB)* isolated from various clinical specimens during the above period was included in the study. Polymerase chain reaction (PCR) was performed for the detection of OXA (*bla*OXA 23 like, *bla*OXA 24 like, *bla*OXA-51 like and *bla*OXA-58 like genes).

PCR was done for the detection of the four families of OXA-type carbapenamases found in *A baumannii*. Sequences of primers used for multiplex PCR for the detection of genes encoding *bla*OXA-23 like, *bla*OXA-24 like, *bla*OXA-51 like and *bla*OXA-58 like genes are given in Table 1.

The PCR conditions slightly modified were as follows: Initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 25 s, 53°C for 40 s and 72°C for 50 s, followed by an elongation step at 72°C for 6 min [(7)].

The PCR products of 501 bp (*bla*OXA-23 like), 353 bp (*bla*OXA-51 like), 246 bp (*bla*OXA-24like) and 599 bp (*bla*OXA-58 like) were visualized by agarose gel electrophoresis. In – house Positive Strain was used as positive control.

Results and Discussion

Of the 190 *Acinetobacter baumannii* (AB) isolates obtained from various clinical samples, 142(75%) were multidrug resistant. Out of 190, 63% (120/190) were CRAB strains of which (63/120) i.e. 53% were found to be MBL producers by MHT. Among 120 *A. baumannii,* OXA genes were detected in 102 isolates.

*Bla*OXA 51 like (n = 100) and *bla*OXA -23 like (n = 98) were the most common and they coexisted in 96 isolates. *bla*OXA 24 and *bla*OXA 58 were not detected in any of the isolates (Table 2 and 3).

Table.1 Details of primers used for detection of OXA carbapenamases gene by PCR with the amplicon size

PRIMER	SEQUENCE	AMPLICON LENGTH (BP)	REFERENCE
OXA23F	5`GATCGGATTGGAGAACCAGA3`	501bp	Woodford et al. (2006)(8)
OXA23R	5`ATTTCTGACCGCATTTCCAT3`		
OXA24F	5`GGTTAGTTGGCCCCCTTAAA3`	246 bp	Woodford et al. (2006)(8)
OXA24R	5`AGTTGAGCGAAAAGGGGATT3`		
OXA51F	5`TAATGCTTTGATCGGCCTTG3`	353 bp	Woodford et al. (2006)(8)
OXA51R	5`TGGATTGCACTTCATCTTGG3`		
OXA58F	5`AAGTATTGGGGCTTGTGCTG3`	599bp	Woodford et al. (2006)(8)
OXA58R	5`CCCCTCTGCGCTCTACATAC3`		

Table.2 Distribution of different types of *bla*OXA genes in the test organisms

OXA type	No. of isolates (n = 102)	Percentage (%)
***bla*OXA-23 like alone**	2	1.96%
blaOXA-24 like alone	-	-
***bla*OXA-51 like alone**	4	3.92
blaOXA-58 like alone	-	-
***bla*OXA-23 like and *bla*OXA-51 like**	96	94.11

Table.3 Detection of OXA genes from various clinical samples (n=120)

Types of samples	Presence of OXA gene	Percentage
Endrotracheal Secretion	44	43.13
Sputum	14	13.72
Pus	13	12.74
Blood	16	15.68
Suction Catheter tip	7	6.86
Tracheostomy tube	6	5.88
Tissue	2	1.96
Total	102	100%

Out of 190 *Acinetobacter baumannii* (AB), 63% (120/190) were CRAB strains. These

120 isolates were subjected to OXA gene detection by PCR. In this study, overall OXA carbapenamases were detected in 85% (*n* = 102) of carbapenem resistant *A. baumannii*. *bla*OXA-51 (*n* = 100) i.e 98% and *bla*OXA-23 (*n* = 98) i.e., 96% were the most common OXA carbapenamases and they coexisted in 96 isolates (94.11%). *bla*OXA-51 was found alone in four isolates (3.92%) and *bla*OXA-23 alone in two isolates (1.96%). *bla*OXA-24 and *bla*OXA-58.were not detected in any of the isolates. In the study by Anusha Karunasagar, Biswajit Maiti *et al.,* (2011) showed slight variation. The prevalence of *bla*OXA-51, *bla*OXA−23-like, *bla*OXA−24-like and *bla*OXA−58-like genes in *A. baumannii* was 100%, 47.9%, 22.9% and 4.2%.

In the study by Atul Khajuria, Ashok Kumar Praharaj *et al.,* (2011) blaOXA-23 like was detected in 55 isolates 55/105 (52.38%), blaOXA-51 like was detected in 47 isolates 47/105 (44.76%), blaOXA-58 like was detected in 15 isolates 15/105 (14.28%) and blaOXA-24 like was detected in 10 isolates 10/105 (9.52%). blaOXA-51 alone was detected in 2 isolates 2/105 (1.9%) while blaOXA-23 alone was detected in 16 isolates 16/105 (15.2%) Coexistence of blaOXA-23 with blaOXA-51 was detected in 33 isolates 33/105 (31.4%), which is slightly lower than our study. The study done by SM Amudhan, Sekar *et al.,* (2014) showed similar result as our study. Among 116 *A. baumannii,* OXA genes were detected in 106 isolates (91.37%). *bla*OXA 51 like (*n* = 99) i.e. 93.39% and *bla*OXA -23 like (*n* = 95) i.e. 89.62% were the most common and they coexisted in 89 (83.96%) isolates. *bla*OXA-24 like gene was detected in two isolates of which one also carried *bla*OXA-51 like and *bla*OXA-58 like genes. *bla*OXA-51 was found alone in nine isolates (8.49%) and *bla*OXA-23 alone in six isolates (5.6%). Through our studies we conclude that AB is a dangerous pathogen as high incidence of Multi drug resistance is common in these organisms. Also presence of high number of CRAB strains have shown that Carbapenems which are considered as life saving drugs for organisms which are resistant to 1st line antibiotics will soon not be useful for treatment of AB, unless judicious use of antibiotics is practiced & strict infection control measures are adopted by each and every health care institute.

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