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Research Article

PREVALENCE OF METALLO-B-LACTAMASES IN CARBAPENEM RESISTANT *ACINETOBACTER BAUMANNII* ISOLATED FROM TRACHEAL SECRETIONS

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ABSTRACT

Introduction: Though, β-lactams have been broadly used as the backbone for treating several bacterial infections, carbapenems are the last line of drug but since 2000, carbapenem resistance due to acquired carbapenemases has emerged and spread particularly from hospital-acquired infections with *Pseudomonas aeruginosa* and *Acinetobacter* spp. **Objective:** To determine the prevalence of MBL in carbapenem resistant *A. baumannii* from tracheal secretions. **Methods:** Totally 89 tracheal secretions from Doctors Diagnostic Centre, Tiruchirappalli were collected and processed based on standard procedures. **Results:** Of 89 tracheal secretions 16 were confirmed as MBL producers by phenotypic methods such as Modified Hodge test 7 (43.8%), Double disc synergy test 9 (56.3%). The antibiotic susceptibility profile showed 100% resistance to ceftriaxone, amoxicillin/clavulanate, co-trimoxazole, meropenem and ofloxacin and 93.8% resistance to ceftazidime, aztreonam, amikacin. Out of 16 isolates, 10 (62.5%) were resistant to both imipenem and meropenem, 6 (37.5%) to imipenem alone. Remarkably, all the 16 MBL producing *A. baumannii* isolates were MDR. **Conclusion:** With the growing clinical prominence of *A. baumannii* and the occurrence of MDR strains, new and novel therapeutics is essential to regulate its spread.

INTRODUCTION

Acinetobacter baumannii is a Gram-negative, pleomorphic bacterium. It is a non-motile, encapsulated, non-lactose fermenting isolates (Berezin and Towner, 1996). Recently *A. baumannii* has appeared as chief opportunistic and nosocomial pathogen globally (Choi *et al*., 2005). It causes variety of infection particularly in ICU patients and is responsible for the occurrence of multidrug-resistant (MDR) strains (Chatterjee *et al*., 2016). The different types of infections caused by *A. baumannii* include pneumonia (both hospital and community acquired), septicaemia, endocarditis, skin and soft tissue infections, urinary tract infections and meningitis. In most of the cases, it is believed that infections are developed after exposure to *A. baumannii* that stick it on contaminated hospital equipment or by interaction with healthcare recruits who exposed to the organism frequently (Rodriguez-Bano *et al*., 2009). In recent years, it has been labelled as a "red alert" human pathogen because it is generating an alarm among the medical community, arising largely from its extensive antibiotic resistance spectrum (Sebeny *et al*., 2008). The problem of resistance can also be happened by the inappropriate recommendation of antibiotic drugs by doctors. The Center for Disease Control and Prevention (CDC) estimates that 50-150 million antibiotic recommendations each year are unwanted and a session led by Levy found that above 80% of physicians had suggested antibiotics on demand against better judgment (Levy, 1998).

Carbapenems are used as last remedying antibiotics to treat the serious nosocomial infections caused by MDR strains (Lee *et*

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al., 2003). Even though carbapenem resistance is triggered by various mechanisms, the resistance due to carbapenem hydrolysing enzymes is now a universal problem (CLSI, 2013). Colistin (also known as Polymyxin E) is the only remaining agent to treat these MDR strains (Li *et al*., 2006), but now colistin-resistant clinical isolates is common (Gales *et al*., 2006). Carbapenem resistant *A. baumannii* (CRAB) is the most common carbapenem resistant organism associated with nosocomial infection where carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) is frequent (Tang *et al*., 2014; Christiansen *et al*., 2010; Kiratisin *et al*., 2012 and Koh, 2008). CRAB is accredited to several reasons including the production of oxacillinases (OXA) and metallo beta lactamases (MBLs), reduced expression of outer membrane proteins (29 kDa, 33-36 kDa) and effective efflux pumps (Higgins *et al*., 2009; Sinha and Srinivasa, 2007).

Production of carbapenemases with high ability and broad spectrum of activity against this organism has been a reason of fear to the clinician and the microbiologist (Peleg *et al*., 2008). The rapid and widespread distribution rate of these resistance genes creates an alarm among the scientists. So, the present study was pointed to assess the resistance pattern of *A. baumannii* to carbapenems and to spot the occurrence of MBL producing *A. baumannii* phenotypically.

METHODOLOGY

Collection of samples

Totally, 89 consecutive, non-repetitive tracheal secretions were collected, analyzed and the isolated cultures obtained from the Doctor's Diagnostic Centre (DDC), Tiruchirappalli. This study did not include any direct human sample collection and was executed only with the clinical isolates and the limited details from the patients reaching the DDC were obtained.

Isolation and Identification of bacteria

The bacterial isolates were screened by standard methods. The obtained isolates were cultured on its selective agar, MacConkey agar and Blood agar to examine their morphological appearances. A pure, well grown colony was taken and species differentiation was done by using conventional microbiological procedures (Collee *et al*., 1996).

Antibacterial disc susceptibility testing

The recovered bacterial isolates were exposed to antimicrobial sensitivity test by Kirby-Bauer disc-diffusion method (Bauer Kirby *et al*., 1966) and the results were recorded based on CLSI (CLSI, 2006). The antibiotic discs used (Hi-Media, India) were ceftazidime (CAZ), ceftazidime+tazobactam (CAT), ceftriaxone (CTR), imipenem (IMP), aztreonam (AT), amoxicillin/clauvlanate (AMC), amikacin (AK), levofloxacin (LE), co-trimoxazole (COT), polymyxin B (PB), colistin (CL), tigecycline (TGC), meropenem (MRP) and ofloxacin (OF). The results were recorded as sensitive and resistant. The strains sowed intermediate sensitive were interpreted as resistant.

Phenotypic methods

Modified Hodge Test (MHT)

One of the major method to identify carbapenemase production amongst the isolates is MHT. In brief, approximately 20ml of sterile Muller Hinton Agar (MHA) was poured on sterile petri plate. To the 5 ml of Muller Hinton broth or saline, 0.5 McFarland dilution of the *E. coli* ATCC 25922 was added and it was diluted to 1:10. From this, a lawn culture was taken and streaked on MHA plate and allowed to dry 3-5 minutes. On the streaked plate, 10µg meropenem antibiotic disc was placed in the center of the plate. Further, the test organisms were streaked from the edge of the disc to the edge of the plate. On the same plate, nearly four isolates can be tested. Then the plates were incubated at 37°C for 16-24 hours. A clover leaf-type indentation at the juncture of the test organism and *E. coli* ATCC 25922 was considered as MBL positive (Miriagou *et al*., 2010).

Combined Disc Test (CDT)

There was no CLSI procedure to detect MBL, the present research had been carried out with Yong *et al*. (2002) and Johann *et al*. (2005). The test bacterial suspension was prepared and incubated at 37°C for 2 hours, further the test organisms were inoculated on MHA. Imipenem (10 µg) and imipenem-EDTA (Ethylenediamine Tetraacetic acid**)** (10 µg + 750 µg) discs were placed at 15 mm distance from edge to edge. For MBL production, there will be a definite difference of ≥7 mm between zone of inhibition between imipenem and Imipenem-EDTA. Imipenem resistant strains showed ≥7 mm increase in zone of inhibition with imipenem-EDTA was considered as MBL producers. Certain isolates showing zone of inhibition < 7 mm or no zone of inhibition or total zone of inhibition < 13 mm with imipenem-EDTA disc were considered as MBL non-producers.

Double Disc Synergy Test (DDST)

The test isolate suspension was prepared and swabbed on MHA plates. Imipenem disc (10 µg) along with a blank filter paper disc which was loaded with 5 µl of 0.5M EDTA solution at 10-25 mm apart was placed on the plate. After 24 hours, the occurrence of an even very small synergistic inhibition zone was taken as positive result for MBL producers (Lee *et al*., 2003). This test has also been with meropenem and ceftazidime disc with 2 µl of concentrated 2-mercaptopropionic acid (Chakraborty *et al*., 2010).

RESULTS

Among 89 tracheal secretion, 31 isolates of *A. baumannii* were resistant to meropenem by the Kirby-Bauer disc diffusion method and only 16 were confirmed as MBL producers by phenotypic methods such as MHT 7 (43.8%), DDST 9 (56.3%) and CDT nil (0%). Of the 16 MBL producers, 15 were from males and 1 from female.

Table 1 Age and gender wise distribution of clinical isolates of MBL producing *A. baumannii*

Age group (years)	Male (no.)	Female (no.)	Total (no.) %
11-20	-	1	1 (6.3)
21-30	4	-	4 (25)
31-40	1	-	1 (6.3)
41-50	2	-	2 (12.5)
51-60	1	-	1 (6.3)
61-70	3	-	3 (18.8)
71-80	4	-	4 (25)

Most of the positive cases were belongs to the age group 21-30 (25%) and 71-80 (25%) (Table1). The location wise distribution demonstrated that most of the positive isolates were from Tiruchirappalli district (12/16), Thanjavur and Dindigul districts shares 3 and 1 isolates respectively.

Antibiogram results were presented in table 2 and the isolates showed 100% resistance to ceftriaxone, amoxicillin/clavulanate, co-trimoxazole, meropenem and ofloxacin and 93.8% resistance were observed to ceftazidime, aztreonam, amikacin. Least number of resistance was recorded to tigecycline (6.25%). All the strains were found to be sensitive to polymyxin B and colistin (100%). Out of 16 isolates, 10 (62.5%) were resistant to both imipenem and meropenem, 6 (37.5%) to imipenem alone. Surprisingly, all the 16 MBL producing *A. baumannii* isolates were MDR.

Table 2 Antimicrobial susceptibility profile of MBL producing *A. baumannii*

S. No.	Antibiotics	Resistant (%)	Sensitive (%)
1	Ceftazidime	15 (93.8)	1 (6.25)
2	Ceftriaxone	16 (100)	-
3	Aztreonam	15 (93.8)	1 (6.25)
4	Amoxicillin/Clavulanate	16 (100)	-
5	Amikacin	15 (93.8)	1 (6.25)
6	Levofloxacin	15 (93.8)	1 (6.25)
7	Co-Trimoxazole	16 (100)	-
8	Polymyxin-B	-	16 (100)
9	Colistin	-	16 (100)
10	Tigecycline	1 (6.25)	15 (93.8)
11	Imipenem	10 (62.5)	6 (37.5)
12	Meropenem	16 (100)	-
13	Ofloxacin	16 (100)	-
14	Ceftazidime/Tazobactam	14 (87.5)	2 (12.5)

DISCUSSION

In recent years the emergence of carbapenem resistant Gram-negative bacilli is a serious problem in hospital settings (Djahmi *et al*., 2014). In particular, MBLs producing *Acinetobacter* isolates are on rise globally due to increased carbapenem usage and selection pressure (Esterly *et al*., 2011; Taneja *et al*., 2011 and Goel *et al*., 2011). This study aimed to evaluate the prevalence of MBL producing *A. baumannii* phenotypically and reported that, 17.9% of MBL producing *A. baumannii* were recovered from tracheal secretion. Some previous reports found that, highest percentage of MBL producing *A. baumannii* from tracheal aspirates, *viz*., Salimizand *et al*.(2015) 82.5% (33 out of 40), Rynga *et al*. (2015) 31% and Noori *et al*. (2014) 52.8%. Kamalraj *et al*. (2015) recorded that, 13% of the meropenem resistant *A. baumannii* isolates were MBL producers, which was almost agreement with the current study where the prevalence of MBL producing *A. baumannii* is 17.9% while Uma *et al*. (2009) stated that, 70.9% MBL producing *A. baumannii* and an another study by Anil *et al*. (2011) from Kerala recorded that, 21% of the *A. baumannii* isolates were found to be MBL producers.

In the present study, the meropenem resistant isolates (31) were screened for the MBL production and 16 isolates were confirmed as MBL producers by phenotypic methods such as Modified Hodge test 7 (43.8%), Double disc synergy test 9 (56.3%) and Combined disc test nil (0%). Similarly, studies conducted by Anwar *et al*. (2016) and Pandya *et al*. (2011) observed that 77.2% and 81.4% were positive by DDST respectively. Kumar *et al*. (2011) reported that 71% of the isolates were carbapenemase producers by the MHT. This result was matched with the results proposed by Lee *et al*. (2003) in Korea, where 73% of the isolates were found to be carbapenemase producers by the MHT whereas, Irfan *et al*. (2008) from Karachi observed that, 96.6% carbapenem resistant strains were MBL producers by CDT. In the same way, Noori *et al*. (2014) identified 86.8% of isolates were MBL producers by CDT.

In this study most of the MBL producing isolates were from the males (93.8%) than females (6.2%). Correspondingly, Alm-El-Din *et al*. (2014) reported that, males (51.5%) are very prone than females (48.8%), Islahi *et al*. (2014) also observed a high percentage of nosocomial infections in males (76.0%) than females (23.9%), Peymani *et al*. (2011) in which 72% were male and 28% were females. Another study by Begum *et al*. (2013) from Islamabad reported that, 42.85% neonates had infections with MDR *A. baumannii*.

From the current study, it was clear that most of the *A. baumannii* isolates were resistant to the antibiotics used except polymyxin B, colistin and tigecycline. Overall, 100% resistance was observed against ceftriaxone, amoxicillin/clauvlanate, co-trimoxazole, meropenem and ofloxacin, 93.8% resistance to ceftazidime, aztreonam, amikacin and levofloxacin. These antibiotic susceptibility patterns was nearly comparable to the results of Islahi *et al*. (2014) who determined a resistance pattern of *Acinetobacter* species in hospitalized patients and they reported 84.7% resistance to amikacin, 86.9% to gentamicin, 89.1% to ceftriaxone and cefotaxime, 86.4% to ciprofloxacin, 10.6% to colistin sulphate and 32.7% to sulbactam/cefoperazone, Jaggi *et al*. (2012) stated that 90.3% were resistant to amikacin, 85.8% to gentamicin, 92.1% to ceftazidime, 89.7% to piperacillin, 91.6% to ciprofloxacin, 87.6% to levofloxacin, and 1.2% to colistin and Noori *et al*. (2014) reported that 95.4% of isolates were resistant to ceftazidime, 40.7% to gentamicin, 80.6% to amikacin, 92.6% to ciprofloxacin, 95.4% to piperacillin/tazobactam, and 1.8% to colistin sulphate.

Manjubhashini *et al*. (2015) stated that, 75% of the *Acinetobacter* were sensitive to colistin and polymyxin B by using Kirby Bauer disc diffusion method which was comparable to our results, this study stated that all the isolates were sensitive to polymyxin B (100%) and colistin (100%) followed by tigecycline (93.8%). Conversely, Abdel (2015) documented that, 99 *A. baumannii* strains showed the highest sensitivity to meropenem (74.4%), imipenem (76.55%), amikacin (65%) and the lowest sensitivity to cefoperazone (9.09%), ceftazidime (11%) and ciprofloxacin (12%) and 23 strains (23.33%) were sensitive to piperacillin/tazobactam. The study of Umadevi *et al*. (2011) showed that the majority of *Acinetobacter* species were susceptible to piperacillin/tazobactam (83%), imipenem (67%) and co-trimoxazole (67%), while being less susceptible to gentamicin (17%), amikacin (50%), ciprofloxacin (67%), tetracycline (50%), ceftriaxone (33%) and ceftazidime (33%). In the current study, 10 (62.5%) isolates of *A. baumannii* were resistant to both imipenem and meropenem and 6 (37.5%) were to imipenem alone. Likewise a study by Abdel (2015) showed that, 26 (26.26%) *A. baumannii* strains out of 99 strains were resistant to at least one or both carbapenem tested. Out of these

26 carbapenem resistant strains, 22 (84.61%) were resistant to both imipenem and meropenem, 3 (11.53%) to meropenem only and one strain (3.84%) to imipenem alone.

CONCLUSION

The present study gives an idea about the common trend of antibiotics resistance in this particular region and dejects the indiscriminate use of antibiotics. Chiefly might help the clinicians to start empirical therapy to manage critically ill cases with intubation prior microbiological culture and sensitivity report arrives. Also guides to develop local antibiotic policy and infection control procedures. Therefore, by this study we strongly recommend that a regular monitoring is required to establish reliable information about resistance pattern of clinical pathogens to restrict further cross resistance development.

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