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Research Article

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Characterization and Antibiotic Susceptibility Testing Of *Acinetobacter* Species in a Tertiary Care Hospital with Special Reference to NDM and OXA Genes

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ABSTRACT

Background-*Acinetobacter* has emerged as a nosocomial pathogen. It was sensitive to most antibiotics, but today it exhibits resistance to most first line antibiotics. Carbapenems are the drug of choice for treating this infection but now resistance to carbapenems is being reported worldwide. Antibiotic susceptibility pattern of *Acinetobacter* may vary geographically and between various units of the same hospital at various point of time. The variation in antibiogram necessitates a periodic surveillance. Hence this study was conducted to understand the difference in phenotypic and genotypic methods by detection of OXA and NDM genes for accurate identification of antibiotic resistance, thus enabling successful implementation of antibiotic policy. **METHODS-** 70 isolates of *Acinetobacter* were collected and speciated. Antibiotic susceptibility pattern of all the isolates was determined and the carbapenem resistant isolates were subjected to real-time PCR for identification of NDM and OXA genes. **RESULTS-** *A baumannii* was the most common (74.3%) species, followed by *A. lwoffii* (25.7%). *A. lwoffii* was 100% sensitive to carbapenems. 50 strains of *A. baumannii* were carbapenems resistant and remaining were susceptible. In Carbapenem resistant *A baumannii* OXA 23 and OXA 51 are the most common gene detected by realtime PCR, followed by OXA 48 and OXA 58, while NDM was detected in 100% strains. **DISCUSSION AND CONCLUSION-** Knowledge of NDM and OXA producing *Acinetobacter* is imperative in formulating Institutional antibiotic stewardship program and infection control practices to control the spread of carbapenem resistant strains of *Acinetobacterbaumanii*. This information is extremely valuable for pharmaceutical companies towards development of newer antibiotics.

Key Words: *Acinetobacter,* Carbapenems, OXA, NDM.

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INTRODUCTION

Acinetobacter species are ubiquitous organisms found in soil, food, water and also as commensals of skin, throat and various secretions of healthy people[1]. *Acinetobacter* has now emerged as an important nosocomial and opportunistic pathogen involved in infections of the urinary tract, wounds and burn and also associated with bacteraemia[2]. *Acinetobacter* generally has low virulence, but infections are common in immune compromised and neutropenic patients as it is becoming increasingly drug resistant. Acinetobacter species have been isolated from a wide range of clinical specimens including tracheal aspirate, blood culture, CSF and pus. A clear association has been found between hospital instrumentation and subsequent infection with *Acinetobacter*. Most of the isolates of *Acinetobacter* are obtained from nosocomial spread and colonization rather than de novo infections.

There is difficulty in treating patients in the intensive care unit as colonization is common and this makes it difficult to distinguish from an infection. In patients who are otherwise healthy, the prognosis of an isolated *Acinetobacter* infection is very good. But the outcome is poor in immune compromised patients[3]. Acinetobacter develop carbapenem resistance by production of class D β-lactamases, which consist of OXA-51, OXA-23, OXA-24 AND OXA-48-like genes and Class B metallo- β-lactamases, mainly VIM, IMP and SIM[4].

The antibiotic susceptibility pattern of various strains varies as per different geographical regions and also difference has been observed in different units of the same hospitals. Hence it is imperative to know the institutional prevalent antibiotic susceptibility pattern. Thus this study was conducted to speciate isolates of *Acinetobacter* from various clinical samples by a simplified phenotypic protocol to determine the antibiotic susceptibility pattern and also to identify carbapenemase resistant NDM and OXA genes.

MATERIALS AND METHODS

Thisprospective study was carried out for a period of one and a half year in theDepartment of Microbiology of a tertiary care hospital and Molecular Laboratory for Infectious diseasesafter approval from the Ethics Committee.70 consecutive non-repititive isolates from blood, pus, respiratory secretions, wound and other body fluids were included in the study.

The samples were processed as per standard protocol and the antimicrobial susceptibilitypattern was done as per latest Clinical and LaboratoryStandards Institute Guidelines[5]. There is no CLSI-validated phenotypic test to confirm the presence of carbapenemase activity in *Acinetobacter*; therefore, a molecular test (real-time PCR) was performed directly on this isolates[6]. Isolates of *Acinetobacter* species which were carbapenem resistant were subjected to real time PCR at Molecular Diagnostic Laboratory for the detection of resistant genes – NDM and OXA.

RESULTS

Of the total 70 isolates of *Acinetobacter*, 55 (78.6%) were isolated from blood, 4(5.7%) were from urine and sputum each and 7 (10.0%) were isolated from othertypes of samples such as tracheal secretion, endotracheal aspirate, wound swab,pleural fluid, central line tip and CBD stent (Table 1).

Table1. Distribution of type of samples studied (N=70)

Type of sample	No. of sample	% of sample
Blood	55	78.6
Urine	4	5.7
Sputum	4	5.7
Other	7	10.0
Total	**70**	**100.0**

Acinetobacterbaumannii(n-52, 74.3%) was the major species isolated in this studyand the remaining 25.7% isolates were *Acinetobacterlwoffii*(n-18, 25.7%)(Table 2).

Table 2. Distribution of Acinetobacter species isolated (N=70)

Acinetobacter species	No. of samples	% of sample
A baumannii	52	74.3
A lwoffii	18	25.7
Total	**70**	**100.0**

Antibiotic susceptibility testing was performed on all the isolates of *Acinetobacter* by Kirby Bauer Disc Diffusion Method and Colistin by E strip. The results were readaccording to CLSI guidelines. All the isolates were sensitive to Colistin (100%).Susceptibility to Netilmycin was seen in 33 isolates (47.1%), followed by 24 isolates(34.3%) each were sensitive to Ampicillin-sulbactam (10/10µg) and Ciprofloxacin(5µg), 23 isolates (32.9%) were senstitive to Piperacillin-tazobactam (100/10µg) and20 isolates (28.6%) each were sensitive to Ceftazidime (30µg), Imipenem (10µg),Meropenem (10g), Gentamicin (10µg), Trimethoprim-sulfamethoxazole(1.25/23.75µg) and Piperacillin (100 µg) respectively. Ceftriaxone susceptibility wasseen in only 16 (22.9%) of the isolates (30µg) (Table 3).

Table 3. Distribution of antibiotic susceptibility testing of Acinetobacter species (CLSI guidelines)

	Resistant		Sensitive	
	N	**%**	**N**	**%**
Ampicillin-sulbactam (10/10 µg)	46	65.7	24	34.3
Ceftazidime (30 µg)	50	71.4	20	28.6
Imipenem (10 µg)	50	71.4	20	28.6
Meropenem (10 µg)	50	71.4	20	28.6
Gentamicin (10 µg)	50	71.4	20	28.6

Ciprofloxacin (5 µg)	46	65.7	24	34.3
Piperacillin-tazobactam (100/10 µg)	47	67.1	23	32.9
Ceftriaxone (30 µg)	54	77.1	16	22.9
Amikacin (30 µg)	48	68.6	22	31.4
Trimethoprim-sulfamethoxazole (1.25/23.75 µg)	50	71.4	20	28.6
Netilmycin	37	52.9	33	47.1
Piperacillin (100 µg)	50	71.4	20	28.6
Colistin (Estrip)	0	0.0	70	100.0

Since all isolates of *A. Lwoffii* were susceptible to Carbapenem (Imipenem andMeropenem) they were not processed for NDM and OXA detection by PCR.

As 2 isolates of *A. Baumannii* were susceptible to carbapenem, they were not processed for PCR. 50 isolates of *A. Baumannii* were included for PCR testing for detection of NDM and OXA genes.

NDM gene was detected in all 50 isolates of Carbapenem resistant *A baumannii*.OXA gene was detected in 49 (98.0%) out of 50 isolates, only one isolate wasdevoid of any OXA gene. Distribution of various types of OXA genes was as follows:OXA 23 was seen in 49 (98.0%) isolates, followed by OXA 51 which was seen in 47isolates (94.0%),OXA 48 in 8 isolates (6.0%) and OXA 58 in only 3 isolates (6.0%)(Table 4 & 5)

Table 4 Distribution of presence of NDM genes (N=50)

NDM genes	**No of samples**	**% of sample**
Negative	0	0.0
Positive	50	100.0
Total	**50**	**100.0**

Table 5 Distribution of prevalence of OXA genes

OXA genes	**No of samples**	**% of sample**
OXA 23	49	98.0
OXA 51	47	94.0
OXA 48	8	16.0
OXA 58	3	6.0

DISCUSSION

In this study a total of 70 isolates of *Acinetobacter*spp were obtained. Most of the isolates of *Acinetobacter* were isolated from blood culture i.e 55 (78.6%),followed byurine and sputum each 4 (5.7%) and the remaining 7 (10.0%) isolates were obtainedfrom other clinical specimen such as tracheal secretion, endotracheal aspirate,wound swab, pleural fluid, central line tip and CBD stent. Our findings are consistentwith a study conducted by Gupta et al who found that *Acinetobacterspp* were predominantly isolated from blood culture sample 41 (36.9%), followed by pus 25(22.5%), respiratory samples 16(14.4%), urine 13 (11.7%), other body fluids 10 (9%)and various catheter tips 6 (5.4%)[7]. Other studies have shown that rate of isolation of *Acinetobacter* from pus samples to be 86.2% and 29%by Oberoi et al and Salmani et al respectively, whereas in a study done by Lahiri etal the maximum isolation (51.3%) was from urine samples[7]. This difference in therate of isolation of *Acinetobacter* in our study could be due to the difference in theinclusion criteria of the study or the study population that was involved. Thedifference can also be due to the smaller sample size of our study.

The most common species isolated in our study is *A. baumannii*(74.3%), followed by*A. lwoffii*(25.7%). In a similar study done by Malathy et al. 77.5% *A. Baumannii* and 22.5% *A. Lwoffii* were isolated[8]. Kalidas and Saha (2014)

reported 74.02%of the total *Acinetobacter* isolates as *A. Baumannii* followed by *A. lwoffii*at 14.2%[4]**.** Our findings are similar to all the studies mentioned above.

Antibiotic susceptibility testing of *Acinetobacter* shows that all the isolates were sensitive to Colistin (100%). Susceptibility to Netilmycin was seen in 33 isolates(47.1%), followed by 24 isolates (34.3%) each were sensitive to Ampicillin-sulbactam(10/10µg) and Ciprofloxacin (5µg), 23 isolates (32.9%) were senstitive to Piperacillintazobactam(100/10µg) and 20 isolates (28.6%) each were sensitive to Ceftazidime(30µg), Imipenem (10µg), Meropenem (10g), Gentamicin (10µg), Trimethoprimsulfamethoxazole(1.25/23.75µg) and Piperacillin (100 µg). Ceftriaxone susceptibility was seen in only 16 (22.9%) of the isolates (30µg). Shareek et al reported that 75%of strains were resistant to carbapenems and only 25% were sensitive tocarbapenem. Even in our study high level of Carbapenem resistance i.e. 71.4% was seen where as only 28.6% of the isolates were sensitive for carbapenems[7].This study shows that isolates of *A. Baumannii* were multi-drug resistant whereas *A.lwoffii* were relatively sensitive species. *A. Baumannii* has been reported to exhibitresistance to carbapenems which is not the case with other *Acinetobacter*species. In our study, *A. Baumannii* was resistant to Imipenem and Meropenem (96.2%),compared to *A. Lwoffii* which recorded a high susceptibility of 100% to theseantibiotics. Our findings are similar to studies conducted by Victor et al who observed72% *A. Baumannii* resistant to Meropenem and 100% *A. Lwoffi* susceptible to it [9]. Low resistance to various antibiotics in *A. Lwoffii* could be due to absence of efficientantibiotic resistance capturing system or as a result of its low dissemination in thehospital environment[10]. The emergence of carbapenem resistance particularly found in *A. Baumannii* can be attributed to antimicrobial inactivating enzymes, reducedaccess to bacterial targets and mutations which change the bacterial targets. The global spread of resistant strains of *A. baumannii*is a major challenge for the healthcare industry[11].

Real-time PCR for identification of NDM and OXA genes was performed onCarbapenem (Imipenem and Meropenem) resistant isolates of *Acinetobacterbaumannii*(N-50).

NDM gene was detected in all 50 isolates of Carbapenem resistant *A baumannii*. Ina study conducted by Yaw AdjeiAnane et al, NDM gene was found to be frequentlyassociated with *A. Baumannii* isolates.

OXA gene was detected in 49 (98.0%) isolates. Distribution of various types of OXAgenes was as follows: OXA 23 was seen in 49 (98.0%) isolates, followed by OXA 51which was seen in 47 isolates (94.0%), OXA 48 in 8 isolates (6.0%) and OXA 58 inonly 3 isolates (6.0%), OXA 23 and 51 coexisted in 47 (94%) of isolates. Mohammed Sami Alhaddad et al reported that carbapenem resistance was very high among *A.baumannii* hospital isolates with predominant detection of OXA 51 and OXA 23genes. Yaowen et al reported OXA-23 gene to be major carbapenemase responsible for resistance in their isolates from a teaching hospital. Merino et al and Zowawi et alalso observed OXA 23 and OXA 51 in carbapenem resistant ICU isolates of *Acinetobacter*[12]. Amudhan et al reported that out of 116 isolates of *Acinetobacter*species, OXA genes were detected in 106 isolates of which OXA 51 (n-99) and OXA23 (n-95) were the most common and they coexisted in 89 isolates[13]. Our findingsare similar to these studies. Coexistence of OXA 23 and OXA 51 results in increased expression of Carbapenemases and presents an emerging threat to theexistence of such resistant strains in the environment.OXA 58 has been reported from Europe, North and South America and West Asia.

The low prevalence in India evident in our study is in agreement with the reports ofMendes et al[14]. OXA producing *A. Baumannii* was first reported in 1993 from theblood culture of a patient at Edinburgh Royal Infirmary. At present, several subtypesof OXA type enzyme have been reported, such as OXA 23, OXA 40/24, OXA 48,OXA 51 and OXA 143. These enzyme coding genes can be detected on theirchromosomes or plasmids. The presence of OXA 23 has been widely reported inclinical isolates in many countries. OXA 23 enzymes have been found in outbreakisolates collected in the UK, East Asia and South America. Rapid acquision of resistance to Meropenem and other carbapenems poses an issuein the treatment of *A. Baumannii* infections. In a report presented in 2007, over 25%of *A. Baumannii* isolates were recorded to be carbapenem resistant. In a tertiary carehospital in North India, Meropenem resistance was reported in 6.4% of *Acinetobacter*spp. tested. In India several workers have reported carbapenemases which result inresistance in *A. Baumannii* to be prevalent. These findings are a pointer to the threatproduced by the treatment of carbapenem resistant *Acinetobacter* in India[14].

Patients with infection due to resistant strains appear to have higher mortality than patients with infection due to susceptible strains. In a systematic review of observational studies that included over 2500 patients with either carbapenemsusceptible or resistant *Acinetobacter* infection, the overall mortality rate was 33%,and carbapenem resistance was associated with a greater risk of death. Patients with carbapenem resistant infection are more likely to have severe underlying illnessor receive inappropriate empiric antibiotic therapy leading to an increase in mortalitydue to this infection[15].

Carbapenems are the drug of choice to treat *A. Baumannii* infections, but due to increased resistance the therapeutic options become limited to Polymyxins and Tigecycline. These drugs have their own limitations and are not indicated as the drugof choice to treat a variety of clinical conditions.

Coexistence of multiple oxacillinases and NDM along with other resistancemechanisms might also result in treatment failure and hence detection methods arerequired for each of these genes. This type of resistance is a significant threat tohospitals and it should be addressed with alternative and newer therapeuticstrategies, strict infection control measures and continuous surveillance[13].

CONCLUSION

To control or limit the spread of these multiresistant strains it is necessary to improve the microbiological techniques for early detection and accurate identification of these organisms along with genotyping. This will enable newer strategies for antibiotic use to be made to reduce selection pressure, including more frequent rotation of antibiotic groups or sequential use of antibiotic classes. Knowledge of the occurrence of NDM and OXA producing bacteria may encourage pharmaceutical companies and the Ministry of Health to facilitate the provision of last resort antibiotics and innovative therapeutic strategies such as bacteriophage therapy and monoclonal antibodies could be considered for future use. Implementation of strict Antimicrobial stewardship program and comprehensive infection control practices can help in controlling the spread of carbapenem resistant *Acinetobacterbaumanii* strains.

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