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Identification, Speciation and Antibiogram along with Detection of Metallo Beta-lactamase Production in Acinetobacter Isolated from Clinical Samples in a Tertiary Care Hospital

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Abstract

Acinetobacter species are gram negative non fermenters, which are important nosocomial pathogens involved in various outbreaks in hospitals due to widespread resistance to majority antibiotics. The aim of this study is to speciate Acinetobacter isolated from clinical samples, to assess the antibiotic sensitivity pattern and to detect the production of metallo-β-lactamase by double disc synergy test. The study was conducted in the department of microbiology, A.J. Institute of Medical Sciences. All clinical samples were subjected to gram stain & cultured; the Acinetobacter isolates obtained were subjected to antibiogram. Those isolates that showed Imipenem resistant were further tested for production of metallo-β-lactamase by double disc synergy test. Out of 6625 culture positive isolates, 414 (36.1%) were identified biochemically to belong to *Acinetobacter* species. Of the 414 cases, 393 (94.9%) were further identified to be *Acinetobacter baumannii* and the remaining 21 (5.1%) to be *Acinetobacter lwoffi. Acinetobacter lwoffii* showed 100% sensitivity to all the drugs. Of the 393 *Acinetobacter baumannii* isolates 109(27.7%) showed resistant to Imipenem. Out of these 109 isolates, 65 (59.63%) were positive for metallo-β-lactamase production by double disk synergy test. The speciation is highly demanding and laborious but it's important to be demonstrated due to difference in the antibiotic susceptibility pattern. Carbapenem resistant *Acinetobacter* nosocomial strains in ICUs are detected to be more resistant to antibiotics. As shown in this study the metallo-β-lactamase producing *A. baumannii* isolates were 59.63% and therapeutic options were limited. Therefore early identification of metallo-β-lactamase producers is of great importance to start appropriate treatment and to control the spread.

Keywords: Non Fermenting Gram Negative Bacilli, *Acinetobacter baumannii*, *Acinetobacter lwoffii*, Metallo-β-lactamase(MBL)

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INTRODUCTION

Acinetobacter species are gram negative non fermenters which are now considered as opportunistic pathogens that colonize patients with reduced host immunity. It causes various clinical infections such as septicemia, pneumonia, urinary tract infections, meningitis and wound infections[1]. Infections caused by Acinetobacter are difficult to treat because of the widespread resistance of these bacteria to majority of antibiotics. They exhibit resistance not only to beta-lactamase and cephalosporin's but also to carbapenems[2].

Carbapenem resistance in Acinetobacter is may be due to various causes such as - reduced expression of outer membrane proteins (29kDa, 33-36kDa) and carbapenamases–beta lactamases [3,30]. Speciation of the isolates and their antibiogram is helpful in epidemiological point of view, at hospitals and community level to create a baseline data for the early management of cases and to prevent further spread of the MDR isolates.

Hence this study was designed to assess the prevalence of Acinetobacter species isolated from clinical samples, their speciation and to determine their antibiotic sensitivity pattern along with detection of MBL production by double disc synergy test.

MATERIALS AND METHODS

This study was conducted in the department of microbiology, A.J Institute of Medical Sciences during the period from 1st November 2016 to 31st October 2017. All clinical samples sent from patients admitted at A.J institute of medical sciences, hospital and research centre were used as source for the study. Samples were collected under aseptic precautions. All samples were subjected to gram stain except urine; simultaneously the specimen were cultured without delay on MacConkey agar and sheep blood agar and were incubated aerobically for 24 hrs at 37°C. On gram stain Acinetobacter species appeared as short gram negative cocco-bacilli ~0.5-1μm. Identification of the isolate was done using standard biochemical tests as described in koneman[4]. Amongst the clinical isolates, those which were non-motile, oxidase negative and non-fermenters were subjected to further biochemical tests as described in a study done by Dimple et al[5] to help in further identification which includes growth at 37°C and 44°C, hemolytic property on blood agar, utilization of citrate, oxidation fermentation of glucose, decarboxylation of arginine, utilization of glucose & sensitivity to chloramphenicol. The clinical isolates of Acinetobacter spp. were subjected to antibiotic sensitivity testing to determine their sensitivity pattern. The antibiotics were selected according to the CLSI guidelines 2015[6] which included Amikacin (30mcg), piperacillin-Tazobactam (100/10mcg), cefoperazone/sulbactam (30mcg/10 mcg), Gentamicin (10 mcg), Imipenem (10mcg), Meropenem (10mcg), ceftazidime (30mcg), cotrimoxazole (25mcg), Levofloxacin (5mcg), Ciprofloxacin (5mcg), Cefepime (30mcg), Aztreonam (30mcg). All the isolates that were showing resistant to Imipenem were selected and tested by double disc synergy test[7] for MBL production.

RESULTS

Total 6625 positive culture samples from exudates, body fluids, urine, blood and sputum were analysed. Out of 6625 culture positive isolates, 1144 (17.2%) were identified as Non Fermenting Gram Negative Bacilli (NFGNB) which included Pseudomonas spp., Acinetobacter spp., and chryseobacterium spp. 414 (36.1%) of these non-fermenters were oxidase negative, non-motile and were further identified biochemically to belong to Acinetobacter species. From a total of 6625 isolates, the prevalence of Acinetobacter spp was 6.2%. Of the 414 Acinetobacter cases, 393 (94.9%) were further identified to be Acinetobacter baumannii and the remaining 21 (5.1%) to be Acinetobacter lwoffii. Antibiogram was done using Kirby-Bauer disc diffusion method as per CLSI 2015 guidelines. The sensitivity pattern of Acinetobacter species were as follows- Amikacin (37%), Ceftazidime (25%), cefoperazone/sulbactam (40%), ciprofloxacin (34%), cotrimoxazole (29%), cefepime (28%), gentamicin (26%), Imipenem (55%), levofloxacin (38%), meropenem (49%), piperacillin/tazobactam (39%), Aztreonam (1%). Of the 393 Acinetobacter baumannii isolates, 141 (35.8%) isolates were multidrug resistant cases. The isolates that showed resistance to Imipenem were further processed using double disc synergy test to detect metallo-β-lactamase production, by

Table 1. *A.baumannii* isolated in various studies

	Isolates speciated	*A.baumannii* (%)
Joshi *et al* (2006)	510	70
Prashanth and badrinath (2006)	49	71.4
Lone *et al* (2009)	258	72
Mindolli *et al* (2010)	200	78
Abhishek Routary *et al* (2013)	65	70.7
Sinha *et al* (2013)	140	92.14
Kalidas&Saha (2014)	154	74.02
Tripathi *et al* (2014)	107	79.3
Raina dimple *et al* (2016)	53	90.6

using IMP-EDTA/IPM disc by HIMEDIA (10mcg/disc +750mcg/disc). Of the 393 A.baumannii isolates, 109(27.7%) isolates were showing resistant to Imipenem and these isolates were further processed by double disc synergy test to detect metallo-β-lactamase production.

Out of these 109 isolates, 65 isolates (59.63%) were positive for metallo-β-lactamase production by double disk synergy test and 44 isolates (40.3%) were negative.

DISCUSSION

The study was conducted from November 2016 to October 2017. A total of 12,605 samples were received for bacteriological culture sensitivity testing during the study period. From these samples, 6625 samples yielded growth, while others were either reported as no growth, contamination/ non-significant growth. From the total non-fermenters that were isolated 1144, 414 (36.1%) isolates accounted for Acinetobacter. A series of biochemical tests were used to sepciate Acinetobacter isolates. Acinetobacter has many species and only a few of them are pathogenic to human beings. Therefore, it is important to at least identify the clinical relevant species. The commonest species pathogenic to human beings is A.baumannii. Speciation reported by a few workers is shown in the table 1.

Our observation is in agreement with other studies that *A.baumannii* is the most common isolated species from clinical samples. Prashanth and Badrinath (2006) reported an isolation rate of 70% for *A.baumannii* followed by

A.lwoffii, isolation rate being 10.2%[8]. Sinha et al (2013) reported A.baumannii as the predominant species accounting for 92.14% followed by *A.lwoffii* at 6.42%[9]. Kalidas and Saha (2014) reported 74.02% of the total Acinetobacter isolates as A.baumannii followed by A.lwoffii at 14.2%[10]. El-Badawy et al reported 56.3% of isolates as Acinetobacter baumannii[11]. The antibiogram was assessed by Kirby-Bauer disc diffusion method. All the isolates were tested using antibiotics as chosen from CLSI guidelines 2015[6]. Highest sensitivity was shown to Imipenem (55%), followed by Meropenem (49%) and piperacillin/tazobactam (39%). Imipenem is a highly popular antibiotic amongst clinicians. However, 109 (27.7%) of the isolates of A.baumannii were showing resistant to Imipenem.

There are several factors leading to carbapenem resistance in Acinetobacter, most important being the acquisition of carbapenem hydrolysing β-lactamases (IMP, VIM, NDM) and Ambler class D oxacillinases[12,13]. Other mechanisms include, reduced expression of outer membrane proteins, altered affinity or expression of penicillin-binding proteins and multidrug-efflux pumps [12,13,14]. Multidrug resistance (MDR) has been described for Acinetobacter. MDR Acinetobacter is defined as those Acinetobacter species that are non-susceptible to at least one agent from 3 antimicrobial classes of the following 6 antimicrobial classes i.e. aminoglycosides, β-lactam/β-lactam β-lactamase inhibitor combination, carbapenems, cephalosporins, fluoroquinolones & sulbactam[15]. The main danger associated with Acinetobacter is

its ability to acquire antimicrobial resistance which has led to the emergence of MDR strains. Hence, the management of Acinetobacter infections today is a major challenge and a significant health problem. In this study we found that the isolates showed resistance to majority of antimicrobials that are used. When resistance to different classes of antibiotics were analysed, we found that 35.8% of the isolates were MDR.

Lone et al[16] reported that the majority of the isolate were sensitive to imipenem (98.5%) and cefoperazone-sulbactam (88.5%), whereas maximum resistance was observed for cefazolin (93.2%) and ampicillin (86.3%). Mindolli et al[17] reported maximum sensitivity to meropenem (90.5%) and piperacillin-tazobactam (90.5%) and maximum resistance to ofloxacin (73.5%) and ceftazidime (38.5%). In another study by Dash et al[18] maximum sensitivity was observed to imipenem (81%) followed by meropenem (78%) and piperacillin-tazobactam (77%). They also reported that majority of them were resistant to ceftazidime (93%), cefepime (89%) and ampicillin-sulbactam (79%). The main danger associated with Acinetobacter is its ability to acquire antimicrobial resistance which has led to the emergence of MDR strains. Hence, the management of Acinetobacter infections today is a major challenge and a significant health problem. The percentage of MDR Acinetobacter is increasing significantly. Resistance to carbapenems is increasing over the years. This may be due to extensive and irrational use of these antibiotics in health care settings[19]. The Acinetobacter isolates which were showing resistance to imipenem were further processed by double disc synergy test to detect metallo-β-lactamase production. In this study, Of the 393 A.baumannii isolates, 109 (27.7%) isolates were imipenem resistant, which were further processed to detect metallo-β-lactamase production by Imipenem/ceftazidime-EDTA double disc synergy test (DDST)[20]. Out of these 109 isolates 65 isolates (59.63%) were positive for MBL production by double disk synergy test and 44 isolates (40.3%) were negative. In a study by Noyal et al[21], of the 46 Acinetobacter baumannii isolates that showed resistance to carbapenems, 6.5% were positive for metallo-β-lactamase by double disc synergy test (DDST). In another study conducted by Pandya et al[22], 7.4% of the Acinetobacter species

isolates were positive for MBL production by DDST. Several studies across India reports rates of MBL production ranging from 72% to 100% among carbapenem resistant NFGNB. The first report of MBL production in India was in 2002 from urban hospital in Bangalore[23]. A study on the Acinetobacter baumannii species done in India, stated that 70.9% of the A.baumannii isolates produced metallo-β-lactamase[24], while a different study from Kerala, India, stated that 21% of the A.baumannii isolates were found to be MBL producers[25].

CONCLUSION

Acinetobacter has now emerged as an established pathogen worldwide, especially in the hospital environment. The infection being high in hospitalized patients and the isolation rate has been increased from clinical sample. Being an opportunistic pathogen Acinetobacter are found involved in causing various infection outbreaks in intensive care units[26]. The speciation is highly demanding and laborious but its importance is demonstrated by the difference in the antibiotic susceptibility and outcome of the patient infected. A.baumannii is the predominant species isolated from pathological specimen and was found to be more resistant to antibiotics than A.lwoffi as shown in this study.

As shown in this study, most of the antibiotics were showing more than 50% resistance, which may be due to injudicious use of antibiotics. Though Imipenem was showing 55% sensitivity, injudicious use of it could lead to complete resistance in upcoming years. Majority of the isolates were found to be multidrug resistant, which shows that it is very important to follow discipline in antibiotic prescribing and re-in force antibiotic stewardship.

Nosocomial strains of A.baumannii that showed Carbapenem resistance were found to be resistant to most of the antibiotics, especially in ICUs. As shown in this study the rate of MBL producing A.baumannii isolates were 59.63% and therapeutic options for these are severely limited.

Although there are several screening methods for detection of MBL production, currently no CLSI guidelines are available for detection of these enzymes. Therefore identification of MBL producers is of great importance and maybe

performed in most of the laboratories to detect resistance at the earliest and to start appropriate treatment of these infections and also to control the spread of resistance. Thus a simple screening test such as IPM/IPM-EDTA DDST is essential to monitor resistance so that appropriate practice of infection control can be placed in order for the better outcome of the patients.

Periodic teaching programmes on infection control practices and re-enforcement of hand hygiene, proper periodic surveillance is utmost important in the control of hospital related Acinetobacter infections.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION
All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

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DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT
The study was conducted after getting institutional ethical clearance certificate.

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