ISSN: 2395 - 0471

International Journal of Clinical and Biomedical Research
Original Article

EMERGENCE OF CARBAPENEM-RESISTANT ACINETOBACTER BAUMANNII IN THE INTENSIVE CARE UNIT OF A REFERRAL HOSPITAL OF EASTERN INDIA AND ITS THERAPEUTIC OPTION

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Received: 26th Apr 2015, Accepted: 21st May 2015.

ABSTRACT

Background and objective: Multidrug-resistant *Acinetobacter baumannii* has one of the most serious nosocomially acquired gram negative infection in Intensive Care Unit (ICU). The gradual increase in incidence of this pathogen reflects their de-novo selection due to antibiotic usages and its ability to spread between patients. This study was undertaken to detect resistance to carbapenems in clinical isolates of *A.baumannii* in our ICU set up and to assess the rate of carbapenemase and MBL production among the isolates with the therapeutic options available against them. **Material and methods**: *A.baumannii* was identified by conventional methodology and susceptibility profile was determined by disc diffusion method. Carbapenem resistant isolates were further checked for mettalo beta lactamases (MBL) assay by EDTA disc synergy test and Minimum inhibitory concentration determination by agar dilution method. **Results**: 71.87% (n=46) of isolates showed resistance to Imipenem by disc diffusion method. 82.6% (n=38) of isolates were MBL producer both by phenotypic EDTA disc synergy test and MIC determination test by agar dilution method. The susceptibility profiles of *A.baumannii* strains towards colistin, polymyxinB and tigecycline were 90.62%, 57.81% and 78.12% respectively. **Conclusion**: Detection as well as awareness of this MBL producing *A.baumannii* in a hospital set up, coupled with judicious antimicrobial therapy based on sensitivity profiles will help us fight against this deadly menace.

KEYWORDS: *Acinetobacter baumannii*, carbapenemases, mettalo beta lactamases.

INTRODUCTION

Acinetobacter baumanii is a ubiquitous pathogen, which has recently emerged as an important causative agent of hospital acquired infection (HAI). It has greater propensity to acquire resistance determinants leading to multidrug resistance (MDR), hence causing therapeutic problems and hospital outbreaks. [1,2] To compounds this problem, *A.baumannii* resistant to all antimicrobial agents have also been reported.[3] So MDR *Acinetobacter* is a burning issue in the current antibiotic era.[4]

Carbapenems are widely used to treat MDR nosocomial infections in mechanically ventilated patients and intravenous devices infections. Mortality rate of these infections ranges from 19-54%.[5] Resistance to carbapenems are now increasing unfortunately. The main mechanism being production of carbapenemase, other non-enzymatic mechanisms are change in outer membrane protein, multidrug efflux pump and alteration in outer membrane binding protein.[6] *Acinetobacter baumannii* is responsible for approximately 2-10% of all gram negative infection in intensive care units (ICUs).[7] Emergence of metallo-β-lactamase (MBL) producing *A. baumannii* is a matter of concern in ICU.[8] MDR Acinetobacter baumanii is defined as those isolates resistant to at least three classes of antimicrobial agents penicillins or cephalosporins with beta-lactamase inhibitor combination, fluroquinolones and aminoglycosides. Extreme-drug-resistant (XDR) are defind as

isolates that are resistant to above mentioned antibiotics plus resistant to carbapenem.[9]

MATERIALS AND METHODS

Research study design

The prospective study was done from March 2014 to December 2014. Necessary ethical approval was taken. The study was carried out in the department of Medical Microbiology. Clinical samples were collected from our tertiary care set up. **Inclusion criteria:** Patients were eligible for participation in the study if they had signs and symptoms of nosocomial surgical site infection, nosocomial urinary tract infection, infected tracheostomy or bacteremia. Full history was obtained from all patients including: a) duration of hospitalization, b) presence of associated risk factors as: diabetes mellitus, previous antibiotic therapy shortly before the occurrence of infection, c) presence of medical devices such as urinary catheters, mechanical ventilation or peripheral intravenous catheters. **Exclusion criteria:** All those persons with disease manifestations during admission or developed within 48 hours of admission were excluded.

Sample size: A total of 2200 samples were examined from different age groups admitted in ICU.

Methodology: Specimen such as blood, endotracheal aspirate, urine, pus and wound swabs were sent to the microbiology laboratory and were processed as per standard methods. Blood was collected in blood culture bottles containing brain heart infusion broth. Subcultures were done on blood agar and MacConkey agar and incubated aerobically at 37^0C for 24 hours. Isolates were identified as *Acinetobacter baumannii* by a battery of tests like morphology, motility, oxidase, catalase, indole, urease, nitrate, citrate tests and oxidation-fermentation reactions of glucose, lactose, xylose, maltose, mannitol (Hugh Leifson method), lysine, arginine decarboxylase test and gelatin lequifaction test.[7] Antibiotics tested for non-fermenter by disc diffusion method were piperacillin (100µg), piperacillin/ tazobactum (100µg/10µg), ceftazidime (30µg), cefepime (30µg), ceftriaxone (30µg),tigecycline (15µg), polymyxin B (300units), colistin (10µg)), amikacin (30µg), ciprofloxacin (5µg), imipenem (10µg) [Hi Media laboratories Mumbai, India], MBL production was detected in imipenem resistant isolates by imipenem-EDTA combined disc method.[11] Zone diameter of >7mm with imipenem (10ug)+ EDTA (750ug) combination disc when compared to only imipenem disc (10ug) is considered carbapenem producer. Also modified Hodge test (MHT) was performed as per CLSI guideline for detection of carbapenemase. Also these patients were clinically reviewed for risk factor assessment and followed till discharge.

RESULTS

During the period of study from March 2014 to December 2014 a total of 1589 samples were examined from different age groups admitted in ICU. Out of total 536 isolates non-fermentors account for 40.29% (216) in this study period. *Pseudomonas* was the most common nonfermenter (140, 64.81%) followed by *Acinetobacter baumannii* (64, 29.62%). Table 1 shows the numbers of *A. baumannii* isolated from different clinical samples.

Table 1. Sources of the *A.baumannii* isolates

Samples	Total	Percentage %
Endotracheal secretion	**23**	**35.9%**
Urine	**21**	**32.8%**
Blood	**10**	**15.6%**
Pus	**10**	**15.6%**
Total	**64**	**100**

The male: female ratio was 1.5:1. Most of these patients had clinical history of COPD, bronchial asthma with features of respiratory failure. Infection in neonates was common in preterm babies. In 81.42% samples growth was monomicrobial. In 18.58% samples growth was polymicrobial. *E. coli* was the most common associated pathogen with *Acinetobacter* in case of urinary tract infection whereas *Staphylococcus aureus* was the associated organism in the case of wound infection, cellulitis and abscess. Out of 64 isolates, 46 (71.87%) *Acinetobacter baumannii* were resistant to imipenem by disc diffusion test. Among the imipenem resistant isolates 38 (82.6%) *A. baumannii* was found to be MBL producer by both imipenem EDTA combined disc method and MHT assay. The sensitivity

profile of MBL positive *A. baumannii* was different from that of MBL nonproducer, but for only colistin maximum no 58 (90.62%) of isolates were found to be sensitive (Table-2).

Table-2 Susceptibility pattern of *Acinetobacter baumannii* to different antimicrobial agents

Antibiotics	Sensitivity pattern	Resistant pattern
Piperacillin	20 (31.25%)	44 (68.75%)
Piperacillin/tazobactum	23 (35.9%)	41 (64.06%)
Polymyxin B	37 (57.81%)	27 (42.18%)
Ceftazidime	15 (23.4%)	49 (76.57%)
Cefepime	17 (26.56%)	47 (73.37%)
Ceftriaxone	14 (21.87%)	50 (78.12%)
Tigecycline	50 (78.12%)	14 (21.87%)
Colistin	58 (90.62%)	06 (9.37%)
Ciprofloxacin	21 (32.81%)	43 (67.18%)
Imipenem	18 (28.12%)	46 (71.87%)
Amikacin	29 (45.31%)	35 (54.68%)

piperacillin (100ug), piperacillin/tazobactum (100ug/10ug), polymyxin B (50ug), ceftazidime (30ug), cefepime (30ug), ceftriaxone (30ug),tigecycline (15ug), polymyxinB (300units), colistin (10ug)), [Hi Media laboratories Mumbai, India], imipenem (10ug).

Acinetobacter infection was more common in persons aged over 40 yrs however the difference was not statistically significant when compared with the distribution among imipenem sensitive *A. baumannii* infected patients (P= 1). (Table 3). Out of the risk factors assessed that were found to be significantly associated with MBL production (p<0.05) like hospital stay for more than 8 days, indwelling urinary catheter, continuous mechanical ventilation for more than 48 hours and prolonged antimicrobial therapy within 30 day of positive culture but underlying co morbid conditions like diabetes mellitus, other endocrinopathies were not significantly associated. The overall mortality in MBL positive patients was found to be 21% and incidence of MBL was more in male patient.

Table 3 Risk factors associated with imipenem resistant *Acinetobacter baumannii* infection

Risk factors		Imipenem resistant A.baumannii (n=46)		Imipenem sensitive A.baumannii (18)		P value
		Number	%	Number	%	
Age	>40	24	52.17	10	55.55	P=1
	<40	22	47.82	8	44.44	
External device	Yes	6	13.04	12	66.66	P<0.001
	No	40	86.95	6	33.33	
Previous antibiotic use	Yes	10	21.73	13	72.22	P=0.003
	No	36	78.26	5	27.77	
Hospitalization >10 days	Yes	11	23.91	11	61.11	P<.008
	No	35	70.08	7	38.88	
Underlying disorders	Yes	22	47.82	11	61.11	P=.4104
	No	24	52.17	7	38.88	

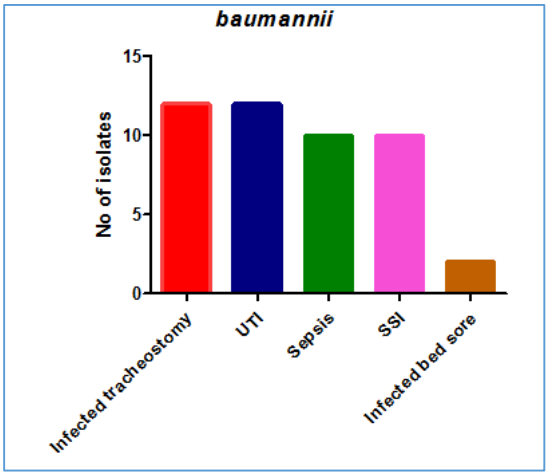
P<0.05 is significant, P<0.001 is highly significant

DISCUSSION

Acinetobacter species has very rapidly emerged as serious MDR pathogen causing life threatening infection both in community and hospital. [12] It is found in nature, soil, and also in skin as commensal. Infection is commonly aerosolly transmitted. Potential risk factors for development of MDR Acinetobacter nosocomial infections are prior use of broad spectrum antibiotics, cross infection by hands of hospital staff, ventilator machine. In our study at our ICU set up though overall *Acinetobacter* infection was 11.94%, but respiratory infection due to *Acinetobacter* in mechanically ventilated patients in ICU was 37.70% (23 isolates of *Acinetobacter* out of 64 isolates isolated from ET tube suction in mechanically ventilated patients). According to one recent study, *Acinetobacter spp* was responsible for 35% of ventilator associated pneumonia making it most conspicuous and dominant pathogen among all bacteria

encountered in that study.[13] This study shows that prevalence rate of MBL producing *Acinetobacter spp* is 7% when isolated from clinical samples in our hospital, which is lower than that of other similar studies in Indian subcontinent.[4,5] Imipenem resistant strains were multidrug resistant except for colistin (90.62% sensitive) and polymyxin B (57.81% sensitive), hence necessitating cautious use of these antibiotics.

A total of 64 *Acinetobacter* isolates were collected from patients in the ICU of our tertiary care set up. *Acinetobacter baumannii* isolates were obtained from different clinical samples including 23 (35.9%) endotracheal aspirate, 21(32.8%) urine, 10 (15.6%) blood, and 10(15.6%) pus (Graph 1). Using disc diffusion method to test susceptibility of Acinetobacter isolates to different antimicrobials, according to the Clinical Laboratory Standard Institute's guidelines (CLSI, 2014) we found that; the highest sensitivity of *A. baumannii* was for colistin (90.62%), followed by tigecycline (78.12%), and then polymyxin B (57.81%). The lowest sensitivity of A.baumannii was ceftriaxone (21.87%), followed by ceftazidime, cefepime and imipenem (Table2). 40 (62.5%) *A.baumannii* isolates were MDR with resistant to ceftriaxone, piperacillin, amikacin, ceftazidime, and ciprofloxacin and 37 (57.81%) were XDR with resistant to imipenem in addition. These findings are well corroborated with the findings of similar studies. [14,15]



Graph 1. Different types of infections caused by Carbapenem resistant *A. baumannii*

High level of imipenem resistance was obtained by disc diffusion method in 46 (71.87%) isolates of Acinetobacter *baumannii*. Of these 46 imipenem resistant *A.baumannii* 38 isolates (82.6%) metallo-β-lactamase producers (increase of ≥7mm in zone diameter of EDTA containing imipenem disc compared to imipenem disc) which is in accordance with similar studies.[16,17] Six isolates were MBL negative.

There were 64 cases of health care associated infection caused by *A.baumannii*, of these cases there were 46 cases caused by imipenem resistant *A.baumannii*, 12 (26.08%) cases of infected tracheostomy, 12 (26..08%) cases of nosocomial urinary tract infection, 10 (21.73%) cases of septicemia, 10 (21.73%) cases of surgical site infection and 2 (4.34%) case of infected bed sore in ICU patient (Graph-1). These findings matches with the findings of Manchanda V et al.[13]

In our study, we found that imipenem resistant *A .baumannii* infection were more common among patients with history of prior exposure to broad spectrum antibiotics shortly before the occurrence of imipenem resistant *A.baumannii* infection with a highly statisticahis rapid sprelly significant difference compared to patient with imipenem sensitive *A.baumannii* infection (p=0.004). We also found that patient who have external devices were more liable to develop imipenem resistant *A.baumannii* infections with a statistically significant effect as a risk factor (p=0.03). There was also a statistically significant association between prolonged hospital stay for 10 days or more and infection with imipenem resistant *A.baumannii* (p=0.002). Imipenem resistant *A.baumannii* infections were more common among patients aged over 40 years, however the difference was not statistically significant when compared with the distribution among imipenem sensitive *A.baumannii* infected patients. (p=0.2)

Rapid emergence of carbapenem resistant MBLs producing *A.baumannii* in our clinical study is alarming and is due to excessive use of that group of antibiotic. Hence, appropriate antibiotic therapy after proper antimicrobial susceptibility testing and reporting and following strict antibiotic policies and hospital infections control measures are mandatory to overcome this rapid spread of carbapenem resistant. As monotherapy carbapenem is often associated with treatment failure and development of antimicrobial resistance

combination therapy with any carbapenem with colistin is more feasible approach. A limitation of the current study was lack of genotypic confirmation for the presence of carbapenem hydrolyzing genes. There is need for genetic analysis of MBLs enzymes. Carbapenem resistant *A.baumannii* is emerging as an important pathogen in our ICU. Antibiotic selective pressure is an important cause of emergence of MDR *A.baumannii* infection in our health care settings. The high prevalence of antibiotic resistance observed in the current study is significant, as only few therapeutic options are available for treatment. New antibiotics are needed to treat MDR *A.baumannii.*

CONCLUSION

The present study provided valuable information about the effect of colistin that can be used in our health care facility. Infection control practices and antibiotic resistance surveillance should be carried out regularly to decrease the spread and impact of *A.baumannii.* Detection and molecular characterization of MBL producing *A.baumannii* strains is recommended for the purposes of infection control and prevention.

ACKNOWLEDGEMENT

We thank Dr. P. K. Maiti, M, Prof & Head. Department of Microbiology, Institute of Post-Graduate Medical Education & Research. 244 AJC Bose Road, Kolkata-700020 for his help and support.

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