ORIGINAL ARTICLE

PREVALENCE OF CARBAPENEM-RESISTANT ACINETOBACTER BAUMANNII (CRAB) IN MEDICAL AND SURGICAL INTENSIVE CARE UNITS (ICUS) OF JPMC, KARACHI

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ABSTRACT

Background: Antimicrobial resistance is one of the research priorities of health organizations due to increased risk of morbidity and mortality. Outbreaks of nosocomial infections caused by carbapenem-resistant *Acinetobacter Baumannii* (CRAB) strains are at rise worldwide. Antimicrobial resistance to carbapenems reduces clinical therapeutic choices and frequently led to treatment failure. The aim of our study was to determine the prevalence of carbapenem resistance in *A. baumannii* isolated from patients in intensive care units (ICUs).

Methods: This cross-sectional study was carried out in the Department of Microbiology, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, from December 2016 to November 2017. Total 63 non-repetitive *A. baumannii* were collected from the patients' specimens, admitted to medical and surgical ICUs and wards of JPMC, Karachi. The bacterial isolates were processed according to standard microbiological procedures to observe for carbapenem resistance. SPSS 21 was used for data analysis.

Results: Out of the 63 patients, 40 (63.5%) were male. The age of the patient ranged from 15-85 year, with average of 43 year. 34.9% patients had been hospitalized for 3 days. Chronic obstructive pulmonary disease was present in highest number with average of 58.7% for morbidity. Number of patients on mechanical ventilation was highest (65.1%). All isolates were susceptible to colistin. The resistance to ampicillin-sulbactam, ceftazidime, ciprofloxacin, amikacin, piperacillin- tazobactam and meropenem was 82.5%, 81%, 100%, 87.3%, 82.5% and 82% respectively. Out of 82% CRAB, 77% were obtained from ICUs.

Conclusion: This study has revealed the high rate of carbapenem resistance in *A. baumannii* isolates in ICUs thus leaving behind limited therapeutic options.

Keywords: *Acinetobacter Baumannii*; Carbapenem Resistant; Nosocomial Infections.

INTRODUCTION

Acinetobacter infections have received great importance in clinical practice due to organism's prolonged environmental survival and strong tendency to develop antimicrobial drug resistance[1]. World Health Organization (WHO) has included the *A. baumannii* in "ESKAPE" group (includes *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) which is the most dangerous and difficult to treat group of organisms[2]. Multidrug resistant *A. baumannii* (MDR-AB) is a serious nosocomial emerging strain, strongly associated with high mortality rate in ICUs patients[3]. Thus, is a research priority for WHO and other health organizations to combat this health issue. Risk factors associated with *A. baumannii* infections are, indwelling catheters, invasive procedures such as central venous catheterization, mechanical ventilation, hands of medical technicians and staff, prolonged hospital stay and wide-

spread use of broad-spectrum antibiotics[4].

A. baumannii is ubiquitous in nature, found mainly in hospitals, causing ventilator associated pneumonia, bloodstream infection, urinary tract infection, vasculitis, meningitis and wound infections[5]. It is Gram-negative aerobe, non-lactose fermenting, oxidase-negative bacteria with a deoxyribonucleic acid (DNA) Guanine + Cytosine (G+C) content of 39% to 47% and grows well at 44ºC[6]. One of most highlighted issues is that this bacterium is naturally resistant to commonly used antibiotics such as amino-penicillins, first-and second-generation cephalosporins and chloramphenicol and Ertapenem, making it a challenge to effectively treat infection caused by this organism[7]. Carbapenems (imipenem, meropenem and doripenem) were considered as drugs of choice for MDR-AB infections. However, recent reports of resistance to even these drugs have emerged with increasing frequency[6]. Multiple mechanisms are utilized by these bacteria to develop resistance, such as production of enzymes (carbapenemases: beta-lactamase), over expression of efflux pumps, porin loss and alteration in pencillin binding proteins[8]. There can be an alarming increase of infections by carbapenem resistant *A. baumannii*; further lack of detection may enhance their rapid spread among clinical isolates. Timely appropriate laboratory investigations are essential to avoid delays in the management of these patients.

The data regarding *A. baumannii* is limited at species level and its resistance pattern needs continuous monitoring for an incident of outbreak. Therefore, this study was design to determine the prevalence and resistance pattern of carbapenem resistance in *A. baumannii* isolated from patients in ICUs. This will help the clinicians in prophylaxis and treatment of infection caused by this bug.

METHODS

This cross-sectional study was conducted in the Department of Microbiology, Basic Medical Sciences Institute (BMSI), JPMC, Karachi after ethical approval from Institutional Review Board, JPMC as a part of M. Phil research. Total 63 non-repetitive *A. baumannii* were collected between December 2016 to November 2017 from the patients admitted to (ICUs), medical and surgical wards of JPMC, Karachi. The samples collected from patients included respiratory secretions, blood, pus and urine. Sample size was calculated by OpenEpi calculator, and confidence level was set at 95%. After taking necessary aseptic measures and verbal consent, the samples were collected and patient history was documented in a proforma. Data collected included age, sex, length of hospital stay, use of invasive medical devices and co-morbidities. *Escherichia coli* ATCC 25922 was used for quality

control of the Gram's stain, biochemical tests, media preparation and susceptibility testing of tetracycline and trimethoprim-sulfamethoxazole and for another antibiotics *Pseudomonas aeruginosa* ATCC 27853 was used[9].

All specimens were processed by standard microbiological procedures including, culture, and biochemical characteristics according to manual of clinical microbiology[10]. *A. Baumannii* was identified by colony morphology (On MacConkey agar colonies of *A. baumannii* appeared as non-lactose fermenter and on blood agar colonies were about 1 to 2 mm in diameter, non- pigmented, domed, and mucoid, with smooth to pitted surfaces).

Specification was confirmed by Analytical Profile Index (API) 20NE (Bio-merieux, France), according to manufacturer's instructions. Antimicrobial susceptibility testing of the isolated organisms was performed by the disc diffusion technique according to the recommendations of the clinical and laboratory standards institute (CLSI, 2015). The minimum inhibitory concentration (MIC) for all isolates to imipenem and meropenem was determined by E-test (Bio-Merieux, France). The results were interpreted according to CLSI guidelines[9]. CRAB was defined as an *A. baumannii* isolate that was resistant to both imipenem and meropenem (MIC of >8чg /ml as resistant) whereas carbapenem- susceptible *A. baumannii* possessed carbapenem MIC of ≤ 2 чg/ml[11].

Colistin and polymyxin B susceptibility was performed by E-test (Biomerieux) and results were confirmed by MIC broth microdilution method. MIC breakpoints for resistant and susceptible were ≥ 4ч g/ml and ≤ 2чg/ml respectively[9]. Data was analyzed by SPSS-21. Required statistical tests were applied and *p*- value <0.05 was considered significant.

RESULTS

Total 63 non-repetitive *A. baumannii* were isolated from patients, admitted in different wards and ICUs of JPMC, Karachi. The subjects included in this study were of both genders, with age ranging from 15 to 85 years (Table 1). Forty (40; 63.5%) positive samples were collected from males while 23 (36.5%) were from females. Among these 8 (12.7%) were received from the surgery wards, 14 (22.2%) from the medical ward whereas 41 (65.1%) were collected from ICUs. Out of 63 samples 6 (9.52%) samples were taken from UTI patients (urine), 8 (12.7%), samples of pus were taken from patient's wounds, 36 (57.1%) samples of respiratory secretions (sputum and tracheal aspirates) were collected from patients of lower respiratory tract infections (LRTIs) and 13 (20.6%) samples of blood were taken from patients with suspected septicemia.

Table 1: Clinical characteristics of 63 patients infected with A. baumannii (N= 63).

Variables	Number	Percent
Gender		
Male	40	63.5
Female	23	36.5
Department		
ICUs	41	65.1
Medical ward	14	22.2
Surgical ward	8	12.7
Samples		
Respiratory secretions	36	57.1
Blood	13	20.6
Pus	8	12.7
Urine	6	9.52

Out of total 63 patients included in this study, majority 41(65.1%) stayed in hospital for more than 3 days while, 22 (34.9%) stayed for < 3 days. Number of patients on mechanical ventilation was higher 41/63 (65.1%), than non-ventilated patients. Of the selected cases, 44 (69.8%) patients were urinary catheterized. 39/63 (61.9%) patients developed hyperpyrexia. CRP (C- reactive protein) of > 8mg/dl was recorded in 45/63 (71.4%) patients while 39/63 (69.1%) patients showed only leukocytosis (WBC >11x10^9/L). COPD (Chronic obstructive pulmonary disease) was present in highest rate 37/63 (58.7%) for morbidity, followed by diabetes mellitus in 33/63 (32.8%) ESRD (End stage renal disease) in 27/63 (42.8%) and hypertension in 19/63 (30.1%) in decreasing order of frequency (Table 2).

Table 2: Demographic characteristics of patients with A. baumannii infection (N=63).

Variables	No. of Patients	Percentage
Hospital stay (hours)		
≤ 3 days	22	34.90%
> 3 days	41	65.10%
On mechanical ventilation	41	65.10%
Urinary Catheterization	44	69.80%
Hyperpyrexia	39	61.90%
C-reactive Protein >8mg/dl	45	71.40%
WBC count >11x10^9/L	39	61.90%
comorbidities:		
Hypertension	19	30.10%
Diabetes mellitus	33	52.80%
End stage renal disease	27	42.80%

Chronic Obstructive Pulmonary Disease

Almost all antibiotics exhibited high resistance against A. baumannii except polymyxin-B and colistin which showed 100% sensitivity. Out of 63 A. baumannii isolates 52(82%) were resistant to ampicillin-sulbactam, piperacillin-tazobactam, imipenem, meropenem and tetracycline. Among the cephalosporin, as less as 51(81%) isolates were resistance to both ceftazidime and cefepime while as much as 62(98.4%) were resistant to ceftriaxone and cefotaxime each. The resistance against other antibiotics was as follows: Amikacin 55 (87.3%), levofloxacin 57(96.8%), doxycycline 55(87.3%), minocycline 46 (73%), while 52 (82.3%) were resistant to tetracycline. Resistance to sulfamethoxazole-trimethoprim was found in 58/63 (92.1%) of the isolates. Whereas, all (100%) were found to be resistant to ciprofloxacin (Figure 1).
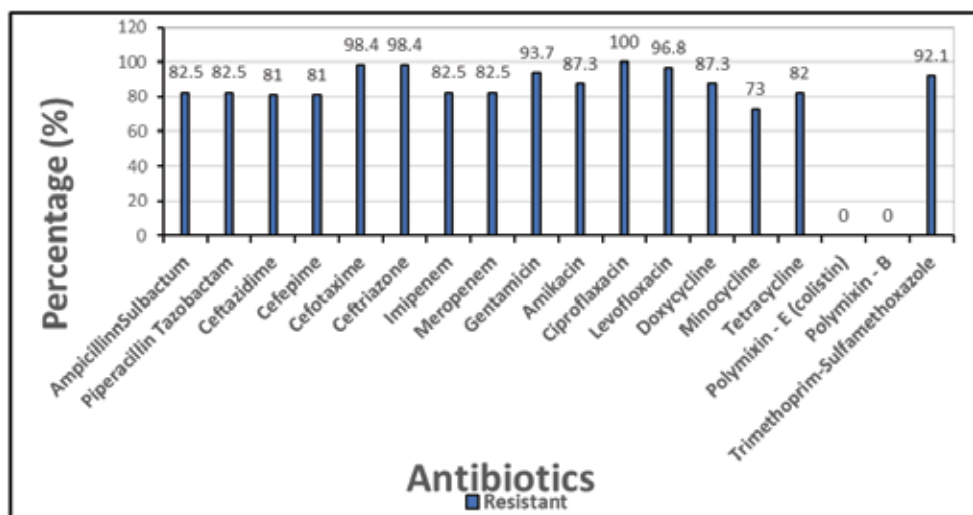

Figure 1: Antimicrobial resistance pattern (%) in A. baumannii (N= 63).

DISCUSSION

Rapid emergence and spread of resistant strains of bacteria are a threat to our health care system. Among serious infections, A. baumannii has emerged as a challenge due to rapid acquisition of resistance to almost all routinely prescribed antimicrobial agents. It has significantly threatened the effectiveness of current available antibiotics[12].

In this study 63 A. baumannii were isolated from different clinical specimens and the commonest source of isolation was respiratory secretion 36 (56%) followed by blood 20.54%, Pus 12.64% and (9.48%) were from urine. This is in accordance with the study conducted by Begum, showed higher prevalence of *A. baumannii* in respiratory secretion which were (54%), (50%) and (41.8%) respectively[13]. This suggested that invasive devices such as tracheal tubes are important reservoir involved in A. baumannii transmission. Al-Sehlawi indicated high prevalence of A. Baumannii in urine samples, which is in contrast to our study[14]. This showed that the site of infection is not specific but may be variable depends upon the different factors including infection control practice, environment and mechanical ventilation. These findings are in agreement with study results by Saleema[15]. The frequency of A. baumannii was higher in ICUs patients, similar findings were reported by Begum et al[13]. This is due to the ICU environment and immunocompromised patients favor the adoption, transmission and infection. The majority of patents (65.1%) showed stayed in hospital for more than 3 days. This indicates that the source of infection was hospital, which is reported everywhere in literature.

In the present study A. baumannii infections were more common in males (63.5%) as compared to females. This may be due to the fact that the males reported more frequently to the hospitals compared to females. Islahi also observed high percentage of nosocomial infections present in males[16], while results from Shoja showed high prevalence in females[17].

Data presented that 100% of isolates were resistant to ciprofloxacin and 96% were resistant to levofloxacin, in accordance to the results reported by Indhar18. Present data showed 82% A. baumannii was resistant to ampicillin-sulbactum and same was in the study conducted by Lowings, showed higher resistance rate of Ampicillin-sulbactam in A. baumannii, again supporting our findings[19]. Resistance to ceftazidime, cefepime, ceftriaxone, cefotaxime showed 81%, 98%, 81% and 98% respectively. The resistance against aminoglycosides was lower, which is similar to former studies[20,21]. This may be due to contraindication and infrequent use of aminoglycosides in comparison to beta-lactam and ciprofloxacin.

Resistance to meropenem and imipenem were 82%, which is in accordance to previous study[21,22].

Carbapenem resistance in A. baumannii is mainly due to expression of (OXA) carbapenemases-types, and uncommon mechanisms are impermeability and altering the expressions of porins or efflux pumps and metallo-beta-lactamase (MBL) carbapenemeses[23]. Different studies from Pakistan conducted by Hasan[24], Sohail[20], and Indhar[16] showed higher level of carbapenem resistance in A. baumannii. Due to the rapid spread of carbapenem resistant A. baumannii, there is dire need of prevention, rapid detection and appropriate management.

CONCLUSION

This study has revealed that rate of carbapenem resistance in A. baumannii isolates in ICUs patients is at a swift rise. Therefore, it is suggested that continuous evaluation in hospitals should be maintained to avoid the irrational use of antibiotics. The delayed isolation of such strains not only leaves behind limited therapeutic options for treatment but also leads to higher rate of mortality.

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CONFLICT OF INTEREST

There was no conflict of interest among the authors.

ETHICS APPROVAL

The study approval was sort from BMSI, JPMC Institutional Board Committee (No.F.2-81-IRB/2017/GEN-L/19809 JPMC).

PATIENTS CONSENT

Verbal and written consent was obtained from all patients.

AUTHORS CONTRIBUTION

ZS conducted the study; RA guided and helped in the write-up, proof read the manuscriptand completed the final version for publication. FU handled the laboratory technical work and helped in the data collection and MAD was the supervisor of the study.

REFERENCES

1. Vashist J, Tiwari V, Das R, Kapil A, Rajeswari MR. Analysis of penicillin-binding proteins (PBPs) in carbapenem resistant Acinetobacter baumannii. Indian J Med Res. 2011; 133(3): 332.

2. Gonzalez-Villoria AM, Valverde-Garduno V. Antibiotic-resistant Acinetobacter baumannii increasing success remains a challenge as a nosocomial pathogen. J Pathog. 2016: 7318075.

3. Cisneros JM, Rodríguez-Baño J. Nosocomial bacteremia due to Acinetobacter baumannii: epidemiology, clinical features and treatment. Clin Microbiol Infect. 2002;8(11):687-93.

4. Almasaudi SB. Acinetobacter spp. as nosocomial pathogens: Epidemiology and resistance features. Saudi J Biol Sci. 2018;25(3):586-96.

5. Lean SS, Yeo CC, Suhaili Z, Thong KL. Comparative genomics of two ST 195 carbapenem-resistant Acinetobacter baumannii with different susceptibility to polymyxin revealed underlying resistance mechanism. Front Microbiol. 2016; 6 :1445-47

6. Abbott I, Cerqueira GM, Bhuiyan S, Peleg AY. Carbapenem resistance in Acinetobacter baumannii: laboratory challenges, mechanistic insights and therapeutic strategies. Expert Rev Anti Infect Ther. 2013;11(4): 395-409.

7. Alfizah H, SARAH OS, Noraziah MZ. Interaction of Imipenem and Tigecycline against Carbapenem-Resistant Acinetobacter baumannii: The Relativity of Synergism. Sains Malaysiana. 2015;44(7):1061-5.

8. Ehlers MM, Hughes JM, Kock MM. Prevalence of Carbapenemases in Acinetobacter baumannii. In Antibiotic Resistant Bacteria-A Continuous Challenge in the New Millennium 2012. IntechOpen.

9. Wayne PA. CLSI (clinical and laboratory standard institute). Performance standards to Antimicrobial susceptibility Testing; 25th informational supplement. M100-S25. 2015.

10. Vaneechoutte M, Dijkshoorn L, Nemec A, Kämpfer P, Wauters G. Acinetobacter, Chryseobacterium, Moraxella, and other non-fermentative Gram-negative rods. InManual Clin Microbiol. 10th Edition 2011(714-738). Am Soc Microbiol.

11. Chang Y, Luan G, Xu Y, Wang Y, Shen M, Zhang C, Zheng W, Huang J, Yang J, Jia X and Ling B. Characterization of carbapenem-resistant Acinetobacter baumannii isolates in a Chinese teaching hospital. Front Microbiol. 2015; 6:910-14.

12. Evans BA, Hamouda A, Abbasi SA, Khan FA, Amyes SG. High prevalence of unrelated multidrug-resistant Acinetobacter baumannii isolates in Pakistani military hospitals. Int J Antimicrob Agents. 2011;37(6):580-1.

13. Begum S, Hasan F, Hussain S, Shah AA. Prevalence of multi drug resistant Acinetobacter baumannii in the clinical samples from tertiary care hospital in Islamabad, Pakistan. Pak J Med Sci. 2013; 29(5):1253-1258.

14. Sehlawi ZSA, Azhar AMA and Thahab AA. Isolation and Identification of Acinetobacter baumannii Clinical isolates using Novel Methods. J Babylon Univ/Pure App Sci. 2014; 22(3):1041-1050.

15. Saleema AE, Saida AM, Melehab MS. Acinetobacter baumannii in ICU patients: A perspective

study highlighting their incidence, antibiotic sensitivity pattern and impact on ICU stay and mortality. Egyptian J Chest Dis Tuber. 2017;6(66): 693-698

16. Islahi S, Ahmad F, Khare V, Singh I. Prevalence and resistance pattern of Acinetobacter species in hospitalized patients in a tertiary care centre. J Evol Med Dental Sci. 2014; 3(17):4629-4635.

17. Shoja S, Moosavian M, Rostami S, Farahani A, Peymani A, Ahmadi K, Ebrahimifard N. Dissemination of carbapenem-resistant Acinetobacter baumannii in patients with burn injuries. J Chin Med Assoc. 2017;80(4):245-52.

18. Indhar F, Durrani MA, Bux A, Sohail M. Carbapenemases among Acinetobacter species isolated from NICU of a tertiary care hospital in Karachi. JPMA. 2017; 67(10):1547-1551

19. Lowings M, Ehlers MM, Dreyer AW, Kock MM. High prevalence of OXA in clinical MDR-AB isolates from South Africa. BMC Infect Dis. 2015; 15:521.

20. Sohail M, Rashid A, Aslam B, Waseem M, Shahid M, Akram M, Khurshid M, Rasool MH. Antimicrobial susceptibility of Acinetobacter clinical isolates and emerging antibiogram trends for nosocomial infection management. Rev Soc Bras Med Trop. 2016; 49(3):300-304.

21. Guo-xin M, Dan-yang S, Xi-zhou G, Jun-chang C, Rui W, Zhi-gang C, Liang-an C. Laboratory to clinical investigation of carbapenem resistant Acinetobacter baumannii outbreak in a general hospital. Jundishapur J Microbial. 2014; 7(1):e13120.

22. Shamim S, Abbas M, Husain M. Prevalence of multidrug resistant Acinetobacter baumannii in hospitalized patients in Lahore, Pakistan. Pak J Mol Med. 2015; 2(1):23-28.

23. Uwingabiye J, Frikh M, Lemnouer A, Bssaibis F, Belefquih B, Maleb A, Dahraoui S, Belyamani L, Bait A, Haimeur C, Louzi L. Acinetobacter infections prevalence and frequency of the antibiotic's resistance: comparative study of intensive care units versus other hospital units. Pan Afr Med J. 2016; 23:191

24. Hasan B, Perveen K, Olsen B and Zahra R. Emergence of carbapenem-resistant Acinetobacter baumannii in hospitals in Pakistan. J Med Microbiol. 2014; 63:50-55.