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Isolation and antibiogram of *Acinetobacter bauminnii* recovered from human clinical specimen in a tertiary care setting from Lahore, Pakistan

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Abstract

Acinetobacter baumannii is non-fermentive oxygen-consuming Gram-negative coccobacilli pathogen that has appeared as an essential bacteria responsible for hospital-acquired infections. It also has proved itself as an opportunistic pathogen and colonises, especially in those patients which were immunocompromised and were admitted in intensive care units (ICUs), orthopaedic wards, gynae wards and medical wards. The present study was designed to isolate the *A.baumannii* recovered from different wards of the hospital from various samples. The biochemical characterisation was done by analytical profile index for non-*Enterobacteriaceae* (API 20NE) system and was subjected for antimicrobial susceptibility pattern. Human clinical specimens like urine, blood, CSF, pus, sputum, HVS and other fluids were examined by the specific methods, and Kirby-Bauer disk diffusion method was adopted according to the Clinical and Laboratory Standards Institute guidelines (CLSI) for the examination of resistivity and susceptibility pattern of pathogens. A total of 150 isolates were recovered out of 912 specimens over the six months (July 2018 to December 2018) in a tertiary care hospital. A high proportion of isolates were resistant against the Ampicillin-Salbactum (69.33%), Cefepime (64.67%), Ceftriaxone (61.33%) and Ceftazidime (60%) whereas Papracillin-Tazobactam (66.67%), Tigecyclin (59.33%), Doxycyclin (56.67%) and Imipenem (55.33%) show maximum efficacy against the isolated pathogens. By adopting the standard policies for the use of antibiotics, increasing resistance of *A. baumannii* against different classes of drugs could be minimised.

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Introduction

Acinetobacter species are non-fermentative oxygen-consumingGram-negativecoccobacilli and high range to colonise the human body and the natural stores (Obeidat *et al.* 2014). *Acinetobacter*istaken from Greek word akinetoswhich means immotile and was used to differentiate it from motile organisms (Howard *et al.* 2012, Mcbride 2010). *Acinetobacter*is short rod size bacteria whichbelongs to *Neisseriaceae* family(Fournier *et al.* 2006).Colonies produce of this organism are smooth but sometimes like greyish-white colour and mucoid colonies (Peleg *et al.* 2008). *Acinetobacter* hasunique character to grow at any pH and different temperature conditions, and it does not require extranutrition for growth. This organism uses various sources of carbon and energy. Because of these abilities *Acinetobacterbaumannii*can easily be transmitted and live inboth moist and dry conditions (Abbo *et al.* 2005).

Acinetobacter species have been segregated from waterbodies, soil, sewage, healing facility fomites and are commensals in people and animals (Visca *et al.* 2011). Its chain of transmission can be broken from one patient to another by adopting suitable disinfection procedures close contact isolation and other protective measures (Kirkgöz *et al.* 2014). The major risk factor of*A. baumannii*infection includes poor overall condition, mechanical ventilation, respiratory system insufficiency, prior antibiotic therapy, circulatory system insufficiency and incidence of foreign materials, venous and arterial catheters (Sieniawski *et al.* 2013). Nosocomial infections that are diagnosed more than 48 hours after admission in hospitals depends on National Nosocomial Infection Surveillance (NNIS) criteria. But infections which occur less than 48 hours of stay in hospitals are called community-acquired infections (Kamble 2015). These microorganisms are commensals for solid skin and considered as contaminants of bacteriological samples which are not responsible for infections (Decré 2012). In the United States, information gathered by healing facilities standard anticipating in the National Nosocomial Infection observation framework 1992–1997 demonstrated that *A. baumannii* caused 1% of nosocomial circulation system disease and 3% of pneumonia cases in the ICU (Rhinehart *et al.* 1999). The ability of this organism to develop resistance against multiple antibiotics is major factor for its growth in hospital environment (Constantiniu *et al.* 2004). Different *Acinetobacter* spp. behave differently in various geographical location (Dash *et al.* 2013). According to Centers for Disease Control and Prevention (CDC), *Acinetobacterbaumannii* developing resistance uncontrollably. According to different reports carbapenems was considered as the gold-standard and last resort of treatment (Rahal 2006). The current study was aimed to determine the anti-microbial susceptibility pattern of Acinetobacter *baumannii.*

Materials and methods

The study was conducted in the Department of Microbiology, King Edward Medical University / Mayo Hospital Lahore over six months from September 2018 to February 2019. A total (n=912) human clinical specimens including urine, pus, blood, Cerebrospinal fluid (CSF), sputum, high vaginal swab (HVS) and endotracheal tube aspirate (ETT-A) were collected from the patients which were admitted in the ICUs and different wards of Mayo Hospital. All human clinical specimens were brought to the hospital microbiological laboratory. Specimen were subjected to microscopy and cultured on blood and MacConkey agar (Sigma-Aldrich) for the isolation of *A.baumannii*. As well as Cysteine Lactose Electrolyte deficient CLED agar (Sigma-Aldrich) was used for the inoculation of urine sample. After growth of bacteria on culture medium at 37℃, API 20NE (biomerieux, France) system was used to confirm the identification of species of the genus *Acinetobacter*. API 20NE biochemical confirmation system consists of 21 tests among which 12 tests belong to carbon assimilation whereas 9 tests were based on enzymatic detection of particular species. This characterising biochemical system was consists of 21 tests which were segmented into 7 parts with three tests in each part. In each part 1, 2 and 4 numbers were designated for positive reaction in well 1, 2 and 3 respectively, whereas 0 is

assigned for negative result in any well. All the values in each part are summed up to produce a digit ranging from 0-7. *A. baumannii* Isolates after biochemical confirmation were added in the study whereas all other isolates were excluded. The antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method,and zones of inhibition were interpreted as per Clinical Laboratory Standards Institutes guidelines (CLSI). Hemolysis pattern on blood agar, citrate utilisation, glucose oxidation, arginine decarboxylation and growth at 37℃ for 18-24 hours contributed to the identification of *A. baumannii*. Different classes of antibiotics (Difco) ciprofloxacin, ceftazidime, ampicillin-sulbactam, piperacillin-sulbactam, gentamicin, doxycycline, cefepime, ceftriaxone, imipenem and tigecycline were used against the isolated pathogens.

Results

Out of 912 clinical specimens, 150 *A. baumannii* recovered from clinical samples. *Acinetobacter baumannii* was identified by Gram staining, colony morphology and biochemical tests. Its colony appears whitish mucoid on solid growth media with diameter of 1.5 to 3 mm; it was gram stain negative coccobacilli, strictly aerobic, non-lactose fermentative,negative oxidase test.*A. baumannii* isolates were observed positive for the assimilation of citrate, glucose, gluconate, malate, caprate, adipate, arabinose and phenylacetate and negative for hydrolysis of gelatin, aesculin, urea and arginine, glucose fermentation, nitrate reduction and for indole production by the API 20NE biochemical characterisation system. Multi-test system API 20NE (bio merieux, France) was established for the biochemical identification of *Acinetobacter* species. For the detection of *A. baumannii* isolates API 20NE system was used. Conventional methods which were used for the biochemical characterisation of *A. baumanni* at species level remained inappropriate therefore, API 20NE system was used for species identification. Hence, API 20 NE system assures the detection of species of *A. baumannii* with minimum changes in the results of biochemical tests (table 1).

Table 1. 7-dogit code generated on API 20NE for *A. baumannii.*

Triades	I			II			III			IV			V			VI			VII		
Wells	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Reactions	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	+	+	+	+	-
Points	0	0	0	0	0	0	0	0	4	1	0	0	0	0	4	1	2	4	1	2	0
Sum		0			0			4			1			4			7			3	
7-digit code	0041473																				

The isolation rate of *A. baumannii* from different clinical samples were as 31 (20.7%) from urine, 20 (13.3%) were from blood, 4 (2.7%) from CSF, 41 (27.3%) from pus, 14 (9.3%) from sputum, 10 (6.7%) from ETTA, 3 (2.0%) from HVS and 27 (18%) from other samples. The isolation rate of *A. bauminnii*from pus was highest (27.3%) whereas the lowest number was recovered from HVS (3.0%) (table 2). The frequency distribution of A. *baumannii* was highest from CICU 49 (32.7%) whereas minimum distribution was observed from paediatric ward 2 (1.3%) (table 3). The susceptibility as well as resistant pattern of recovered *A. baumannii* were as 89(59.33%) strains were sensitive and 61(40.67%) were found to be resistant against Tigicycline, 83(55.33%) sensitive whereas 67(44.67%) were resistant to Imipenem, 100(66.67%)were sensitive whereas 50(33.33%) wereresistant to Piperacillin - Tazobactum, 75(50%) were sensitive and 75(50%) resistant to Ciprofloxacin, 60 (40%) sensitive while 90(60%) were resistant to Ceftazidime, 73(48.67%) sensitive and 77(51.33%) were resistant to Gentamicin. 85(56.67%) were sensitive, and 65(43.33%) were resistant to Doxycycline, 53(35.33%) were sensitive, and 97(64.67%) were resistant to Cefipime, 58(38.67%) were sensitive, and

92(61.33%) were resistant to Ceftriaxone, 46(30.67%) were sensitive and 104(69.33%) were resistant against Ampicillin-Sulbactam.It was recorded that Piperacilline –Tazobactum showed maximum efficacy against 100 (66.67%) A. *baumannii* strains whereas the most ineffective antibiotic against *A. baumannii* was Ampicillin-Sulbactam as shown in (table 4).

Table 2. Prevalence of *A. baumannii*in various clinical samples.

Samples	No. of isolates recovered from specimen	Percentage of isolated pathogens
Urine	31	20.7 %
Blood	20	13.3 %
CSf	4	2.7 %
Pus	41	27.3 %
Sputum	14	9.3 %
ETT	10	6.7 %
HVS	3	2 %
Other fluids	27	18 %

Discussion

Acinetobacter species shows a critical cause of nosocomial infections involve meningitis, bacteremia and pneumonia. It is a microorganism that is usually harmless but can become pathogenic when the patient's resistance to disease is impaired,especially when the patient is critically ill (Bergogne-Berezin *et al.* 1996). Multidrug-resistant *Acinetobacter* species shows an increase in the number of hospital-acquired cases of disease among patients above the expected number of cases which have been described a short time ago in various countries (Corbella *et al.* 2000, Manikal *et al.* 2000). In current study 150 *Acinetobacter baumannii* recovered from different clinical samples, in which highest isolation percentage were observed as 31(20.7%) from urine, 20(13.3%) from blood, 41(27.3%) from pus respectively, which is slightly different from the study conducted by the Abbo A *et al* in which isolation rate of *A.* baumannii from different samples were as 38(32%) from respiratory tract, 23(19.5%) from wounds, 22(19%) from urine, 19(16%) from blood and 16(13.5%) from sterile fluids and catheter tips respectively (Abbo *et al.* 2005).

Table 3. Frequency distribution of isolates recovered from different wards.

Wards	No. of Recovered isolates	Percentages
CICU	49	32.7%
MICU	36	24.0%
Orthopaedic Ward	28	18.7%
Gynae Ward	13	8.7%
Medical Ward	9	6.0%
Neuro Ward	8	5.3%
Surgical Ward	5	3.3%
Paediatric Ward	2	1.3%
Total	150	100%

This difference in isolation percentage may be due to the type and number of clinical specimens included in the present study. A similar observation also noted by Akula S *et al.* in which maximum *A.baumanii* isolated from respiratory aspirates (58.1%), as in present study most of the patients admitted in the ICUs also suffer from urinary tract infections and other systemic infections because *A. baumannii* is one of the opportunistic pathogen responsible for the hospital-acquired infections like septicemia, wound sepsis, meningitis and urinary tract infections (UTI) (Akula *et al.* 2017).

In the present study all clinical specimens included from the patients which were admitted in ICUs and different wards of the tertiary care hospital. Out of 150 isolated *A. baumannii* most of the pathogens 49 (32.7%) were recovered from the patients which were admitted in the cardiac intensive care unit (CICU) followed by the medical intensive care unit (MICU) from which 36 (24%) isolates were recovered from patients table 2. In another research conducted by Anupurba S *et al.* in 2005 revealed that 20.8% of pathogens recovered from ICUs mimic the high percentage of current study(Anupurba *et al.* 2005). As most of the patients which were admitted in these wards had treated with surgical procedures like angiography, mechanical ventilation and surgery also explained that high percentage of *A. baumannii*pathogens involve in nosocomial infections day by day (Anupurba *et al.* 2005). While observations concluded by Lone R *et al.* in 2009 explained that mechanical ventilation and admission in ICU were the most critical factors which contributes the infections caused by *A. baumannii*. So these factors in intensive care units contribute for emerging *A. baumannii* as the most prominent nosocomial pathogens explained by notable research study (Lone *et al.* 2009).

Table 4. Resistant and sensitivitybehaviourof *A. baumannii* against different antibiotics.

Sr No.	Antibiotics	Abbreviations	Resistant	% of Resistant Samples	Sensitive	% of Sensitive Samples
1	CIPROFLOXACIN	CIP	75	50	75	50
2	CEFTAZIDIM	CAZ	90	60	60	40
3	AMPICILLIN-SULBACTAM	SAM	104	69.33	46	30.67
4	PIPRACILLIN-TAZOBACTAM	TZP	50	33.33	100	66.67
5	GENTAMICIN	CN	77	51.33	73	48.67
6	DOXYCYCLINE	DO	65	43.33	85	56.67
7	CEFIPIME	FEP	97	64.67	53	35.33
8	CEFTRIAXONE	CRO	92	61.33	58	38.67
9	IMIPENEM	IPM	67	44.67	83	55.33
10	TIGECYCLINE	TGC	61	40.67	89	59.33

In the present study susceptibility pattern of *A. baumannii* isolates were examined against 10 clinically used antibiotics. Among all antibiotics,Piperacillin-tazobactam, Tigecycline, doxycycline and imipenem show 66.67%, 59.33%, 56.67% and 55.33% efficacy against *A. baumannii*respectively whereas high pattern of resistance of *A. baumannii* was observed against Ampicillin-sulbactam (69.33%), Cefepime (64.67%), Ceftriaxone (61.33%) and Ceftazidime (60%). A study conducted by Dimple R *et al.* in 2016 showed that resistance of *A. baumannii* against Ampicillin-sulbactam, Ceftazidime, Aztreonam, Cefuroxime and Ampicillin was (96%), (91%), (94%), (92%) and (94%) respectively (Dimple *et al.* 2016). These observations argued with this study in which high percentage of Ampicillin-sulbactam and Ceftazidime were found to be ineffective against *A. baumannii* whereas certain drugs like Gentamicin (51.33%), Ciprofloxacin (50%), Imipenem (44.67%) and Doxycycline (43.33%) showed considerable high inefficacy against the *A.baumannii*.The research work generated by Taneja *et al.* in 2011 also discussed that *A. baumannii* were found to be resistant against Gentamicin (79.5%), Amikacin (73.2%) and Ciprofloxacin (72.8%) (Taneja *et al.* 2011). These results are slightly different from current studies in which *A. baumannii* though showed considerable high resistance pattern against the Gentamicin (51.33%) and Ciprofloxacin (50%) but had low percentage of resistance as compared to previous study. This difference in resistance pattern of *A. baumannii* against antibiotics may be due to the

difference of high number of isolates recovered from the earlier studies.

Conclusion

Acinetobacter spp., specifically *A. baumannii* isolated from different human clinical specimens established its importance as a causative agent of nosocomial infections as a human pathogen *A. baumannii* proved its notorious repute which is increasing day by day. It is revealed that *A.baumannii* infections occur with high ratio to the patients which were admitted in the ICUs. Due to the use of wide range of drugs to treat the infections, *A. baumannii* has become multidrug-resistant. Piperacillin -Tazobactum and Tigecyclin are the drugs of preference for the treatment of *A. baumannii* infections. So, increasing resistance of *A. baumannii* against different classes of drugs have been challenging task but could be minimised by adopting a standard policies and procedures for antibiotic use to control the nosocomial infections.

Conflict of interest

Authors declare no conflict of interest.

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