

ANTIMICROBIAL RESISTANCE PATTERN OF ACINETOBACTER BAUMANNII AMONG ICU PATIENTS, IN KHARTOUM STATE-SUDAN

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ABSTRACT

Background: *Acinetobacter baumannii* has become a significant nosocomial pathogen because of its remarkable ability to acquire antibiotic resistance and to survive in nosocomial environments. The aim of this study was to investigate the prevalence and antimicrobial susceptibility of *Acinetobacter baumannii* among ICU Patients, in Khartoum state-Sudan. **Methods:** One hundred isolates suspected of *A.baumannii* were collected from ICU patients, from the RoyalCare International Hospital and Ribbat National Hospital, then identified by PCR method, The disc diffusion of antimicrobial susceptibility testing according to the (CLSI, using Muller-Hinton agar (Hi-Media, Mumbai) and antimicrobial discs (bioanalyse, Turkey and Hi-Media, Mumbai).

The following antimicrobial agents (µg/ml) were used: Ceftazidim (30), cefuroxime (30), gentamicin (10), cefixime (30), ciprofloxacin (5), amoxiclav (30), meropenem (10), ceftriaxone (30) and colistin (10). **Result:** a total of 100 non-fermenting short Gram-negative bacilli isolates obtained from hospital microbiology laboratory were re-identified as

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Acinetobacter spp by PCR method. *A.baumannii* was identified in 39 isolates and *Acinetobacter* species in 13 isolates while the remaining 48 were identified as non-*Acinetobacter* species, All 39 *A.baumannii* isolates were subjected to 9 type of the commonly used antibiotic at Khartoum State microbiology laboratory, Among *A.baumannii* the resistance rates were extremely high for all types except 16 isolates were susceptible toward colistin. MDR was seen in most of isolates. **Conclusion:** Antibiotic resistance is considered to be a major future challenge in Sudan, No doubt the emergence and spread of the resistance to carbapenem in *A. baumannii* will further limit clinical therapeutic options and threaten the public health of the region.

INTRODUCTION

Over the last few years, *Acinetobacter baumannii* (*A. baumannii*) has become an important opportunistic pathogen that is widespread in hospitals and other healthcare settings. It has the ability to colonize a large variety of surfaces and can survive for prolonged periods under a wide range of environmental conditions.^[1] The emergence of *A. baumannii* strains resistant to broad-spectrum antimicrobial agents in hospital settings has become a major health burden, due to the limited treatment options for infections caused by this pathogen.^[2,3] Several highly successful MDR clones have disseminated on a global scale and are associated with particular mechanisms of resistance.

Carbapenems have been used as the most appropriate choice for the treatment of infections due to MDR strains of *A. baumannii*. Unfortunately, the prevalence of carbapenem-resistant isolates is increasing.^[4] Since carbapenems play a vital role in the treatment of nosocomial infections caused by *A. baumannii*, evaluation of the sensitivity rate of *A. baumannii* to carbapenem is crucial for proper antibiotic therapy and for preventing the emergence of MDR strains.^[4,5] Extensive administration of broad-spectrum cephalosporins and/or carbapenems is a significant risk factor for the development of colonization or infection with carbapenemase-producing *A. baumannii*.

MATERIALS AND METHODS

One hundred *A. baumannii* isolates were collected from the Microbiology laboratory department at the Royal Care International Hospital (RCIH) and the National Ribat Hospital (NRH) as identified by both laboratories, Both hospitals located in Khartoum city, Sudan. The clinical specimens were sputum, blood, urine, wound swabs, central-line catheter and tips then plated out on MacConkey agar.

Isolation and identification of *A. baumannii*: was carried out based on cultural characteristics, Gram stains, oxidase test and conventional biochemical tests following standard assay of gram negative rods at microbiology laboratory at both hospitals. Then genotypes identification of the isolate, was performed by amplification of DNA-based testing PCR was used to confirm the identification of *Acinetobacter* species and *A. baumannii* from other gram negative isolates. Depending on the site of infections and types of specimens, significant growth of each pathogen was identified and processed for antimicrobial susceptibility testing. Every single significant growth of *Acinetobacter* species and *A. baumannii* were included in this study.

Antimicrobial susceptibility was performed by disc diffusion method as per the (CLSI) guidelines^[6], using Muller-Hinton agar (Hi-Media, Mumbai) and antimicrobial discs (bioanalyse, Turkey and Hi-Media, Mumbai). The following antimicrobial agents ($\mu\text{g/ml}$) were used: Ceftazidim (30), cefuroxime (30), gentamicin (10), cefixime (30), ciprofloxacin (5), amoxiclav (30), meropenem (10), ceftriaxone (30) and colistin (10). The diameter of inhibition zones was measured and reported as susceptible or resistant. Quality control of the disks were checked by using reference strains.

RESULT

Intensive care unit patients are susceptible to several opportunistic infections due to their immune-suppressive state. Therefore; collection of a total of 100 non-fermenting Gram-negative short bacilli cultures that obtained from hospital microbiology laboratory was done and were re-identified as *Acinetobacter* spp, Due to the high similarities among *A. baumannii* and non-*Acinetobacter* species as routine biochemical tests for identification Gram negative bacteria was poorly distinguish between them, therefore Genotyping identification by PCR, served to identify the *Acinetobacter* species as well as confirm the *A. baumannii* isolates. (fig1).

A. baumannii was identified in 39 isolates and *Acinetobacter* species in 13 isolates while the remaining 48 were identified as non-*Acinetobacter* species, Most of specimen of the isolates were from sputum specimen (table1), Resistance to the antibiotics used was very high, The lowest percentage of resistance observed was to colistin (table2).

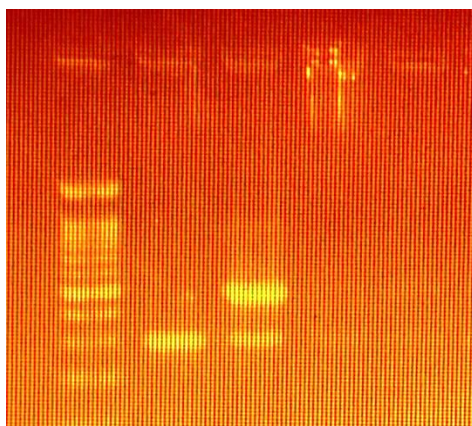


Fig.1: Gel-electrophoresis result of *Acinetobacter* isolates identification. Lane 1: DNA ladder 100bp; lane 2: a 293 bp band with the Pf/Pr1 primer pair for identification *Acinetobacter* Spp; lane 3: two bands one at 293bp and one at 500 bp with the Pf/Pr2 primer pair for identification of *A.baumannii*; lane 4&5: non-*Acinetobacter* Spp.

Table 1: Distribution pattern of *A.baumannii* isolates by their respective sources of specimens from ICU patients at Khartoum state selected hospitals.

Source of specimens	<u><i>A.baumannii</i> (n=39)</u> N (%)
Sputum (n=37)	29 (74.4%)
Urine (n=4)	3 (7.7%)
Wound (n=2)	1 (2.6%)
Blood (n=3)	3 (7.7%)
Bed sore (n=1)	1 (2.6%)
Central line (n=2)	1 (2.5%)
Tip (n=3)	2 (5.1%)

Table 2: Antimicrobial susceptibilities profiles of *A.baumannii* isolates and CP-AB from ICU patients at Khartoum state selected hospitals.

Antibiotic disc µg/ml	<i>A.baumannii</i> (n=39)	
	Resistance N (%)	Sensitive N (%)
Ciprofloxacin	39 (100%)	0 (0.0%)
Cefixime	39 (100%)	0 (0.0%)
Ceftazidime	39 (100%)	0 (0.0%)
Gentamycin	39 (100%)	0 (0.0%)
Ceftriaxone	39 (100%)	0 (0.0%)
AMC*	39 (100%)	0 (0.0%)
Cefuroxime	39 (100%)	0 (0.0%)
Colistin	23 (59.0%)	16 (41.0%)
Meropenem	38 (97.4%)	1 (2.6%)

*Antibiotic disc concentrations in µg/ml, AMC; Amoxicillin/Clavulanic Acid, *Sig. P value<0.05,

DISCUSSION

A.baumanii is an important opportunistic pathogen that is responsible for health-care infection mainly by MDR strain. *A.baumannii* is highly resistant to commonly used antibiotics such as penicillins, cephalosporins, aminoglycosides and fluoroquinolones by intrinsic and acquired mechanisms. They are also gradually becoming resistant to carbapenems, as this study has shown, in Khartoum state. Elsewhere the isolation of MDR *A.baumannii* from ICU samples as seen in the present study is had been reported earlier by Josheghani SB, Moniri R, Firoozeh F, Sehat M, Dastehgoli K and Koosha H.^[7]

Hospitals have long served as reservoirs for the transmission of pathogenic bacteria, and this has become a problem in Khartoum state. Among the source of the isolates the vast majority of positive cultures were from respiratory specimens (74.4%) followed by urine and tip specimens, This is consistent with other studies.^[8,9,10] Local Infections with *A. baumannii* sometimes may end up with bacteraemia especially in patient received mechanical ventilation.^[11]

The results of the present study showed that there was an extreme increase at our hospitals in the resistance rate of *A. baumannii* to meropenem which, from 89% in 2015 to 100% in 2019.^[12] In addition, the resistance rate to *A. baumannii* to colistin was 59%, which is higher than in previous reports in Khartoum state and other studies.^[13,14,15] The present study showed 100% resistant rates of the most clinically applicable antibiotics for the treatment of infections caused by *A. baumannii*, except for colistin, which may be used as the final options in the management of infections caused by this bacterium. In this study, the high resistance rate of *A. baumannii* against carbapenems may indicate the outcome of overuse and misuse of carbapenems in our hospitals.

Several studies on *A. baumannii* in different parts of the world have also shown huge drug-resistance rates; for example, there is 70% resistance to imipenem in Egypt^[16], 64.3% resistance to meropenem in Nigeria^[17], 32.6% resistance to carbapenem in Saudi Arabia⁽¹⁸⁾, and 17.6% resistance to imipenem in Chinese hospitals.^[19] Variations in reports between the studies could be influenced by ecological factors and different administration of antimicrobial plans.

Colistin is currently the most frequently administered antimicrobial agents as a result of the decrease sensitivity to the carbapenems which explain the current high resistance to

colistin.^[20] Nonetheless, it is outstanding that colistin was not used empirically. The concern about the toxicity of colistin and the lack of clinical trials which evaluate the empirical use of this antibiotic could explain this finding.^[21] Motaouakkil et al.^[22] reported better clinical response rates (56–61%) for intravenous colistin treatment of multidrug-resistant *Acinetobacter ventilator-associated pneumonia*.

REFERENCES

1. Ben-Chetrit E, Wiener-Well Y, Lesho E, Kopuit P, Broyer C, Bier L, et al. An intervention to control an ICU outbreak of carbapenem-resistant *Acinetobacter baumannii*: long-term impact for the ICU and hospital. *Critical Care*, 2018; 22(1): 319.
2. Kempf M, Rolain J-M. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. *International journal of antimicrobial agents*, 2012; 39(2): 105-14.
3. Sileem AE, Said AM, Meleha MS. *Acinetobacter baumannii* in ICU patients: A prospective study highlighting their incidence, antibiotic sensitivity pattern and impact on ICU stay and mortality. *Egyptian Journal of Chest Diseases and Tuberculosis*, 2017; 66(4): 693-8.
4. BAGHERI JS, MONIRI R, FIROOZEH F, SEHAT M, DASTEHGOLI K, KOOSHA H, et al. EMERGENCE OF BLAOXA-CARRYING CARBAPENEM RESISTANCE IN MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII IN THE INTENSIVE CARE UNIT, 2017.
5. Josheghani SB, Moniri R, Firoozeh F, Sehat M, Dastehgoli K, Koosha H, et al. Emergence of bla OXA-Carrying Carbapenem Resistance in Multidrug-Resistant *Acinetobacter baumannii* in the Intensive Care Unit. *Iranian Red Crescent Medical Journal*, 2017; 19(5).
6. Patel JB, Cockerill FR, Bradford PA. Performance standards for antimicrobial susceptibility testing: twenty-fifth informational supplement, 2015.
7. Josheghani SB, Moniri R, Firoozeh F, Sehat M, Dastehgoli K, Koosha H, et al. Emergence of bla OXA-Carrying Carbapenem Resistance in Multidrug-Resistant *Acinetobacter baumannii* in the Intensive Care Unit. *Iranian Red Crescent Medical Journal*, 2017; 19(5).
8. Sileem AE, Said AM, Meleha MS. *Acinetobacter baumannii* in ICU patients: A prospective study highlighting their incidence, antibiotic sensitivity pattern and impact on

- ICU stay and mortality. *Egyptian Journal of Chest Diseases and Tuberculosis*, 2017; 66(4): 693-8.
9. Reddy D, Morrow BM, Argent AC. *Acinetobacter baumannii* infections in a South African paediatric intensive care unit. *Journal of Tropical Pediatrics*, 2015; 61(3): 182-7.
 10. Elabd FM, Al-Ayed MS, Asaad AM, Alsareii SA, Qureshi MA, Musa HA-A. Molecular characterization of oxacillinases among carbapenem-resistant *Acinetobacter baumannii* nosocomial isolates in a Saudi hospital. *Journal of infection and public health*, 2015; 8(3): 242-7.
 11. Hong KB, Oh HS, Song JS, Lim J-h, Kang DK, Son IS, et al. Investigation and control of an outbreak of imipenem-resistant *Acinetobacter baumannii* infection in a pediatric intensive care unit. *The Pediatric infectious disease journal*, 2012; 31(7): 685-90.
 12. Omer MI, Gumaa SA, Hassan AA, Idris KH, Ali OA, Osman MM, et al. Prevalence and resistance profile of *acinetobacter baumannii* clinical isolates from a private hospital in Khartoum, Sudan. *Am J Microbiol Res.*, 2015; 3(2): 76-9.
 13. Bakour S, Olaitan AO, Ammari H, Touati A, Saoudi S, Saoudi K, et al. Emergence of colistin-and carbapenem-resistant *Acinetobacter baumannii* ST2 clinical isolate in Algeria: first case report. *Microbial Drug Resistance*, 2015; 21(3): 279-85. Qureshi ZA, Hittle LE, O'Hara JA, Rivera JI, Syed A, Shields RK, et al. Colistin-resistant.
 14. *Acinetobacter baumannii*: beyond carbapenem resistance. *Clinical infectious diseases*, 2015; 60(9): 1295-303.
 15. Machado D, Antunes J, Simões A, Perdigão J, Couto I, McCusker M, et al. Contribution of efflux to colistin heteroresistance in a multidrug resistant *Acinetobacter baumannii* clinical isolate. *Journal of medical microbiology*, 2018; 67(6): 740-9.
 16. Al-Agamy MH, Khalaf NG, Tawfick MM, Shibl AM, El Kholy A. Molecular characterization of carbapenem-insensitive *Acinetobacter baumannii* in Egypt. *International Journal of Infectious Diseases*, 2014; 22: 49-54.
 17. Josheghani SB, Moniri R, Firoozeh F, Sehat M, Dastehgoli K, Koosha H, et al. Emergence of bla OXA-Carrying Carbapenem Resistance in Multidrug-Resistant *Acinetobacter baumannii* in the Intensive Care Unit. *Iranian Red Crescent Medical Journal*, 2017; 19(5).
 18. Ibrahim ME. Prevalence of *Acinetobacter baumannii* in Saudi Arabia: risk factors, antimicrobial resistance patterns and mechanisms of carbapenem resistance. *Annals of clinical microbiology and antimicrobials*, 2019; 18(1): 1.

19. Hu F-P, Guo Y, Zhu D-M, Wang F, Jiang X-F, Xu Y-C, et al. Resistance trends among clinical isolates in China reported from CHINET surveillance of bacterial resistance, 2005–2014. *Clinical microbiology and infection*, 2016; 22: S9-S14.
20. Livermore DM. Fourteen years in resistance. *International journal of antimicrobial agents*, 2012; 39(4): 283-94.
21. Montero M, Horcajada J, Sorli L, Alvarez-Lerma F, Grau S, Riu M, et al. Effectiveness and safety of colistin for the treatment of multidrug-resistant *Pseudomonas aeruginosa* infections. *Infection*, 2009; 37(5): 461-5.
22. Garnacho-Montero J, Amaya-Villar R. Multiresistant *Acinetobacter baumannii* infections: epidemiology and management. *Current opinion in infectious diseases*, 2010; 23(4): 332-9.