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ABSTRACT

Background:**The emergence of multidrug resistant *A.baumannii* as an opportunistic nosocomial pathogen in intensive care units is of great public health concern worldwide. Carbapenems such as meropenem and imipenem have been used as antibiotics of last resort, the main mechanisms of *A. baumannii* are the production of carbapenemases.**

Objective: **To investigate the prevalence carbapenemase genes among MDR *A.baumannii* in clinical isolates from Critical Care Unit of KNH.**

Design: **December 2014 and May 2015 cross sectional study.At KNH Microbiology and KAVI Institute of clinical Research (KAVI-ICR) laboratories.**

Setting: **Susceptibility of 53 isolates of MDR *A. baumannii* from CCU was determined. However only 22 were analyzed for carbapenemase genes due to cost. Real time polymerase chain reaction (RT PCR)) was used to detect the common carbapenemase genes: Veron integrin metallo-beta-lactamases (VIM), imipenemase (IMP) and New Delhi metallo-beta-lactamase (NDM), OXA-48 and *Klebsiella pneumonia*e carbapenemase(KPC).**

Results: **The findings indicated high levels of *A. baumannii* resistance to antibiotics used in CCU. Resistance to meropenemwas 84.9% (n=45) and 73.6% (n=39) to imipenem. Prevalence of carbapenemase genes among MDR *A. baumannii* isolates was 100% (n=22). The genes were heterogeneously distributed among the isolates. The most predominant carbapenemase gene detected was OXA48 with 100% (n= 22) followed by NDM with 90.9% (n=20).**

Conclusion: **Carbapenem resistance conferring genes were detected in all multidrug resistant *A. baumanii,* with OXA48 always present. Strengthening of antibiotic stewardship programs which will contribute to enhancement of infection control policies.**

INTRODUCTION

Acinetobacter baumannii is a gram-negative coccobacillus that is non-motile, non-fastidious, aerobic, oxidase negative and glucose non fermanter (1). It is an important opportunistic pathogen mainly in critical care units (CCU) responsible for a wide range of diseases which affects human respiratory tract, bloodstream, surgical sites, wounds and urinary tract (1)(2). The potentiality of *A. baumannii* to accumulate antibiotic resistant determinants in response to antibiotic interventions and it's environmental resilience are associated with patients initial colonization and subsequent infection (3).

The recommended regimen for the treatment of *A. baumanii* include Sulbactam, aminoglycosides, carbapenems, tetracyclines, tigecycline and polymyxins (4). The carbapenem antibotics have been used as the drug of choice in treatment of MDR *A. baumannii* due to their low toxicity and effective antibacterial activity, however resistance to this drugs is creating major therapeutic challeges (5).

In South Africa resistance on MDR *A. baumanii* was reported to be higher than 86% in meropenem and imipenem out of 345 isolates studied (6)(7). A systematic review in Eastern Africa reported 64.8% resistance of MDR *A. baumannii* to carbapenems in 30 isolates (8). Multidrug resistance among *A. baumannii* refers to resistance to at least three of the following antimicrobials: ampicillin, augmentin, ceftazidime, ciprofloxacin, gentamicin, and/or trimethoprim-sulfamethoxazole (9). The major mechanisms of β-lactam acquisition of antibiotic resistance in *A. baumannii* is the production of carbapenemases, mainly Ambler class D β-lactamases and to a lesser degree class B β-lactamases metallo-β-lactamases (7)

A.baumannii resistance to carbapenem in Europe has been widely attributed to the production of IMP-types and OXA-type carbapenemases (10). Acinetobacter species possessing NDM-1 genes have been reported in various countries including India, Israel, Egypt, Germany, Spain, Switzerland, the United Arab Emirates and China (11)

In Kenya a study on carbapenem resistant *A.baumannii* (CRAB) indicated that all isolates harbored Oxa type carbapenemase genes, with one isolate also harboring $bla_{\text{NDM-1}}$gene (12). Interestingly to note that presence of a carbapenemase gene is not determined by prior exposure to carbapenem therapy but also with use of other antibiotics such as aminoglycosides and fluoroquinolones. This has been demonstrated by detection of OXA-23 in *A. baumannii* even where carbapenems are not used (13). It is against this back ground that we sought to determine Carbapenemase genes among MDR *A. baumannii* in the CCU of Kenyatta National Hospital (KNH).

METHODS

Study area and design: From December 2014 to May 2015 a cross-sectional study was conducted on urine and tracheal aspirate samples sent to KNH Microbiology Laboratoryand KAVI Institute of clinical Research KAVI-ICR from ICU patients at the KNH. The Laboratories are ISO 15189: 2012 accredited.

Sample size determination and sampling: A total of 289 urine and 450 tracheal aspirates were received in the Microbiology Laboratoryfor analysis.Urine specimens were cultured in CystineLactose Electrolyte Deficient (CLED) medium while tracheal aspirates were culture on Sheep blood agar and MaCconkey agar.The plates were incubated at 37^0C for 18-24 hours. Microbial identification and susceptibility profiles of *A. baumannii* isolates were performed byVITEK® 2 automated system (bioMérieuxFrance) automatic analyzer. Viteck 2 analyzer is controlled by a program under CLSI guidelines.. *A. baumannii* isolates were identified using Gram negative card (2 GN), while susceptibility testing was

determined by Gram negative sensitivity cards (AST-GN83).Escherichia coli ATTC 25922 was used as control organismfor susceptibility determination.

For the purpose of this study the resistance pattern for the MDR was determined byresistance to at least three of the following antimicrobials: ampicillin, augmentin, ceftazidime, ciprofloxacin, gentamicin, and/or trimethoprim-sulfamethoxazole. The isolates were non repetitive.

The following following sixteen antibiotics were tested: ampicillin, augumentin, cefuroxime, piperacillin-tazobactum, imipenem, cefepine, ceftriaxone, ceftazidime, cefotaxime, meropenem, aztreonam, cefoxitin, ciprofloxacin, gentamycin, amikacin and trimethoprim – sulfamethoxazole.

After identification of MDR*A. baumannii*isolates colonies were put in vials containing brain heart infusion broth supplemented with 20% glycerol and then stored at -70^0 c. The isolates were pooled and later removed from the freezer, thawed and sub cultured on MaCconkey agar, incubated for 18-24 hours to check viability. All 53 isolates successfully grew. 22 isolates were randomly selected and taken for real time PCR at KAVI Laboratory.

Real time PCR: DNA extraction and Detection of carbapenemase genes were carried out according to kit manufacturer's instructions (MDR MBL and MDR KPC/OXA kits- Sacace biotechnologies Italy).

The results were interpreted by the real-time PCR instrument software by the crossing or not crossing of the threshold line by the fluorescence curve. The results of amplification were considered positive if the fluorescence curve was characteristic of real time PCR (S-shaped) crossed the threshold line (the Ct value present). The result of amplification was considered negative if the fluorescence curve was not S-shaped and if it did not cross the threshold line (the Ct value is absent). Quality control was in accordance with ISO -Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

Ethical considerations: The approval of this research was sought from KNH/ UoN ethical board following approval by all the supervisors: Ref no: P668/11/2014.

Data management and analysis: Data was entered and managed in MS Excel spread sheet study population was described by summarizing the age into mean with standard deviation and sex into proportions. The type of microbial agent and antimicrobial sensitivities were analyzed and presented as proportions of the total sample size. In addition, antimicrobial sensitivities were stratified according to microbial agents. Gene expressions were presented as percentages and stratified by the type of microbial agents.

RESULTS

Growth was obtained in 108 (37.4%) samples of urine and 322 (71.6%) samples of tracheal aspirates. Of the growth obtained, 12 (11.1%) isolates from urine and 77 (23.9%) isolates from tracheal aspirates were identified as *A. baumannii*. A total of 53 isolates were MDR *A. baumanni*; 9 (75.0%) isolates from urine and 44 (57.1%) isolates from tracheal aspirates.

Susceptibility to various antibiotics

The isolates exhibited very high resistance to antibiotics used in the hospitals CCU. Amikacin was the most sensitive drug with resistance levels of n= 37 (32.1%) followed by imipenem n = 39 (73.6%), gentamycin n=43(81.1%), ciprofloxacin n=44 (83%) meropenem n=45 (84.9%), trimethoprim-sulfamethoxazole n= 45 (84.9%), piperacillin/tazobactamnn=49 (92.5%), cefoxitinn=53 (19.4%),cefepine n=46 (86.8%), aztreonamn=51 (92.2%), ceftazidimen=50 (94.3%), augmentinn=52 (98.1%), cefuroxime n=53 (98.1%), cefotaximen=53 (100%), ampicillin n=53 (100%) and ceftriaxone n=52 (100%).

Table 4

Antimicrobial resistance forMDRA.baumannii

Antimicrobial agent	**Microbial agent**	
	***Acinetobacter baumannii* (n=53)**	
	Count	**%**
Ampicillin	53	100.0
Augmentin	52	98.1
Pip/tazobactam	49	92.5
Cefuroxime	53	100.0
Cefoxitin	53	100.0
Cefotaxime	53	100.0
Ceftazidime	50	94.3
Cefriaxone	52	98.1
Cefepime	46	86.8
Aztreonam	51	96.2
Meropenem	45	84.9
Amikacin	37	32.1
Gentamicin	43	81.1
Ciprofloxacin	44	83.0
Trime/sulfamethoxazole	45	84.9
Imipenem	39	73.6

Prevalence of carbapenemase genes: A total of 22 samples of *A. baumannii* were analyzed by real-time PCR for VIM, IMP, NDMA48 and KPC genes.

Distribution of carbapenemase genes: Heterogeneous distribution of carbapenemase genes was observed in *A. baumannii* More than one carbapenemase genes were detected in single isolates. Distribution was as follows; *A. baumannii:* (22*)*: NDM 20 (90.9 %), IMP 7 (31.8%), OXA 48 22 (100%), VIM 0 (0%), KPC 0 (0%).

Table 6

Gene expression

	NDM	IMP	VIM	OXA48	KPC
A. baumannii (n=22)	20 (90.9%)	7(31.8%)	0	22(100%)	0

Table 7

Co-existence of genes in isolates

Gene	*A.baumannii* (n=22)
NDM,OXA 48	20
NDM,IMP	7
NDM, IMP,OXA48	7

DISCUSSION

Resistance mechanism among multidrug resistant *A. baumannii* in CCU is mainly mediated by NDM and oxa48 genes which indicate danger of limited treatment options. A great treatment challenge caused by MDR *A. baumannii* is highly exhibited by the results of this investigation. The isolation of extremely resistant *A.baumannii* isolates from CCU is anticipated as such scenarios have been reported by previous studies.

The high resistance rates among MDR *A.baumannii* could be as a result of prolonged antibiotic use and CCU stays (1)(2) and presence of multiple carbapenemases (14). Amikacin was found to be more sensitive than carbapenems (67.9%) and this could be attributed to its limited use in the hospitals CCU. The same scenario was also noted in Egypt, South Africa and Nigeria on carbapenem resistant *A. baumannii* (15) (6)(7)(16).

Isolates were more resistant to meropenem (84.9%) than imipenem (73.6). This discordance in carbapenem susceptibilities have been described in other studies (4). This could be attributed to the fact that meropenem is the commonly used carbapenem in the hospitals CCU. This finding contrasts with study in South Africa where imipenem was more resistant than meropenem (7). Due to the high resistance observed in carbapenems testing of antibiotics such as colistin and tigecycline is important in order to provide alterative treatment to these MDR *A. baumannii* isolates.

The findings in this study indicates that all the isolates harbored almost similar genes, This could be attributed to the fact that the study was conducted over a short period of time and all the patients were in the same confinement thus obvious similar environmental exposures, this may have led to gene exchanges between different bacterial species.

In this study all isolates possessed OXA 48 gene. This scenerio was observed in a neibouring hospital where all CRAB isolates possessed OXA type carbapnemase(12). NDM and OXA 48 genes coexisted in nearly all the samples. The findings also agrees with another study in Morroco which repoted OXA type in all MDR *A.baumanii* studied and its co occurrence with NDM in some isolates (17). Coexistence of carbapenemase genes provides selection advantage to this pathogens(18). This probably explains the high level of resistance observed.

The prevalence of VIM was zero, this concurs with a research carried out in Poland on MBL producing *A. baumannii* which indicated that 10.3% of 78 isolates studied carried $bla_{\text{IMP-like}}$ gene and none carried bla_{VIM} (19). None of the isolates haboured KPC genes. A similar finding was documented in Egypt KPC and VIM were absent IN 30 CRAB isolates investigated (20).

CONCLUSION

Resistance mechanism among multidrug resistant *A. baumannii* in CCU is mainly mediated by NDM and oxa48 genes which indicate danger of limited treatment options.

LIMITATIONS

This study had a number of limitations. For instant Due to cost of RT-PCR not all known genes were analyzed; only commonly expressed genes were analyzed. Thus, there is a probability that some carbapenemase producing pathogens could not be adequately characterized. The study was not able to differentiate between carbapenemase gene variants e.g. NDM-1 and NDM-2. A better representation of resistance would have been revealed if the study was carried out in all major hospitals with CCU. This was not possible due to limited resources.

REFERENCES

1. Visca P, Seifert H, Towner KJ. Acinetobacter infection - An emerging threat to human health. IUBMB Life. 2011;63(12):1048–54.
2. Arhoune B, Oumokhtar B, Hmami F, Fakir S El, Moutaouakkil K, Chami F, et al. Intestinal carriage of antibiotic resistant Acinetobacter baumannii among newborns hospitalized in Moroccan neonatal intensive care unit. PLoS One. 2019;14(1):1–12.
3. Fournier PE, Richet H, Weinstein RA. The Epidemiology and Control of Acinetobacter baumannii in Health Care Facilities. Clin Infect Dis. 2006;42(5):692–9.
4. Fishbain J, Peleg AY. Treatment of Acinetobacter Infections. 2010;51(Table 1):79–84.
5. Alhaddad MS, AlBarjas AK, Alhammar LE, Al Rashed AS, Badger-Emeka LI. Molecular Characterization and Antibiotic Susceptibility Pattern of Acinetobacter Baumannii Isolated in Intensive Care Unit Patients in Al-Hassa, Kingdom of Saudi Arabia. Int J Appl basic Med Res [Internet]. 2018;8(1):19–23. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29552530
6. Lowings M, Ehlers MM, Dreyer AW, Kock MM. High prevalence of oxacillinases in clinical multidrug-resistant Acinetobacterbaumannii isolates from the Tshwane region, South Africa - an update. BMC Infect Dis [Internet]. 2015;15(1):1–10. Available from: http://dx.doi.org/10.1186/s12879-015-1246-8
7. Lowe M, Ehlers MM, Ismail F, Peirano G, Becker PJ, Pitout JDD, et al. Acinetobacter baumannii: Epidemiological and beta-lactamase data from two tertiary academic hospitals in Tshwane, South Africa. Front Microbiol. 2018;9(JUN):1–9.
8. Geteneh A, Demissew A, Adimas A, Alemu D, Girma L. Therapeutic Challenges of Multidrug Resistant Acinetobacter baumannii in Eastern Africa : Systematic Review. 2019;1–20.
9. Pop-Vicas AE, D'Agata EMC. The Rising Influx of Multidrug-Resistant Gram-Negative Bacilli into a Tertiary Care Hospital. Clin Infect Dis. 2005;40(12):1792–8.
10. Walsh TR, Toleman MA, Poirel L, Nordmann P. the Quiet Before Storm. 2005;18(2):306–25.
11. Cherkaoui A, Emonet S, Renzi G, Schrenzel J. Characteristics of multidrug - resistant Acinetobacter baumannii strains isolated in Geneva during colonization or infection. Ann Clin Microbiol Antimicrob. 2015;1–7.
12. Revathi G, Siu LK, Lu PL, Huang LY. First report of NDM-1-producing Acinetobacter baumannii in East Africa. Vol. 17, International Journal of Infectious Diseases. 2013.
13. Manenzhe RI, Zar HJ, Nicol MP, Kaba M. The spread of carbapenemase-producing bacteria in Africa: A systematic review. J Antimicrob Chemother. 2015;70(1):23–40.
14. Vali L, Dashti K, Opazo-Capurro AF, Dashti AA, Al Obaid K, Evans BA. Diversity of multi-drug resistant Acinetobacter baumannii population in a major hospital in Kuwait. Front Microbiol. 2015;6(JUL):1–8.
15. Al-Agamy MH, Khalaf NG, Tawfick MM, Shibl AM, El Kholy AA. Molecular characterization of carbapenem-insensitive Acinetobacter baumannii in Egypt. Int J Infect Dis [Internet]. 2014;22:49–54. Available from: http://dx.doi.org/10.1016/j.ijid.2013.12.004
16. Nwadike VU, Ojide CK, Kalu EI. Multidrug resistant acinetobacter infection and their antimicrobial susceptibility pattern in a Nigerian tertiary hospital ICU. African J Infect Dis. 2014;8(1):14–8.

17. Uwingabiye J, Lemnouer A, Roca I, Alouane T, Frikh M, Belefquih B, et al. Clonal diversity and detection of carbapenem resistance encoding genes among multidrug-resistant Acinetobacter baumannii isolates recovered from patients and environment in two intensive care units in a Moroccan hospital. 2017;1–9.

18. Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F. Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. Biomed Res Int. 2014;2014.

19. Szejbach A, Mikucka A, Bogiel T, Gospodarek E. Usefulness of phenotypic and genotypic methods for metallo-beta-lactamases detection in carbapenem-resistant Acinetobacter baumannii strains. Med Sci Monit Basic Res. 2013;19:32–6.

20. Ramadan RA, Gebriel MG, Kadry HM, Mosallem A. Carbapenem-resistant acinetobacter baumannii and pseudomonas aeruginosa: Characterization of carbapenemase genes and E-test evaluation of colistin-based combinations. Infect Drug Resist. 2018;11:1261–9.