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Short Report

Virulence genes and plasmid replicon profiles of selected β-lactamase-producing *Acinetobacter baumannii* from orthopaedic patients and the environment in a tertiary referral hospital in Tanzania, East Africa

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SUMMARY

Acinetobacter baumannii has emerged as an important nosocomial pathogen due to its high resistance to multi-drugs and disinfectants plus its ability to survive in hospital environments. Rectal swabs were collected for screening β-lactamases-producing Acinetobacter baumannii among hospitalized orthopedic patients at a tertiary referral hospital in Tanzania. Swabs were also taken from patients' caretakers, healthcare workers, and the neighboring inanimate environment. A total of 26 confirmed β-lactamases producing Acinetobacter baumannii were isolated, of which 4 representative isolates (two from patients and two from hospital environment) underwent whole-genome sequencing (WGS) to detect sequence types (ST), β-lactamases genes, plasmid replicon types, and virulence genes. All four isolates harbored multiple β-lactamases genes including blaADC-25(3), blaOXA(4), blaCTX-M-15(2) and blaNDM-1(2). Furthermore, isolates harbored virulence

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genes encoding outer membrane protein (ompA), curli protein (csg), siderophore biosynthesis systems (enterobactin [entABCDEFS, fepABCDG, fes]; yersiniabactin [ybtAEPQSTUX, irp1, irp2, fyuA] and aerobactin [iucABCD, iutA]), transport secretion system type II (T2SS) and type III (T3SS), E. coli common pilus (ecpRABCDE operon), type 1 fimbriae (fim), arylsulfatase (aslA) and adhesions (fedC). Only isolates from patients harbored 4 plasmid replicons each, with the most common plasmid replicons being IncFIA_1; IncY_1 and IncFIB(AP001918)_1. Admitted orthopedic patients and the hospital environment act as a reservoir of multiple β-lactamases producing Acinetobacter baumannii (including those against carbapenems like blaOXA and blaNDM-1) endowed with virulence genes, highlighting the necessity to routinely screening of orthopedic patients with open fractures on admission as well as reinforcing infection prevention and control measures to reduce the dissemination of nosocomial infection within the hospital environment.

Acinetobacter baumannii is an important nosocomial pathogen due to its high resistance to multi-drugs and disinfectants that are detrimental to most pathogens, while enduring in the hospital environment [1]. Unlike numerous investigations on the drug-resistant epidemiology of *Acinetobacter baumannii*, virulence determinants are less studied [2]. Understanding the virulence genes among β-lactamase-producing *Acinetobacter baumannii* isolates from patients and the hospital environment is crucial for surveillance and devising the strategies for control and prevention of this pathogen. Therefore, from January to May 2020, we conducted a cross-sectional study to unravel the sequence type, plasmid replicons, and virulence determinant genes from the selected β-lactamase-producing *Acinetobacter baumannii* isolated from orthopaedic patients and the hospital environment of Bugando Medical Centre, a tertiary hospital in Mwanza, Tanzania. Rectal swabs were collected from hospitalized orthopaedic patients and swabs were also taken from the patients' neighbouring inanimate environment for screening β-lactamase-producing *Acinetobacter baumannii*. We obtained 26 β-lactamase-producing *Acinetobacter baumannii* isolates (nine (34.6%) from patients and 17 (65.4%) from the hospital environment).

We purposely selected two isolates from patients, and two from the surfaces that were commonly shared by the patients. Microbiological culture and DNA extraction were carried out followed by whole-genome sequencing on the Illumina NovaSeq 6000 platform generating paired-end sequencing reads of 150bp. The analysis of genomic data was performed using rMAP, the Rapid Microbial Analysis pipeline [3], with *Acinetobacter baumannii* reference (NZ_CP018254.1).

Of these four β-lactamase-producing *Acinetobacter baumannii* isolates, two were ST1 (patient and environmental isolates), and others were ST103 (patient) and ST52 (environment). All isolates harboured multiple β-lactamase genes including $bla_{ADC\text{-}25}$ (three), $bla_{OXA\text{-}1,\ 69,\ 70,\ 98,}$ and $_{420}$ (four), $bla_{CTX\text{-}M\text{-}15}$ and $bla_{NDM\text{-}1}$ (two). All isolates harboured virulence genes encoding outer membrane protein (*ompA*), curli protein (*csg*), siderophore biosynthesis systems (enterobactin (*entABCDEFS, fepABCDG, fes*), yersiniabactin (*ybtAEPQSTUX, irp1, irp2, fyuA*) and aerobactin (*iucABCD, iutA*)), transport secretion system type II (*T2SS*) and type III (*T3SS*), *Escherichia coli* common pilus (*ecpRABCDE* operon), type 1 fimbriae (*fim*), arylsulfatase (*aslA*) and adhesions (*fedC*).

Notably, isolates from the patients harboured more virulence genes than isolates from inanimate surfaces. All clinical isolates possessed four plasmid replicons while all isolates from the inanimate environment did not possess any of the plasmid replicons, with the most common plasmid replicons being *IncFIA_1; IncY_1* and *IncFIB(AP001918)_1* (Table I).

Worryingly, these β-lactamase-producing *Acinetobacter baumannii* are implicated in hydrolyzing third-generation cephalosporins (commonly used agents in admitted patients) and carbapenems (last-resort antibiotic options for critically ill patients). Of note, β-lactamase genes delineated in this study were alarmingly conferring resistance to both third-generation cephalosporins ($bla_{ADC\text{-}25}$ and $bla_{CTX\text{-}M\text{-}15}$), and carbapenems ($bla_{OXA}$ and $bla_{NDM\text{-}1}$).

We demonstrated that clinical isolates have a huge arsenal of virulence factors compared with isolates collected from the hospital environment. The virulence determinants in *Acinetobacter baumannii* from patients predispose these patients with open fractures to the acquisition of severe nosocomial infections and therefore raise an alarm for strengthening routine genomic surveillance and nosocomial infection prevention and control.

All isolates harboured the *ompA* gene encoding for outer membrane protein (*OmpA*), which induces host tissue damage, cell death, and effluxing antibiotics out of bacterial cells promoting multi-drug-resistant phenotype [4]. *OmpA* performs several functions to facilitate infectivity and pathogenicity, such as the formation of biofilm, adhesion to host epithelial cells, and resistance against complement killing [5]. We highlight that all β-lactamases producing *Acinetobacter baumannii* harboured one or more genes encoding for siderophores biosynthesis (enterobactin, yersiniabactin, and aerobactin). Two isolates harboured genes encoding for iron acquisition from heme (*chu* and *shu*).

The clinical isolates harboured genes encoding for protein secretion systems type 2 and 3 (*T2SS* and *T3SS*) which form a potential virulent strategy [6]. We showed that all isolates studied have one or more genes for adhesions (*csg, fedC, fimH, ecp*). Adhesion and biofilm formation are important for attachment and hence invasion of host cells. Adhesins or type 1 fimbriae protein encoded by the *fimH* gene facilitate adhesions and biofilm formation. The isolates from patients harboured *aslA* gene encoding arysulfatase *A* enzyme, which breaks down sulfatides (cerebroside 3-sulfate) into cerebroside and sulfate and is used for invasion. Studies revealed that *aslA* contributes to the invasion of brain microvascular endothelial cells causing meningitis by *E. coli* [7].

Table I
Distribution of four beta-lactamase-producing Acinetobacter baumannii isolates by source, sequence type (ST), drug-resistant genes, virulence determinant genes,- and plasmid replicons

SN	ID	Source	ST	Beta-actamase genes	Other drug-resistant genes	Virulence genes	Plasmid replicon type
1	ORTHO 015E	Wheel chair	52	*blaADC-25; blaNDM-1; blaOXA-98*	*floR; mph(E); msr(E); sul2; tet(39); tet(B)*	*csgG; entB; ompA*	—
2	ORTHO 037E	Ward floor	1	*blaADC-25; blaOXA-69*	*ant(2")-la; sul2*	*csgG; entB; ompA*	—
3	ID092	Patient	103	*blaCTX-M-15; blaNDM-1; blaOXA-1; blaOXA-70*	*aac(3)-lla; aac(6')-lb-cr; aadA5; aph(3")-lb; aph(6)-ld; dfrA17; dfrA8; mdf(A); mph(A); gnrS1; sul1; sul2; tet(B)*	*afaA; afaB-I; afaC-I; afaE-V; aslA; chuS; chuT; chuU; chuV; chuW; chuY; cnf1; csgB; csgD; csgG; daaF; draD; draP; entA; entB; entD; entE; entF; entS; espL1; espR4; espX1; espX4; espX5; espY1; espY2; espY4; fdeC; fepA; fepB; fepC; fepD; fepG; fes; fimB; fimC; fimD; fimE; fimF; fimH; fimI; gspC; gspD; gspE; gspF; gspG; gspH; gspI; gspJ; gspK; gspL; gspM; hlyA; hlyC; hlyD; iucA; iucB; iucC; iucD; iutA; kpsD; ompA; papB; papC; papE; papF; papG; papH; papI; papJ; papK; papX; shuA; shuX; yagV/ecpE; yagX/ecpC; yagZ/ecpA; ybtA; ybtT; ybtU; ykgK/ecpR*	*IncFIA_1; IncFIB(AP001918)_1; IncFII(pRSB107)_1_pRSB107; IncY_1*
4	ID267	Patient	1	*blaADC-25; blaCTX-M-15; blaOXA-420; blaOXA-69*	*aadA5; ant(2")-la; aph(3")-lb; aph(3')-la; aph(6)-ld; dfrA17; dfrA20; erm(B); mdf(A); mph(A); mph(E); msr(E); sul1; sul2*	*aslA; chuV; chuW; chuY; csgB; csgD; csgF; csgG; entA; entB; entC; entD; entE; entF; entS; espL1; espL4; espR1; espR4; espX1; espX4; espX5; espY1; espY2; espY3; espY4; fdeC; fepA; fepB; fepC; fepD; fepG; fes; fimA; fimB; fimC; fimD; fimE; fimF; fimG; fimH; fimI; fyuA; gspC; gspD; gspE; gspF; gspG; gspH; gspI; gspJ; gspK; gspL; gspM; irp1; irp2; iucA; iucB; iucC; iucD; iutA; kpsD; kpsM; ompA; papC; papC; papD;papF; papG; papH; papI; papJ; papK; papX; sat; shuA; shuS; ShuT; shuX; yagV/ecpE; yagW/ecpD;yagX/ecpC; yagY/ecpB; yagZ/ecpA; ybtA;ybtE; ybtP; ybtQ;ybtS; ybtT; ybtU;ybtX;ykgK/ecpR*	*Col156_1; IncFIA_1; IncFIB(AP001918)_1; IncY_1*

SN, sample number.

These virulence genes can be transmitted from one bacterium to another by horizontal gene transfer among different species residing in the same niche such as in the gastrointestinal tract [8]. The survivability of *Acinetobacter baumannii* in adverse conditions such as desiccation and extreme pH makes it difficult to manage its infections [1].

In conclusion, admitted orthopaedic patients and hospital environments act as reservoirs of multiple β-lactamase-producing *Acinetobacter baumannii* (including those against carbapenems such as bla_{OXA} and bla_{NDM-1}) endowed with arsenals of virulence genes. This study highlights the necessity to strengthen screening of patients with prolonged hospital stays following admission, such as orthopaedic patients with open fractures, as well as the need to reinforce infection prevention and control measures to reduce the dissemination of nosocomial *Acinetobacter baumannii* infections within hospitals.

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Joint CUHAS/BMC Research and Ethics Committee (CREC/409/2019) and the National Health Research Ethics Review Committee of the National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/3322) in Tanzania. All information obtained was coded and kept confidential.

Author contributions

B.R.K., G.M., I.S. and J.S. made substantial contributions to the conception, design, coordination and execution of the study; I.S., S.K., B.R.K., E.N. and G.M. participated in the analysis and interpretation of the data; I.S., G.M., J.S. and B.R.K. wrote the initial draft of the manuscript, which was critically revised by all authors. All authors reviewed and approved the final manuscript.

Conflict of interest

The authors declare no competing interests.

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