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Using WGS to identify antibiotic resistance genes and predict antimicrobial resistance phenotypes in MDR Acinetobacter baumannii in Tanzania

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Background: Reliable phenotypic antimicrobial susceptibility testing can be a challenge in clinical settings in low- and middle-income countries. WGS is a promising approach to enhance current capabilities.

Aim: To study diversity and resistance determinants and to predict and compare resistance patterns from WGS data of Acinetobacter baumannii with phenotypic results from classical microbiological testing at a tertiary care hospital in Tanzania.

Methods and results: MLST using Pasteur/Oxford schemes yielded eight different STs from each scheme. Of the eight, two STs were identified to be global clones 1 (n=4) and 2 (n=1) as per the Pasteur scheme. Resistance testing using classical microbiology determined between 50% and 92.9% resistance across all drugs. Percentage agreement between phenotypic and genotypic prediction of resistance ranged between 57.1% and 100%, with coefficient of agreement (κ) between 0.05 and 1. Seven isolates harboured mutations at significant loci (S81L in gyrA and S84L in parC). A number of novel plasmids were detected, including pKCRI-309C-1 (219000 bp) carrying 10 resistance genes, pKCRI-43-1 (34935bp) carrying two resistance genes and pKCRI-49-1 (11681bp) and pKCRI-28-1 (29606bp), each carrying three resistance genes. New ampC alleles detected included ampC-69, ampC-70 and ampC-71. Global clone 1 and 2 isolates were found to harbour ISAba1 directly upstream of the ampC gene. Finally, SNP-based phylogenetic analysis of the A. baumannii isolates revealed closely related isolates in three clusters.

Conclusions: The validity of the use of WGS in the prediction of phenotypic resistance can be appreciated, but at this stage is not sufficient for it to replace conventional antimicrobial susceptibility testing in our setting.

Introduction

The burden of antimicrobial resistance is drastically increasing globally.1 MDR Gram-negative bacterial pathogens especially pose major health challenges worldwide.2 Acinetobacter baumannii is an important nosocomial pathogen and has been reported to cause serious health problems due to its resistance to multiple antimicrobial agents.3 Its ability as a non-lactose-fermenter to grow with low nutritional requirements enables the pathogen to survive in a wide range of environments.4 Historically, A. baumannii infections have mainly been associated with severely ill patients in ICUs and surgical wards and in people exposed to medical apparatus such as ventilators and catheters.5–7 Today, A. baumannii is found across hospital wards and associated with various diseases and/or surgical sites such as respiratory tract, bloodstream, urinary tract and wound infections.8,9 It is becoming a leading cause of nosocomial infections worldwide, with high mortality and morbidity rates.5 In many cases, the infections involve MDR strains of the pathogen.10

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A. baumannii possesses several mechanisms to resist a wide range of existing antibiotic classes. These include production of enzymes that can hydrolyse β-lactams, the presence of efflux pumps and modifications of the outer membrane. The pathogen has great capability to acquire new resistance determinants, which notoriously increases the threat it poses to global health.

European and other medically advanced countries started monitoring antimicrobial resistance about 20 years ago. The majority of these countries have formulated strategies to combat this life-threatening disaster. Regular data generation on infection patterns, aetiological agents and their susceptibility patterns over time is a core aspect of these strategies.

In developing countries, data to monitor trends and susceptibility patterns of common aetiological agents are infrequently produced, despite these data being crucial for formulating control strategies and treatment guidance. Challenges encountered in resource-limited countries include, for example, low-capacity diagnostic tools, which hamper efforts to analyse and combat antimicrobial resistance, and low availability of reliable phenotypic drug susceptibility testing resources in clinical laboratories, resulting in untraceable resistance patterns for disease-causing agents.

High-throughput diagnostic tools such as WGS hold great promise in medical diagnostics and have proven to be invaluable in the control of antimicrobial resistance. The use of such high-throughput technologies to study pathogens has brought a breakthrough in surveillance and monitoring in medically advanced countries. Unfortunately, such tools are rarely available in resource-limited countries.

In this study, we present what WGS can offer in tackling antimicrobial resistance by applying this technology in Tanzania where, as in the rest of the world, antimicrobial resistance is a serious and fast-growing health concern. We used WGS to identify resistance determinants and predict phenotypic resistance in A. baumannii from patients who were admitted to Kilimanjaro Christian Medical Centre (KCMC), a referral hospital in northern Tanzania. The isolates were sequenced and analysed locally at the Christian Medical centre (KCMC), a referral hospital in northern Tanzania from patients who were admitted to Kilimanjaro.

Materials and methods

Study design, location and sample collection

This study was part of a larger study that collected 286 wound/pus swabs from 263 inpatients admitted to the medical or surgical departments of the hospital. The study was a descriptive analysis to characterize isolates from clinical specimens using WGS. This study was conducted at KCMC, a tertiary healthcare facility for the northern zone of Tanzania. The clinical specimens were collected during 2013 to 2015 from patients who were admitted to the medical and surgical departments of the hospital. Informed consent was obtained for all participants. Patient hospital files were used to obtain sociodemographic and clinical characteristics of the study participants. Data were then recorded on designated case report forms.

Laboratory methods and data analysis

Clinical samples were collected during routine clinical care and transported to the Microbiology Unit of KCRI Biotechnology Laboratory for routine microbiological analysis as previously described. The isolates were stored at −80°C before extraction of genomic DNA using the MasterPure™ Complete DNA and RNA Purification Kit (Cat. No. MC85200; Epicentre, Illumina). The quality and quantity of genomic DNA were confirmed using a Qubit 2.0 fluorometer (Life Technologies). Library preparation (dual indexing) was done using the Nextera XT DNA Preparation Kit (Illumina Inc., San Diego, CA, USA). WGS of the library was completed on an Illumina MiSeq using the 2×250 bp paired-end protocol. Patient characteristics were double data-entered in OpenClinica. Excel sheets were extracted and exported to STATA 13 (StataCorp LP, TX, USA) for analyses.

MLST, antimicrobial resistance genes and SNP analysis

The raw reads were de novo assembled using SPAdes 3.6.0. The assembled sequences were analysed to identify MLST STs and antimicrobial resistance genes using offline versions of the MLST (version 1.7.21) and ResFinder (version 2.1.22) tools available online from the Centre for Genomic Epidemiology (CGE) at the Technical University of Denmark (http://cge.cbs.dtu.dk/services/). Phylogenetic distances were computed using an offline version of the CSI phylogeny pipeline available at CGE (https://cge.cbs.dtu.dk/services/CSIPhylogeny/). Phylogenetic trees were generated with PhyML (version 3.1, bootstrap count 100). The reference genome used was AYE (NC_010410.1). MLST and their allelic profiles are indicated in Table S5.

Determination of plasmids, resistance regions and ISs

DNA gyrase gene sequences (gyrA, gyrB, parC and parE) were extracted from the A. baumannii assemblies using gene-cutter, part of blast-galley (commit 0d87ef, https://github.com/zwets/blast-galley), reference proteins used for A. baumannii were GyrA (DQCBH9) and ParC (DQCB90). Nucleotide sequences were obtained from Ensembl Genomes (https://ensemblegenomes.org). Alignment of the protein sequences was done using MAFFF (version 7.271).

Detection of fluoroquinolone mutations in A. baumannii

DNA gyrase gene sequences (gyrA, gyrB, parC and parE) were extracted from the A. baumannii assemblies using gene-cutter, part of blast-galley (commit 0d87ef, https://github.com/zwets/blast-galley). Reference proteins used for A. baumannii were GyrA (DQCBH9) and ParC (DQCB90). Nucleotide sequences were obtained from Ensembl Genomes (https://ensemblegenomes.org). Alignment of the protein sequences was done using MAFFF (version 7.271).

Prediction of phenotypic drug resistance pattern using WGS

ResFinder was used to predict resistance based on phenotypes from origin. Published studies of resistance-conferring genes. Prediction of fluoroquinolone resistance was done based on mutations in gyrA and parC, as there were no genes for fluoroquinolone resistance detected by ResFinder. Intrinsic genes were included if mobile genetic elements such as ISAba1 were found directly upstream of the genes. Predictions were made for antimicrobials including amoxicillin/clavulanate, ampicillin, cefazolin, cefazolin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid and trimethoprim/sulfamethoxazole. These antimicrobials are commonly used in our setting. Correlation between the phenotypically tested antibiotic resistance and predicted antibiotic resistance was determined using Cohen’s Kappa coefficient of agreement (κ) in eight drugs. Two drugs were dropped as they were used in combination.

Ethics

Ethics approval for the study was obtained from the National Institute for Medical Research, with certificate number NIMR/HQ/R.8/4/Vol.IX/2080, and from Kilimanjaro Christian Medical University College, with certificate number 891.
The age range of the patients involved in this study was 11 to 92 years. Thirteen out of 14 patients were admitted due to wound infections. The causes of wounds included surgery, burns, cuts, diabetes mellitus, cancer, chronic ulcers and bedsores. The length of hospitalization, where known, was from 7 to 44 days, with most of the patients recovering from their conditions. One patient died after 20 days of hospitalization. We could not trace hospitalization length and outcome for four patients. One patient prematurely left the hospital by absconding treatment. See Table 1.

### Antimicrobial resistance pattern using classical microbiology

The isolates were tested by conventional microbiology using a panel of 10 antimicrobial agents. The overall resistance levels ranged between 50% and 92.9% across all drugs. The highest resistance level was 92.9% for cefazolin and ceftriaxone, followed by ampicillin (85.7%), ceftazidime (78.6%), chloramphenicol (71.43%) and trimethoprim/sulfamethoxazole (71.4%), gentamicin (64.3%), amoxicillin/clavulanate and nalidixic acid (57.1%) and ciprofloxacin (50%). See Table 2.

### MLST STs, antimicrobial resistance genes and plasmids

MLST yielded eight different STs. The most prevalent ST (n=4) was global clone 1 (Pasteur scheme ST1). One isolate was of global clone 2 (Pasteur scheme ST2). These global clones 1 and 2 were identified as ST405 and ST848, respectively, in the Oxford scheme. Other recurring STs as per Pasteur/Oxford schemes were: ST499/345 (n=3), ST625/860 (n=2), followed by single isolates for ST979/1848, ST374/1325, ST1232/1846 and ST1233/1847. The STs ST1232/1846 and ST1233/1847 were novel in both schemes, whereas ST979/1848 was novel in the Oxford scheme. More information on the allelic profiles of the isolates is in Table S4.

### Results

#### Characteristics of the study participants

The age range of the patients involved in this study was 11 to 92 years. Thirteen out of 14 patients were admitted due to wound infections. The causes of wounds included surgery, burns, cuts, diabetes mellitus, cancer, chronic ulcers and bedsores. The length of hospitalization, where known, was from 7 to 44 days, with most of the patients recovering from their conditions. One patient died after 20 days of hospitalization. We could not trace hospitalization length and outcome for four patients. One patient prematurely left the hospital by absconding treatment. See Table 1.

### Table 1. Sociodemographic characteristics associated with 14 A. baumannii bacterial isolates from patients who were admitted to medical and surgical departments of KCMC hospital

<table>
<thead>
<tr>
<th>ID</th>
<th>Collection date</th>
<th>Age (years)</th>
<th>Ward</th>
<th>Gender</th>
<th>Disease</th>
<th>Source of sample</th>
<th>Length of hospitalization (days)</th>
<th>Patient outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>033</td>
<td>31 July 2013</td>
<td>49</td>
<td>medical</td>
<td>male</td>
<td>respiratory infection</td>
<td>sputum</td>
<td>NI</td>
<td>NI</td>
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<td>043</td>
<td>1 August 2013</td>
<td>45</td>
<td>SICU B</td>
<td>female</td>
<td>burst abdomen</td>
<td>wound swab</td>
<td>20</td>
<td>death</td>
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<td>wound swab</td>
<td>NI</td>
<td>NI</td>
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<tr>
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<td>92</td>
<td>surgical</td>
<td>female</td>
<td>bedsores</td>
<td>wound swab</td>
<td>9</td>
<td>recovery</td>
</tr>
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<td>518B</td>
<td>30 May 2015</td>
<td>29</td>
<td>surgical</td>
<td>female</td>
<td>burn wound</td>
<td>wound swab</td>
<td>32 abscended</td>
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</tr>
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<td>wound swab</td>
<td>9</td>
<td>recovery</td>
</tr>
<tr>
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<td>11 March 2015</td>
<td>11</td>
<td>SICU B</td>
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<td>NI</td>
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<td>44</td>
<td>recovery</td>
</tr>
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<td>female</td>
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<tr>
<td>363B</td>
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<td>22</td>
<td>recovery</td>
</tr>
<tr>
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<td>surgical</td>
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<td>cancerous wound</td>
<td>wound swab</td>
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<td>recovery</td>
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<td>chronic ulcer</td>
<td>wound swab</td>
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<tr>
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<td>76</td>
<td>surgical</td>
<td>male</td>
<td>DM wound</td>
<td>wound swab</td>
<td>34</td>
<td>recovery</td>
</tr>
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</table>

DM, diabetes mellitus; NI, no information; SICU, surgical ICU.

### Table 2. Phenotypic antimicrobial resistance pattern of the 14 A. baumannii isolates

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<tr>
<th>ID</th>
<th>AMC</th>
<th>AMP</th>
<th>CFZ</th>
<th>CAZ</th>
<th>CRO</th>
<th>CHL</th>
<th>CIP</th>
<th>GEN</th>
<th>NAL</th>
<th>SXT</th>
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<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>I</td>
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</tr>
<tr>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<tr>
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<td>R</td>
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<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td></td>
</tr>
</tbody>
</table>

| R (%) | 57.1 | 85.7 | 92.9 | 78.6 | 92.9 | 71.4 | 50   | 64.3 | 57.1 | 71.4 |
| S (%) | 14.3 | 14.3 | 7.1  | 14.3 | 7.1  | 21.43| 42.9 | 35.7 | 28.6 | 28.6 |
| I (%) | 28.6 | 0.0  | 0.0  | 7.1  | 7.1  | 0.0  | 7.1  | 7.1  | 14.3 | 0.0  |

AMC, amoxicillin/clavulanate; AMP, ampicillin; CFZ, cefazolin; CAZ, ceftazidime; CRO, ceftriaxone; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; NAL, nalidixic acid; SXT, trimethoprim/sulfamethoxazole; R, resistant; S, susceptible; I, intermediate.
either chromosomally or on plasmids. Chromosomally located genes included aacC1, aadA1, aphA1, catA1, sul1, tet(A), tet(B), strA and strB, while bla\textsubscript{OXA-23}, mph(E), msr(E), tet39, floR, sul2 and aadB were found on plasmids. β-Lactamase genes included class D (OXA-type) genes that are intrinsic factors in A. baumannii, bla\textsubscript{PER-7} and the cephalosporinase-encoding gene ampC.

The three isolates of ST499/ST345 (116, 123C, 309C) were found to carry an ~219 kb plasmid exhibiting 93% identity with Table 3. STs, resistance genes, mobile genetic elements and plasmids

<table>
<thead>
<tr>
<th>ID</th>
<th>STs (Pasteur/Oxford)</th>
<th>Mobile genetic elements</th>
<th>Intrinsic genes</th>
<th>Chromosomal resistance genes</th>
<th>Plasmids, size and associated drug resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>116</td>
<td>499/345</td>
<td>CR (IS91), Intg1</td>
<td>bla\textsubscript{OXA-95}, ampC-70\textsuperscript{a}, ampC-181\textsuperscript{a}</td>
<td>ND</td>
<td>plasmid: putative plasmid pKCRI-309C-1 Size: ~219 kb genes: arr-3, strB, armA, bla\textsubscript{PER-7}, cmlA1, mph(E), msr(E), strA, sul1, dfrA26</td>
</tr>
<tr>
<td>123C</td>
<td>499/345</td>
<td>CR (IS91), Intg1</td>
<td>bla\textsubscript{OXA-95}, ampC-70\textsuperscript{a}, ampC-181\textsuperscript{a}</td>
<td>ND</td>
<td>plasmid: putative plasmid pKCRI-309C-1 Size: ~219 kb genes: arr-3, strB, armA, bla\textsubscript{PER-7}, cmlA1, mph(E), msr(E), strA, sul1, dfrA26</td>
</tr>
<tr>
<td>309C</td>
<td>499/345</td>
<td>CR (IS91), Intg1</td>
<td>bla\textsubscript{OXA-95}, ampC-70\textsuperscript{a}, ampC-181\textsuperscript{a}</td>
<td>ND</td>
<td>plasmid: putative plasmid pKCRI-309C-1 Size: ~219 kb genes: arr-3, strB, armA, bla\textsubscript{PER-7}, cmlA1, mph(E), msr(E), strA, sul1, dfrA26</td>
</tr>
<tr>
<td>43</td>
<td>625/860</td>
<td>IS3 and IS6, ISAba1</td>
<td>bla\textsubscript{OXA-90}, ampC-4</td>
<td>ND</td>
<td>plasmid: pKCRI-43-1 Size: 34 935 bp genes: floR, sul2 plasmid: PRAY genes: aadB</td>
</tr>
<tr>
<td>432B</td>
<td>625/860</td>
<td>IS3 and IS6, ISAba1</td>
<td>bla\textsubscript{OXA-90}, ampC-71\textsuperscript{a}</td>
<td>ND</td>
<td>plasmid: pKCRI-432-1 Size: 34 935 bp genes: floR, sul2 plasmid: PRAY genes: aadB</td>
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<tr>
<td>49</td>
<td>374/1325</td>
<td>ND</td>
<td>bla\textsubscript{OXA-180}, ampC-56</td>
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<td>plasmid: pKCRI-49-1 Size: 11 681 bp genes: mph(E), msr(E), tet39</td>
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<tr>
<td>33</td>
<td>979/1848\textsuperscript{a}</td>
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<td>bla\textsubscript{OXA-51}, ampC-50, bla\textsubscript{OXA-69}, ampC-4</td>
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<td>ISAba1</td>
<td>bla\textsubscript{OXA-51}, ampC-50</td>
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<td>bla\textsubscript{OXA-69}, ampC-4</td>
<td>bla\textsubscript{OXA-69}, ampC-4</td>
<td>ND</td>
</tr>
<tr>
<td>518B</td>
<td>1/405</td>
<td>ISAba1</td>
<td>bla\textsubscript{OXA-69}, ampC-4</td>
<td>bla\textsubscript{OXA-69}, ampC-4</td>
<td>presence suggestive of novel plasmids, not carrying known resistance genes</td>
</tr>
<tr>
<td>558</td>
<td>1/405</td>
<td>ISAba1, ISAba4</td>
<td>bla\textsubscript{OXA-69}, ampC-4</td>
<td>bla\textsubscript{OXA-69}, ampC-4</td>
<td>plasmid: pAB14 genes: bla\textsubscript{OXA-23}</td>
</tr>
<tr>
<td>28</td>
<td>2/848</td>
<td>ISAba1</td>
<td>bla\textsubscript{OXA-66}, ampC-2</td>
<td>bla\textsubscript{OXA-66}, ampC-2</td>
<td>plasmid: pKCRI-28-1 Size: 29 606 bp genes: mph(E), msr(E), tet39</td>
</tr>
<tr>
<td>164C</td>
<td>1232/1846\textsuperscript{a}</td>
<td>ND</td>
<td>bla\textsubscript{OXA-64}, ampC-68\textsuperscript{a}, bla\textsubscript{OXA-51}, ampC-69\textsuperscript{a}</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>186</td>
<td>1233/1847\textsuperscript{a}</td>
<td>ND</td>
<td>bla\textsubscript{OXA-64}, ampC-68\textsuperscript{a}, bla\textsubscript{OXA-51}, ampC-69\textsuperscript{a}</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not detected.
Note: pKCRI = new plasmid described in this study.
\textsuperscript{a}New STs identified in this study.
\textsuperscript{b}New ampC ST.
the pliOMTU433 plasmid (AP014650) and 94% with the pA297-3 plasmid (KU744946). The plasmid hosted the arr-3, strB, armA, blaPER-7, cmlA1, mph(E), msr(E), strA, sul1 and dfrA26 resistance genes. See Table 3. The plasmid appeared structurally identical to the pliOMTU433 plasmid, with differences concentrated in two regions, each flanked bilaterally by ISs. The first region was adjacent to the class 1 integron-integrase gene intI1. Where pliOMTU433 contains the sul2 gene, this was replaced in pKCI- 309C-1 by a smaller segment containing the dfrA26 resistance gene. This segment is present identically on plasmid pAB04-1 (AP012007). The second difference was an ~20 kb insert containing, among other genes, the mercury resistance operon. This transposon is present nearly identically (99.94%) on pA297-3, a plasmid that shares much of its sequence with pliOMTU433, but lacks the class 1 integron and its downstream gene cassette that pliOMTU433 and our ST499 isolates carry.

It was not possible to assemble the pKCI-309C-1 plasmid into a single contig, due to the inability of short-read sequencing to ‘read through’ larger repeated sequences. We hope to perform a detailed follow-up analysis when long-read sequencing becomes available to our laboratory in the future. The plasmid has been submitted to GenBank as an assembly of six unplaced contigs (accession pending).

The mph(E), mrs(E) and tet39 genes, encoding macrolide, lincosamide, streptogramin B and tetracycline resistance in isolates 28 and 49, seemed to be located on the novel plasmids pKCI-28-1 (LR026972; 29606bp) and pKCI-49-1 (LR026974; 11681bp), respectively. The plasmids also harboured a higA/higB toxin–antitoxin gene pair. This gene combination has been described for other plasmids isolated from a global clone 2 strain, where the tet39 and mph(E)-mrs(E) were found to be in mobile modules flanked by pdif sites.36 As in that study, tet39 was found in both plasmids to be in a 2001 bp region flanked by inverted pdif sequences. The mph(E)-msr(E) gene pair was found, together with a predicted resolvase/recombinase product (similar to WP_015060246), in 4219 and 4300 bp regions, respectively, flanked by pdif sites. The novel plasmid carried by isolate 28 additionally encoded a putative cmlA/floR chloramphenical efflux system, located directly upstream of an ISL3-family transposase separating it from the tet39 element.

The floR and sul2 resistance genes in isolates 43 and 432B were determined to be on a novel 34 935 bp plasmid, pKCI-43-1 (LR026973). Both genes were flanked by transposases: the floR gene by IS3 and IS6-family (IS1008) transposases; and the sul2 gene by ISAb1 upstream and by glmM followed by IS91 and IS6-family transposases downstream. The aadB gene in isolates 43 and 432B was found to be located on a pRAY plasmid present in both isolates; the plasmid and its variants are widely distributed and the most common cause of gentamicin and tobramycin resistance in Acinetobacter spp.37

The blaOXA-23 gene of isolate 558 was found to be located on plasmid pAB14, directly downstream of ISAb4, suggesting the presence of transposon Tn2007 and the possibility of elevated carbapenemase production.38

Various other plasmids were identified in the isolates; no resistance genes were detected on these plasmids. See Table 3.

### AboR resistance regions

The comM gene that is a prevalent location for insertion of AboR resistance regions39 was found to be interrupted in the four ST1 isolates (363, 423, 518B and 558) and in the ST2 (28) isolate. The comM gene was found intact in all other isolates. In silico PCR also detected the presence of the left-hand side of Tn6019 in the ST1 and ST2 isolates and the right-hand side in the ST1 isolates.

### ISs

Several isolates were found to have ISs upstream of their chromosomal β-lactamase genes. The four ST1 isolates (363, 423, 518B and 558) and isolate 28 (ST2) had ISAba1 directly upstream of the ampC gene. In the other isolates, no IS was found directly upstream of the ampC gene. In the three ST499 isolates, a common region (CR) (IS91) was found directly upstream of the blaPER-2 gene in the pKCI-309C-1 plasmid. As pointed out above, in isolate 558 the plasmidic blaOXA-23 gene was found directly downstream of ISAb4, constituting Tn2007. Few ISs overall were found in isolates 164C, 49 and the four ST1 isolates, whereas numerous ISs were found in the three ST499 isolates (10–15 ISs) and notably isolate 186 (26 ISs).

### Detection of fluoroquinolone mutations in A. baumannii

Mutations in gyrase/topoisomerase genes may confer resistance by inhibiting fluoroquinolone binding. Half (7/14) of the A. baumannii isolates were found to have mutations in gyrA (S81L) and in parC (S84L), all exhibiting resistance to ciprofloxacin and nalidixic acid. Among the other seven isolates without the double mutation, only one was found to be fluoroquinolone resistant. All other isolates had no relevant mutations. See Table 4.
Prediction of phenotypic drug resistance pattern using WGS

Percentage agreement between phenotypic and genotypic prediction of resistance was 57.1% for ampicillin, 64.3% for cefazolin and ceftiraxone, 71.4% for cefazidime and nalidixic acid, 85.7% for chloramphenicol and amoxicillin/clavulanate and 92.9% for trimethoprim/sulfamethoxazole and gentamicin. Ciprofloxacin was the only drug with resistance predicted at 100%. The eight drugs for which \( \kappa \) was computed were cefazolin, ceftazidime, ceftiraxone, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid and ampicillin. \( \kappa \) ranged between 0.05 and 1, i.e. between slight and perfect agreement. Tables S1, S2, S3 and S4 summarize the correlation between phenotypic and WGS-predicted resistance as determined by Cohen’s \( \kappa \).

SNP phylogenetic tree

From the SNP phylogenetic tree, three clusters of Tanzanian isolates were observed. Cluster I contained the three isolates with ST499/345. All three were isolated in 2014 (14 February, 18 February and 16 September). All had the T82S mutation in gyrA and no mutations in parC. Isolates from this cluster all possessed the plasmids pKCRI-309C-1 (accession pending), containing multiple antibiotic resistance genes, and pAC450, on which no resistance genes were found. Cluster II contained the two isolates with ST625/860. The isolates were collected on the same ward (surgical ICU B), but 2 years apart, on 1 August 2013 and 11 May 2015. Both isolates had the fluoroquinolone mutations S81L in gyrA and S84L in parC and were ciprofloxacin resistant and nalidixic acid resistant. The isolates both contained the prAY plasmid carrying aadB and pK4RI-43-1 (LR026973) containing flor and sul2. Cluster III contained the four ST1/405 isolates. See Figure 1. Isolation dates were 4 December 2014, 4 March 2015, 30 March 2015 and 14 July 2015. They all had the fluoroquinolone mutations S81L in gyrA and S84L in parC and were all found to be resistant to ciprofloxacin and nalidixic acid. One of the four isolates possessed plasmid pAB14 containing the \( \text{bla}_{OXA-23} \) gene. The five isolates that were not part of the above-mentioned clusters were located at independent positions in the tree. The ST374/1325 isolate clustered closely with an Australian isolate of the same ST, while the ST2/848 isolate clustered with an isolate of the same ST collected in 2003 on an American hospital ship. The remaining three isolates, which were of novel STs, were all located phylogenetically larger distances from other isolates. Raw sequence data of the 16 A. baumannii from our study have been submitted to the European Nucleotide Archive (https://www.ebi.ac.uk/ena) under study accession number PRJEB26612. Plasmid accession numbers are: pKCR1-28-1 (LR026972); pKCR1-43-1 (LR026973); pKCR4-9-1 (LR026974); and pKCR1-309C-1 (accession pending).

Discussion

The current study aimed to investigate the diversity and resistance determinants of A. baumannii isolates collected at a tertiary hospital in Tanzania and to compare predicted resistance patterns from WGS data with phenotypic results from classical microbiological testing. The majority of patients investigated in this study were hospitalized with wound infections and had relatively long hospital stays despite the fact that many recovered. Hospitalized patients with open wounds and long hospital stays are among those who are vulnerable to A. baumannii infections. The pathogen can persist on patients’ body surfaces or in the environment for a considerable number of days, as the infections are difficult to treat.40 The pathogen has the ability to resist many antibiotics leading to fewer treatment options and sometimes death.9 It was noted from the study that some patients abscond hospitalization before recovery. In low-resource settings, patients who cannot afford hospital charges will sometimes withdraw from treatment to avoid further costs. Occasionally such patients will return to the hospital with worsened conditions. Aforesaid behaviour could cause hospital-based infections to be disseminated to the community and vice versa, with severe consequences when these patients carry drug-resistant strains, which could then easily spread to other susceptible persons.

MLST is one of the gold standards for determination of epidemiological relatedness of organisms.41 In this study MLST was performed, based on both Pasteur and Oxford schemes. Considerable genetic variation among A. baumannii strains was observed. The observed variation included STs that represent the global clones 1 and 2 of A. baumannii, as well as novel STs first identified in this study. The observed global clones are of great concern as they are disseminated worldwide and have been highly associated with MDR. They have notably been associated with carbapenem resistance and outbreaks in hospital settings.42 Among the global clones, one isolate was confirmed to possess the \( \text{bla}_{OXA-23} \) gene located on plasmid pAB14, directly downstream from ISAba4, suggesting the presence of transposon Tn2007 and the possibility of elevated carbapenemase production.18 Plasmid-mediated class D \( \beta \)-lactamase genes, particularly \( \text{bla}_{OXA-23} \), play a large role in mediating resistance.43 These findings are in contrast to a study conducted in Tanzania that identified predominantly IMP-type (class B) carbapenemases in A. baumannii isolates.44

SNP-based phylogenetic analysis of the A. baumannii further confirmed the diversity by revealing three major clusters containing clades of closely related isolates. This observation, particularly in the light of the time separation between isolation events, suggests existence of specific strains of A. baumannii at the KCMC hospital. The origin of these clones is not known, but it seems likely that they dwell in the hospital environment and cause infections over an extended period of time. Previous studies have reported on A. baumannii isolates from medical apparatus, water systems and handwashing sinks in the hospital environment, including ICUs and surgical wards.45,46 These isolates tend to be MDR and hence can persist for a long time if the reservoir is not destroyed, serving as a potential source for recurrent outbreaks. Isolates in clusters I and II were found to possess plasmid-mediated resistance genes. The majority of these genes were situated in novel plasmids (pKCR1-309C-1 for cluster I and pKCR1-43-1 for cluster II). Cluster III consisted of global clone 1, for which in some isolates resistance genes were found in both plasmids and chromosomes. The coexistence of chromosomally and plasmid-encoded \( \beta \)-lactamases and other genes in global clone isolates has been observed in other studies.47,48 The isolate belonging to global clone 2 in this study was found to carry chromosomally mediated genes, as well as mph(E), mra(E) and tet39 genes encoding macrolide, lincosamide, streptogramin B and tetracycline resistance on a novel plasmid pKCR1-28-1.
ISs have been associated with antimicrobial resistance through multiple mechanisms: disruption of coding regions, mobilization of gene cassettes and up-regulation of gene expression by additional promoters. Our findings show that all global clone 1 and 2 isolates studied were resistant to ceftriaxone despite the absence of the genes responsible for resistance. However, the isolates were found to harbour ISAba1 directly upstream of the $\text{ampC}$ gene. This configuration has been demonstrated to elevate $\text{ampC}$ expression levels, conferring resistance to third-generation cephalosporins. Isolates in Cluster I were identified as belonging to ST499/345 as per Pasteur/Oxford schemes. Recent reports from other parts of the world, including Egypt in Africa, reported isolates with these STs to be associated with carbapenem resistance. In the current study, these isolates were not confirmed to have resistance to carbapenem.

Figure 1. SNP phylogeny analysis of 31 $A. baumannii$ genomes that were isolated in 10 countries: Tanzania (TZ; current study), USA, UK, China, Malaysia, Togo, Australia, Czech Republic, Iraq and Germany. From the SNP phylogenetic tree, three clusters for Tanzanian isolates were observed. Cluster I was composed of three isolates with ST499/345 as per Pasteur/Oxford schemes. All had the T82S mutation in $\text{gyrA}$ and had no mutations in $\text{parC}$. Isolates from this cluster possessed pIOMTU433-like plasmids, which contained several antibiotic resistance genes, and pAC450, which did not carry any resistance genes. Cluster II was composed of two isolates with ST625/860 as per Pasteur/Oxford schemes. Both isolates were isolated in the surgical ICU B ward. They both had fluoroquinolone mutations S81L and S84L in $\text{gyrA}$ and $\text{parC}$, respectively, and were ciprofloxacin resistant and nalidixic acid resistant. The isolates contained more than one plasmid with no resistance genes on them with the exception of two: pA297-1, which carried $\text{aadB}$; and pKCRI-43-1, containing the genes $\text{floR}$ and $\text{sul2}$. Cluster III was composed of four isolates with ST1/405 as per Pasteur/Oxford schemes. They all had fluoroquinolone mutations S81L and S84L in $\text{gyrA}$ and $\text{parC}$, respectively. They were all resistant to ciprofloxacin and nalidixic acid. Three possessed a novel plasmid and one possessed plasmid pAB14 containing $\text{blaOXA-23}$.50
carbapenems; however, they were found to harbour the novel ampC allele ampC-70, encoding the novel amino acid sequence AmpC-181. These isolates were further confirmed to possess CR (IS911) directly upstream of the blaoxac-7 gene in the pKCRI-309C-1 plasmid. These findings corroborate the observed phenotypic resistance to ceftazidime and other cephalosporins that have been reported from other studies.\(^5^3\)

Antibiotic resistance to commonly prescribed antibiotics was found to be high, with alarming resistance rates to fluoroquinolones and third-generation cephalosporins. The high resistance rates are of great concern when it comes to A. baumannii, which is a notorious nosocomial pathogen. Overprescription of antibiotics, over-the-counter availability, self-medication, premature treatment cessation, counterfeit drugs, poor diagnostic facilities and other factors prevalent in low-income settings contribute greatly to antibiotic resistance. Empirical treatment in such settings becomes compromised and treatment options are reduced.\(^5^4\)–\(^5^6\)

Prediction of phenotypes from WGS data could be a powerful potential application of next-generation sequencing technology in low-resource settings, although limited studies have demonstrated this.\(^5^7\) Given that sequencing technology is scarcely deployed, especially in resource-limited settings, it is almost impossible to assess its power. However, the process of predicting phenotypes is complex and it is currently in limited use.\(^1^5\)

In the current study, the percentage agreement between phenotypic and genotypic prediction of resistance ranged between 57.1% and 100%, with \(\kappa\) ranging between 0.05 and 1. Our prediction did not rely on the presence or absence of resistance genes alone, but considered several other aspects. In some cases, genes conferring resistance to drugs such as ciprofloxacin, nalidixic acid and cefazolin were not detected by ResFinder, yet phenotypic resistance was observed. In this case, other resistance mechanisms, such as mutations in DNA gyrase/tapeoisomerase IV genes, were responsible for resistance phenotypes. In the prevailing report we detected significant mutations at 81/84 in gyrA/parC in the A. baumannii isolates. Mutations at residues 81 and 84 in gyrA and parC, respectively, are most frequently observed among the strains with nalidixic acid and ciprofloxacin resistance, respectively. Other studies have reported the existence of triple mutations (gyrA, gyrB and parC) that also contribute to development of a high level of fluoroquinolone resistance in clinical isolates of A. baumannii.\(^5^8\) However, for convenient determination of such mutation trends, especially for the fluoroquinolones, a larger sample number is crucial in future studies.

WGS techniques have great promise in providing sufficient and reliable data for surveillance and monitoring of antimicrobial resistance. Despite the technology being robust, limited knowledge of bioinformatics data analysis is one of the major challenges. In resource-limited settings, cost challenges and a lack of sequencing expertise widen the gap to its potential utilization. These study findings support the implementation in disease-endemic regions of high-throughput techniques, to replace or add value to existing technology, and hence take a step ahead in medical diagnostics.

**Conclusions**

The validity of the use of WGS in prediction of phenotypic resistance can be appreciated, but at this stage is not sufficient to replace conventional antimicrobial susceptibility testing in our setting. Classical microbiology still complements the genotypically predicted resistance, as it provides better resolution of what is happening between organisms and their environment, taking into consideration that observed phenotypes are a result of interaction between genotypes and the environment.

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**Supplementary data**

Tables S1 to S5 are available as Supplementary data at JAC Online.

**References**


