Molecular Epidemiology of Carbapenem-Resistant *Acinetobacter baumannii* From Khartoum State, Sudan

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Carbapenem resistant *Acinetobacter baumannii* (CRAb) is an important global pathogen contributing to increased morbidity and mortality in hospitalized patients, due to limited alternative treatment options. Nine international clonal (IC) lineages have been identified in many countries worldwide, however, data still lacks from some parts of the world, particularly in Africa. We hereby present the molecular epidemiology of MDR *A. baumannii* from four hospitals in Khartoum, Sudan, collected from 2017 to 2018. Forty-two isolates were whole-genome sequenced, and subsequent molecular epidemiology was determined by core genome MLST (cgMLST), and their resistomes identified. All isolates had an array of diverse antibiotic resistance mechanisms conferring resistance to multiple classes of antibiotics. We found a predominance (88%) of IC2 (with the intrinsic OXA-66 and acquired OXA-23), and some with NDM-1. IC2 isolates were sub-divided into 4 STs separated by 5 to 431 allelic differences, and with evidence of seven transmission clusters. Isolates belonging to IC1, IC5, and IC9 were also identified. These data illustrate that MDR IC2 *A. baumannii* are widely distributed in Khartoum hospitals and are in possession of multiple antibiotic resistance determinants.

Keywords: antibiotic resistance, cgMLST, OXA, NDM-1, Sudan, international clones

INTRODUCTION

Acinetobacter baumannii is an important nosocomial pathogen that causes a variety of infections including but not limited to ventilator-associated pneumonia and bloodstream infection (Peleg et al., 2008). Treatment options have been compromised by the emergence of multi-drug resistant (MDR) isolates. The prevalence of MDR *A. baumannii* in hospitals has put the organism on

the "ESKAPE" pathogens list: an acronym developed by the Infectious Diseases Society of America for a group of MDR nosocomial pathogens with limited remaining treatment options (Boucher et al., 2009). *A. baumannii* is also considered as priority 1 ("critical") on the World Health Organization list of priority organisms for research and development of new antibiotics (WHO, 2017). Of particular concern is carbapenem resistance, as carbapenems are considered the drugs of last resort in treating *A. baumannii* infections, and resistance is commonly attributed to acquired carbapenemases (Poirel and Nordmann, 2006).

For the investigation of local outbreaks, pulsed field gel electrophoresis (PFGE) has been the traditional typing method, but given the global distribution of *A. baumannii,* current typing methods must be reproducible and portable, both of which PFGE lacks. Multi-locus sequence typing (MLST) is a relatively easy method to perform but it lacks the resolution for outbreak investigation (Higgins et al., 2017). The advent of relatively cheap whole-genome sequencing (WGS) technology gives the opportunity to perform high resolution typing and the ability to compare whole genomes rather than only a few loci. WGS also allows for the identification of antimicrobial resistance determinants, both intrinsic and acquired, and can aid in our understanding of the circulating resistome.

Molecular epidemiological studies have assigned nine major International Clones (IC1-9) of *A. baumannii*, the most widespread of which is IC2 commonly harboring the acquired carbapenemase OXA-23 (Higgins et al., 2010a; Zarrilli et al., 2013; Hamidian and Nigro, 2019; Müller et al., 2019; Tomaschek et al., 2019). However, there is a significant lack of epidemiological and genomic data from low- and middle-income countries (LMICs), mainly due to limited research infrastructure and resources. Given that antimicrobial resistance does not respect borders, there is therefore an urgent need to support local initiatives in LMICs to generate epidemiological and genomic data in order to assess the burden of infectious diseases, and track resistance globally (Okeke et al., 2005).

This study aimed to explore the molecular epidemiology and antimicrobial resistance mechanisms using WGS of carbapenem resistant *A. baumannii* (CRAb) isolated from patients in Khartoum State in Sudan.

MATERIALS AND METHODS

Bacterial Strains and Antimicrobial Susceptibility

A total of 71 consecutive *A. baumannii* isolates were collected between 2017 and 2018 from hospitalized patients in four different hospitals in Khartoum, Sudan: Soba University Hospital ($n = 37$), Ibrahim Malik Hospital ($n = 3$), The Military Hospital ($n = 16$), and Al Baraha Hospital ($n = 16$). These were isolated from a variety of samples including blood, urine, sputum, wound, cerebrospinal fluid, and catheter tips (**Table 1**). Isolates were initially identified at the clinical microbiology laboratories of the hospitals using conventional methods. For the purpose of this study, all isolates were confirmed as *A. baumannii* by the *gyrB* multiplex PCR method and MALDI-TOF prior to whole genome

sequencing (WGS) (Higgins et al., 2010b). Presence of OXA and metallo- carbapenemase-encoding genes was determined by PCR (OXA-23, -40,-51, -58, -143, and -235, and NDM, IMP,VIM, GIM, KPC, and GES) (Woodford et al., 2006; Ellington et al., 2007; Higgins et al., 2013).

Antimicrobial susceptibility (AST) was initially performed by disk-diffusion at the clinical microbiology laboratories and interpreted according to CLSI guidelines. Upon species confirmation, the minimum inhibitory concentration (MIC) was determined by MICRONAUT-GN, an automated microtiter broth dilution susceptibility testing system (Merlin Diagnostika, Germany). Colonies from a pure overnight subculture were used to prepare a 0.5-McFarland-standard suspension in 0.9% saline as recommended by the manufacturer (Merlin Diagnostika, Germany). This system allows the determination of the MIC for a panel of antibiotics with two concentrations (mg/L) based on the EUCAST MIC breakpoints for sensitivity (S) or resistance (R) (EUCAST, 2020b): meropenem (MER), gentamicin (GEN), amikacin (AMK), trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CIP), and colistin (COL) in a single microtitre plate. After the addition of the bacterial suspension and rehydration of the antibacterial agents, the plates were incubated at 37°C for 18–24 h. Bacterial growth in the wells was monitored photometrically at 620 nm, and a density of >50% above the cutoff value for the growth control. The results were also observed visually for turbidity to confirm the results. Additionally, MIC for imipenem (IMP) was performed by broth microdilution (BMD) method according to the EUCAST guidelines V2, 2020 (EUCAST, 2020a). Quality control strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used in all AST experiments.

Whole Genome Sequencing and Molecular Typing

Total DNA was extracted from the bacterial isolates using the MagAttract HMW DNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Sequencing libraries were prepared using the Nextera XT library prep kit (Illumina GmbH, Munich, Germany) for a 250-bp paired-end sequencing run on an Illumina MiSeq platform. Genomes were assembled *de novo* using Velvet v1.1.04. Molecular epidemiology of the isolates was investigated by core-genome MLST (cgMLST) based on a core genome of 2,390 alleles using Ridom® SeqSphere+ v. 7.2.3 (Higgins et al., 2017).

Multi-locus sequence typing was performed on the pubMLST database https://pubmlst.org/abaumannii/ to identify sequence types (STs) for both the Pasteur and Oxford schemes. Antimicrobial resistance determinants were identified using the Resfinder software v3.2 https://cge.cbs.dtu.dk/services/ResFinder/. Beta-lactamases were identified using the online beta-lactamase database[1] (Naas et al., 2017).

All assembled genomes were submitted to GenBank under BioProject number PRJNA628907. All accession numbers are listed in **Table 1**.

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[1]http://www.bldb.eu/

TABLE 1 | Details of isolates included in the study.

Isolate	Genotype	Transmission cluster	Year	Specimen	Hospital	IMP	MEM	GEN	AMK	SXT	CIP	CST	ST Oxf	ST Pas	Accession No.
						MIC mg/L									
AC-36	IC1		2018	urine	Military	<2	≤2	≥4	≤8	≥4	≥0.5	≤2	2270	1	JABETE000000000
SUH-4	IC1		2016	blood	Soba	128	≥8	≥4	≥16	≥4	≥0.5	≤2	231	1	JABESK000000000
AC-12	IC2	TC-1	2018	sputum	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	2	JABESV000000000
AC-17	IC2	TC-1	2018	wound	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	2	JABESX000000000
AC-19	IC2	TC-1	2018	sputum	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	2	JABESY000000000
AC-2	IC2	TC-1	2018	sputum	Soba	16	≥8	≥4	≥16	≥4	≥0.5	≤2	195	2	JABESQ000000000
AC-33	IC2	TC-1	2018	sputum	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	2	JABETC000000000
AC-35	IC2	TC-1	2018	sputum	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	2	JABETD000000000
AC-37	IC2	TC-1	2018	wound	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	2	JABETF000000000
AC-9	IC2	TC-1	2018	blood	Soba	32	≥8	≥4	8	≥4	≥0.5	≤2	195	2	JABETI000000000
SUH-10	IC2	TC-1	2017	urine	Soba	256	≥8	≥4	≥16	≥4	≥0.5	≤2	208	2	JABERU000000000
SUH-12	IC2	TC-1	2017	blood	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	632	JABERX000000000
SUH-13	IC2	TC-1	2017	CSF	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	632	JABERY000000000
SUH-15	IC2	TC-1	2016	urine	Soba	128	≥8	≥4	≥16	≤2	≥0.5	≤2	208	2	JABESA000000000
SUH-20	IC2	TC-1	2017	blood	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	208	632	JABESC000000000
SUH-23	IC2	TC-1	2017	wound	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	632	JABESD000000000
SUH-26-1	IC2	TC-1	2017	CSF	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	632	JABESE000000000
SUH-28	IC2	TC-1	2017	wound	Soba	64	≥8	≥4	≥16	≥4	≥0.5	≤2	208	2	JABESH000000000
SUH-3	IC2	TC-1	2016	blood	Ibrahim Malik	64	≥8	≥4	≥16	≤2	≥0.5	≤2	208	2	JABESJ000000000
SUH-5	IC2	TC-1	2016	catheter	Soba	256	≥8	≥4	≥16	≤2	≥0.5	≤2	208	2	JABESL000000000
SUH-8	IC2	TC-1	2016	urine	Soba	256	≥8	≥4	≥16	≥4	≥0.5	≤2	195	2	JABESO000000000
SUH-9	IC2	TC-1	2017	blood	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	195	632	JABESP000000000
SUH-29	IC2		2019	blood	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	1632	600	JABESI000000000
AC-5	IC2	TC-2	2018	blood	Military	16	≥8	≥4	≥16	≤2	≥0.5	≤2	208	2	JABESS000000000
AC-7	IC2	TC-2	2018	sputum	Military	32	≥8	≥4	≥16	≥4	≥0.5	≤2	208	2	JABESU000000000
AC-3	IC2	TC-3	2018	sputum	Military	16	≥8	≥4	≥16	≤2	≥0.5	≤2	208	2	JABESR000000000
AC-6	IC2	TC-3	2018	sputum	Military	32	≥8	≥4	≥16	≤2	≥0.5	≤2	208	2	JABEST000000000
SUH-11-1	IC2	TC-4	2017	blood	Ibrahim Malik	64	≥8	≥4	≥16	≥4	≥0.5	≤2	208	2	JABERV000000000
SUH-11-2	IC2	TC-4	2017	blood	Ibrahim Malik	64	≥8	≥4	≥16	≥4	≥0.5	≤2	208	2	JABERW000000000
SUH-26-2	IC2	TC-4	2017	CSF	Soba	64	≥8	≥4	≥16	4	≥0.5	≤2	195	2	JABESF000000000
AC-26	IC2	TC-5	2018	sputum	Soba	64	≥8	≥4	≥16	≤2	≥0.5	≤2	1632	600	JABETB000000000
AC-27	IC2	TC-5	2018	sputum	Soba	128	≥8	≥4	≥16	≤2	≥0.5	≤2	1632	600	JABETJ000000000
SUH-6	IC2	TC-5	2016	wound	Soba	256	≥8	≥4	≥16	≤2	≥0.5	≤2	1632	600	JABESM000000000
SUH-27	IC2	TC-6	2017	wound	Soba	64	≥8	≥4	≥16	≥4	≥0.5	≤2	208	2	JABESG000000000
SUH-7	IC2	TC-6	2016	wound	Soba	64	≥8	≥4	≥16	≥4	≥0.5	≤2	195	2	JABESN000000000
AC-14	IC2	TC-7	2018	sputum	Al-Braha	128	≥8	≥4	≥16	≤2	≥0.5	≤2	1701	570	JABESW000000000
AC-23	IC2	TC-7	2018	catheter	Al-Braha	128	≥8	≥4	≥16	≤2	≥0.5	≤2	1701	570	JABETA000000000
AC-40	IC2	TC-7	2018	sputum	Al-Braha	64	≥8	≤2	≥16	≤2	≥0.5	≤2	1701	570	JABETG000000000
AC-45	IC2	TC-7	2018	sputum	Al-Braha	128	≥8	≥4	≥16	≤2	≥0.5	≤2	1701	570	JABETH000000000
SUH-14	IC5		2016	urine	Soba	128	≥8	≥4	≥16	≥4	≥0.5	≤2	732	602	JABERZ000000000
AC-20	IC9		2018	tissue	Military	128	≥8	≥4	≥16	≤2	≥0.5	≤2	1089	85	JABESZ000000000
SUH-2	S		2016	wound	Soba	4	≤2	≥4	≤8	≤2	≥0.5	≤2	1208	584	JABESB000000000

Isolate number, genotype, transmission clusters, year of collection, type of specimen, hospital from which it was collected, MIC, ST (Oxford and Pasteur schemes), and GenBank accession numbers.

IC, international clone; S, singleton, TC, transmission cluster; CSF, cerebrospinal fluid; IMP, imipenem; MEM, meropenem; GEN, gentamicin; AMK, amikacin; SXT, trimethoprim/sulfamethoxazole; CIP, ciprofloxacin; CST, colistin.

Some isolates have two different STs in the Oxford scheme due to two gdhB alleles detected, we give only the first one.

SUH-29 was not part of a transmission cluster.

AC-36 contained a novel gpi allele (assigned number 388), and subsequently a novel ST was assigned: ST-2270 in the Oxford MLST scheme.

RESULTS

Of the 71 isolates initially collected, a total of 42 isolates were confirmed as *A. baumannii* by *gyrB*, presence of $bla_{OXA-51-like}$, and MALDI-TOF, and subsequently included in this study. The remaining isolates were other Gram negative organisms misidentified as *A. baumannii* and excluded from the study.

Antimicrobial susceptibility results showed that all isolated were MDR (**Table 1**) and that 95% of the isolates ($n = 40$) were CRAb, with an MIC for imipenem ranging from 16 to 256 mg/L (**Table 2**). Resistome analysis revealed that bla_{OXA-23}

TABLE 2 | Resistome data for all isolates.

Antibiotic Class	Beta-lactam														Aminoglycoside and Fluoroquinolone														Other antibiotics											
Resistance genes	blaNDM-1	blaOXA-23	blaOXA-58	blaOXA-66	blaOXA-69	blaOXA-1	blaOXA-98	blaOXA-94	blaOXA-91	blaCTX-M-15	blaTEM-1D	blaGES-11	blaADC	blaTEM-199	aacA4-like	AadA1	AadA2	aadB	aph(3')-Ic	aph(3')-VIa	aph(3')-Ia	aph(6')-Id	aph(3')-Ib	armA	strA	StrB	aac(3)-IIa	aac(6')Ib-like	mph(E)	msr(E)	sul1	Sul2	dfrA1	DfrA7	tet(B)	tet(B)-like	tet(39)	cmlA1-like	catB8	floR-like
AC-12		D		D							D		D								D	D	D	D					D	D					D					
AC-14	D	D		D							D		D							D	D		D						D	D	D									
AC-17		D		D							D		D								D	D		D					D	D					D	D				
AC-19		D		D							D		D								D	D	D	D					D	D					D	D				
AC-2		D		D							D		D								D	D	D	D					D	D										
AC-20	D			D				D					D							D			D						D	D		D								
AC-23	D	D		D							D		D							D		D	D						D	D	D									
AC-26		D		D							D		D									D	D						D	D										
AC-27	D	D		D									D							D			D						D	D										
AC-3		D		D							D		D		D	D				D		D	D									D	D			D			D		
AC-33		D		D							D		D								D		D						D	D					D		D			
AC-35		D		D							D		D						D		D	D	D	D					D	D					D					
AC-36					D								D		D	D													D						D			D		
AC-37		D		D							D		D								D	D	D	D					D	D										
AC-40	D	D		D							D		D							D	D								D	D	D									
AC-45	D	D		D							D		D							D	D		D						D	D										
AC-5		D		D									D		D	D					D	D	D	D				D	D	D					D				D	
AC-6		D		D			D						D						D		D	D									D	D			D					
AC-7		D		D									D		D	D			D				D						D	D	D	D			D				D	
AC-9		D		D							D		D								D	D	D	D					D	D							D			
SUH-10		D	D	D							D		D							D			D	D	D	D			D	D	D	D	D		D				D	D
SUH-11-1		D		D							D		D		D	D				D		D	D	D	D			D	D	D	D	D							D	
SUH-11-2		D		D							D		D		D	D				D		D	D	D	D			D	D	D	D	D							D	
SUH-12		D		D							D		D									D	D	D	D			D	D						D					
SUH-13		D		D							D		D									D	D	D	D			D	D						D					
SUH-14		D							D		D	D	D			D	D		D			D	D	D	D		D	D	D	D	D		D		D				D	
SUH-15		D		D							D		D								D	D	D	D				D	D						D					
SUH-2				D	D				D				D								D			D	D								D		D		D		D	
SUH-20		D		D							D		D									D	D		D				D	D					D					
SUH-23		D		D							D		D									D	D	D				D	D					D						
SUH-26-1		D		D							D		D		D	D						D	D	D				D	D					D						

(Continued)

TABLE 2 | Continued

Antibiotic Class	Resistance genes	SUH-26-2	SUH-27	SUH-28	SUH-29	SUH-3	SUH-4	SUH-5	SUH-6	SUH-7	SUH-8	SUH-9

D, Detected.

was the most prevalent carbapenemase, present in 39 isolates (92%), with three isolates co-harboring bla_{OXA-58} (**Table 2**). Other β-lactamases detected included NDM-1 ($n = 10$), GES-11 ($n = 2$), CTX-M-15 ($n = 1$), OXA-1 ($n = 1$), TEM-1D ($n = 30$) and TEM-199 ($n = 1$). Multiple aminoglycoside modifying enzymes were identified: *armA, aadA1, aad-B-like, aac(3)-Ia-like, aph(3')-VIa-like, aph(3')-Ia, aph(6)-Id, aph(3'')-Ib, aph(3')-Ic, strA, strB,* and *aac(6')-Ib-like.* Genes contributing to macrolide resistance *mphE* and *msrE* were present in 33 isolates, some of which co-localized in a gene cassette with aminoglycoside resistance genes. Twenty-five isolates possessed *tet(A), tet(B)* or *tet(39)* contributing to tetracycline and erythromycin resistance, found on the same contigs as aminoglycoside modifying enzymes. All isolates had the chromosomally encoded ADC cephalosporinase.

The isolates belonged to seven distinct STs according to the Pasteur scheme: ST1, ST2, ST85, ST570, ST584, ST600, ST602, and ST632, which belonged to IC1, IC2, IC5, IC9, and a singleton. **Table 1** lists the STs identified in both the Pasteur and Oxford schemes. OXA-66, OXA-69, OXA-91, OXA-94, and OXA-51 were the intrinsic OXA-51-like enzymes identified in the study (**Table 2**).

Molecular epidemiology by cgMLST confirmed that IC2 with the intrinsic OXA-66 and acquired OXA-23 was the major clonal lineage found across the four hospitals in Khartoum ($n = 37$, 88%). As seen in **Figure 1**, IC2 is sub-divided into 4 STs (ST-2, −570, −600, and −632) separated by 5–431 allelic differences. Within IC2, we found evidence of seven transmission clusters (TC) (defined at ≤10 allelic differences between isolates) one of which included two Pasteur STs (ST-632 and ST-2), including two inter-hospital transmissions (**Figure 1**). The largest transmission cluster (TC-1, $n = 20$) comprises two Pasteur STs: ST-2 and ST-632 (single-locus variants at the *rpoB allele*), and two Oxford STs: ST-195 and ST-208 (single locus variants at the *gpi* allele).

Two isolates belonging to IC1 (ST-1) and harboring OXA-69 were from two different hospitals (Soba and the Military Hospital), separated by >200 allelic differences, and with distinct resistomes. The two isolates shared the presence of aminoglycoside modifying enzymes *aadA1* and *aac(3)-Ia*, and *sul1*. Isolate SUH-4 was carbapenem resistant, and harbored OXA-23, NDM-1, and additional aminoglycoside modifying enzymes *aadB-like, aph(3')VIa, aph(3')Ic, strA, strB*, and *dfrA1* contributing to trimethoprim resistance. Isolate AC-36, on the other hand, was carbapenem susceptible.

The remaining isolates SUH-14 (OXA-91, ST-602) and AC-20 (OXA-94, ST-85) belonged to IC5 and IC9, respectively, while SUH-2 (OXA-98, ST-584) was a singleton not belonging to an IC.

These data also gave an indication of the local inter-hospital transmissions. Soba University Hospital had a large diversity of isolates belonging to IC1, IC2, IC5 and the singleton ST-584. As seen in **Figure 1**, the majority of these isolates were part of a large transmission cluster (TC-1) that included an isolate from the Ibrahim Malik hospital, as well as having three other smaller transmission clusters (TC-4, TC-5, and TC-6), of which TC-4 comprised three identical isolates, two of which were from the Ibrahim Malik hospital. Isolates from the other two hospitals, Al-Baraha and the Military Hospital, in this study were part

The row labels (Resistance genes) top to bottom: floR-like, catB8, cmlA1-like, tet(39), tet(B)-like, tet(B), DfrA7, dfrA1, Sul2, sul1, msr(E), mph(E), aac(6')Ib-like, aac(3)-IIa, StrB, strA, armA, aph(3')-Ib, aph(6)-Id, aph(3')-Ia, aph(3')-VIa, aph(3')-Ic, aadB, AadA2, AadA1, aacA4-like, blaTEM-199, blaADC, blaGES-11, blaTEM-1D, blaCTX-M-15, blaOXA-91, blaOXA-94, blaOXA-98, blaOXA-1, blaOXA-69, blaOXA-66, blaOXA-58, blaOXA-23, blaNDM-1.

Antibiotic Class groupings: Other antibiotics, Aminoglycoside and Fluoroquinolone, Beta-lactam.

FIGURE 1 | Ridom SeqSphere+ minimum spanning tree (MST) for 42 samples based on 2,390 alleles. Isolates grouped by color indicating the different STs (Pasteur scheme). Eight different STs were identified, belonging to IC1, IC2, IC5, IC9, and a singleton. Numbers between the nodes indicate the number of allelic differences. Shaded nodes represent transmission clusters (TC), and are numbered TC1-7.

of transmission clusters TC-2, TC-3, and TC-7, but with no inter-hospital spread.

With regards to carbapenem-resistance, all but three isolates possessed bla_{OXA-23} which was the sole carbapenemase in 26 isolates. In addition to bla_{OXA-23}, two isolates had bla_{GES-11}, eight bla_{NDM-1}, two bla_{OXA-58}, and one had both bla_{NDM-1} and bla_{OXA-58} (**Table 2** and **Supplementary Figure 2**). One isolate had only NDM-1. There were two carbapenem susceptible isolates, one of which was positive for the ESBLs CTX-M-15 and bla_{OXA-1}. The plasticity of the isolates is demonstrated by them being identical by cgMLST, but differing in their resistomes as for of isolate SUH-8 differing from SUH-28 by having an additional bla_{NDM-1}.

Three isolates from Ibrahim Malik Hospital all fall within IC2 (ST-2) but one of which was separated from the other two by >192 allelic differences. The Military Hospital, on the other hand, had isolates belonging to IC1, IC2, and IC9. A single cluster represented isolates from Al-Baraha Hospital (ST-570) belonging to IC2 and all co-harbored OXA-23 and NDM-1.

DISCUSSION

We hereby present the molecular epidemiology of carbapenem-resistant *A. baumannii* collected from patients from Khartoum,

Sudan. A total of 71 isolates from four different hospitals were collected in 2017–2018, out of which 42 were confirmed as *A. baumannii*. Routine identification in clinical microbiology laboratories in Sudan relies primarily on phenotypic methods in combination with simple biochemical methods, which are not always reliable for accurate bacterial species identification, and 29 isolates (40%) were excluded in the study due to misidentification. Similar findings were reported in a study in Sudan on *Klebsiella pneumoniae* where 40% of isolates were misidentified (Osman et al., 2020). These data highlight an urgent need to improve diagnostic facilities across LMICs such as Sudan, in order to obtain accurate information of the local burden of infections and resistance, and to select optimal treatment options. However, restrictions in funding and capacity pose great limitations in implementing molecular diagnosis in clinical microbiology laboratories. Genomic data from Sudan is scarce, despite the indicative high burden of MDR in hospitals (Hamdan et al., 2015). One study that has performed WGS on an *A. baumannii* isolate from Khartoum, identified it as $ST^{OXF}164$, with OXA-91 as the intrinsic OXA-51-like enzyme (Mohamed et al., 2019), however, the genome of this isolate had over 2,000 allelic differences when compared to our isolates (data not shown).

Core-genome MLST analysis revealed seven transmission clusters, with a predominance of IC2-OXA-23 accounting

for 88% of the isolates across Khartoum Hospitals. This is in accordance with previously published data showing that IC2-OXA-23 is the most successfully disseminating clone globally (Hamidian and Nigro, 2019), however, there are regional variation such as IC5 and IC7 being more predominant in South and Central America (Higgins et al., 2010a). IC2 in Sudan is composed of four sequence types (ST^{PAS}2, ST^{PAS}570, ST^{PAS}600, and ST^{PAS}632). The IC2 isolates were further delineated by cgMLST showing that the ST2 isolates were in some cases more distant than to other STs. For example >190 allelic differences are found between isolates within ST2 (SUH-10 and SUH-11), whereas SUH-3 and SUH-23 which belong to ST2 and ST632, respectively, only have 5 allelic differences. A recent comparison of the two MLST schemes suggested that the Pasteur scheme was more stable than the Oxford scheme (Gaiarsa et al., 2019); while this might be true in most cases, these data from Sudan highlight that a reliance on only 7-loci can lead to a false sense of strain divergence, when in fact, isolates differ in five alleles out of a total 2,390, one of which is the *rpoB* MLST allele. Furthermore, the seven transmission clusters, included inter-hospital spread of two clones (TC-1 and TC-4), which was not apparent from their STs. Similarly, isolates belonging to ST1 have >200 allelic differences between them. These findings illustrate the low resolution of traditional 7-loci typing, particularly for local outbreak and transmission investigations.

Although transmission clusters are clearly evident (**Figure 1**), and despite the relatively conserved dissemination of carbapenemases in the current study, there are some differences, particularly in acquired genes such as NDM-1. For example, SUH-28 and SUH-8 were identical by cgMLST and both had OXA-23, but SUH-28 harbored additional NDM-1 and OXA-58. Furthermore, NDM-1 was present in only three of the four ST-600 isolates. Relying on characterisation of the resistome for determining clonality can be misleading and illustrate the genome plasticity of MDR *A. baumannii*. It is therefore important that discriminatory genomic typing methods are applied in combination with the resistome, clinical and epidemiological data to obtain an accurate picture of outbreaks and transmission links. As *A. baumannii* is an emerging nosocomial pathogen with extended antibiotic resistance, it's important to note the availability of online resources and databases offering rapid typing and phylogenetic relatedness to use when investigating local and global outbreaks, which is increasingly important in a globalized community.

Three isolates (SUH-2, AC20 and AC36) did not harbor OXA-23, but on the other hand harbored other β-lactamases: CTX-M-15, OXA-1, and/or NDM-1. To our knowledge, this is the first report of OXA-1 in *A. baumannii*. OXA-1 is commonly found in *Enterobacteriaceae*, and frequently co-carried with CTX-M-15, as also present in our data (Sugumar et al., 2014).

Our study complements previous studies on the successful dissemination and possible endemicity of specific STs and resistance determinants across North Africa and the Middle East. ST-85 (IC9) is a common clone in Africa and the Middle East as it has been reported as a major clone spreading the NDM-1 gene in Tunisia, among Syrian refugees in Lebanon and Turkey, in France from patients with a history of hospitalization in Algeria, Tunisia and Egypt (Bonnin et al., 2013; Jaidane et al., 2018; Salloum et al., 2018), and recently Spain in a strain harboring NDM-6 (Xanthopoulou et al., 2020). ST6, a single locus variant of ST85, has also been identified among Lebanese patients (Rafei et al., 2014). A single isolate from the Military Hospital was identified as ST-85 harboring OXA-94 and NDM-1 similar to the isolates from Tunisia and Lebanon. With Oxford MLST scheme, this isolate (AC20) was assigned to ST-1089^{OXF}, which has also been reported in Egypt (Al-Hassan et al., 2019; Al-Hassan and Al-Madboly, 2020). Two isolates, SUH-14 and SUH-7, assigned to ST602 (IC5) and ST-2 (IC2), respectively, were positive for GES-11 ESBL. GES-11 has been identified in a Belgian outbreak and was found associated with travel to Egypt, Turkey, and Gaza, thereby indicating a possible geographic dissemination (Moubareck et al., 2009; Bogaerts et al., 2010). Travel and medical tourism are among the major contributors to the acceleration of spread of resistance globally (Ostholm-Balkhed et al., 2013; Tatem, 2014).

CONCLUSION

Our study illustrates that CRAb IC2 *A. baumannii* are widely distributed in Khartoum hospitals and are in possession of multiple antibiotic resistance determinants. We are also able to confirm the spread of specific clones, such as IC9 (ST-85) globally, and across the Middle East and North Africa region specifically. Despite advances in genomic technology, and the relative cheap price of conducting WGS on bacterial genomes, it remains a challenge to implement routine molecular typing methods in Sudan, particularly due to the need of advanced technology and expertise. It is, however, essential to support local efforts to obtain epidemiological data on the burden of resistance in major hospital-acquired infections such as those caused by *A. baumannii*. For local outbreak investigations, molecular epidemiology must be combined with patient clinical and demographic data in order to track transmission and resistance.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in GenBank, under BioProject PRJNA628907. All accession numbers are listed in **Table 1**.

AUTHOR CONTRIBUTIONS

LA-H, HE, EO, DC, and PH contributed to the design of the experiments. HE, EO, SA, and KE were responsible for the collection of isolates from the hospitals. LA-H, HE, EO, DC, and JW performed the experiments. LA-H, HE, EO, JW, HS, and PH analyzed and interpreted the data. LA-H, HE, and PH wrote

the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.628736/full#supplementary-material

Supplementary Figure 1 | Ridom SeqSphere+ minimum spanning tree (MST) for 42 samples based on 2,390 alleles, Numbers between the nodes indicate the number of allelic differences. Isolates grouped based on the hospital they were collected from.

Supplementary Figure 2 | Ridom SeqSphere+ minimum spanning tree (MST) for 42 samples based on 2,390 alleles. Color of nodes indicate the different β-lactamases they harbor. Numbers between the nodes indicate the number of allelic differences. OXA-23 is the main carbapenemase. NDM-1 is present in 10 isolates representing IC1, IC2, and IC9. Several isolates harbor multiple β-lactamases.

REFERENCES

Al-Hassan, L., Zafer, M. M., and El-Mahallawy, H. (2019). Multiple sequence types responsible for healthcare-associated *Acinetobacter baumannii* dissemination in a single centre in Egypt. *BMC Infect. Dis.* 19:829. doi: 10.1186/s12879-019-4433-1

Al-Hassan, L. L., and Al-Madboly, L. A. (2020). Molecular characterisation of an *Acinetobacter baumannii* outbreak. *Infect. Prev. Pract.* 2:100040. doi: 10.1016/j.infpip.2020.100040

Bogaerts, P., Naas, T., El Garch, F., Cuzon, G., Deplano, A., Delaire, T., et al. (2010). GES extended-spectrum β-lactamases in *Acinetobacter baumannii* isolates in Belgium. *Antimicrob. Agents Chemother.* 54, 4872–4878. doi: 10.1128/AAC.00871-10

Bonnin, R. A., Cuzon, G., Poirel, L., and Nordmann, P. (2013). Multidrug-resistant *Acinetobacter baumannii* clone. *France. Emerg. Infect. Dis.* 19, 822–823. doi: 10.3201/eid1905.121618

Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B., et al. (2009). Bad bugs, no drugs: no ESKAPE! an update from the infectious diseases Society of America. *Clin. Infect. Dis.* 48, 1–12. doi: 10.1086/595011

Ellington, M. J., Kistler, J., Livermore, D. M., and Woodford, N. (2007). Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. *J. Antimicrob. Chemother.* 59, 321–322. doi: 10.1093/jac/dkl481

EUCAST (2020a). *EUCAST Reading Guide for Broth Microdilution (v2) 2 March 2020.* Available online at: https://www.eucast.org/ast_of_bacteria/mic_determination/ (accessed September 1, 2020)

EUCAST (2020b). *Testing Breakpoint Tables for Interpretation of MICs and Zone Diameters (v10.0).* Available online at: https://eucast.org/clinical_breakpoints/ (accessed September 1, 2020).

Gaiarsa, S., Biffignandi, G. B., Esposito, E. P., Castelli, M., Jolley, K. A., Brisse, S., et al. (2019). Comparative analysis of the two *Acinetobacter baumannii* multilocus sequence typing (MLST) schemes. *Front. Microbiol.* 10:930. doi: 10.3389/fmicb.2019.00930

Hamdan, H. Z., Kubbara, E., Adam, A. M., Hassan, O. S., Suliman, S. O., and Adam, I. (2015). Urinary tract infections and antimicrobial sensitivity among diabetic patients at Khartoum, Sudan. *Ann. Clin. Microbiol. Antimicrob.* 14:26. doi: 10.1186/s12941-015-0082-4

Hamidian, M., and Nigro, S. J. (2019). Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*. *Microb. Genomics* 5:e000306. doi: 10.1099/mgen.0.000306

Higgins, P. G., Dammhayn, C., Hackel, M., and Seifert, H. (2010a). Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* 65, 233–238. doi: 10.1093/jac/dkp428

Higgins, P. G., Lehmann, M., Wisplinghoff, H., and Seifert, H. (2010b). gyrB multiplex PCR to differentiate between acinetobacter calcoaceticus and *Acinetobacter genomic* species 3. *J. Clin. Microbiol.* 48, 4592–4594. doi: 10.1128/JCM.01765-10

Higgins, P. G., Pérez-Llarena, F. J., Zander, E., Fernández, A., Bou, G., and Seifert, H. (2013). OXA-235, a novel class D β-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 57, 2121–2126. doi: 10.1128/AAC.02413-12

Higgins, P. G., Prior, K., Harmsen, D., and Seifert, H. (2017). Development and evaluation of a core genome multilocus typing scheme for whole-genome sequence-based typing of *Acinetobacter baumannii*. *PLoS One* 12:e0179228. doi: 10.1371/journal.pone.0179228

Jaidane, N., Naas, T., Oueslati, S., Bernabeu, S., Boujaafar, N., Bouallegue, O., et al. (2018). Whole-genome sequencing of NDM-1-producing ST85 *Acinetobacter baumannii* isolates from Tunisia. *Int. J. Antimicrob. Agents* 52, 916–921. doi: 10.1016/j.ijantimicag.2018.05.017

Mohamed, S. B., Hassan, M., Munir, A., Kambal, S., Abdalla, N. I., Hamad, A., et al. (2019). Whole-genome sequence of *Acinetobacter baumannii* strain NUBRI-A, isolated from a hospitalized patient in Khartoum, Sudan. *Microbiol. Resour. Announc.* 8, 9–10. doi: 10.1128/MRA.00542-19

Moubareck, C., Brémont, S., Conroy, M.-C., Courvalin, P., and Lambert, T. (2009). GES-11, a novel integron-associated GES variant in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 53, 3579–3581. doi: 10.1128/AAC.00072-09

Müller, C., Stefanik, D., Wille, J., Hackel, M., Higgins, P. G., and Seifert, H. (2019). "Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* clinical isolates and identification of the novel international clone IC9: results from a worldwide surveillance study (2012 – 2016)," in *Proceedings of the Abstract Book of the 29th European Congress of Clinical Microbiology and Infectious Diseases, P0947*, Amsterdam.

Naas, T., Oueslati, S., Bonnin, R. A., Dabos, M. L., Zavala, A., Dortet, L., et al. (2017). Beta-lactamase database (BLDB)–structure and function. *J. Enzyme Inhib. Med. Chem.* 32, 917–919. doi: 10.1080/14756366.2017.1344235

Okeke, I. N., Laxminarayan, R., Bhutta, Z. A., Duse, A. G., Jenkins, P., O'Brien, T. F., et al. (2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. *Lancet Infect. Dis.* 5, 481–493. doi: 10.1016/S1473-3099(05)70189-4

Osman, E. A., El-Amin, N., Adrees, E. A. E., Al-Hassan, L., and Mukhtar, M. (2020). Comparing conventional, biochemical and genotypic methods for accurate identification of *Klebsiella pneumoniae* in Sudan. *Access Microbiol.* 2:acmi000096. doi: 10.1099/acmi.0.000096

Ostholm-Balkhed, A., Tärnberg, M., Nilsson, M., Nilsson, L. E., Hanberger, H., and Hällgren, A. (2013). Travel-associated faecal colonization with ESBL-producing

Enterobacteriaceae: incidence and risk factors. *J. Antimicrob. Chemother.* 68, 2144–2153. doi: 10.1093/jac/dkt167

Peleg, A. Y., Seifert, H., and Paterson, D. L. (2008). *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin. Microbiol. Rev.* 21, 538–582. doi: 10.1128/CMR.00058-07

Poirel, L., and Nordmann, P. (2006). Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin. Microbiol. Infect.* 12, 826–836. doi: 10.1111/j.1469-0691.2006.01456.x

Rafei, R., Dabboussi, F., Hamze, M., Eveillard, M., Lemarié, C., Gaultier, M. P., et al. (2014). Molecular analysis of *Acinetobacter baumannii* strains isolated in lebanon using four different typing methods. *PLoS One* 9:e115969. doi: 10.1371/journal.pone.0115969

Salloum, T., Tannous, E., Alousi, S., Arabaghian, H., Rafei, R., Hamze, M., et al. (2018). Genomic mapping of ST85 blaNDM-1 and blaOXA-94 producing *Acinetobacter baumannii* isolates from syrian civil war victims. *Int. J. Infect. Dis.* 74, 100–108. doi: 10.1016/j.ijid.2018.07.017

Sugumar, M., Kumar, K. M., Manoharan, A., Anbarasu, A., and Ramaiah, S. (2014). Detection of OXA-1 β-lactamase gene of *Klebsiella pneumoniae* from blood stream infections (BSI) by conventional PCR and in-silico analysis to understand the mechanism of OXA mediated resistance. *PLoS One* 9:e91800. doi: 10.1371/journal.pone.0091800

Tatem, A. J. (2014). Mapping population and pathogen movements. *Int. Health* 6, 5–11. doi: 10.1093/inthealth/ihu006

Tomaschek, F., Higgins, P. G., Stefanik, D., Wisplinghoff, H., Seifert, H., Hatcher, J., et al. (2019). Head-to-Head comparison of two multi-locus sequence typing (MLST) schemes for characterization of *Acinetobacter baumannii* outbreak and sporadic isolates. *PLoS One* 11:e0153014. doi: 10.1371/journal.pone.0153014

WHO (2017). *Global Priority List of Antibiotic-Resistant Batceria to Guide Research, Discovery, and Development of New Antibiotics*. Geneva: WHO, 7.

Woodford, N., Ellington, M. J., Coelho, J. M., Turton, J. F., Ward, M. E., Brown, S., et al. (2006). Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int. J. Antimicrob. Agents* 27, 351–353. doi: 10.1016/j.ijantimicag.2006.01.004

Xanthopoulou, K., Urrutikoetxea-Gutiérrez, M., Vidal-Garcia, M., Diaz de Tuesta del Arco, J. L., Sánchez-Urtaza, S., Wille, J., et al. (2020). First report of New Delhi Metallo-β-Lactamase-6 (NDM-6) in a clinical *Acinetobacter baumannii* isolate from Northern Spain. *Front. Microbiol.* 11:589253. doi: 10.3389/fmicb.2020.589253

Zarrilli, R., Pournaras, S., Giannouli, M., and Tsakris, A. (2013). Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. *Int. J. Antimicrob. Agents* 41, 11–19. doi: 10.1016/j.ijantimicag.2012.09.008