International Journal of Infectious Diseases 91 (2020) 94–100

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Clonal expansion of colistin-resistant *Acinetobacter baumannii* isolates in Cape Town, South Africa

Yolandi Snyman[a,*], Andrew Christopher Whitelaw[a,b], Sandra Reuter[c], Angela Dramowski[d], Motlatji Reratilwe Bonnie Maloba[e,f], Mae Newton-Foot[a,b]

[a] Division of Medical Microbiology, Department of Pathology, Stellenbosch University, Cape Town, South Africa
[b] National Health Laboratory Service, Tygerberg Hospital, Cape Town, South Africa
[c] Institute for Infection Prevention and Hospital Epidemiology, Medical Centre, University of Freiburg, Freiburg, Germany
[d] Department of Paediatrics and Child Health, Stellenbosch University, Cape Town, South Africa
[e] Department of Medical Microbiology, University of the Free State, Bloemfontein, South Africa
[f] National Health Laboratory Service, Universitas Hospital, Bloemfontein, South Africa

ARTICLE INFO

Article history:
Received 28 October 2019
Received in revised form 17 November 2019
Accepted 18 November 2019

Keywords:
Colistin resistance
Acinetobacter baumannii
Clonal expansion
South Africa

ABSTRACT

Objectives: To describe colistin-resistant *Acinetobacter baumannii* isolates in Cape Town, South Africa.
Methods: *A. baumannii* isolates identified on Vitek 2 Advanced Expert System were collected from Tygerberg Hospital referral laboratory between 2016 and 2017. Colistin resistance was confirmed using broth microdilution and SensiTest. *mcr-1–5* were detected using PCR and strain typing was performed by rep-PCR. Whole genome sequencing (WGS) was performed on a subset of isolates to identify chromosomal colistin resistance mechanisms and strain diversity using multilocus sequence typing (MLST) and pairwise single nucleotide polymorphism analyses.
Results: Twenty-six colistin-resistant and six colistin-susceptible *A. baumannii* were collected separately based on Vitek susceptibility; 20/26 (77%) were confirmed colistin-resistant by broth microdilution. Four colistin-resistant isolates were isolated in 2016 and 16 in 2017, from five healthcare facilities. Thirteen colistin-resistant isolates and eight colistin-susceptible isolates were identical by rep-PCR and MLST (ST1), all from patients admitted to a tertiary hospital during 2017. The remaining colistin-resistant isolates were unrelated.
Conclusions: An increase in colistin-resistant *A. baumannii* isolates from a tertiary hospital in 2017 appears to be clonal expansion of an emerging colistin-resistant strain. This strain was not detected in 2016 or from other hospitals. Identical colistin-susceptible isolates were also isolated, suggesting relatively recent acquisition of colistin resistance.
© 2019 The Author(s). Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Acinetobacter baumannii is a gram-negative coccobacillus that is responsible for a substantial proportion of bacterial hospital-acquired (HA) infections in intensive care units (ICUs) and is emerging as a leading healthcare-associated pathogen. A. baumannii may colonize the skin, oropharynx, and gastrointestinal tract without causing infection (Maragakis and Perl, 2008). However, among immunocompromised hosts, particularly patients in ICU settings, *A. baumannii* may cause serious infections including bacteraemia, meningitis, pneumonia, urinary tract infections, and wound infections. This organism can survive on dry surfaces, potentiating its ability for nosocomial spread (Maragakis and Perl, 2008).

A. baumannii is challenging to treat because it can easily upregulate innate resistance mechanisms and acquire a wide array of antimicrobial resistance genes (Ewers et al., 2016; Perez et al., 2007). Carbapenems represent one of the last therapeutic options for the treatment of infections due to multidrug-resistant (MDR) *A. baumannii*.

The emergence of carbapenem resistance has further driven the need to use colistin as a last therapeutic option to combat MDR infections. The emergence of colistin resistance has been widely reported, leading to global concern for the treatment of these

* Corresponding author at: Division of Medical Microbiology, Department of Pathology, Stellenbosch University, Tygerberg Hospital, Fransie van Zyl Avenue, Cape Town, South Africa.
E-mail address: 17770467@sun.ac.za (Y. Snyman).

https://doi.org/10.1016/j.ijid.2019.11.021
1201-9712/© 2019 The Author(s). Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

infections. Colistin resistance among *A. baumannii* is still uncommon, but resistance has been reported worldwide (Cai et al., 2012; Asif et al., 2018).

To date only chromosomally encoded colistin resistance mechanisms have been reported in *A. baumannii*. Mutations in the *pmrB/pmrA/pmrC* operon and genes encoding lipid A biosynthesis (*lpxA, lpxC, lpxD*) confer colistin resistance through modification of the lipid A component of the lipopolysaccharide (LPS) and through complete loss of LPS, respectively (Moffatt et al., 2010; Adams et al., 2009; Mu et al., 2016). The presence of the insertion sequence ISAba11 in *lpxC* or *lpxA* also causes inactivation of LPS production (Moffatt et al., 2011). Alternatively, phosphoethanolamine transferase overexpression can result from the integration of the ISAbaI upstream of a *pmrC* homolog *eptA* leading to colistin resistance (Trebosc et al., 2019).

Recently, colistin resistance due to the plasmid-mediated *mcr-1–9* genes has been described in numerous bacterial species, but not *A. baumannii* (Liu et al., 2016; Xavier et al., 2016; Yin et al., 2017; Carattoli et al., 2017; Borowiak et al., 2017; Yang et al., 2018; AbuOun et al., 2017; Wang et al., 2018; Carroll et al., 2019). The *mcr-4.3* variant has been described in an *A. baumannii* isolate from China; however this variant does not confer colistin resistance (Ma et al., 2019). In South Africa, the prevalence of *mcr-1* has only been investigated in *Escherichia coli* and *Klebsiella pneumoniae* (Newton-Foot et al., 2017; Coetzee et al., 2016; Poirel et al., 2016).

It appears that no previous studies have described the clonal diversity of colistin-resistant *A. baumannii* isolates in southern Africa. The objective of this study was to characterize the colistin resistance mechanisms and molecular diversity of colistin-resistant A. baumannii isolates recovered from patients with clinical infections from hospitals in the Western Cape of South Africa. Surveillance of colistin resistance mechanisms present in a population is vital for advising effective treatment options and for monitoring the development and spread of resistance.

Methods

Clinical setting

The National Health Laboratory Service (NHLS) Microbiology Laboratory at Tygerberg Academic Hospital receives specimens from Tygerberg Hospital and various regional and district hospitals in the Western Cape. Areas from Cape Town, Cape Winelands, West Coast, and Overberg rural districts are part of the Tygerberg Hospital catchment area. This hospital serves as a referral centre for more than 120 primary health care clinics, 17 district hospitals, and four regional hospitals. Tygerberg Hospital itself is a 1384-bed tertiary academic hospital, including 176 paediatric beds, 124 neonatal beds, and general and specialist medical and surgical services.

Isolate collection

A. baumannii isolates were collected from clinical specimens processed routinely between May 2016 and August 2017; these isolates were chosen on the basis of being colistin-resistant. In addition, colistin-susceptible, carbapenem-resistant *A. baumannii* isolates from the neonatal unit at a tertiary hospital were collected separately between March and May 2017 as part of an outbreak investigation. Microbial identification and antimicrobial susceptibility testing were performed as part of routine diagnostic procedures, using the Vitek 2 Advanced Expert System (BioMérieux). All isolates were initially selected based on carbapenem and colistin susceptibility results as determined by the Vitek 2 system.

Hospital- or community-acquired isolates/infection

Isolates from the tertiary hospital were classified as community- or hospital-acquired (CA or HA) if they were cultured from specimens collected within 3 days (CA) or after 3 days (HA) of admission. If a patient had been previously hospitalized for two or more days within the past 90 days, the isolates were also classified as hospital-acquired (Kieninger and Lipsett, 2009). A 72-h cut-off was opted for instead of a 48-h cut-off, as only date of admission was available, not the specific time. Isolates from outside of the academic hospital were not classified this way, as admission dates were not available.

Phenotypic resistance testing

Although isolates were initially selected based on colistin and carbapenem susceptibility results as determined by the Vitek 2 system, colistin susceptibility was confirmed using SensiTest Colistin (Liofilchem, Italy) and by broth microdilution (BMD) following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and breakpoints version 8 (ISO-standard broth microdilution method 20776- 1) (Eucast, 2016). Isolates with a minimum inhibitory concentration (MIC) of $\leq$2 μg/ml were classified as colistin-susceptible and those with a MIC > 2 μg/ml as colistin-resistant. *A. baumannii* EUCAST-20 (MIC ~0.5 μg/ml) and *E. coli* NCTC 13846 (MIC 2–4 μg/ml) were included as controls.

PCR detection of mcr *genes*

PCR detection of *mcr-1*, *-2*, *-3*, *-4*, and *-5* was performed on colistin-resistant isolates using previously described primers (Liu et al., 2016; Xavier et al., 2016; Yin et al., 2017; Carattoli et al., 2017; Borowiak et al., 2017). The *rpoB* gene product was used as an internal amplification control (Khosravi et al., 2015), and several *mcr* amplification controls were used (NCTC 13846, KP37-BE (Xavier et al., 2016), WJ1(13), CAC13 (MG948623.2), PLA35 (unpublished sequenced PCR products)). PCR assays were done using the KAPA Taq ReadyMix PCR Kit (Kapa Biosystems) and amplicons were separated on a 1.5% w/v agarose gel and detected using the Alliance 2.7 imaging system (UVITec).

Molecular typing using rep-PCR

The clonal relationship of all *A. baumannii* isolates was analysed by rep-PCR using previously described primers: REP 1F 5′-IIIGCGCCGICATCAGGC-3′ and REP 2R 5′-ACGTCTTATCAGGCCTAC-3′ (Vila et al., 1996). GelCompar II version 7.5 (Applied Maths) was used for dendrogram construction (http://www.applied-maths.com/bionumerics). Banding patterns were normalized to the KAPA Universal Ladder (Kapa Biosystems) and similarity between the profiles calculated with the band matching Dice similarity coefficient with 1% position tolerance (Domenech de Cellès et al., 2012). Dendrograms were generated using the unweighted pair group method with arithmetic mean (UPGMA) (Domenech de Cellès et al., 2012). Isolates with $\geq$98% similarity were defined as identical, $\geq$95% as closely related, and <94.9% as unrelated (Higgins et al., 2012).

Whole genome sequencing (WGS)

Four rep-PCR identical isolates were chosen for WGS: two were colistin-resistant (CAC12 and CAC38) and two colistin-susceptible (CAC37 and CAC29). Genomic DNA was extracted using the Roche Pure PCR Template Preparation Kit (Roche Diagnostics, Germany). The DNA concentration was determined using the Qubit dsDNA BR

Assay Kit and the Qubit 2.0 Fluorometer (Life Technologies, USA) and diluted to 0.2 ng/μl. DNA libraries were prepared using the Nextera DNA Flex Library Preparation Kit (Illumina, Germany) and sequenced with the Illumina MiSeq Reagent Kit V2.

Sequencing reads were assessed for sufficient coverage (>30 × based on mapping to reference genome A1 (CP010781)) and assembly statistics (N50 > 100 000 bp; length ~4 Mbp; total number of contigs <100) (Supplementary Material Table S1). Taxonomic identification was done using Kraken version 0.10.5-beta with the 4 GB MiniKraken database (Wood and Salzberg, 2014). Assemblies were generated using SPAdes (Bankevich et al., 2012) and annotated using Prokka (Seemann, 2014). Roary was used to determine shared gene presence and absence (Page et al., 2015).

The raw reads have been deposited in the European Nucleotide Archive (ENA) (PRJEB34692) and the assemblies have been deposited in the National Center for Biotechnology Information (NCBI) (Supplementary Table 1).

Genotypic detection of chromosomal resistance mechanisms using WGS data

Acquired antimicrobial resistance genes and mutations in the *lpxA*, *lpxC*, *lpxD*, and *pmrB/pmrA/pmrC* genes were detected using ARIBA (Hunt et al., 2017) using the CARD (McArthur et al., 2013) and ResFinder (Zankari et al., 2012) databases. Additionally, Artemis (Rutherford et al., 2000) was used to manually screen the annotated assemblies for known colistin resistance mutations and gene differences (sequence variants and the presence/absence of genes) in the colistin resistance pathway.

Strain typing using WGS data

Sequence type (ST) was determined from the assemblies (https://github.com/tseemann/mlst) using the Pasteur scheme (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, *rpoB*). Reads were mapped against the first collected colistin-susceptible *A. baumannii* isolate (CAC29) and single nucleotide polymorphisms (SNPs) were identified. From the resulting alignment, a pairwise SNP distance matrix was generated using snp-dists 0.6.3 (https://github.com/tseemann/snp-dists). This presented the relatedness of these isolates with the highest possible resolution. SNPs were manually viewed in Artemis to check for their quality to eliminate those called due to errors in sequencing or mapping accuracy.

RT-PCR detection of the I527N mutation

Real-time PCR (RT-PCR) was performed on all colistin-resistant and susceptible isolates using two sets of primers that are specific for the I527N mutation, I527N1-YF 5′-CTAAAGAGGCACCAGCTT-TAAATAA-3′ and I527N1-YR 5′-ACCAAGACGGAACTGTGAA-3′, and wild-type sequence I527N2-YF 5′-AGTTGTCCTTGCATTTTATAAA-TATGC-3′ and I527N2-YR 5′-CGAACAATCGCCTCATGAA-3′. RT-PCR assays were done using the KAPA SYBR Fast qPCR Kit (Kapa Biosystems) on the Rotor Gene Q (Qiagen) and analysed using the Rotor Gene Q software.

Results

Characteristics of colistin-resistant and susceptible A. baumannii *isolates*

Twenty-six non-duplicate colistin-resistant *A. baumannii* isolates were collected based on Vitek 2 antimicrobial susceptibility testing. Of these, 20 (62.5%) were confirmed to be colistin-resistant by both BMD and SensiTest (Figure 1, Supplementary Material Table S2). These isolates were collected from various microbiological specimens including urine, blood culture, tracheal aspirate, bronchial aspirate, abscess (superficial) aspirate, and tissue, and samples were obtained from numerous wards. Four colistin-resistant isolates were isolated in 2016 and 16 in 2017, from tertiary ($n = 15$) and district/regional level ($n = 4$) hospitals and a primary healthcare clinic ($n = 1$).

Six colistin-susceptible isolates (AB2, AB3, AB4, AB5, AB9, and AB11) analysed as part of an outbreak of carbapenem-resistant, colistin-susceptible *A. baumannii* in the neonatal unit at a tertiary hospital, were collected separately between March and May 2017 (Figure 1). These isolates were all collected from blood cultures.

Rep-PCR typing

Thirteen colistin-resistant *A. baumannii* isolates as well as eight colistin-susceptible isolates (including five from the neonatal unit outbreak) were identical by rep-PCR (Figure 2). One neonatal isolate (AB3) had 88.9% similarity to this strain. The remaining isolates were all unrelated based on rep-PCR.

The related isolates were all from patients admitted to a tertiary hospital between May 2016 and August 2017, except for one isolate (CAC23) from a regional hospital. This isolate was

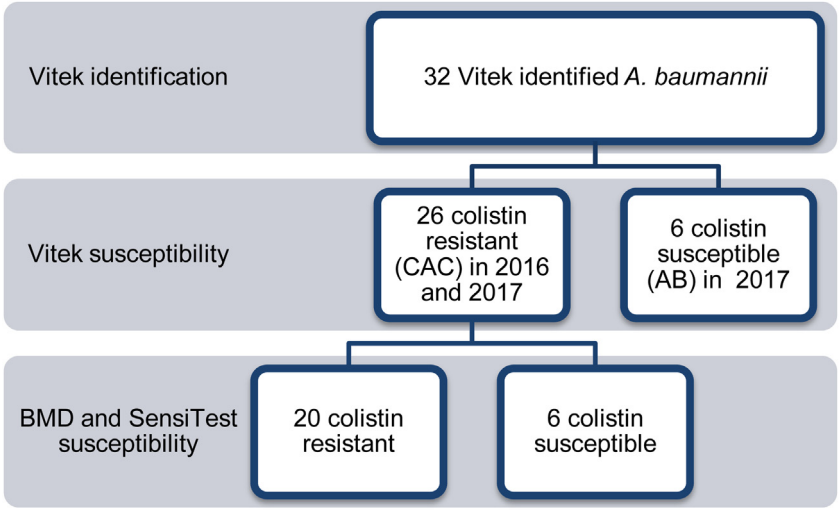
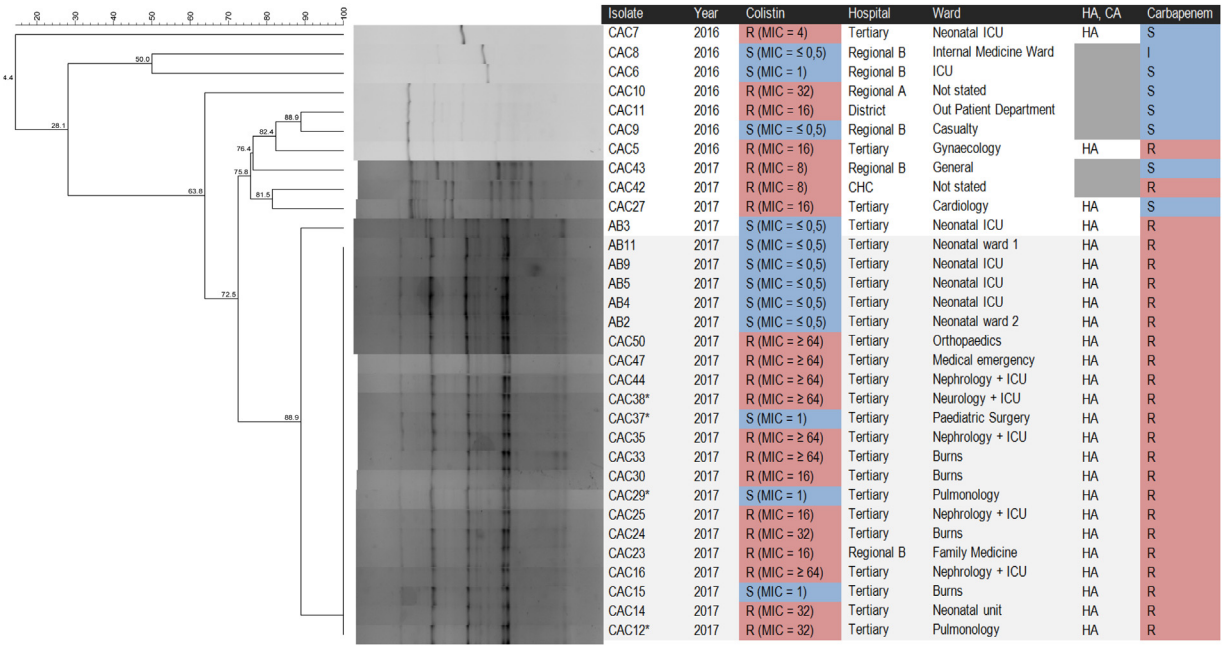


Figure 1. Colistin resistant and susceptible *A. baumannii* isolates.



Isolate	Year	Colistin	Hospital	Ward	HA, CA	Carbapenem
CAC7	2016	R (MIC = 4)	Tertiary	Neonatal ICU	HA	S
CAC8	2016	S (MIC = ≤ 0,5)	Regional B	Internal Medicine Ward		I
CAC6	2016	S (MIC = 1)	Regional B	ICU		S
CAC10	2016	R (MIC = 32)	Regional A	Not stated		S
CAC11	2016	R (MIC = 16)	District	Out Patient Department		S
CAC9	2016	S (MIC = ≤ 0,5)	Regional B	Casualty		S
CAC5	2016	R (MIC = 16)	Tertiary	Gynaecology	HA	R
CAC43	2017	R (MIC = 8)	Regional B	General		S
CAC42	2017	R (MIC = 8)	CHC	Not stated		R
CAC27	2017	R (MIC = 16)	Tertiary	Cardiology	HA	S
AB3	2017	S (MIC = ≤ 0,5)	Tertiary	Neonatal ICU	HA	R
AB11	2017	S (MIC = ≤ 0,5)	Tertiary	Neonatal ward 1	HA	R
AB9	2017	S (MIC = ≤ 0,5)	Tertiary	Neonatal ICU	HA	R
AB5	2017	S (MIC = ≤ 0,5)	Tertiary	Neonatal ICU	HA	R
AB4	2017	S (MIC = ≤ 0,5)	Tertiary	Neonatal ICU	HA	R
AB2	2017	S (MIC = ≤ 0,5)	Tertiary	Neonatal ward 2	HA	R
CAC50	2017	R (MIC = ≥ 64)	Tertiary	Orthopaedics	HA	R
CAC47	2017	R (MIC = ≥ 64)	Tertiary	Medical emergency	HA	R
CAC44	2017	R (MIC = ≥ 64)	Tertiary	Nephrology + ICU	HA	R
CAC38*	2017	R (MIC = ≥ 64)	Tertiary	Neurology + ICU	HA	R
CAC37*	2017	S (MIC = 1)	Tertiary	Paediatric Surgery	HA	R
CAC35	2017	R (MIC = ≥ 64)	Tertiary	Nephrology + ICU	HA	R
CAC33	2017	R (MIC = ≥ 64)	Tertiary	Burns	HA	R
CAC30	2017	R (MIC = 16)	Tertiary	Burns	HA	R
CAC29*	2017	S (MIC = 1)	Tertiary	Pulmonology	HA	R
CAC25	2017	R (MIC = 16)	Tertiary	Nephrology + ICU	HA	R
CAC24	2017	R (MIC = 32)	Tertiary	Burns	HA	R
CAC23	2017	R (MIC = 16)	Regional B	Family Medicine	HA	R
CAC16	2017	R (MIC = ≥ 64)	Tertiary	Nephrology + ICU	HA	R
CAC15	2017	S (MIC = 1)	Tertiary	Burns	HA	R
CAC14	2017	R (MIC = 32)	Tertiary	Neonatal unit	HA	R
CAC12*	2017	R (MIC = 32)	Tertiary	Pulmonology	HA	R

Figure 2. Genetic relatedness of colistin resistant and carbapenem resistant *A. baumannii* isolates from 2016 and 2017. CAC and AB: *A. baumannii*, R: Resistant, S: Susceptible, MIC's were determined using BMD. HA: Hospital-acquired, CA: Community-acquired. CHC: Community health Centre. CA or HA was only determined for the Tertiary Hospital, no information about HA or CA at the regional, district and CHC. * WGS was performed.

indistinguishable by rep-PCR, and an epidemiological investigation showed that this patient had been admitted to the same tertiary hospital 2 weeks previously. All of the related isolates were not only carbapenem-resistant but also resistant to a further nine antibiotics (amikacin, cefotaxime, cefepime, ceftazidime, ciprofloxacin, gentamicin, piperacillin–tazobactam, tobramycin, trimethoprim–sulfamethoxazole) as determined by Vitek 2 susceptibility testing, giving this strain type an extensively drug-resistant (XDR) profile.

Genomic investigation into resistance mechanisms, in particular colistin resistance

WGS was performed on four of the rep-PCR identical isolates: two colistin-resistant (CAC12 and CAC38) and two colistin-susceptible (CAC37 and CAC29). All four isolates were identified as ST1 using the Pasteur scheme. The four isolates were very closely related with a maximum of 11 SNPs between isolates (Table 1).

None of the known *mcr-1–5* genes were detected in the colistin-resistant isolates using PCR; this was confirmed in the sequenced isolates. No *mcr-6–9* genes were detected in the WGS data. No previously described colistin resistance mutations were detected in the *pmr* or *lpx* genes of the colistin-resistant isolates CAC12 and CAC38, and the sequences of these genes were identical in the two colistin-resistant and two susceptible isolates. Additional acquired resistance genes were detected in all four XDR *A. baumannii* isolates (Table 2), which correlated with the susceptibilities of the Vitek 2 tested antibiotics. Phenotypic testing for macrolides, rifampicin, sulphonamides, and phenicols is not performed routinely.

A single non-synonymous mutation was identified in both colistin-resistant isolates that was absent in both colistin-susceptible isolates. The I527N mutation was identified in the 2196-bp DctM-like transporter family protein (tripartite ATP-independent periplasmic transporter); however this protein has not previously been associated with colistin resistance. Ten of the 13 clonal colistin-resistant isolates had the I527N mutation. Three clonal colistin-resistant isolates (CAC14, CAC30, and CAC47) and all of the other unrelated colistin-resistant isolates did not have the I527N mutation. The I527N mutation was not detected in any of the colistin-susceptible isolates. The gene presence/absence search with Roary did not show any relevant differences between the colistin-resistant and colistin-susceptible isolates. Roary only showed that CAC38 had a missing phage compared to the other three isolates. This phage was determined to be similar to *Psychrobacter* phage Psymv2 (NC_023734) using the PHAge Search Tool Enhanced Release (PHASTER) (Arndt et al., 2016).

Table 1
Number of SNP differences between two colistin-susceptible and two colistin-resistant *Acinetobacter baumannii* isolates using snp-dists 0.6.3.

Colistin		Resistant		Susceptible	
		CAC12	CAC38	CAC37	CAC29
Resistant	CAC12	0	2	2	9
	CAC38		0	4	11
Susceptible	CAC37			0	9
	CAC29				0

SNP, single nucleotide polymorphism.

Discussion

The increase in colistin-resistant *A. baumannii* isolates from a tertiary hospital in the Western Cape in 2017 appears to be due to clonal expansion of an emerging colistin-resistant strain within the hospital. This strain was not detected in 2016 or in isolates from other hospitals.

A 2016 surveillance study in South Africa reported that carbapenem resistance in *A. baumannii* in South Africa is high (81%), with consistent findings across sentinel health care facilities, as well as increasing levels of resistance over time (Perovic et al., 2018). This limits treatment options, with colistin being the most commonly used alternative antimicrobial agent. To date no surveillance data on colistin resistance among *A. baumannii* laboratory isolates are available in South Africa.

Table 2
Additional acquired resistance genes in CAC12, CAC38, CAC37, and CAC29.

Resistance	Acquired resistance genes
Beta-lactam	bla_{OXA-23}, bla_{ADC-25}, bla_{PER-7}, bla_{OXA-69}, bla_{NDM-1}
Macrolide	*mphE*
Trimethoprim	*dfrA1*
Sulphonamide	*sul1*, *sul2*
Tetracycline	*tet(B)*
Phenicol	*cmlA1*
Rifampicin	*ARR-2*
Macrolide, lincosamide and streptogramin B	*msrE*
Aminoglycoside	*aac(3)-Ia*, *aph(3")-Ib*, *aph(3')-Ia*, *aadA1*, *aph(6)-Id*, *armA*

All of the clonal *A. baumannii* isolates were resistant to commonly available antibiotics, and most were resistant to colistin. The presence of contemporaneous identical colistin-susceptible strains isolated from the same hospital suggests either relatively recent acquisition of the colistin resistance mechanism or perhaps a heteroresistant population in which the respective mutations have not (yet) become fixed and are thus difficult to trace genomically. If more than four isolates were sequenced, we might have found more genetic modifications between the outbreak isolates.

The absence of plasmid-mediated *mcr* genes and any known chromosomal mutations suggests that the colistin resistance in these isolates may be due to an undescribed mechanism of colistin resistance. Functionality studies are planned to investigate the single I527N non-synonymous mutation that was identified in 10 of the 13 clonal colistin-resistant isolates to determine its putative role in colistin resistance. However, the fact that three of the clonal colistin-resistant isolates did not have the I527N mutation may cast doubt on the role of this mutation in colistin resistance.

WGS has been recognized to be more effective than traditional methods for characterizing outbreaks caused by gram-negative bacteria; however in this study, rep-PCR showed an accurate representation of the sequencing results. Various publications have shown rep-PCR to be more discriminatory than multilocus sequence typing (MLST) (Runnegar et al., 2010; Hojabri et al., 2014). The four isolates that underwent WGS showed low SNP diversity, yet were obtained from different wards in the tertiary hospital. The sequence type of these clonally related isolates is ST1, which is part of global clone 1, one of the dominating clones worldwide (Ewers et al., 2016; Martins et al., 2012; Ghaith et al., 2017; Handal et al., 2017; Hamidian et al., 2014). Given the discriminatory power of rep-PCR, it is almost certain that all of the clonal isolates belong to ST1. ST1 has not been described previously amongst colistin-resistant *A. baumannii*.

The phylogenetic relationship of the strains was critical in guiding infection control measures and restricting the outbreak. The infection prevention hospital staff were immediately informed and outbreak intervention measures were intensified, including active surveillance, hand hygiene, and contact precautions. Restricting the spread of *A. baumannii* to reduce rates of hospital-acquired infections is challenging, because the bacteria can survive in the environment for long periods. Thorough environmental cleaning of potential reservoir sources in hospital environments, e.g. ventilators, humidifiers, suction containers, and moist articles, is highly recommended.

Limitations

Information regarding whether isolates were hospital- or community-acquired was only available for patients from the tertiary hospital. However, the clonally related isolates were all from this hospital, and the isolates from other hospitals were unrelated. Some colistin-resistant isolates may have been missed, as only isolates shown to be resistant using Vitek were collected, and the limitations of the Vitek for colistin susceptibility testing have been described (Girardello et al., 2018). Likewise, colistin-susceptible isolates belonging to the same clone may have been missed, since the primary selection criterion was colistin resistance and the neonatal isolates were included fortuitously. At the time when isolate collection was initiated, the limitations of colistin susceptibility testing using automated platforms were not widely recognized, and it would have been impractical to collect all *A. baumannii* isolates, given the large number of isolates coming through the NHLS laboratory.

Conclusions

A clonal expansion in 2017 of an emerging colistin-resistant *A. baumannii* was detected in a tertiary hospital in the Western Cape of South Africa. The presence of genetically closely related colistin-susceptible strains in the same hospital during the same time frame suggests that the acquisition of colistin resistance was relatively recent. This is the first report of a colistin-resistant clonal expansion of *A. baumannii* in South Africa.

Ethical approval

The study was approved by the Health Research Ethics Committee at Stellenbosch University (S18/10/259). Informed consent was waived by the HREC, since the study was observational and patient care was not influenced.

Funding

This study was supported by the NHLS Research Trust (grant number 94610) and Stellenbosch University Library's Open Access Publication Fund (grant number FFB007P). Personal funding was obtained by the NRF Innovation Masters Scholarship (grant number SFH160627174441). The funders had no role in the study design, data collection, analysis and interpretation of the data, decision to submit the work for publication, or preparation of the manuscript.

Transparency declaration

Part of this work was presented at the 2018 ECCMID Conference in Spain: "Clonal expansion of colistin-resistant *Acinetobacter baumannii* isolates in Cape Town, South Africa" (P4643).

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

YS performed all experiments, interpreted and organized the project, and drafted the manuscript. MNF, AW, and MRBM designed the study, supervised, interpreted the results, and edited the manuscript. AD interpreted the results and edited the manuscript. YS and SR performed bioinformatics analyses. All authors read and approved the final manuscript.

Acknowledgements

The authors are grateful to Miss Sarah Klassen for extracting the DNA and sequencing the four isolates at the University of Freiburg

Medical Centre, Germany. We also thank Prof. Hajo Grundmann for providing funding for the sequencing. The authors are also thankful to Prof. Surbhi Malhotra-Kumar from the University of Antwerp, Belgium, for providing us with KP37-BE (mcr-2 control strain) and Timothy Walsh from Cardiff University, Wales, for providing us with WJ1 (*mcr-3* control strain).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.11.021.

References

AbuOun M, Stubberfield EJ, Duggett NA, Kirchner M, Dormer L, Nunez-Garcia J, et al. mcr-1 and mcr-2 variant genes identified in Moraxella species isolated from pigs in Great Britain from 2014 to 2015. J Antimicrob Chemother 2017;72 (October (10))2745–9. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/29091227.

Adams MD, Nickel GC, Bajaksouzian S, Lavender H, Murthy AR, Jacobs MR, et al. Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the PmrAB two-component system. Antimicrob Agents Chemother 2009;53 (September (9))3628–34. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/19528270.

Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, et al. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 2016;44(W1):W16–21.

Asif M, Alvi IA, Ur Rehman S. Insight into *Acinetobacter baumannii*: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infect Drug Resist 2018;11:1249–60.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19(5)455–77, doi:http://dx.doi.org/10.1089/cmb.2012.0021 Available from:.

Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. Identification of a novel transposon-associated phosphoethanolamine transferase gene, mcr-5, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. enterica serovar Paratyphi B. J Antimicrob Chemother 2017;72(December (12))3317–24. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/28962028.

Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of *Acinetobacter baumannii*: clinical reports, mechanisms and antimicrobial strategies. J Antimicrob Chemother. 2012;67(7):1607–15.

Carattoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppi A, et al. Novel plasmid-mediated colistin resistance mcr-4 gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Available from:. . p. 1–5. https://www.ncbi.nlm.nih.gov/pubmed/28797329.

Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO, Wiedmann M. Identification of novel mobilized colistin resistance gene mcr-9 in a multidrug-resistant, colistin-susceptible *Salmonella enterica* serotype typhimurium isolate. Turner MS, editor. MBio 2019;10(May (3))1–6, doi:http://dx.doi.org/10.1128/mBio.00853-19 Available from:.

Coetzee J, Corcoran C, Prentice E, Moodley M, Mendelson M, Poirel L, et al. Emergence of plasmid-mediated colistin resistance (MCR-1) among *Escherichia coli* isolated from South African patients. S Afr Med J 2016;106(April (5))35–6. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/27138657.

Domenech de Cellès M, Salomon J, Marinier A, Lawrence C, Gaillard J-L, Herrmann J-L, et al. Identifying more epidemic clones during a Hospital Outbreak of Multidrug-Resistant *Acinetobacter baumannii*. Adler B, editor. PLoS One 2012;7 (September (9))e45758, doi:http://dx.doi.org/10.1371/journal.pone.0045758 Available from:.

Eucast. Recommendations for MIC determination of colistin (polymyxin E) as recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group. 2016; (March, 22). 2016.

Ewers C, Klotz P, Scheufen S, Leidner U, Göttig S, Semmler T. Genome sequence of OXA-23 producing *Acinetobacter baumannii* IHIT7853, a carbapenem-resistant strain from a cat belonging to international clone IC1. Gut Pathog 2016;8(1):1–7.

Ghaith DM, Zafer MM, Al-Agamy MH, Alyamani EJ, Booq RY, Almoazzamy O. The emergence of a novel sequence type of MDR *Acinetobacter baumannii* from the intensive care unit of an Egyptian tertiary care hospital. Ann Clin Microbiol Antimicrob 2017;16(1):1–8.

Girardello R, Cury AP, Franco MRG, Di Gióia TR, de Almeida JN, de Araújo MRE, et al. Colistin susceptibility testing and Vitek-2™: is it really useless?. Diagn Microbiol Infect Dis 2018;91(4):309–11.

Hamidian M, Holt KE, Pickard D, Dougan G, Hall RM. A GC1 *Acinetobacter baumannii* isolate carrying AbaR3 and the aminoglycoside resistance transposon TnaphA6 in a conjugative plasmid. J Antimicrob Chemother 2014;69(April (4))955–8. . Available from: https://academic.oup.com/jac/article-lookup/doi/10.1093/jac/dkt454.

Handal R, Dawodi R, Marzouqa H, Hindiyeh M, Qunibi L, Sahouri I, et al. Characterization of carbapenem-resistant *Acinetobacter baumannii* strains isolated from hospitalized patients in Palestine. Int J Microbiol 2017;2017:.

Higgins PG, Hujer AM, Hujer KM, Bonomo Ra, Seifert H. Interlaboratory reproducibility of DiversiLab rep-PCR typing and clustering of *Acinetobacter baumannii* isolates. J Med Microbiol 2012;61(Pt 1)137–41. . Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3347881&tool=pmcentrez&rendertype=abstract.

Hojabri Z, Pajand O, Bonura C, Aleo A, Giammanco A, Mammina C. Molecular epidemiology of *Acinetobacter baumannii* in Iran: endemic and epidemic spread of multiresistant isolates. J Antimicrob Chemother 2014;69(9):2383–7.

Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J, Keane JA, et al. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb Genomics 2017;3(10)e000131. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/29177089.

Khosravi AD, Sadeghi P, Shahraki AH, Heidarieh P, Sheikhi N. Molecular methods for identification of acinetobacter species by partial sequencing of the rpoB and 16S rRNA Genes. J Clin Diagn Res 2015;9(July (7)):DC09–13.

Kieninger AN, Lipsett PA. Hospital-acquired pneumonia: pathophysiology, diagnosis, and treatment. Surg Clin North Am 2009;89:439–61.

Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 2016;16(February (2))161–8, doi:http://dx.doi.org/10.1016/S1473-3099(15)00424-7 Available from:.

Ma F, Shen C, Zheng X, Liu Y, Chen H, Zhong L, et al. Identification of a novel plasmid carrying mcr-4.3 in an *Acinetobacter baumannii* Strain in China. Antimicrob Agents Chemother 2019;63(April (6))1–7, doi:http://dx.doi.org/10.1128/AAC.00133-19 Available from:.

Maragakis LL, Perl TM. Antimicrobial resistance: *Acinetobacter baumannii*: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis 2008;46(8):1254–63.

Martins N, Martins IS, de Freitas WV, de Matos JA, Magalhães ACG, Girão VBC, et al. Severe infection in a lung transplant recipient caused by donor-transmitted carbapenem-resistant *Acinetobacter baumannii*. Transpl Infect Dis 2012;14 (3):316–20.

McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, et al. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother 2013;57(July (7))3348–57. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/23650175.

Moffatt JH, Harper M, Harrison P, Hale JDF, Vinogradov E, Seemann T, et al. Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. Antimicrob Agents Chemother 2010;54(December (12))4971–7. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/20855724.

Moffatt JH, Harper M, Adler B, Nation RL, Li J, Boyce JD. Insertion sequence ISAba11 is involved in colistin resistance and loss of lipopolysaccharide in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2011;55(June (6))3022–4. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/21402838.

Mu X, Wang N, Li X, Shi K, Zhou Z, Yu Y, et al. The Effect of colistin resistance-associated mutations on the fitness of *Acinetobacter baumannii*. Front Microbiol 2016;7(November)1715. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/27847502.

Newton-Foot M, Snyman Y, Maloba MRB, Whitelaw AC. Plasmid-mediated mcr-1 colistin resistance in *Escherichia coli* and *Klebsiella* spp. clinical isolates from the Western Cape region of South Africa. Antimicrob Resist Infect Control 2017;6:78. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/28785405.

Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, et al. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 2015;31(22):3691–3.

Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant *Acinetobacter baumanni*. Antimicrob Agents Chemother 2007;51(10):3471–84.

Perovic O, Ismail H, van Schalkwyk E. Antimicrobial resistance surveillance in the South African public sector. S Afr J Infect Dis 2018;1–12. . Available from: http://www.tandfonline.com/action/showCitFormats?doi=10.1080/23120053.2018.1469851.

Poirel L, Kieffer N, Brink A, Coetze J, Jayol A, Nordmann P. Genetic features of MCR-1-producing colistin-resistant *Escherichia coli* isolates in South Africa. Antimicrob Agents Chemother 2016;60(July (7))4394–7. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/27161623.

Runnegar N, Sidjabat H, Goh HMS, Nimmo GR, Schembri MA, Paterson DL. Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* in a single institution over a 10-year period. J Clin Microbiol 2010;48(November (11))4051–6. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/20739495.

Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream MA, et al. Artemis: sequence visualization and annotation. Bioinformatics 2000;16(10):944–5.

Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics 2014;30 (July (14))2068–9. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/24642063.

Trebosc V, Gartenmann S, Tötzl M, Lucchini V, Schellhorn B, Pieren M, et al. Dissecting colistin resistance mechanisms in extensively drug-resistant *Acinetobacter baumannii* clinical isolates. Wright GD, editor. MBio 2019;10(July (4))1–12. . Available from: https://doi.org/10.1128/mBio.01083-19.

Vila J, Marcos MA, Jimenez de Anta MT. A comparative study of different PCR-based DNA fingerprinting techniques for typing of the *Acinetobacter calcoaceticus-A. baumannii* complex. J Med Microbiol 1996;44(June (6))482–9. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/8636966.

Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, et al. Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing *Klebsiella pneumoniae*. Emerg Microbes Infect 2018;7(1)122. . Available from: http://www.nature.com/articles/s41426-018-0124-z.

Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 2014;15(March (3))R46. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/24580807.

Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, et al. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in *Escherichia coli*, Belgium, June 2016. Euro Surveill 2016;21(July (27))6–11. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/27416987.

Yang YQ, Li YX, Lei CW, Zhang AY, Wang HN. Novel plasmid-mediated colistin resistance gene mcr-7.1 in *Klebsiella pneumoniae*. J Antimicrob Chemother 2018;73(7):1791–5.

Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, et al. Novel plasmid-mediated colistin resistance Gene mcr-3 in *Escherichia coli*. MBio 2017;8(3)4–9. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/28655818.

Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67(November (11))2640–4. . Available from: http://www.ncbi.nlm.nih.gov/pubmed/22782487.