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Genomic Analysis of Two Drug-Resistant Clinical *Morganella morganii* Strains Isolated from UTI Patients in Pretoria, South Africa

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Significance and impact of study

We report on the first clinical *Morganella morganii* draft genomes from Africa. The isolates were found in the urine of patients presenting with urinary tract infections (UTIs). Notably, they were resistant to important clinical antibiotics, including those used to treat UTIs. Due to the common occurrence of UTIs, particularly among pregnant women for whom drug options are limited, the presence of antibiotic-resistant uropathogens such as *M. morganii* is a serious public health concern. We therefore characterised the resistance mechanisms and epidemiology of these isolates to provide further insights into their dissemination and background data for future studies.

Abstract

Morganella morganii is an opportunistic bacterial pathogen of the Enterobacteriaceae family that is occasionally isolated from clinical (animal and human) specimens with varying resistance profiles. Detailed genomic analyses of drug-resistant *M. morganii* strains are relatively limited, particularly in Africa, which is also due to their relatively low isolation rates from clinical settings. Here, we report on two multidrug-resistant clinical *M. morganii* isolates from urine specimens of two hospitalised patients in South Africa who presented with urinary tract infections in 2013. The isolates, M006 and E042, were only susceptible to carbapenems, amikacin and tigecycline. One strain, M006, had a novel class 1 integron, *ln1484*, associated with *aadA7*, *sull* and *gcuD* gene cassettes and a Col3M plasmid replicase gene. The *ln1484 int11:aadA7:sull* genes were bracketed by a *TnAs3* composite transposon while a *tet(B)* gene was found on an *IS4* family transposon. The rare *bla*_{DHA-4} and *bla*_{DHA-1} AmpC β -lactamase genes were identified on the isolates' chromosome. The isolates were phylogenetically distant and closely related to other international strains, suggesting that they were not obtained from a single epidemiological source. Further molecular surveillance is necessary to establish the prevalence of these MDR strains in the tertiary hospital. Moreover, antibiotic stewardship and antibiotic sensitivity testing of all clinical isolates should be undertaken after empirical treatment to inform tailored therapy as well as reduce escalation of resistance and associated morbidities and mortalities.

Keywords: *Morganella morganii*; DHA-5; DHA-1; AmpC; integron; Enterobacteriaceae; UTI; South Africa

Introduction

Urinary tract infections (UTIs) are a common clinical occurrence, particularly among pregnant women worldwide (Bitew, Molalign & Chanie, 2017). Notably, women, immunocompromised patients such as diabetics and cancer patients, persons with urinary catheters, intensive-care unit (ICU) patients, and those in long-term care facilities are most at risk of developing UTIs (Singla *et al.*, 2010; Bitew *et al.*, 2017). Recommended first-line treatment for UTIs include nitrofurantoin, fosfomycin, penicillins, cephalosporins, sulphonamides and in certain cases, fluoroquinolones and aminoglycosides (Osei Sekyere, 2018). Common uropathogens mainly implicated in UTIs include *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Citrobacter freundii*, *Staphylococcus aureus*, *Streptococcus spp.* etc. However, *Morganella morganii*, an opportunistic Enterobacteriaceae species and an intestinal denizen in humans and animals, is not a common cause of UTIs, making their isolation from urine a rare occurrence. Moreover, the intrinsic resistance of *M. morganii* to several important antibiotics such as colistin and the ubiquitous presence of inducible AmpC enzymes in this species that confer resistance to cepheids, limit therapeutic arsenals available for treating *M. morganii* infections (Singla *et al.*, 2010; Bitew *et al.*, 2017).

Besides intrinsic and vertical resistance, *M. morganii* can also exchange resistance genes horizontally from same or different species (Rojas *et al.*, 2006; Hsieh *et al.*, 2015; Liu *et al.*, 2016). Lateral gene transfer enables acquired antimicrobial resistance in bacteria, which has led to the rapid spread of drug resistance in clinical isolates (Pedersen *et al.*, 2018). In recent years, multidrug-resistant (MDR) *Morganella morganii* cases having mobile genetic elements (MGEs) such as plasmids and integrons, have been reported in the literature (Liu *et al.*, 2016). The mobilization of multiple resistance genes by MGEs into pathogens has resulted in clinical failure even with recommended treatment regimens (Liu *et al.*, 2016; Pedersen *et al.*, 2018). Broad-spectrum plasmid-mediated AmpC β -lactamases such as CMY, MIR, MOX, ACT, ACC, FOX, LAT, CFE and DHA enzymes, which confer broad-spectrum resistance to β -lactams, have been described in several species in the literature (Liu *et al.*, 2016; Logan *et al.*, 2016). The plasmid-mediated DHA enzyme was first described in Saudi Arabia. It originated from the chromosome of *M. morganii* and is most commonly described in *Klebsiella pneumoniae* isolates (Castanheira *et*

al., 2014; Liu *et al.*, 2016). While the DHA-1 enzyme is the most frequently reported, usually in *K. pneumoniae*, DHA-2 and DHA-3 enzymes have been also reported, particularly in *K. pneumoniae* in countries such as France and Taiwan (Liu *et al.*, 2016). The DHA-2 enzyme has been shown to be a point mutation of DHA-1. A novel AmpC β -lactamase, DHA-23, was recently described in one *Escherichia coli* and two *K. pneumoniae* isolates from Taiwan (Hsieh *et al.*, 2015).

Herein, we report for the first time in Africa, the draft genome sequences of two MDR clinical *M. morgani* isolates from a tertiary hospital in Pretoria, South Africa, which were phylogenetically distant from each other but closely related to international strains. Unfortunately, these strains, which are intrinsically resistant to oxacillin, ampicillin, amoxicillin, most of the first- and second-generation cephalosporins, macrolides, lincosamides, glycopeptides, fosfomycin, fusidic acid, and colistin (Liu *et al.*, 2016), were also not sensitive to recommended first-line drugs such as nitrofurantoin, and β -lactams except carbapenems. They were also resistant to important antibiotics including fluoroquinolones, aminoglycosides except amikacin, sulphonamides, chloramphenicol and tetracyclines (Osei Sekyere, 2018). The presence of such MDR uropathogens is very concerning and requires further investigations to institute better infection control practices and revise empirical antibiotic treatment for such cases.

Results and discussion

With the exception of intrinsic resistance to 1st and 2nd generation cephalosporins, colistin, lincosamides and some penicillins (Liu *et al.*, 2016), the isolates were resistant to almost all types and classes of antibiotics including piperacillin, 3rd and 4th generation cephalosporins, tetracyclines, aminoglycosides, fluoroquinolones, monobactams, sulphonamides, and chloramphenicol (Table 1). They were susceptible only to carbapenems and amikacin. E042 was also susceptible to fosfomycin and nitrofurantoin, antibiotics recommended for uncomplicated urinary tract infections (UTIs). M006 was intermediate resistant to tigecycline. With the exception of amoxicillin-clavulanate, both isolates were non-resistant i.e., either susceptible or intermediate-resistant to antibiotic- β -lactamase combinations: piperacillin-tazobactam (M006 was intermediate resistant), ceftazidime-clavulanate and cefotaxime-clavulanate (Table 1). The MDR phenotype of these isolates make it very concerning, particularly in a UTI. The absence of ceftaxime resistance in both strains is quite intriguing as *Morganella spp.*, *Citrobacter spp.*, *Serratia spp.* etc are known to have inducible AmpC or derepressed resistance to ceftaxime (cephamycins) (Liu *et al.*, 2016).

The isolates were confirmed by NCBI's ANI (average nucleotide identity) as *M. morganii*. The genomic characteristics of the isolates were similar, with both isolates having similar GC content of ~51%. There were no CRISPR arrays in either strain, but E042 had more contigs and RNAs than M006 (Table 2). The absence of a plasmid replicase gene in E042 strongly suggests the absence of a plasmid in that isolate, which could also explain the presence of only two resistance genes, i.e., *bla*_{DHA-1} and *catA2*, in E042 (Table 3). Contrarily, M006, which had a Col3M plasmid had six resistance genes conferring resistance to aminoglycosides (*aadA7*), fluoroquinolones (*qnrD*), β-lactams (*bla*_{DHA-4}), sulfonamide (*sulI*), tetracycline (*tet B*) and phenicol (*catA2*). *bla*_{DHA-4} was found on the chromosome of M006 and *bla*_{DHA-1} was found on the chromosomes of E045; this is not surprising as *bla*_{DHA} are known to originate from *M. morganii* (Liu *et al.*, 2016). In addition, it is interesting to note that although E042 had no plasmid replicon and had only one resistance gene, it was multidrug resistant and conferred resistance to several antibiotics (Table 1). Although *M. morganii* is known to be intrinsically less susceptible to tigecycline, this was not observed in these strains just as fosfomycin resistance was not observed in E042, albeit *M. morganii* are intrinsically resistant to fosfomycin (Table 1) (Liu *et al.*, 2016; Osei Sekyere *et al.*, 2016).

A novel In1484 class 1 integron, harbouring *aadA7*₍₁₅₄₈₄₋₁₆₃₄₅₎-*attC*₍₁₅₄₇₈₋₁₅₅₃₇₎-*gcuD* Δ ₍₁₅₁₆₄₋₁₅₄₈₃₎-*attC*₍₁₅₁₅₈₋₁₅₂₁₇₎-3'CS cassette array, was identified in M006 on contig 14 (accession number NXXH000000000) (Tables 4 and 5). This integron and associated gene cassettes (*sulI* and *aadA7*) were found on a *TnsA3* composite transposon, which had a 9.99% sequence homology and 24% query cover with *Edwardsiella tarda* plasmid p9.2(MG228256.1). On the other hand, *tet(B)* was found on an IS4 composite transposon (Table 4). The Col3M plasmid replicase gene was found on the same contig as the *qnrD1* resistance gene, suggesting that the *qnrD1* is found on this plasmid (Table 4). The genetic environment of *catA2* highly suggests that it is chromosomally borne while that of *sulI* and *aadA7* suggests that they are plasmid borne. Interestingly, the genetic context of *tet(B)* is such that it could be on either the chromosome or on a plasmid. (Table 4) The role of class 1 integrons, transposons and Col3M plasmids in the dissemination of antibiotic resistance in the clinical environment among pathogens cannot be gainsaid; however, the transferability/mobility of the genes on these mobile genetic elements was not confirmed using conjugation assays. This is a major limitation of this study.

Contrary to this study, Mahrouki et al. (2013) identified *bla*_{DHA-1} in class-1 integrons in *M. morganii* in clinical specimens collected between 2004 and 2009 in Tunis, Tunisia, which also harboured *qnrA6* and *aac(6')-Ib-cr* quinolone resistance determinants (Mahrouki *et al.*, 2013). Ndiaye et al. (2010) also reported on a *M. morganii* isolate from the cerebrospinal fluid of a 12-year old boy with acute meningoencephalitis in Dakar, Senegal (Ndiaye *et al.*, 2010), confirming the presence of this opportunistic pathogen in African healthcare settings. Nevertheless, a whole-genome analysis of *M. morganii* from Africa is non-existent.

An all-by-all BLAST phylogenomic comparison was performed using all 59 (there were 72 genomes on PATRIC, but only 59 were good, eight were poor) *M. morganii* genomes at Genbank/PATRIC with our isolates. It can be seen on the tree (Figure 1) that both strains are very distant from each other and must be of different clones, suggesting that different *M. morganii* clones are circulating in the same hospital. The clonal difference between the two isolates is further confirmed by the genomic and antibiogram differences between them. M006 was of the same clone/clade as FDAARGOS_63 from the United States of America (*bla*_{DHA-4}, *catA2*, *tet(B)*), GN28 from China (*ere(B)*, *mph(A)*, *aac(6')-Ib-cr*, *tet(B)*, *catA1*, *catA2*, *catB3*, *aac(3)-IIId*, *aac(6')-Ib-cr*, *aadA2b*, *ARR-3*, *bla*_{CARB-2}, *bla*_{DHA-4}, *bla*_{OXA-1}), F675 (*aadA2*, *aph(3')-VI*, *bla*_{CARB-2}, *bla*_{DHA-4}, *bla*_{NDM-1}, *ble*, *catA1*, *dfrA19*, *ere(A)*, *ere(B)*, *mph(A)*, *tet(B)*), MH16-367M from Vietnam (*aadA2*, *ant(2'')-Ia*, *aph(3')-Ia*, *bla*_{CARB-2}, *bla*_{DHA-4}, *bla*_{NDM-1}, *bla*_{TEM-1}, *ble*, *catA1*, *catA2*, *catB3*, *dfrA19*, *qacEdelta1*, *qepA1*, *qnrD1*, *rmtB*, *sull1*, *tet(A)*, *tet(B)*), and CQ-M7 from China (*bla*_{DHA-4}, *catA2*) while E042 was closely related to a clinical strain, FDAARGOS_438, from Canada (*aac(3)-IIId*, *aadA5*, *aph(3'')-Ib*, *aph(6)-Id*, *bla*_{DHA-1}, *bla*_{NDM-1}, *bla*_{TEM-1}, *ble*, *catA1*, *catA2*, *dfrA17*, *mph(A)*, *qacEdelta1*, *rmtC*, *sull1*, *sul2*, *tet(B)*) (Figure 1; Supplementary metadata).

The isolates that were closely related to M006 and E042 all had *bla*_{DHA-4/1} gene, with F675, MH16-367M, and FDAARGOS_438 strains having NDM-1 carbapenemases. The repertoire of resistance genes in these closely related strains show the potential of *M. morganii* to serve as hosts and reservoirs of resistance genes in animal, humans and environmental strains (<https://www.ncbi.nlm.nih.gov/pathogens/isolates#/search/Morganella%20morganii>).

In conclusion, we show that MDR *M. morganii* strains are implicated in UTIs in a tertiary hospital in South Africa, confirming the clinical importance of this species as a human pathogen worldwide (Liu *et al.*, 2016). The chromosomally encoded *bla*_{DHA-1} and *bla*_{DHA-5} were also

detected in the isolates, with a novel integron enclosed by a *TnsA3* composite transposon being identified in M006. This work, using whole-genome analysis on clinical *M. morgannii* strains in Africa, is the first to report on a draft *M. morgannii* genome, and shows the epidemiological association between the strains and other international isolates. Moreover, the correlation between the resistome and antibiotic sensitivity results (phenome) as well as the genetic context of the resistance genes are also characterised through in-depth sequence analyses, further supporting the importance of whole-genome sequencing-based clinical diagnosis and molecular surveillance of antibiotic resistance. The presence of such drug-resistant uropathogens is very concerning and requires further investigations to institute better infection control practices and revise empirical antibiotic treatment for such cases. Further molecular surveillance is necessary to establish the prevalence of these MDR strains in the tertiary hospital and inform a revision of current UTI empirical antibiotic therapy and stewardship.

Materials and Methods

Isolate collection, sensitivity and host demographics

Two clinical *M. morgannii* strains, M006 and E042, were isolated from the urine of two patients admitted to a referral hospital in South Africa. One patient was a female of 53 years presenting with a confusion after a 2nd degree fall and the other was a male of 90 years. The isolates were cultured on blood agar at 37°C overnight prior to determining their identity and antimicrobial susceptibility using the MicroScan® WalkAway automated Instrument (Beckman Coulter, California, USA) following the manufacturer's instructions. The Clinical Laboratory Standard Institute (CLSI)'s recommended clinical breakpoints were used to interpret the results as resistant or susceptible.

Whole-genome sequencing and bioinformatics

Genomic DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research, Epigenetics, USA). Genomic DNA was sheared to 200 or 400bp libraries and sequenced on the Ion Proton whole genome-sequencing platform (ThermoFisher, USA). Raw sequence reads of the isolate was adaptor- and quality-trimmed using Trimmomatic (Bolger, Lohse & Usadel, 2014). The raw reads were de-novo assembled using the SPAdes version 3.10.1 assembler (Bankevich *et al.*, 2012). The resulting FASTA files were deposited at Genbank under the bioproject PRJNA355910 with accession number: **NXKH00000000**. NCBI Prokaryotic Genome Annotation

Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genome/annotation_ptok/) version 4.1 and SEED subsystems in the RAST (rapid annotation using subsystem technology) server were used to annotate the genome (Brettin *et al.*, 2015). The resistome of the isolates were identified using ResFinder 3.0 (<https://cge.cbs.dtu.dk/services/ResFinder>). Integrall (integrall.bio.ua.pt) was used to identify and classify the novel class 1 integron in M006, while RAST 2.0 and PGAP were used to characterize the resistance genes' genetic context. The genomes of all *M. morganii* strains deposited at Genbank/PATRIC i.e., 72, were downloaded from Genbank/PATRIC and used to draw a core genome phylogeny tree using Parsnp, with the -c 1000 flag, and annotated with Figtree (<http://tree.bio.ed.ac.uk/software/figtree/>). However, eight isolates having poor genomes were excluded from the phylogenetic analyses, resulting in 59 isolates. *E. coli* MGH57 (PRJNA234256) was used as reference strain (Supplementary metadata S1). The resistance genes of the respective genomes were also obtained from the NCBI's Pathogen Detection database (<https://www.ncbi.nlm.nih.gov/pathogens/isolates#/search/>).

Conflict of Interest

NM, CF, JOS, DA, NM, LM, SYE None to declare

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References

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A. V, Sirotkin, A. V, Vyahhi, N., Tesler, G., Alekseyev, M.A. and Pevzner, P.A. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol.* **19**, 455–77.

- Bitew, A., Molalign, T. and Chanie, M. (2017) Species distribution and antibiotic susceptibility profile of bacterial uropathogens among patients complaining urinary tract infections. *BMC Infect Dis.* **17**, 654.
- Bolger, A.M., Lohse, M. and Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics.* **30**, 2114–20.
- Brettin, T., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Olsen, G.J., Olson, R., Overbeek, R., Parrello, B., Pusch, G.D., Shukla, M., Thomason 3rd, J.A., Stevens, R., Vonstein, V., Wattam, A.R. and Xia, F. (2015) RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep.* **5**, 8365.
- Castanheira, M., Farrell, S.E., Krause, K.M., Jones, R.N. and Sader, H.S. (2014) Contemporary diversity of beta-lactamases among Enterobacteriaceae in the nine U.S. census regions and ceftazidime-avibactam activity tested against isolates producing the most prevalent beta-lactamase groups. *Antimicrob Agents Chemother.* **58**, 833–838.
- Hsieh, W.-S., Wang, N.-Y., Feng, J.-A., Weng, L.-C. and Wu, H.-H. (2015) Identification of DHA-23, a novel plasmid-mediated and inducible AmpC beta-lactamase from Enterobacteriaceae in Northern Taiwan. *Front Microbiol.* **6**, 436.
- Liu, H., Zhu, J., Hu, Q. and Rao, X. (2016) *Morganella morganii*, a non-negligent opportunistic pathogen. *Int J Infect Dis.* **50**, 10–17.
- Logan, L.K., Hujer, A.M., Marshall, S.H., Domitrovic, T.N., Rudin, S.D., Zheng, X., Qureshi, N.K., Hayden, M.K., Scaggs, F.A., Karadkhele, A. and Bonomo, R.A. (2016) Analysis of beta-Lactamase Resistance Determinants in Enterobacteriaceae from Chicago Children: a Multicenter Survey. *Antimicrob Agents Chemother.* **60**, 3462–3469.
- Mahrouki, S., Perilli, M., Bourouis, A., Chihi, H., Ferjani, M., Ben Moussa, M., Amicosante, G. and Belhadj, O. (2013) Prevalence of quinolone resistance determinant *qnr* A6 among broad- and extended-spectrum beta-lactam-resistant *Proteus mirabilis* and *Morganella morganii* clinical isolates with *sul* 1-type class 1 integron association in a Tunisian Hospital. *Scand J Infect Dis.* **45**, 600–605.
- Ndiaye, M., Sène, M.S., Sow, A.D., Seck, L.B., Coulibaly, T., Diagne, N.S., Touré, K., Diop,

A.G. and Ndiaye, M.M. (2010) Méningoencéphalite à *Morganella morganii* : à propos d'un cas. *Bull la Société Pathol Exot.* **103**, 230–232.

Osei Sekyere, J. (2018) Genomic insights into nitrofurantoin resistance mechanisms and epidemiology in clinical Enterobacteriaceae. *Futur Sci OA.* **4**, FSO293.

Osei Sekyere, J., Govinden, U., Bester, L.A. and Essack, S.Y. (2016) Colistin and Tigecycline Resistance In Carbapenemase-Producing Gram Negative Bacteria: Emerging Resistance Mechanisms And Detection Methods. *J Appl Microbiol.* **121**, 601–617.

Pedersen, T., Osei Sekyere, J., Govinden, U., Moodley, K., Sivertsen, A., Samuelsen, Ø., Essack, S.Y. and Sundsfjord, A. (2018) Spread of Plasmid-Encoded NDM-1 and GES-5 Carbapenemases among Extensively Drug-Resistant and Pandrug-Resistant Clinical Enterobacteriaceae in Durban, South Africa. *Antimicrob Agents Chemother.* AAC.02178-17.

Rojas, L., Vinuesa, T., Tubau, F., Truchero, C., Benz, R. and Viñas, M. (2006) Integron presence in a multiresistant *Morganella morganii* isolate. *Int J Antimicrob Agents.* **27**, 505–512.

Singla, N., Kaistha, N., Gulati, N. and Chander, J. (2010) *Morganella morganii* could be an important Intensive Care Unit pathogen. *Indian J Crit Care Med.* **14**, 154–5.

Table 1: Antibiotic susceptibility characteristics of the *Morganella morganii* isolates (n=2)

Antibiotics	MIC (mg/L)		Associated resistance genes
	E042	M006	
Penicillins			
ampicillin (AM)	>16 (R)	>16 (R)	<i>bla_{DHA-1}</i> (E042)
ampicillin/sulbactam (A/S)	>16/8 (R)	>16/8 (R)	<i>bla_{DHA-4}</i> (M006)
amoxicillin / clavulanate (AUG)	16/8 (I)	16/8 (R)	intrinsic resistance
piperacillin (PI)	>64 (R)	>64 (R)	
piperacillin / tazobactam (P/T)	<8 (S)	64 (I)	
Monobactams			
aztreonam (AZT)	16 (R)	>16 (R)	<i>bla_{DHA-1}</i> (E042), <i>bla_{DHA-5}</i> (M006),
Cephalosporins			
cefepime (CPE) 4	>16 (R)	>16 (R)	<i>bla_{DHA-1}</i> (E042), <i>bla_{DHA-4}</i> (M006), intrinsic resistance
cefotaxime (CFT) 3	>32 (ESBL)	>32(ESBL)	
cefotaxime/clavulanate (CFT/CA) 3	<0.5	4	

cefoxitin (CFX) 2	<8 (S)	<8 (S)	
ceftazidime (CAZ) 3	8 (ESBL)	16 (ESBL)	
ceftazidime / clavulanate (CAZ/CA) 3	2	>2	
cefuroxime axetil/sodium (CRM) 2	>16	>16	
cephalothin (CF) 1	>16	>16	
Carbapenems			
doripenem (DOR)	<1 (S)	<1 (S)	No carbapenemase
ertapenem (ETP)	<0.5 (S)	<0.5 (S)	
imipenem (IMP)	<1 (S)	2 (I)	
meropenem (MER)	<1 (S)	<1 (S)	
Aminoglycosides			
amikacin (AK)	<8 (S)	<8 (S)	<i>aadA7</i> (M006)
gentamicin (GM)	>8 (R)	>8 (R)	
tobramycin (TO)	>8 (R)	8 (I)	
Quinolones			
norfloxacin (NXN)	>1 (R)	>1 (R)	<i>qnrD1</i> (M006)
levofloxacin (LVX)	>4 (R)	>4 (R)	
ciprofloxacin (CP)	>2 (R)	>2 (R)	
nalidixic acid (NA)	>16	>16	
Tetracyclines			
tetracycline (TE)	>8 (R)	>8 (R)	<i>tet(B)</i> (M006)
minocycline (MIN)	8 (I)	>8 (R)	intrinsic resistance to tigecycline
tigecycline (TGC) (glycylcyclines)	<1 (S)	2 (I)	
Lincosamides			
Clindamycin (CL)	4	>4	Unknown resistance mechanism
Sulfonamides			
trimethoprim / sulfamethoxazole (T/S)	>4/76 (R)	>4/76 (R)	<i>Sul1</i> (M006)
Miscellaneous Agents			
chloramphenicol (C)	>16 (R)	<8 (S)	<i>catA2</i> (M006)
colistin (CL)	4 (R)	>4 (R)	Intrinsic resistance
fosfomycin (FOS)	<32 (S)	>64 (R)	Intrinsic resistance
nitrofurantoin (FD)	64 (S)	>64 (R)	Unknown resistance mechanism

* Taxonomy determined by NCBI by comparing to proxytype strains in GenBank using the average nucleotide identity (ANI) test (75). †EUCAST resistant breakpoints (v 7.1) are used. Abbreviations are used for all antibacterial agents as follows: GEN=gentamicin (R>4mg/L); TOB= tobramycin (R>4mg/L);

AMK=amikacin (R>16mg/L); TZP=piperacillin-tazobactam (R>16mg/L); ETP=ertapenem (R>1mg/L); IMI=imipenem (R>8mg/L); MEM=meropenem (R>8mg/L); DOR=doripenem (R>2mg/L); CXM=cefuroxime (R>8mg/L); CTX=cefotaxime (R>2mg/L); CAZ=ceftazidime (R>4mg/L); FEP=cefepime (R>4mg/L); CZA=ceftazidime avibactam (R>8mg/L); FOX=cefoxitin (R>8mg/L); CIP=ciprofloxacin (R>0.5 mg/L); SXT=trimethoprim-sulfamethoxazole (R>4mg/L); TGC=tigecycline (R>2mg/L); ATM=aztreonam (R>4mg/L); AMC=amoxicillin-clavulanic acid (R>8mg/L); FOF=fosfomycin (R>32mg/L); CST=colistin (R>2mg/L)

Table 2: Genomic features of *Morganella morganii* M006 and E042

Attribute	Value	
	M006	E042
Contigs	120	292
Genome size (bp)	4.1	3.9
DNA G + C (%)	51.1	51.0
Genome coverage (X)	94	97
Number of tRNAs genes	61	70
Number of RNAs genes	77	96
N50	255229	382227
L50	6	4
Genes assigned to COGs	3848	3658
Confirmed CRISPRs	0	0

Table 3: Genetic and phenotypic characteristics of the DHA-encoding *Morganella morganii* isolates from Tshwane Academic Hospital (n=2)

Strain ID	Sex	Age	Host disease	Isolation source	Cluster	ESBLs	β - lactamase	Other resistance genes/enzymes	Plasmid replicon type	GenBank accession
E042	Female	53	Confusion 2-degree fall	Urine		+	DHA-1	catA2	-	NXIQ00000000
M006	Male	90	-	Urine		+	DHA-4	aadA7, QnrD1, catA2, sul1, tet(B)	Col3M	NXKH00000000

Table 4. Genetic environment of resistance genes found in M006 strain

Contig/Node number	Resistance gene (location on contig/node)	Genetic environment/ context of resistance gene (ARG)	Closest Chromosome /plasmid (accession number) aligned to contig
3 (NXKH01000003.1)	bla_{DHA4} (22878-24017)	No mobile genetic element (MGE) flanking ARG	Aligns closely with <i>M. morganii</i> FDAARGOS_63's chromosome (CP026046.1): 100% query cover and 99.99% homology
14 (NXKH01000014.1)	aadA7 (15539-16336), Sul1 (13875-14714)	Hg(II) reductase: merD::resolvase:transposase:transposase:N-acetyl transferase:sul1:qacE:aadA7:Int1:DNA resolvase:Tn3 family transposase TnAs3	99.99% sequence ID and 24% query cover with <i>Edwardsiella tarda</i> plasmid p9.2 (MG228256.1)
17 (NXKH01000017.1)	catA2 (17123-17596)	XRE family transcriptional regulator: catA2:α/β hydrolase:tetR/acrR transcriptional regulator:DNA polymerase II	Aligns closely with <i>M. morganii</i> FDAARGOS_63's chromosome (CP026046.1): 96% query cover and 99.99% homology
37 (NXKH01000037.1)	Tet(B) (1343-2548)	Transposase:tetC:tet(B):tetR:::IS4 family transposase	100% query cover and 99.98% homology with <i>S. enterica</i> FDAARGOS.313 chromosome (CP022069.2); 100% query cover and 99.98% homology with <i>Escherichia coli</i> pFAM21845_1 plasmid (CP017221.1)
50 (NXKH01000050.1)	qnrD1 (1698-2342), Col3M replicon (2436-2592)	Col3M plasmid replicon found on contig	100% cover and 99.92% homology with plasmid p3M-2B (JX514066.1) from <i>Proteus spp.</i> and plasmid KVHS-004 (KJ685892.1) of <i>Salmonella</i> Hadar

Table 5. Cassette array for the novel integron, *lnI484*, carrying the *aadA7* and *gcuD* gene cassettes in M006 (contig 14).

Coding sequence of gene cassettes (contig 14)	<i>attC</i>	3'CS

<i>aadA7</i> ₍₁₅₅₃₉₋₁₆₃₃₆₎	<i>attC</i> _{aadA6(15484-15537;16340-16345)}	13247-15163
<i>gcuD</i> Δ ₍₁₅₁₇₂₋₁₅₄₅₀₎ truncated	<i>attC</i> _{gcuDD(15164-15217; 15478-15483)}	<i>qacE</i> Δ 1: 14708-15055
		<i>sul1</i> : 16875-14714
		<i>orf5</i> : 13247-13747
Array of gene cassettes: <i>aadA7</i> ₍₁₅₄₈₄₋₁₆₃₄₅₎ - <i>attC</i> ₍₁₅₄₇₈₋₁₅₅₃₇₎ - <i>gcuD</i> Δ ₍₁₅₁₆₄₋₁₅₄₈₃₎ - <i>attC</i> ₍₁₅₁₅₈₋₁₅₂₁₇₎ -3'CS		

Figure 1. A neighbour joining phylogenetic tree of *M. morgani* isolates deposited at GenBank as at April 2019. The tree shows the isolates of different countries being of the same clone or very close sequence similarity. Isolates belonging the same clone are highlighted with the same colour while those of the same clade are also highlighted with the same colour from their branches. The tree shows that isolates of the same clone and clades are circulating in different countries and hosts. The two clinical isolates, M006 and E042, were distantly related albeit M006 was of the same clone as FDAARGOS_63 isolated from a human wound in 2013 in the USA.

Supplementary metadata S1. Metadata of isolates included in phylogenomic analysis

