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Genome note

Genome sequences of two *Salmonella enterica* strains (MEZSAL74 and MEZSAL81) harbouring multiple antimicrobial resistance genes isolated from livestock in South Africa

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ABSTRACT

Objectives: Antimicrobial-resistant livestock-associated *Salmonella enterica* infections pose a significant public-health threat worldwide. Here we report for the first time the draft genome sequences of two multidrug-resistant livestock-associated *S. enterica* strains isolated from a chicken and a cow in South Africa.
Methods: Genomic DNA of *S. enterica* strains MEZSAL74 and MEZSAL81 was sequenced using an Illumina MiSeq platform. The generated reads were trimmed and de novo assembled. The assembled contigs were analysed for antimicrobial resistance genes, chromosomal mutations and extrachromosomal plasmids. Multilocus sequence typing (MLST) was also performed. In order to compare isolates MEZSAL74 and MEZSAL81 with other previously sequenced *S. enterica* isolates, raw read sequences were downloaded and all sequence files were treated identically to generate a bootstrapped maximum likelihood phylogenetic tree.
Results: Extrachromosomal plasmids and genetic determinants of antimicrobial resistance were detected in both sequenced bacterial isolates to aminoglycosides and fluoroquinolones. By MLST, strain MEZSAL74 belonged to an unknown sequence type (ST) and strain MEZSAL81 belonged to ST33.
Conclusion: The genome sequences of strains MEZSAL74 and MEZSAL81 reported here will serve as a reference for molecular epidemiological studies of antimicrobial-resistant livestock-associated *S. enterica* in Africa.

Salmonella enterica is a facultative intracellular foodborne pathogen of global importance causing morbidity and mortality in humans and that can be transmitted through different distinct routes [1]. Zoonotic salmonellosis is a great public-health concern. Detection of *S. enterica* in livestock and food-chain animals has frequently been reported worldwide and recently in South Africa [1]. An increasing number of studies have reported the acquisition of multiple antimicrobial resistances in *S. enterica*, including resistance to colistin, posing a serious threat to human and animal health. The genome of *S. enterica* will provide important comparative genomic information to help understand its pathogenicity and to monitor its antimicrobial resistance characteristics. There is scare information on the genome sequence of *S. enterica* isolates from Africa.

S. enterica strains MEZSAL74 and MEZSAL81 were isolated from a cow and a chicken, respectively, in KwaZulu-Natal Province, South Africa, in May 2018. Agar slants of bacterial cultures were further analysed as part of the GenomeTrakr project. Isolates were identified using conventional microbiological methods for *Salmonella* and were confirmed by colony PCR for the *invA* gene as previously described [1]. Samples were further processed for DNA isolation and whole-genome sequencing. DNA isolation was performed using a MasterPure™ DNA Isolation Kit (Lucigen Corp., Middleton, WI, USA) according to the manufacturer's protocol. Sequencing libraries were prepared using a Nextera XT Library Prep Kit (Illumina Inc., San Diego, CA, USA). Sequencing was performed on an Illumina MiSeq platform using a v2 Reagent Kit (Illumina Inc.), which yielded 250-bp paired-end reads.

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A total of 862.6 Mb and 869.5 Mb raw data reads were generated for MEZSAL74 and MEZSAL81, respectively. Sequences were assembled using Unicycler v.0.4.7 [2] into 38 contigs for MEZSAL74 and into 118 contigs MEZSAL81. Assembly quality was assessed using QUAST [2] for both isolates, yielding the following: for MEZSAL74, a total of 4 767 316 bp with a GC content of 52.12%, an N_{50} of 349 646 bp and an L_{50} of 4; and for MEZSAL81, a total of 4 846 596 bp with a GC content of 52.19%, an N_{50} of 150 971 bp and an L_{50} of 10. Prokka v.1.13 [3] was used for annotation to provide values on coding sequences (CDS), tRNAs, tmRNAs and RNAs: for the annotated MEZSAL74 genome there were 4465 CDS and 81 tRNA, 1 tmRNA and 3 rRNA genes; and for the annotated MEZSAL81 genome there were 4554 CDS and 82 tRNA, 1 tmRNA and 3 rRNA genes. To assess the isolates for genomic epidemiological factors, the contig FASTA files were uploaded and analysed by the bacterial analysis pipeline-batch uploader [4]. From the results, only MEZSAL81 had a known sequence type (ST33), therefore we included for both strains the closest known sequenced bacterial strain and its accession number; both bacterial isolates also have a detected extrachromosomal plasmid (Table 1). Antimicrobial susceptibility testing by the disk diffusion method revealed that MEZSAL74 was susceptible to amoxicillin/clavulanic acid (AMC), azithromycin, ceftriaxone and chloramphenicol, intermediate-resistant to ciprofloxacin and resistant to sulfamethoxazole, tetracycline and ampicillin. MEZSAL81 was susceptible to ampicillin, AMC, azithromycin, ciprofloxacin and chloramphenicol, intermediate-resistant to ceftriaxone and resistant to sulfamethoxazole and tetracycline. For more in-depth characterisation, ResFinder 3.2 [5] was utilised for detection of acquired antimicrobial resistance genes and chromosomal mutations. Both strains contained acquired antimicrobial resistance genes to aminoglycosides as well as a previously reported chromosomal mutation in *parC* (T57S) that confers resistance to fluoroquinolones [6]; other chromosomal mutations were also detected that could also further contribute to aminoglycoside and fluoroquinolone resistance but have not been previously reported (Table 1). The presence of the mutations did not induce phenotypic resistance to fluoroquinolones; the only isolate to show decreased susceptibility to ciprofloxacin was MEZSAL74, which had intermediate resistance. No colistin or tetracycline resistance-conferring mutations or colistin or tetracycline plasmid-mediated resistance mechanisms were identified in either strain despite both stains exhibiting phenotypic tetracycline resistance. This exemplifies that genetic determinants cannot always be demonstrated for all resistance phenotypes.

In order to generate a maximum likelihood phylogenetic tree to establish how the two isolates compared with other previously sequenced *S. enterica* isolates, raw read files with origins from Africa from BioProject PRJNA293224 (Appendix A; Supplementary Table S1) were downloaded. All raw read isolate files were processed by the same methods of trimming and de novo assembly as mentioned above. All bacterial isolate files were then annotated using Prokka v.1.13. Core genome alignments were done using Roary pangenome pipeline with default setting. RAxML v.8.2.12 was used for inference of bootstrapped maximum likelihood phylogenetic tree, and visualisation was done using the webtool Interactive Tree of Life (iTOL) [7] (Fig. 1).

Strain MEZSAL74 was phylogenetically related to the *S. enterica* subsp. *enterica* serovar Braenderup strain SA20026289 complete genome and was of unknown sequence type, whilst strain MEZSAL81 was phylogenetically related to the *S. enterica* strain FDAARGOS_313 complete genome and was ST33. The genome sequence predicted the serotype of strain MEZSAL81 as serotype Hadar and strain MEZSAL74 as serotype Alachua.

Detection of antimicrobial resistance genes in livestock-associated pathogens of zoonotic potential is a serious increasing public-health concern. To the best of our knowledge, the two draft genomes of strains MEZSAL74 and MEZSAl81 reported in this study are the first non-human, livestock-associated strains from South Africa. Similar studies worldwide have reported the detection of antimicrobial resistance in livestock-associated *S. enterica* from diverse sources of avian (chicken and turkey), swine, bovine and ovine hosts as well as their products and the environment [8]. This report highlights the significance of continued genomic surveillance of nontyphoidal *Salmonella*, which continue to pose a serious global threat to human health and food security, primarily a

Table 1
Overview of *Salmonella enterica* strains MEZSAL74 and MEZSAL81, detected antimicrobial resistance genes (ARGs) and associated chromosomal mutations with predicted phenotypes.

Strain	Closest known sequenced bacterial strain accession number	Closest known sequenced bacterial strain	Detected plasmid	Acquired ARGS	Identity (%)	Acquired ARG accession no.	Chromosomal mutation	Nucleotide change	Predicted resistance phenotype	Chromosomal mutation PMID reference
MEZSAL74	**NZ_CP022490.1**	*S. enterica* subsp. *enterica* serovar Braenderup strain SA20026289 chromosome, complete genome	IncY	*aac(6′)-Iaa*	97.26	NC_003197			Aminoglycosides	
							16S *rrsD* 1133A > G	A→G	Aminoglycosides	
							16S *rrsD* 1139T > C	T→C	Aminoglycosides	
							parC T57S	ACC→AGC	Fluoroquinolones	15388468
							parC T255S	ACC→TCC	Fluoroquinolones	
MEZSAL81	**NZ_CP022069.2**	*S. enterica* strain FDAARGOS_313 chromosome, complete genome	ColRNAI	*aph(3)-Ib*	100	AF024602			Aminoglycosides	
				aph(6)-Id	100	M28829			Aminoglycosides	
							16S *rrsD* 92C > T	C→T	Aminoglycosides	
							16S *rrsD* 249T > A	T→A	Aminoglycosides	
							16S_*rrsD* 1133A > G	A→G	Aminoglycosides	
							16S_*rrsD* 1139T > C	T→C	Aminoglycosides	
							parC T57S	ACC→AGC	Fluoroquinolones	15388468
							parC T255S	ACC→TCC	Fluoroquinolones	
							parC N395S	AAC→AGC	Fluoroquinolones	
							parC S469A	TCC→GCC	Fluoroquinolones	

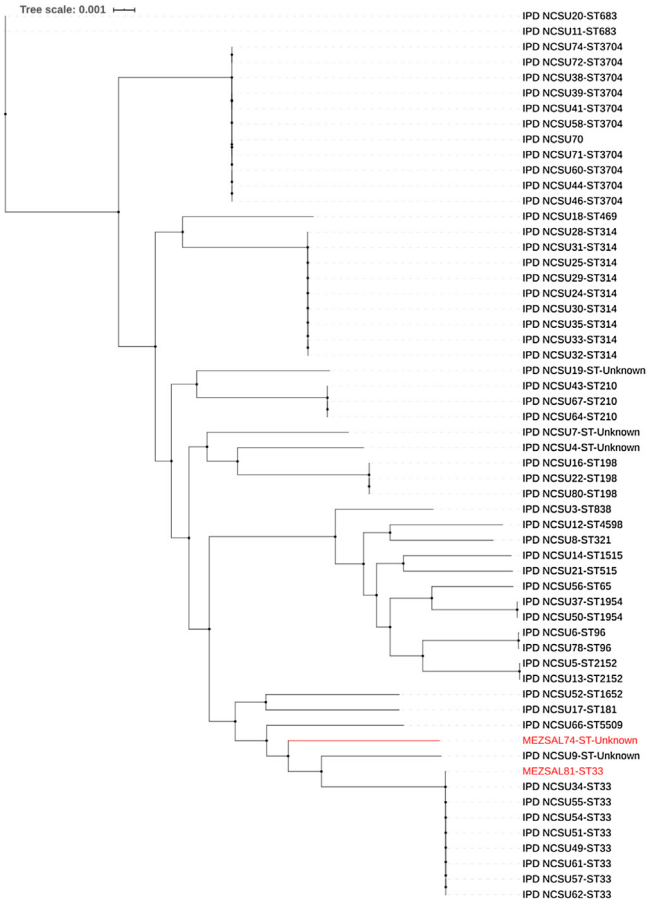

Fig. 1. Phylogenetic positioning of strains MEZSAL74 and MEZSAL81 (ST33) (highlighted in red) within *Salmonella enterica*. Representative genomes of *S. enterica* were used to compare the two novel isolates. Maximum likelihood phylogeny was inferred from 3346 core genes and 16 085 total genes. Black circles represent bootstrap branch support values >90% based on 100 replicates. ST, sequence type.

leading cause of foodborne and invasive illnesses in humans. This study highlights the importance of food-producing animals as principal reservoirs of many multidrug-resistant pathogenic *S. enterica* strains.

This whole genome sequencing project has been deposited at GenBank/NCBI under BioProject no. PRJNA293224, with BioSample accession nos. SAMN11636367 and SAMN12058707 and GenBank accession nos. AAEAXB000000000 and AAKADM000000000 for strains MEZSAL74 and MEZSAL81, respectively. The versions described in this paper are the first versions **AAEAXB000000000.1** and **AAKADM000000000.1**. The sequences have been submitted to the Sequence Read Archive (SRA) under the accession nos. SRR9050339 and SRR9050348.

Ethical approval

This study was approved by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Durban, South Africa) [reference nos. AREC/051/017M, AREC 071/017 and AREC 014/018; Act No. 35 of 1984 Section 20 approval reference no. 12/11/1/5].

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Competing interests

None declared.

Authors' contribution

M.E.Z. conceived, coordinated, and supervised the research project, prepared and wrote the manuscript, and submitted the strain for WGS. M.P. and M.E.Z. conducted samples collection and bacterial isolation. R.A.H. conducted the phylogenetic analysis. R.A.H. and J.D.J. contributed to manuscript writing. O.T.Z., A.E.Z, and J.D.J. reviewed the manuscript. M.E.Z. critically revised the manuscript. All authors approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2020.04.001.

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