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Identification of multidrug-resistant monophasic *Salmonella* Typhimurium ST34 and other NTS from animal-environmental origins in South Africa

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Non-typhoidal *Salmonella* (NTS) causes approximately 155 000 deaths annually and poses significant risks to both human and animal health. Antimicrobial resistance (AMR) in NTS is a growing global public health threat. Using a One Health approach, this study investigated NTS in swine, poultry, and wastewater in Gauteng Province, South Africa. From May 2019 to August 2020, 507 samples were collected, including animal faeces ($n = 388$), hand swabs ($n = 104$), abattoir and farm run-off ($n = 10$), and hospital ($n = 1$) and municipal wastewater ($n = 4$). Whole genome sequencing of recovered isolates revealed a 2.37% (12/507) NTS prevalence, identifying four serovars: *Salmonella* Enteritidis sequence type (ST) 11 ($n = 3$), *S*. Infantis ST32 ($n = 4$), *S*. Irumu ST2026 ($n = 2$), and multidrug-resistant *S*. Typhimurium monophasic variant 1,4,[5],12:i:- ST34 ($n = 3$). The ST34 strains, detected in swine, exhibited ASSuT (ampicillin, streptomycin, sulfamethoxazole, tetracycline) resistance pattern and marked the first detection of ST34 from an animal source in Africa. The strains harboured a novel *sopE*-phage (AmTI) and SGI-4. Phylogenetic analysis linked these strains to human cases in South Africa and the UK, which could indicate transmission of MDR *S*. Typhimurium between animals and humans, underscoring the importance of enhanced AMR surveillance using a One Health approach.

Non-typhoidal *Salmonella* (NTS) infections present a global public health threat, notably in sub-Saharan Africa (SSA), where specific serovars and sequence types (STs) are prevalent, infecting diverse species[1]. In 2017, 79% of the estimated 535,000 worldwide cases of invasive NTS (iNTS) occurred in SSA[2]. These infections, often foodborne, have a case fatality rate of 20%–25% and are linked to bacteraemia and meningitis in infants and immunocompromised adults, particularly those with HIV, tuberculosis, or malaria[1,3]. The predominant iNTS serovars include *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) ST 313, *S*. Enteritidis ST11, *S*. Dublin, *S*. Isangi, and, to a lesser extent, *S*. Infantis and *S*. Irumu, with *S*. Typhimurium and *S*. Enteritidis accounting for 65.2% and 33.1% of iNTS infections in Africa, respectively[1]. *Salmonella enterica* 4,[5],12:i:- of ST34 (monophasic *S*. Typhimurium ST34), emerged in Europe in the 1990s and has spread globally[4]. It is associated with multidrug-resistance (MDR), high transmission potential, and adaptability across hosts and environments, with the pandemic strain having the capacity to cause significant iNTS outbreaks[1,5]. Two factors contributing to the bacterial fitness and pathogenicity of ST34 monophasic pandemic strains include the acquisition of the transferable *sopE* virulence gene via the mTmV (in UK strains)[6,7] or mTmV2 (in Italian strains)[4] prophages, and the presence of the *Salmonella* Genomic Island 4 (SGI-4), which enhances resistance to heavy metals[8].

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The SSA iNTS strains are more human-adapted, pathogenic, and harbour antimicrobial resistance (AMR) genes[2,9]. Resistance to third-generation cephalosporins and fluoroquinolones in iNTS is rising in this region, limiting treatment options[9]. Resistant NTS from livestock, domestic, and wild animals can spread to humans through direct contact or contaminated food, accelerating AMR development in the human gut[10]. Reducing antimicrobial use in livestock is critical to controlling the spread of AMR between animals, humans, and the environment, requiring a One Health approach.

Since 2003, the Centre for Enteric Diseases, National Institute for Communicable Diseases (NICD), South Africa, has monitored enteric pathogens of public health concern through its GERMS-SA laboratory surveillance network[11]. In 2017, it adopted whole-genome sequencing (WGS) to enhance outbreak surveillance and epidemiological investigations[12]. Surveillance has confirmed *S.* Typhimurium and *S.* Enteritidis as the primary iNTS strains causing infections in clinical settings[11]. Although studies report MDR NTS in human, swine, poultry, and environmental sources in South Africa[13–16], no research has focused on NTS from commercial abattoirs, animal sources and their environment. This study employed a One Health Approach to investigate the virulence factors and AMR profiles of zoonotic NTS from swine, poultry, human contacts, and wastewater in Gauteng Province (GP), South Africa.

Results

The overall prevalence of *Salmonella* isolates was 2.37% (12/507), distributed as follows: swine [41.7% (5/12)], poultry [50% (6/12)], and poultry abattoir effluent [8.3% (1/12)]. These confirmed isolates were recovered from swine and poultry abattoirs and a swine farm in GP. No *Salmonella* growth was detected in human hand swabs from the abattoirs and farm, nor in effluents collected from municipal wastewater treatment plants (WWTPs) and the hospital setting.

Subtyping and assigning serovars for *Salmonella* strains

WGS identified four serovars: (i) *S.* Typhimurium monophasic variant 1,4,[5],12:i:- ST34 [25% (3/12)], (ii) *S.* Enteritidis ST11 [25% (3/12)], (iii) *S.* Infantis ST32 [33.3% (4/12)], and (iv) *S.* Irumu ST2026 [16.7% (2/12)] (Table 1). The *S.* Enteritidis strain was isolated from poultry and a poultry abattoir effluent, the *S.* Infantis from poultry, and the *S.* Irumu from swine.

Phenotypic and genotypic antimicrobial resistance testing of *Salmonella* strains

NTS strains were susceptible (100%) to amoxicillin/clavulanic acid, cefotaxime, ceftazidime, cefepime, ertapenem, imipenem, meropenem, tigecycline, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole (Fig. 1) (Appendix Table A).

Conversely, all strains (100%) were resistant to amikacin, cefoxitin, and gentamicin. Among the NTS strains, *S.* Typhimurium (3/12) from swine exhibited resistance to ampicillin, while *S.* Infantis (4/12) from poultry was resistant to ciprofloxacin. These findings correlate with our WGS results, which detected the extended-spectrum β-lactamase (ESBL) $bla_{\text{TEM-1B}}$ gene in *S.* Typhimurium strains and *gyrA*(p.S83Y) and *parC*(p.T57S) gene mutations in the quinolone resistance-determining region (QRDR) in *S.* Infantis strains. Additionally, all four *S.* Infantis strains, along with one *S.* Typhimurium strain (PSA-34-3), showed resistance to nitrofurantoin (Fig. 1) (Appendix Table A). Colistin resistance was detected in three (3/12) *S.* Enteritidis strains, two of which were isolated from poultry and one from wastewater in a poultry abattoir. Broth-microdilution (BMD) testing revealed MIC_{50} values of 4 µg/mL for the two poultry isolates (LPA-34-1 and LPA-34-2) and 8 µg/mL for the environmental run-off water isolate (RPA-RO1). However, M-PCR and WGS confirmed that none of the colistin-resistant *S.* Enteritidis strains carried the *mcr-1* to *mcr-9* genes or harboured colistin resistance-associated mutations in the *pmrABCEK*, *mgrB, phoP/phoQ, and acrAB-tolC* efflux genes.

Virulome and pathogenicity of non-typhoidal *Salmonella*

Virulome and pathogenicity/genomic islands results are found in Table 1. The WGS result observed that all 12 NTS strains harboured the major SPI-1 to 5, SPI-9, SPI-13 and SPI-14, said to be conserved in iNTS strains, with the exception of the *S.* Enteritidis strains, which harboured the SPI-10. This study also detected the pathogenicity islands (PI) centisome 54 (CS54_island) conserved in all NTS strains, 83.3% (10/12) except *S.* Irumu strains. In addition, the centisome 63 (C63PI) was conserved in only *S.* Enteritidis and *S.* Irumu strains, 41.7% (5/12). Screening of NTS isolates with the virulence factor database (VFDB) identified 137 virulence factors, with 37 virulence factors conserved across all NTS strains. Adhesion operons, particularly the *lpfABCDE* and type 1 fimbriae genes (*fimA* and *fimH*), were found in 83.3% (10/12) of NTS strains, excluding the *S.* Irumu strain. The *csg* fimbriae operon, *phoP/phoQ* two-component system that promotes virulence and resistance to a variety of antimicrobial peptides and invasive genes, including the plasmid-encoded virulence genes, *spvBCD*, *pefBACD*, *rck*, and *mig-5* genes, was exclusively detected in the *S.* Enteritidis strains [25% (3/12)]. The *Gifsy-1* prophage encoded gene *gogB* was detected in the *S.* Typhimurium strains [25% (3/12)], while the *Gifsy-2* prophage gene, *sodC-1*, was detected in both *S.* Enteritidis [16.7% (2/12)] and *S.* Typhimurium [25% (3/12)] strains. All *S.* Typhimurium strains carried the invasion plasmid antigen H *ipaH* gene. The *S.* Infantis strains [33.3% (4/12)] harboured the virulent yersiniabactin operon; *irp1*, *irp2*, *fyuA, ybtQ* and *ybtP*, while both *S.* Typhimurium and *S.* Infantis strains harboured the *ibeB* [58.3% (7/12)] and *ail* [41.7% (5/12)] invasion genes. Macrophage inducing gene *mig-14* was detected in *S.* Typhimurium 8.3% (1/12), *S.* Irumu 8.3% (1/12) and *S.* Infantis 25% (3/12) strains except the *S.* Enteritidis, which harboured the *mig-5* [25% (3/12)] gene. Iron uptake-regulating gene *fur* (50%, 6/12) was also reported in *S.* Irumu and *S.* Infantis strains.

ST34 strain from South Africa harbouring SGI-4 and *sopE*-phage region

All three *S.* Typhimurium ST34 strains contained the SGI-4 that encodes genes associated with resistance to heavy metals, characteristic of the ST34 monophasic pandemic. Each ST34 genome harboured two copies of *sopE*-prophages. BLAST identified a complete prophage (31.7 Kb) in the study ST34 genomes we designated AmTI (African monophasic Typhimurium I) (Fig. 2), similar to the one found in a clinical *S.* Typhimurium strain 3018683606 (CP094332) from China. One additional partially assembled region, an incomplete mtmV (UK *sopE*-phage) like phage from the reference genome *S.* Typhimurium S04698-09 (GCF_001540845.1), was detected in our ST34 study isolates (data not shown).

Phylogenetics and transmission dynamics of *S.* Typhimurium monophasic variant ST34

Phylogenetic analysis using core-genome multilocus sequence typing (cgMLST) based on single-nucleotide polymorphism (SNP) alignment of study isolates assigned to the cgMLST HC10-2 cluster, along with international isolates (HC10-2 and HC20-2), revealed genetic relatedness among 49 *S.* Typhimurium ST34 strains from South Africa and 13 other countries spanning six continents. These strains were collected from diverse sources between 2015 and 2022 (Fig. 3).

All three *S.* Typhimurium study strains isolated in 2020 clustered together and shared a common ancestry with two clinical strains from GP, South Africa. These three strains differed from the two clinical strains by four (isolated in 2020) and ten (isolated in 2021) SNPs, suggesting the recent spread of the pandemic ST34 strain rather than direct transmission (highlighted in figure 3). Swine and clinical isolates in this clade were closely related to a more distantly rooted clinical strain from the UK collected in 2018, with a 44 SNP difference (Fig. 3). The *S.* Typhimurium strains in this clade exhibited comparable STs, resistomes, and mobilome, displaying the ASSuT (ampicillin, streptomycin, sulfamethoxazole, tetracycline) resistance pattern. The strains harboured the AMR genes encoding the ESBL $bla_{\text{TEM-1B}}$; aminoglycosides *aph(6)-Id* and *aph(3″)-Ib*; sulphonamide *sul2*;

Table 1 | Genomic characteristics of NTS isolates

SampleID	Source	Virulence factors	Sequence type (ST)	Serovar	*Salmonella* pathogenicity/genomic islands
RPA-RO1	Environmental	*misL, shdA, ratB, sinH, pefBACD, mig-5, spvBCD, rck, sseK2, sseK1, sspH2, invA, hilA, mgtCB, sopA, sopB/sigD, sseI/srfH, fimA, fimH, lpfABCDE, csgABCDEFG, sifA. phoQ/phoP, sopE,*	11	Enteritidis	CS54_island, C63PI, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-10, SPI-13, SPI-14
KPA-13-2	Poultry	*irp1, irp2, fyuA, ybtQ, ybtP, sifB, ratB, shdA, sinH, hilA, sopA, sopB/sigD, sseI/srfH, invA, lpfABCDE, sopD2, mgtCB, fur, misL, sseK2, sseK1, fimA, fimH, mig-14, sspH2, ibeB*	32	Infantis	CS54_island, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14
KPA-13-3	Poultry	*irp1, irp2, fyuA, ybtQ, ybtP, invA, hilA, ratB, shdA, sinH, sifB, misL, mgtCB, fur, lpfABCDE, sopA, sopB/sigD, fimA, fimH, mig-14, sseK2, sseK1, sspH2, sseI/srfH, sopD2, ail, ibeB*	32	Infantis	CS54_island, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14
KPA-24-1	Poultry	*irp1, irp2, fyuA, ybtQ, ybtP, sopA, sopB/sigD, ratB, shdA, sinH, invA, hilA, misL, lpfABCDE, mgtCB, sspH2, sseK2, sseK1, sseI/srfH, fimA, fimH, fur, mig-14, sopD2, ibeB, ail*	32	Infantis	CS54_island, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14
KPA-24-2	Poultry	*irp1, irp2, fyuA, ybtQ, ybtP, sifB, ratB, shdA, sinH, sopA, sopB/sigD, invA, misL, hilA, mgtCB, fur, lpfABCDE, sspH2, sseK2, sseK1, sseI/srfH, fimA, fimH, sopD2, ibeB*	32	Infantis	CS54_island, C63PI, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14
LAF-25-1	Swine	*sopD, sopA, sopB/sigD, shdA, sinH, ratB, fimD, invA, sspH2, sseK2, fur, mgtCB, tssF-5, sseI/srfH, misL, hila*	2026	Irumu	C63PI, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14
LAF-25-2	Swine	*sopA, sopB/sigD, sopD, shdA, sinH, ratB, misL, mig-14, sseK2, invA, mgtCB, fur, hilA, sspH2, sspH1, sseI/srfH, tssF-5*	2026	Irumu	C63PI, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14
PSA-22-1	Swine	*shiB, misL, sopA, sopB/sigD, lpfABCD, fimA, fimH, sseK2, sseK1, invA, hilA, mgtCB, ratB, shdA, sinH, ail, ipaH, sopD, sopD2, sseI/srfH, sspH2, ibeB, gogB, sodC-1, sopE*	34	Typhimurium I 4,[5],12:i:-	CS54_island, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14, sgi4*
PSA-22-2	Swine	*shiB, sopA, sopB/sigD, misL, sopD, sopD2, shdA, sinH, lpfABCDE, ratB, mgtCB, hilA, sseK2, sseK1, mig-14, invA, sspH2, sspH1, sseI/srfH, fimA, fimH, ail, ipaH, ibeB, gogB, sodC-1, sopE*	34	Typhimurium I 4,[5],12:i:-	CS54_island, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14, sgi4*
PSA-22-3	Swine	*shiB, misL, mgtCB, lpfABCDE, fimA, fimH, sseK2, invA, hilA, ratB, shdA, sinH, sifA, sifB, sopD, sopD2, sopA, sopB/sigD, sseI/srfH, sspH2, sseK1, ipaH, ail, ciaB, ibeB, gogB, sodC-1, sopE*	34	Typhimurium I 4,[5],12:i:-	CS54_island, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-13, SPI-14, sgi4*
LPA-34-1	Poultry	*invA, sspH2, sifA, fimA, fimH, sopD2, sseI/srfH, sopA, sopB/sigD, misL, hilA, lpfABCDE, shdA, ratB, sinH, csgBDG, pefBACD, mig-5, rck, mgtCB, spvBCD, sseK1, phoQ/phoP, sodC-1, sopE*	11	Enteritidis	CS54_island, C63PI, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-10, SPI-13, SPI-14
LPA-34-2	Poultry	*sopA, sopB/sigD, sopD2, sseI/srfH, sspH2, sifA, mgtCB, hilA, fimA, fimH, ratB, shdA, sinH, misL, lpfABCDE, csgBDG, invA, pefBACD, spvBCD, mig-5, rck, sseK1, phoQ/phoP, sodC-1, sopE*	11	Enteritidis	CS54_island, C63PI, Un-named SPI, SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-10, SPI-13, SPI-14

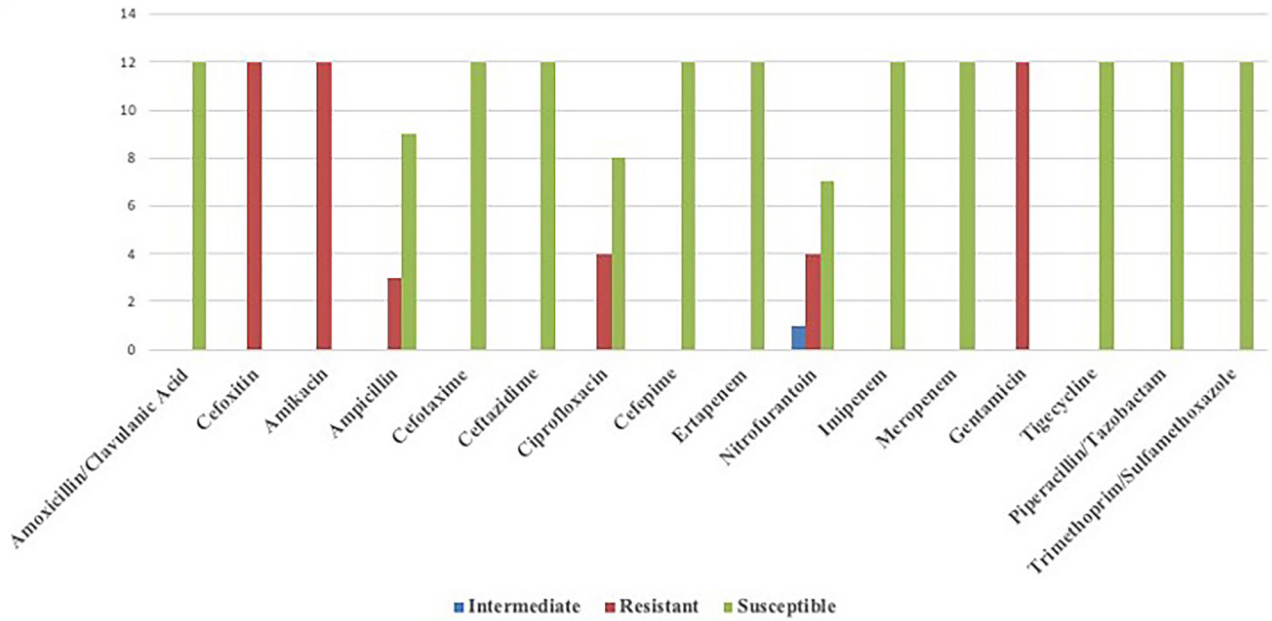

Fig. 1 | Phenotypic resistance in NTS strains. Antibiotic resistance profiles of Salmonella NTS strains using the VITEK®-2 automated system (bioMérieux, France).

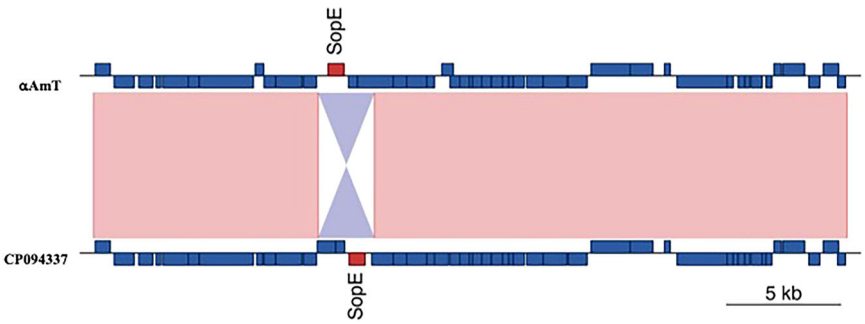

Fig. 2 | Comparison of the AmTI prophage with the *Salmonella* Typhimurium CP094337 prophage. Nucleotide sequence (horizontal lines) of novel *sopE*-prophage aligning to *Salmonella* CP094337 prophage isolated from an *S.* Typhimurium monophasic variant 1,4,[5],12:I:- strain in China. Each box on the line represents a predicted gene on the positive (above the line) or negative strand (below the line). The pink shading between lines indicates sequence conservation (>90% identity) in conserved order, while the blue shading indicates the sequence similarity but in reverse orientation. The *sopE* gene has been coloured in red for visibility.

and tetracycline *tet(B)* genes, along with the IncQ1 plasmid. In addition, the three study isolates harboured the aminoglycoside cryptic *aac(6′)-Iaa* gene conferring resistance to tobramycin, kanamycin, and amikacin, with two isolates (PSA-22-2 and PSA-22-3) co-harbouring the *tetA(P)* and *tetB(P)* genes encoding efflux proteins, conferring low-level resistance to tetracycline and minocycline. Some ST34 strains collected at different points in time from Africa, America, and Europe showed additional resistance to phenicol, quinolone, and trimethoprim, harbouring the IncHI2A or IncFII(S)/IncFIB(S) extra plasmids (Fig. 3). None of the strains harboured the pSLT plasmid.

An ancestral network analysis using PastML revealed an unresolved route of transmission for ST34 strains in this study, with South Africa, Germany, and the UK identified as potential countries of ancestral origin (Appendix Fig. A). However, a cluster of human strains from South Africa predicted a direct spread from the UK. Additionally, the strain source transmission network displayed an unresolved source for ST34 study strains, which are rooted in human and swine sources but show a connected root with environmental and food sources up the tree, underscoring the interconnected nature of the ST34 spread (see Appendix Fig. B).

Phylogenetics and transmission dynamics of *S.* Enteritidis ST11

The maximum-likelihood core SNPs phylogeny of two *S.* Enteritidis ST11 poultry strains and one wastewater strain, which belong to HC5-410 and HC5-402086, respectively, along with 108 similar cgMLST cluster strains isolated from 10 countries, revealed a close relationship. The study isolates clustered with local and international strains in two separate clades (Fig. 4).

Poultry isolates collected in 2019 showed 100% identity and shared ancestry with a clade of South African clinical strains collected between 2020 and 2022 across five provinces, with pairwise SNP distance of eight to 19 SNPs. The wastewater strain, recovered from a different poultry site, differed from the poultry (LPA-34-1 and LPA-34-2) strains by ~ 55 SNPs and grouped with clinical isolates from South Africa and the UK. Phylogenetic analysis confirmed *S.* Enteritidis ST11 strains in South Africa originated from poultry, clinical, food, and environmental sources and harboured the cryptic *aac(6′)-Iaa* gene. Local and international strains harboured the IncFII(S) and IncFIB(S) plasmids, with 6.3% (7/111) co-harbouring a Col plasmid, including the cryptic ColpVC plasmid identified in poultry isolates from this study. SRST2 confirmed the presence of a sequence with homology to an 83 kb IncF plasmid from *S.* Enteritidis strain NCCP 16206 (CP041972.1) in all three ST11 genomes detected in this study (data not shown).

The ST11 ancestral transmission network analysis predicted a direct spread from the UK to a cluster of South African strains (including the study isolates) before spreading back to the UK and onward to the USA, and Australia (Appendix Fig. C). However, strains from this study were predicted to originate from South Africa, with the two poultry strains showing an unresolved origin in human and poultry sources and the environmental strain a direct spread from human sources (see Appendix Figs. C and D).

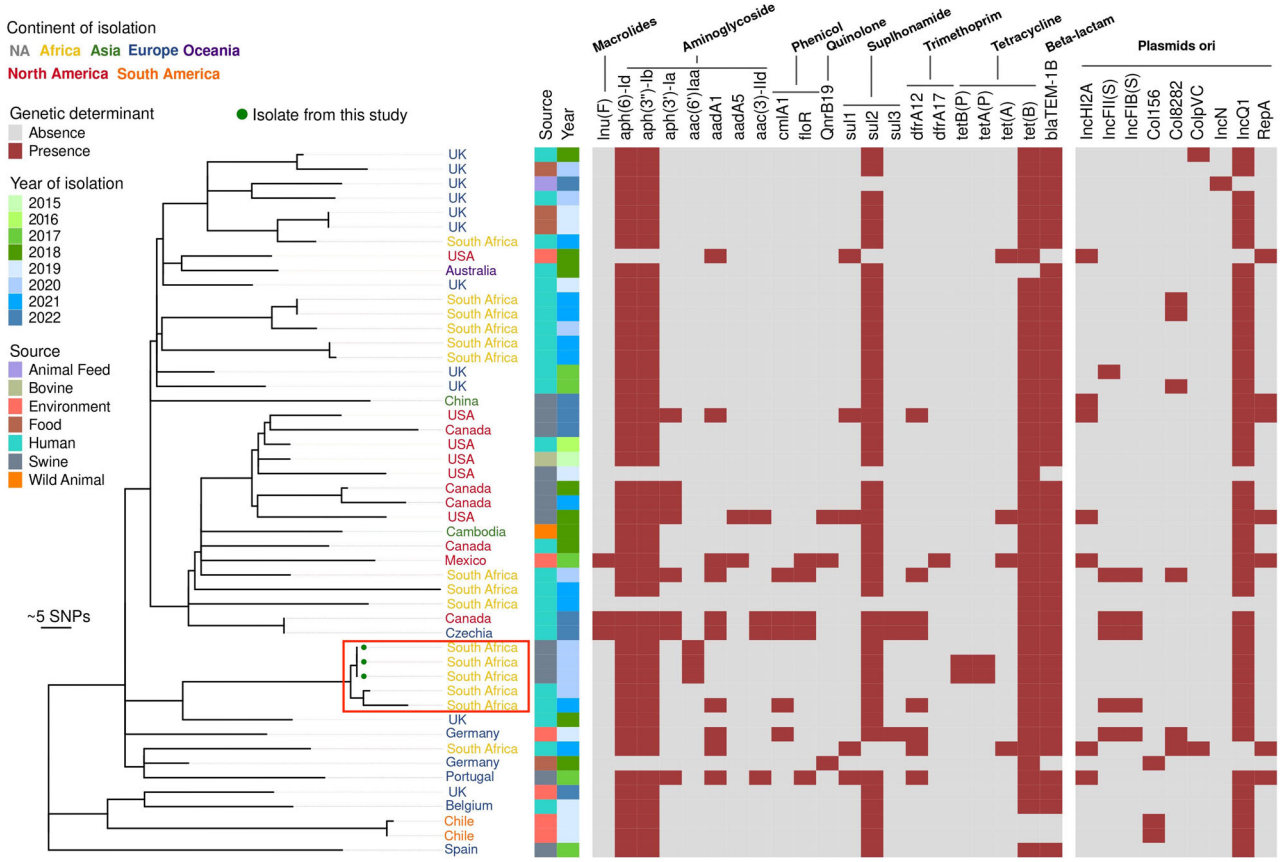

Fig. 3 | A maximum likelihood phylogenetic core SNP tree showing the relationship between study strains and international *Salmonella* Typhimurium ST34 based on core-genome SNPs. Antimicrobial resistance genes and plasmids are presented in heatmaps. The tree was rooted at the inferred position of the outgroup.

Phylogenetics and transmission dynamics of *S.* Infantis clonal group ST32

Phylogenetic analysis revealed genetic diversity among the four study *S.* Infantis ST32 strains assigned to similar cgMLST HC20-256857, with SNP distances ranging from six to 33. These strains, which were collected in Gauteng, clustered with three clinical strains from different time points originating from KwaZulu-Natal and Western Cape Provinces, South Africa (Fig. 5).

All four ST32 strains harboured the cryptic *aac(6′)-Iaa* gene, with one poultry-associated strain (KPA-24-2) additionally harbouring the *tetA(P)* gene encoding an efflux protein. A large cluster of African and European strains exhibited *gyrA*(p.S83Y) and *parC*(p.T57S) mutations in the QRDR, with 75.6% showing resistance to aminoglycosides, sulphonamides, and tetracyclines. A separate cluster of American strains showed MDR to *β*-lactams, trimethoprim, florfenicol, and fosfomycin while presenting with a *gyrA*(p.D87Y) and *parC*(p.T57S) QRDR mutation. All strains in both clades harboured the IncFIB (pN55391) conjugative plasmid.

The location of the ancestral root for ST32 strains in this study is unresolved between three regions: Germany, Hungary, and the UK (Appendix E). Furthermore, the source transmission predicted a direct spread of ST32 from human to poultry strains in this study. (see Appendix Fig. F).

Phylogenetics and transmission dynamics of *S.* Irumu clonal group ST2026

The phylogenetic analysis of 104 local and international *S.* Irumu ST2026 strains revealed that the two swine strains from this study with the cgMLST HC50-120836 profile nested among clinical strains from South Africa and the UK, with the closest, a UK strain, differing by 51 SNPs (Fig. 6).

South African clinical strains collected between 2020 and 2023 exhibited MDR, contrasting with strains from other regions around the world, which harboured only a single AMR gene. Swine strains in this study only harboured the cryptic *aac(6′)-Iaa* gene. A number of North American strains harboured IncF variants 65.5% (36/55) and IncI 25.5% (14/55) plasmids, while MDR clinical strains from South Africa harboured the IncH plasmid variant 66.7% (4/6), with one strain each harbouring the IncF 16.7% (1/6) and IncI 16.7% (1/6) plasmids.

An ancestral network analysis predicted a bidirectional spread of ST2026 between the USA and a cluster of South African strains (including study strains), and a uni-directional spread between South Africa and the UK (Appendix G). Additionally, swine strains in this study were predicted to originate from either swine or human sources (see Appendix Fig. H).

Discussion

iNTS infections remain a challenge in SSA[2,3], and innovative strategies[17] are needed to reduce their burden using a One Health Approach. Despite extensive studies on salmonellosis, data on foodborne NTS (including iNTS) in South Africa and Africa are limited; thus, the dynamics of zoonotic transmission are poorly understood. Using phenotypic and genotypic techniques, this study investigated the molecular epidemiology of NTS isolates from swine, poultry, and wastewater collected from abattoirs and livestock farms across GP in South Africa.

The overall *Salmonella* spp. prevalence of 2.37% in this study falls within the wide range of rates reported in other South African studies. It is higher than the 1.3% reported for beef products by Naidoo et al.[18], but considerably lower than prevalence rates reported in livestock production systems (29.4%)[19] and poultry (32.1%)[20]. Globally, prevalence also varies widely, from 4.3% in retail meat in California, USA, to 20% observed in retail chicken in China[21,22]. The prevalence of *Salmonella* serovars worldwide is

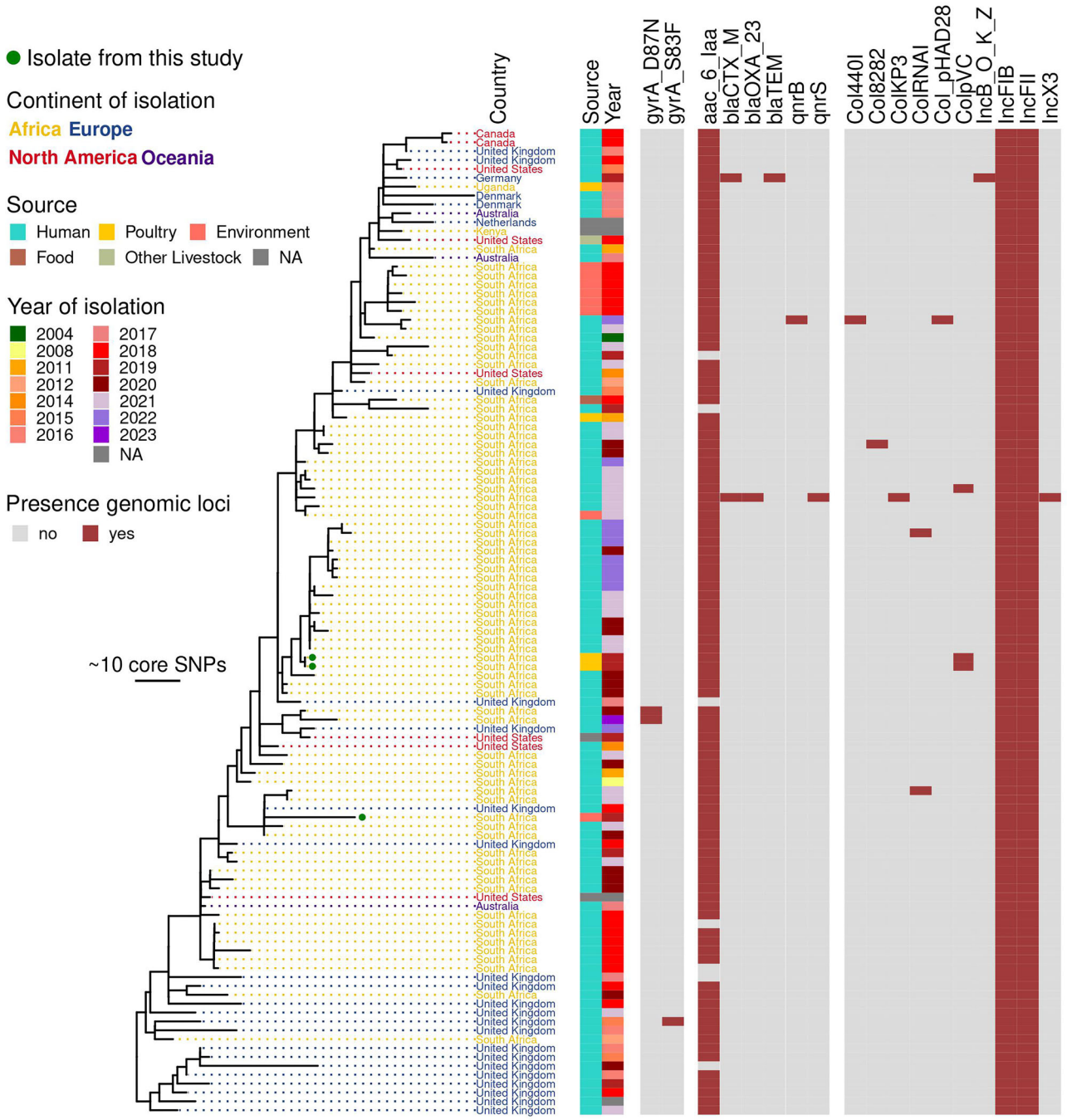

Fig. 4 | Core genome SNPs-based phylogenetic relationships of study strains and international *Salmonella* Enteritidis ST11 strains with heatmaps of mutations, antimicrobial resistance genes and plasmids. The tree was rooted at the inferred position of the outgroup.

known to be affected by geographical, environmental, seasonal risk factors, sources and surveillance gaps in healthcare settings[23]. The low prevalence observed in this study indicates a minimal but notable risk of human infection from meat products in Gauteng province. This study did not detect *Salmonella* spp. in the sampled effluents from wastewater treatment plants (WWTPs) and the tertiary hospital visited. In contrast, a study by Teklehaimanot et al.[24], reported an 86.8% presumptive *Salmonella* positivity rate in 272 wastewater and receiving water-body samples collected from the same WWTPs in GP over a 10-month study period (August 2011 and May 2012). This discrepancy may be due to differences in sampling size, sampling period, sampling locations, and seasonal variations, as against the once-off sampling approach used in this study, emphasising the need for further investigations into factors influencing *Salmonella* spp. presence in wastewater.

This study identified diverse *Salmonella* serovars using WGS, including, based on an extensive literature search, and to the best of our knowledge, the first African report of the *S.* Typhimurium monophasic variant 1,4,[5],12:i:- ST34 from an animal or food source. This variant of *S.* Typhimurium was first identified in clinical isolates in South Africa in 2020 and has continued to be identified in clinical settings to date (Dr Anthony Smith, NICD, personal communication). In addition, a literature search on Scopus, PubMed, and Google Scholar (as of September 9, 2025) found no prior reports of this variant in animal or food sources from Africa. The *S.* Typhimurium monophasic variant 1,4,[5],12:i:- ST34 is the third leading cause of human salmonellosis outbreaks in the European Union (EU) and the fifth leading cause in the USA[25].

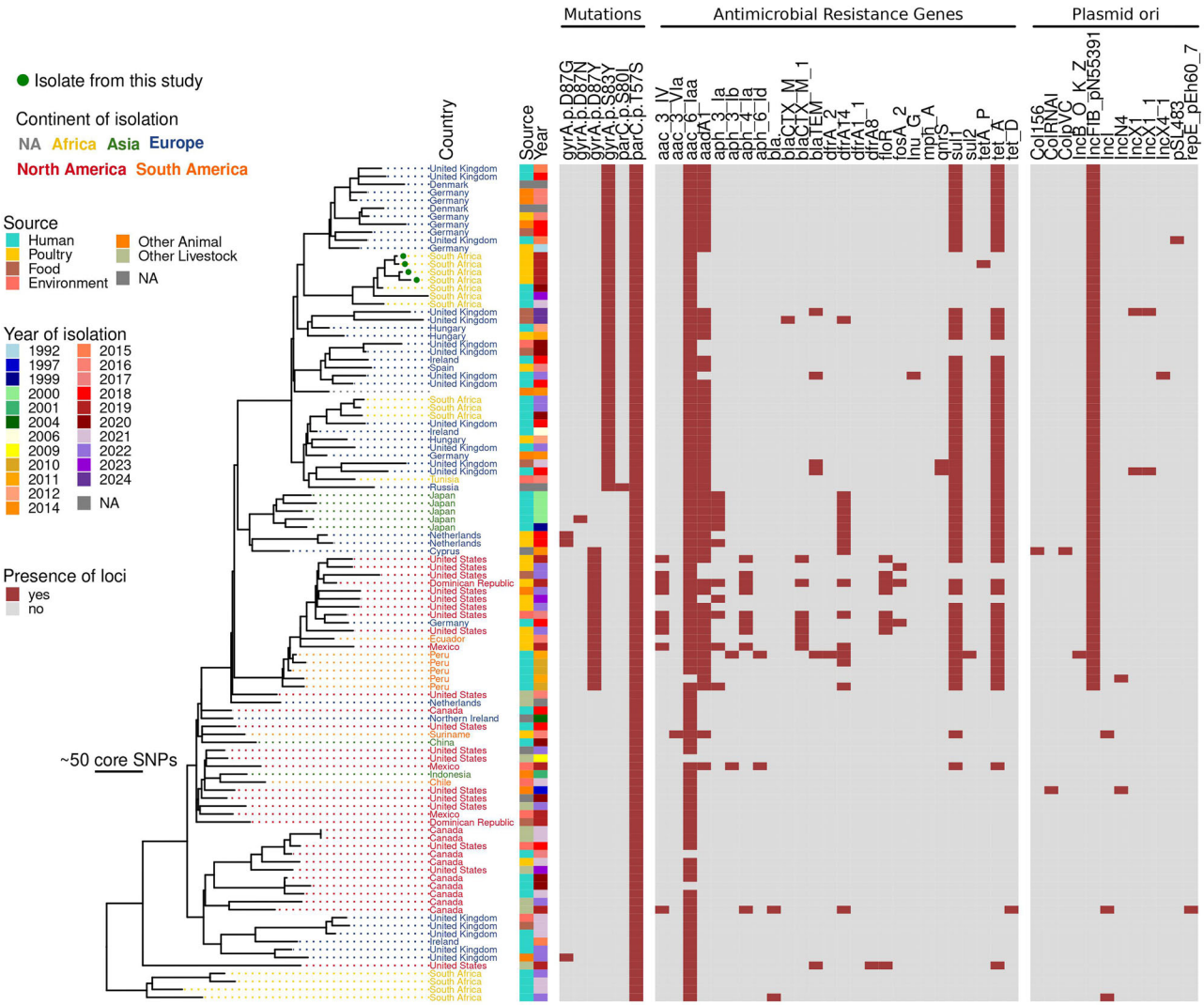

Fig. 5 | Core genome SNPs-based phylogenetic relationships of study strains and international *Salmonella* Infantis ST32 strains with heatmaps of mutations, antimicrobial resistance genes and plasmids. The tree was rooted at the inferred position of the outgroup.

Phenotypic resistance to amikacin, cefoxitin, and gentamicin was observed in all NTS strains examined in this study. Additionally, all three *S.* Typhimurium strains exhibited resistance to ampicillin, while four *S.* Infantis strains were resistant to ciprofloxacin and nitrofurantoin. Similar resistance patterns have been documented in *Salmonella* isolates from poultry and swine in both African and European studies[26,27]. The widespread and indiscriminate use of antimicrobials in animal husbandry in South Africa may be a key factor driving the emergence and dissemination of AMR in NTS populations. Two poultry and one wastewater *S.* Enteritidis strains exhibited phenotypic resistance to colistin, but neither M-PCR assay nor WGS confirmed the presence of *mcr* genes or known mutations promoting colistin resistance in the three strains. As such, these strains may carry new or underreported mutations leading to colistin resistance. Findings by Rule et al.[28], in South Africa, reported a fatal case of a rare *mcr*-negative colistin-resistant *S.* Enteritidis in an immunocompromised HIV patient with invasive disease and meningitis. The observed resistance to critical antimicrobials, including ampicillin, cefoxitin, ciprofloxacin, and colistin, which are essential for treating iNTS and other Gram-negative bacterial infections in humans, highlights the urgent need to enhance public health surveillance efforts to combat iNTS infections effectively.

A genomic study alone is not sufficient to assess whether the strains isolated here have the potential to cause invasive disease. However, detection of *Salmonella* pathogenicity islands (SPIs) linked to invasive human disease is consistent with findings from a study in Israel, where all iNTS serovars were found to carry SPIs 1-5, SPI-9, SPI-13, and SPI-14[29]. These SPIs encode type III secretion systems (T3SSs), which are essential for bacterial invasion, growth, survival, and disease progression within the host, and often contain clusters of virulence factors located on mobile genetic elements (MGEs) such as plasmids and insertion sequences[30]. Invasive genes, including plasmid-encoded operons (*spvBCD*, *pefBACD*, *rck*, and *mig-5* genes) and the fimbriae operon *csg* gene, were only recovered from the *S.* Enteritidis strains. These genes enable the *S.* Enteritidis strains to attach, evade immune response mechanisms, and colonise the host to cause invasive disease[31]. The Gifsy-1 phage-encoded gene, *gogB*, was detected only in the *S.* Typhimurium strains, while the Gifsy-2 prophage gene, *sodC-1*, was recovered from the *S.* Enteritidis and *S.* Typhimurium strains. These phage-encoded genes aid in the colonisation of the small intestine and survival of *Salmonella* inside the macrophages, causing enteritis and invasive disease in humans[29,32]. Interestingly, the *ibeB* and *ail* genes were recovered from *S.* Typhimurium strains and *S.* Infantis strains. These genes may enable adherence to and invasion of brain endothelial and epithelial cells, initiating colonisation, tolerance, and persistence of these strains within the host during infection, with implications for transmission and chronicity[32].

Notably, the study identified the SGI-4 in ST34 strains, a genomic island first reported in UK ST34 strains[4]. This integrative conjugative

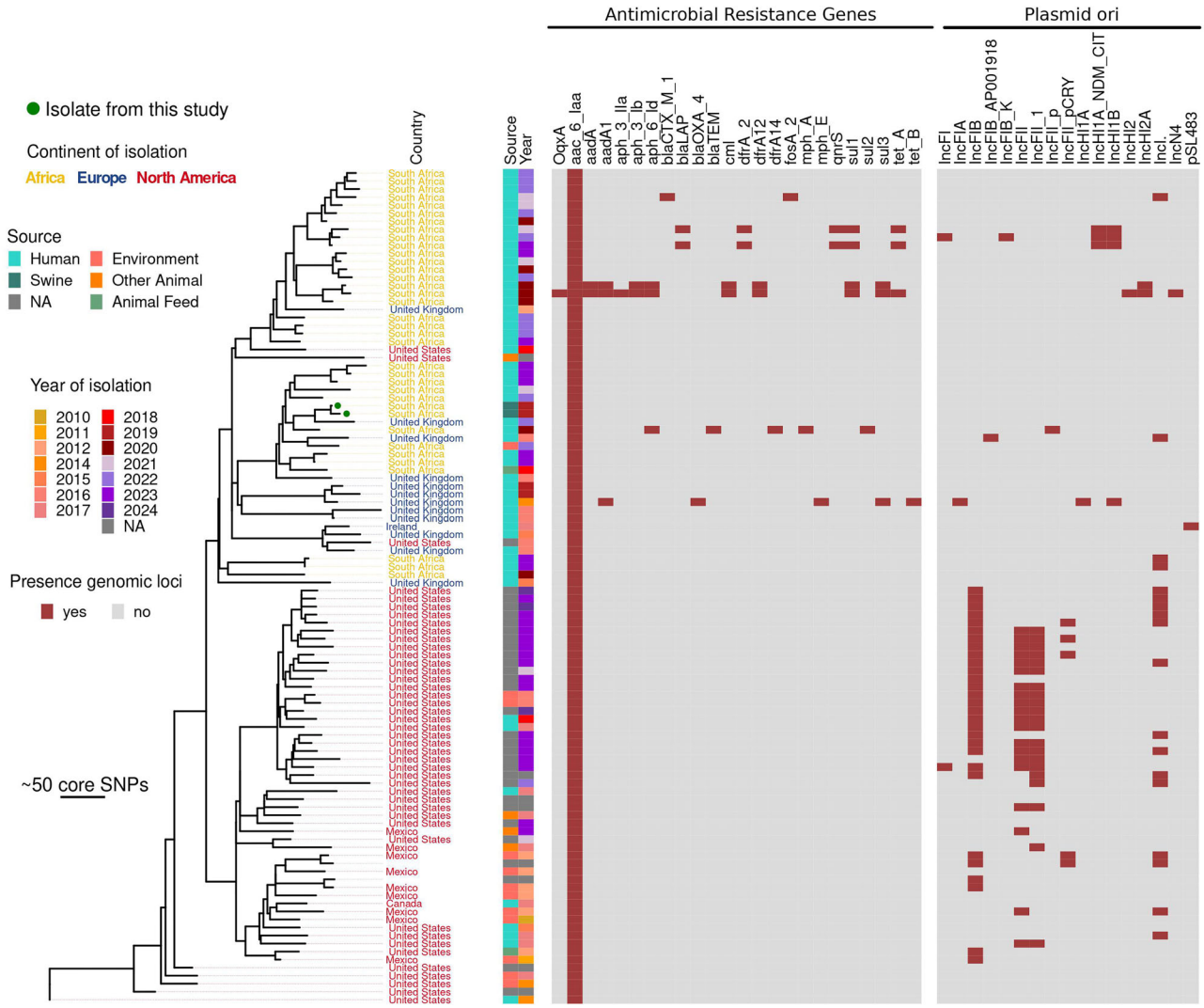

Fig. 6 | Whole-genome SNPs-based phylogenetic relationships of study strains and international *Salmonella* Irumu ST2026 strains with heatmaps of antimicrobial resistance genes, bar charts of plasmids and shape plots of insertion sequences/transposons. The tree was rooted at the inferred position of the outgroup.

element confers resistance to heavy metals, including copper and zinc, and is considered the hallmark for the spread of the *S.* Typhimurium monophasic pandemic strains worldwide[4]. Due to their antimicrobial activity, these metals are often used as growth promoters in the livestock industry, following restrictions on the use of antimicrobials in animal feed[8]. Its presence in South African animal strains could enhance their environmental adaptability, thereby complicating and facilitating the community spread of *S.* Typhimurium monophasic pandemic strains, increasing their persistence and potential transmission to vulnerable hosts. Further investigation into SGI-4's presence in human strains in South Africa is warranted.

The ST34 genomes in the study harboured a novel *sopE*-prophage, designated AmTI, homologous to a prophage detected in a clinical ST34 strain isolated from a 2-year-old in China[33]. An additional incomplete mTmV-like phage was also reported. Multiple copies of *sopE*-phage have been reported previously in ST34 strains[4], although our study strains harboured a different *sopE*-prophage, the AmTI. Long-read sequencing is recommended to explore multiple *sopE*-phage copies and their genetic context in all ST34 strains detected in this study. Expression of the *sopE* gene, a type III effector typically acquired during an epidemic spread via horizontal gene transfer, enhances NTS strain virulence, fitness and transmission between hosts by promoting host nitrate production, which facilitates respiration, invasion, and colonisation of the intestinal mucosa[4,6].

Phylogenetic analysis based on hierarchical clustering of ST34 strains collected in South Africa between 2020 and 2021 revealed that ST34 swine isolates and human clinical strains shared a close ancestry, differing by just four to ten SNPs. This suggests potential zoonotic transmission between animals and humans. These strains belonged to a distinct clade with a more deeply rooted UK isolate, suggesting a shared evolutionary origin at a specific point in time and implying localised microevolution following an initial international transmission. Ancestral reconstruction of study ST34 strains predicted an unresolved origin from South Africa, Germany, and the UK. This suggests the possibility of an intercontinental dissemination of the MDR *S.* Typhimurium ST34 since its emergence in European swine populations in the late 1990s. Worldwide, transboundary diseases have been introduced into new regions through several routes, such as international travel and legal and illegal trades[34,35]. According to a report published by the South African Department of Agriculture, Forestry and Fisheries (DAFF) in 2021, 80% of South African pork and pork product imports are from the EU, with Germany, the Netherlands, France, and the UK accounting for the largest imports[36]. Although all swine abattoirs and farm in this study sourced their animals within South Africa[37], with the rise of global markets, the cross-border trade of animals and animal products has become more frequent. Previous studies have also shown multiple transboundary transmission of the ST34 strains into the USA from Europe through sources including human travellers and imported food products[38,39]. Therefore, whether trade

and travel routes contribute to the transmission of *S.* Typhimurium monophasic variant ST34 strains into South Africa needs further investigation. This emphasises the importance of implementing surveillance using a One Health approach at both local and global levels to effectively monitor, prevent, and respond to emerging zoonotic threats. All strains in this clade exhibited MDR ASSuT resistance patterns and harboured the IncQ1 plasmid. Two swine ST34 study strains in this clade harboured the *tetA(P)* and *tetB(P)* genes encoding intracellular efflux proteins, which confer ribosomal protection for tetracycline and minocycline. The monophasic *S.* Typhimurium ST34 clone has been reported to acquire genes that confer resistance to multiple antimicrobials[4,8], supporting why only two of the three ST34 study strains in this clade harboured the *tetA(P)* and *tetB(P)* genes.

The *S.* Enteritidis poultry strains in this study differed from clinical strains across five South African provinces by 8–19 SNPs. Globally, *S.* Enteritidis ST11 outbreaks report genetic variances of <20 SNPs[40], including South African outbreaks with <6 allelic differences[15], suggesting that strains within this clade may belong to the same outbreak. Wastewater strain from a different site clustered with clinical strains from South Africa and the UK. Most strains in these clades harboured only the cryptic *aac(6′)-Iaa* gene, aligning with a study reporting a pan-susceptible *S.* Enteritidis ST11 clade from diverse sources in South Africa and globally[10]. This study found that the absence of AMR genes does not necessarily correlate with susceptibility to antimicrobials, as all ST11 strains examined exhibited phenotypic resistance to amikacin, gentamicin, cefoxitin, and colistin. Ancestral evolutionary analysis predicted a bidirectional spread of ST11 strains between South Africa and the UK, with direct transmission events to Australia and the USA. These findings illustrate the global establishment of the *Salmonella Enteritidis* ST11 clone, which circulates among animals, humans, and the environment. While an unresolved ancestral root was identified in poultry-associated ST11 strains, the environmental strain analysed in this study was traced directly to a human origin. Although humans were the primary source of ST11 dissemination in this dataset, it is likely influenced by the study's data size and composition, as 82.9% (92/111) of isolates selected for this study under HC5-410 and HC5-402086 clusters were of human origin. This likely created a bias, even though poultry remains the major global reservoir of ST11[41].

The *S.* Infantis study strains and some African strains nested within European clades, with ancestral construction predicting an unresolved origin from Germany, Hungary, and the UK. All ST32 strains exhibited the *gyrA*(p.S83Y) and *parC*(p.T57S) gene mutations in the QRDR and harboured the IncFIB(pN55391) plasmid, characteristic of the 'parasitic' pESI-like megaplasmid[16]. These mutations, associated with reduced susceptibility to nalidixic acid and ciprofloxacin[42], align with earlier findings of phenotypic ciprofloxacin resistance observed in ST32 strains detected in this study. Similar resistance traits have been observed in *S.* Infantis strains from human, animal, and food sources in South Africa and countries in Europe and America[16,43].

First reported in 1956 in frozen eggs in South Africa, *S. Irumu* strains have since spread globally[44,45]. The two study strains clustered with clinical, environmental, and animal feed isolates from South Africa and the UK but showed no relatedness <10 SNPs (i.e. no strains <10 SNPs distance). Ancestral transmission predicted a bidirectional spread of ST2026 strains between the USA and South Africa and onward spread from South Africa to the UK. Historical reports link South African and UK outbreaks, with the first *S.* Irumu case outside South Africa recorded in the UK in 1969[45]. Genetic analysis showed strains from South Africa harboured more AMR genes than strains originating from around the world. Genomic information on Irumu is limited, and a robust study linking characterised outbreaks of this serovar to SNP distance is not currently available.

Interpretation of SNP distances in *Salmonella* requires consideration of serovar-specific genetic diversity, as cut-off values vary. For example, in *S.* Enteritidis, outbreak-related isolates are mainly defined by <20 SNPs between genomes, with some *S.* Enteritidis reported outbreaks containing strains on average 32 SNPs away from each other[15,40]. In *S.* Typhimurium, <5 SNPs are typically considered strong evidence of direct transmission[38], while *S.* Agona has reported average distances from 126 - 240 SNPs within an outbreak[40]. Another study reported that *Salmonella* serovars with <5 SNPs usually indicate direct transmission, and <10 SNPs indicate related strains worth investigating[46]. Using the upper thresholds of SNP distance in the absence of epidemiological information on the strains is, however, risky as it may lead to the mis-grouping of strains for separate outbreaks into a single transmission network.

In conclusion, this study detected the ST34 for the first time in animal and food sources in Africa associated with a novel AmTI prophage. The large importation of pork and its products through trade routes or travel may contribute to the introduction of some emerging NTS strains into Africa, warranting further investigation. However, the limited dataset used for ancestral reconstruction in this study poses a constraint, underscoring the necessity for more comprehensive genomic analyses to confirm these findings. Additional limitations include the one-time collection of effluents from heavily contaminated sample sites such as the WWTPs and tertiary hospital, and not using selective enrichment (Rappaport-Vassiliadis broth) for isolation of *Salmonella* from these samples, as recommended by the International Organisation for Standardisation. This may have reduced the likelihood of detecting NTS, potentially underestimating its prevalence in this study. Animal and environmental strains in this study clustered with clinical strains both locally and internationally, harbouring diverse MGEs and showing resistance to critical antimicrobials, including colistin. SNP distances between our strains and genomes reported from other studies fell within the contextual thresholds, which strengthens the interpretation that animal and environmental strains identified in this study are closely related to human clinical isolates, with potential zoonotic implications. All NTS strains harboured SPIs and virulence factors essential for invasion, colonisation, and intracellular multiplication in infected human hosts. It is imperative to continue monitoring and implementing adequate control measures across the farm-to-fork chain using a One Health Approach to limit the spread of these NTS strains to humans globally. Future research should consider spatiotemporal and ecological niche modelling to identify high-risk regions for NTS transmission in South Africa. This can aid in identifying potential control strategies and informing public health interventions.

Methods

Ethics

The University of Pretoria Animal Ethics Committee (H012-18), Research Ethics Committee (485/2018), Gauteng Province National Health Research Database (GP 201903 032), South African Department of Agriculture, Forestry and Fisheries Section 20 [12/11/1/1/19 (1314)], and the City of Tshwane Utility Services Department (W9/1/2/1) provided approvals for the study. Approval was also acquired from the Department of Veterinary Public Health of the Gauteng Department of Agriculture and Rural Development for sampling in abattoirs and farms. The abattoir/farm owners and workers completed consent forms indicating their understanding and approval of the study objectives, as well as their voluntary participation in the study.

Sample collection

The study was conducted in commercial swine and poultry abattoirs and farms in GP. Location sites and sources of supply of the abattoirs, farms and WWTPs in this study are described in an earlier study[37]. A majority of these abattoirs source their animals from within and outside the province of study. A total of 507 samples were collected between May 2019 and August 2020, from swine abattoirs ($n = 4$), swine farms ($n = 1$), and poultry abattoirs ($n = 5$). Cloacal swabs from poultry ($n = 220$), swine faecal samples ($n = 168$), and hand swabs from abattoir and farm workers ($n = 104$) were collected. A single round of effluent sampling was conducted at each abattoir and farm visited ($n = 10$). Similarly, once-off effluent samples were collected from a tertiary hospital ($n = 1$), a referral health facility that provides specialised care, and municipal WWTPs ($n = 4$) serving the sampled locations. Poultry and hand swabs were collected in Amies media (without charcoal)

(Oxoid Ltd, UK), while swine faeces were collected in sterile (screw-cap) containers (Greiner Bio-One, Germany). Eight litres of effluent were aseptically collected in sterile glass bottles from each abattoir, farm, and WWTP visited. All samples were labelled, transported on ice, and processed within 3 h of arrival in the laboratory.

Laboratory processing of samples and NTS isolation

Swine faecal samples and swabs were homogenised in 10 mL tryptone soy broth (Oxoid Ltd, UK) and incubated overnight at 37 °C in an orbital shaker (Si500, Bibby Scientific Group, UK) at 230 rotation per minute (rpm). Enriched samples (100 µl) were plated on XLD agar (Thermo Fisher Scientific, USA) and incubated (Vacutec, South Africa) for 18 h to 24 h at 37 °C. Effluent samples (100 mL) were filtered through 0.45 µM Millipore™ membrane filters (Merck, Germany) repeatedly until the sample was exhausted. Membrane filters were placed aseptically on XLD agar plates (Thermo Fisher Scientific, USA) and incubated as described earlier. Red bacterial colonies with black centres were identified as presumptive *Salmonella*. Five colonies randomly selected from each positive plate were subcultured in brain heart infusion (BHI) broth (Lab M Limited, UK) and incubated similarly. Presumptive *Salmonella* isolates were confirmed using the *Salmonella* latex agglutination test (Oxoid Ltd, UK) and the Lysine decarboxylase test (Merck, South Africa). Confirmed isolates with a purple hue were stored in 50% glycerol (Merck, Germany).

Whole-genome sequencing of *Salmonella* spp. isolates

WGS was performed on 12 *Salmonella* isolates at the Sequencing Core Facility, NICD, South Africa. Genomic DNA was extracted from overnight cultures on blood agar plates (Oxoid Ltd, UK) using the ZR Fungal/Bacterial DNA MiniprepTM kit (Zymo Research, USA). The NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA) was used to measure the concentration of DNA. Libraries were prepared using the Nextera DNA Flex Kit (Illumina), and each *Salmonella* isolate was sequenced on the Illumina NextSeq 500 platform (Illumina, San Diego, CA) with 2 × 150 bp paired-end runs at 80× coverage. The JEKESA bioinformatics pipeline (v1.0) (https://github.com/stanikae/jekesa) was used for read analysis and typing. Details on serotypes determination, multilocus sequence typing, virulence factors, acquired AMR genes, chromosomal mutations, plasmid replicons, and pathogenic/genomic islands are described in the Appendix (pp 3–4).

Determining phenotypic and genotypic resistance of *Salmonella* strains

Following the manufacturer's instructions, the VITEK®2 automated system (bioMérieux, France) was used to identify and analyse the antimicrobial susceptibility (AST) of all confirmed *Salmonella* strains. Fresh colonies of *Salmonella* isolates grown overnight on sheep blood agar (Oxoid Ltd, UK) and incubated (Vacutec, South Africa) at 37 °C for 16 to 18 h were suspended in saline (0.85%) (DMP-NHLS, South Africa) to prepare a 0.5 McFarland suspension for each *Salmonella* isolate. The minimum inhibitory concentration (MIC) for each isolate was interpreted according to the CLSI guidelines[47]. Rapid Colistin NP test, BMD test, and *mcr* resistance genes analysis were performed on all *Salmonella* strains as described in the Appendix (pp 2–3).

Phylogenomic reconstruction and transmission networks analysis

Selection of isolates for phylogenetic reconstruction and transmission networks was dependent on the available data, so strategy is not the same for all STs analysed. Short reads were uploaded to EnteroBase[48], and additional isolates from various sources, including humans, animals, food and the environment, were selected based on cgMLST HierCC clusters and available metadata. Overall, the selection prioritised isolates closely related to our study isolates, and other isolates from South Africa, even if they are more distantly related. Raw reads from the additional isolates were downloaded using srahunter (https://github.com/GitEnricoNeko/srahunter) and used to generate core SNP alignments with snippy 4.6.0 (https://github.com/tseemann/snippy) using SL1344 [GCA_000210855] as a reference. Recombination regions were detected and masked using gubbins 2.4.1[49], and snp-dist (0.8.2) (https://github.com/tseemann/snp-dists) was used to generate SNP distance matrices. Maximum likelihood trees were built using RAxML-ng (version 1.2.0) with the GTR-CAT substitution model[50]. The number of bootstraps was selected using the autoMRE option, and the SL1344 [GCA_000210855] reference was used as an outgroup to root the trees. Figures were plotted in R (4.3.3) (R Core Team 2024) using R-studio and the packages ggtree (3.10.1)[51], ggplot2 (3.5.0)[52], ape (5.7.1)[53], and pheatmap (1.0.12)[54]. To analyse the NTS ancestry and transmission dynamics, we utilised PastML (version 1.9.50) in default settings to infer ancestral characters as previously described[55], using the Marginal Posterior Probabilities Approximation prediction method and the F18 evolutionary model.

Data availability

The nucleotide sequences of all strains detected in this study have been deposited in the NCBI under BioProject ID PRJNA1170267 and accession numbers SAMN44102839, SAMN44102838, SAMN44102837, SAMN44102836, SAMN44102835, SAMN44102834, SAMN44102833, SAMN44102832, SAMN44102831, SAMN44102830, SAMN44102829, and SAMN44101722. Additional data generated or analysed during this study are provided in the strain-associated figures in the Appendix.

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References

1. Stanaway, J. D. et al. The global burden of non-typhoidal *Salmonella* invasive disease: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Infect. Dis.* **19**, 1312–1324 (2019).
2. Tack, B., Vanaenrode, J., Verbakel, J. Y., Toelen, J. & Jacobs, J. Invasive non-typhoidal *Salmonella* infections in sub-Saharan Africa: a systematic review on antimicrobial resistance and treatment. *BMC Med.* **18**, 1–22 (2020).
3. Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S. & Gordon, M. A. Invasive non-typhoidal *Salmonella* disease: an emerging and neglected tropical disease in Africa. *Lancet* **379**, 2489–2499 (2012).
4. Tassinari, E. et al. Whole-genome epidemiology links phage-mediated acquisition of a virulence gene to the clonal expansion of a pandemic *Salmonella enterica* serovar Typhimurium clone. *Microb. Genom.* **6**, e000456 (2020).
5. Larkin, L. et al. Investigation of an international outbreak of multidrug-resistant monophasic *Salmonella* Typhimurium associated with chocolate products, EU/EEA and United Kingdom, February to April 2022. *Eurosurveillance* **27**, 2200314 (2022).
6. Petrovska, L. et al. Microevolution of monophasic *Salmonella* typhimurium during epidemic, United Kingdom, 2005–2010. *Emerg. Infect. Dis.* **22**, 617 (2016).
7. Bawn, M. et al. Evolution of *Salmonella enterica* serotype Typhimurium driven by anthropogenic selection and niche adaptation. *PLoS Genet.* **16**, e1008850 (2020).
8. Branchu, P. et al. SGI-4 in monophasic *Salmonella* Typhimurium ST34 is a novel ICE that enhances resistance to copper. *Front. Microbiol.* 1118. https://doi.org/10.3389/fmicb.2019.01118 (2019).
9. Gilchrist, J. J. & MacLennan, C. A. Invasive non-typhoidal *Salmonella* disease in Africa. *EcoSal Plus* **8**, https://doi.org/10.1128/ecosalplus.esp-0007-2018 (2019).
10. Carroll, L. M., Pierneef, R., Mathole, M. & Matle, I. Genomic characterisation of endemic and ecdemic non-typhoidal *Salmonella enterica* lineages circulating among animals and animal products in South Africa. *Front. Microbiol.* **12**, 748611 (2021).

11. Meiring, S. et al. Unlocking insights: key findings from GERMS-SA Annual Surveillance Review 2022. Available: https://www.phbsa.ac.za/key-findings-from-germs-surveillance-review-2022/. Accessed 28 October 2024.
12. Brümmer, B. et al. Whole genome sequencing assisted outbreak investigation of *Salmonella enteritidis*, at a hospital in South Africa, September 2022. *Access Microbiol.* **6**, 000835–v000833 (2024).
13. Magwedere, K., Rauff, D., De Klerk, G., Keddy, K. H. & Dziva, F. Incidence of non-typhoidal *Salmonella* in food-producing animals, animal feed, and the associated environment in South Africa, 2012–2014. *Clin. Infect. Dis.* **61**, S283–S289 (2015).
14. Ramatla, T., Taioe, M. O., Thekisoe, O. M. & Syakalima, M. Confirmation of antimicrobial resistance by using resistance genes of isolated *Salmonella* spp. in chicken houses of North West, South Africa. *World's Vet. J.* **9**, 158–165 (2019).
15. Smith, A. M. et al. Whole-genome sequencing to investigate two concurrent outbreaks of *Salmonella* Enteritidis in South Africa, 2018. *J. Med. Microbiol.* **69**, 1303–1307 (2020).
16. Mattock, J. et al. A One Health Perspective on *Salmonella enterica* Serovar Infantis, an emerging human multidrug-resistant pathogen. *Emerg. Infect. Dis.* **30**, 701 (2024).
17. Kariuki, S. & Owusu-Dabo, E. Research on invasive non-typhoidal *Salmonella* disease and developments towards better understanding of epidemiology, management, and control strategies. *Clin. Infect. Dis.* **71**, S127–S129 (2020).
18. Naidoo, S. et al. Virulence factors and antimicrobial resistance in *Salmonella* species isolated from retail beef in selected KwaZulu-Natal municipality areas, South Africa. *Appl. Sci.* **12**, 2843 (2022).
19. Mthembu, T. P., Zishiri, O. T. & El Zowalaty, M. E. Detection and molecular identification of *Salmonella* virulence genes in livestock production systems in South Africa. *Pathogens* **8**, 124 (2019).
20. Ramtahal, M. A. et al. Molecular epidemiology of Salmonella enterica in poultry in South Africa using the farm-to-fork approach. *Int. J. Microbiol.* **2022**, https://doi.org/10.1155/2022/5121273 (2022).
21. Li, X. et al. Whole-genome sequencing identification of a multidrug-resistant *Salmonella enterica* serovar Typhimurium strain carrying bla_{NDM-5} from Guangdong, China. *Infect., Genet. Evol.* **55**, 195–198 (2017).
22. Lee, K. Y. et al. Antimicrobial resistance profiles of non-typhoidal *Salmonella* from retail meat products in California, 2018. *Front. Microbiol.* **13**, 76 (2022).
23. Morgado, M. E. et al. Climate change, extreme events, and increased risk of salmonellosis: foodborne diseases active surveillance network (FoodNet), 2004-2014. *Environ. Health* **20**, 1–11 (2021).
24. Teklehaimanot, G. Z., Genthe, B., Kamika, I. & Momba, M. Prevalence of enteropathogenic bacteria in treated effluents and receiving water bodies and their potential health risks. *Sci. Total Environ.* **518**, 441–449 (2015).
25. Ferrari, R. G. et al. Worldwide epidemiology of *Salmonella* serovars in animal-based foods: a meta-analysis. *Appl. Environ. Microbiol.* **85**, e00591–00519 (2019).
26. Abd El-Aziz, N. K. et al. Extensive drug-resistant *Salmonella enterica* isolated from poultry and humans: prevalence and molecular determinants behind the co-resistance to ciprofloxacin and tigecycline. *Front. Microbiol.* **12**, 738784 (2021).
27. Lauteri, C., Festino, A. R., Conter, M. & Vergara, A. Prevalence and antimicrobial resistance profile in *Salmonella* spp. isolates from swine food chain. *Ital. J. Food Saf.* **11**, 9980 (2022).
28. Rule, R. et al. A rare case of Colistin-resistant *Salmonella* Enteritidis meningitis in an HIV-seropositive patient. *BMC Infect. Dis.* **19**, 1–7 (2019).
29. Suez, J. et al. Virulence gene profiling and pathogenicity characterisation of non-typhoidal *Salmonella* accounted for invasive disease in humans. *PLoS ONE* **8**, e58449 (2013).
30. Cheng, R. A., Eade, C. R. & Wiedmann, M. Embracing diversity: differences in virulence mechanisms, disease severity, and host adaptations contribute to the success of non-typhoidal *Salmonella* as a foodborne pathogen. *Front. Microbiol.* **10**, 1368 (2019).
31. Koczerka, M. et al. The invasin and complement-resistance protein *Rck* of *Salmonella* is more widely distributed than previously expected. *Microbiol. Spectr.* **9**, e01457–01421 (2021).
32. Wahl, A., Battesti, A. & Ansaldi, M. Prophages in *Salmonella enterica*: a driving force in reshaping the genome and physiology of their bacterial host?. *Mol. Microbiol.* **111**, 303–316 (2019).
33. Wei, J. et al. Emergence of a clinical *Salmonella enterica* serovar 1,4,[5],12:i:- isolate, ST3606, in China with susceptibility decrease to ceftazidime-avibactam carrying a novel $bla_{CTX-M-261}$ variant and a bla_{NDM-5}. *Eur. J. Clin. Microbiol. Infect. Dis.* 1–12. https://doi.org/10.1007/s10096-024-04765-3 (2024).
34. Beltran-Alcrudo, D., Falco, J. R., Raizman, E. & Dietze, K. Transboundary spread of pig diseases: the role of international trade and travel. *BMC Vet. Res.* **15**, 64 (2019).
35. Herrera-Ibatá, D. M., Martínez-López, B., Quijada, D., Burton, K. & Mur, L. Quantitative approach for the risk assessment of African swine fever and Classical swine fever introduction into the United States through legal imports of pigs and swine products. *PLoS ONE* **12**, e0182850 (2017).
36. DAFF. A profile of the South African pork market value chain. South African Department of Agriculture, Land Reform and Rural Development. http://webapps1.daff.gov.za/AmisAdmin/upload/Pork%20Market%20Value%20Chain%20Profile%202021.pdf. Accessed 12 November 2024.
37. Ogundare, S. T. et al. Epidemiology and antimicrobial resistance profiles of pathogenic *Escherichia coli* from commercial swine and poultry abattoirs and farms in South Africa: A One Health approach. *Sci. Total Environ.* **951**, 175705 (2024).
38. Elnekave, E. et al. *Salmonella enterica* serotype 4,[5], 12: i:-in swine in the United States Midwest: an emerging multidrug-resistant clade. *Clin. Infect. Dis.* **66**, 877–885 (2018).
39. Elnekave, E. et al. Transmission of multidrug-resistant *Salmonella enterica* subspecies *enterica* 4,[5],12:i:- sequence type 34 between Europe and the United States. *Emerg. Infect. Dis.* **26**, 3034 (2020).
40. Leekitcharoenphon, P., Nielsen, E. M., Kaas, R. S., Lund, O. & Aarestrup, F. M. Evaluation of whole genome sequencing for outbreak detection of *Salmonella enterica*. *PLoS ONE* **9**, e87991 (2014).
41. Li, S., He, Y., Mann, D. A. & Deng, X. Global spread of *Salmonella* Enteritidis via centralised sourcing and international trade of poultry breeding stocks. *Nat. Commun.* **12**, 1–12 (2021).
42. Askoura, M. & Hegazy, W. A. H. Ciprofloxacin interferes with *Salmonella* Typhimurium intracellular survival and host virulence through repression of *Salmonella* pathogenicity island-2 (SPI-2) genes expression. *Pathog. Dis.* **78**, ftaa011 (2020).
43. Mattock, J. et al. Genetic characterisation of *Salmonella* Infantis from South Africa, 2004–2016. *Access Microbiol.* **4**, https://doi.org/10.1099/acmi.0.000371 (2022).
44. Kidanemariam, A., Engelbrecht, M. & Picard, J. Retrospective study on the incidence of *Salmonella* isolations in animals in South Africa, 1996 to 2006. *J. South Afr. Vet. Assoc.* **81**, 37–44 (2010).
45. Christie, A. Salmonellosis. *J. R. Coll. Gen. Pract.* **18**, 27 (1969).
46. Chattaway, M. A., Painset, A., Godbole, G., Gharbia, S. & Jenkins, C. Evaluation of genomic typing methods in the *Salmonella* reference laboratory in Public Health, England, 2012–2020. *Pathogens* **12**, 223 (2023).
47. CLSI: Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI Document M100-Ed30. (2020).
48. Zhou, Z. et al. The EnteroBase user's guide, with case studies on *Salmonella* transmissions, *Yersinia pestis* phylogeny, and *Escherichia* core genomic diversity. *Genome Res.* **30**, 138–152 (2020).

49. Croucher, N. J. et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res.* **43**, e15–e15 (2015).
50. Kozlov, A. M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* **35**, 4453–4455 (2019).
51. Yu, G., Smith, D. K., Zhu, H., Guan, Y. & Lam, T. T. Y. ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods Ecol. Evol.* **8**, 28–36 (2017).
52. Wickham, H. & Wickham, H. *Data Analysis* (Springer, 2016).
53. Paradis, E. & Schliep, K. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**, 526–528 (2019).
54. Kolde, R. Pheatmap: Pretty Heatmaps. R. Package Version 1.0.12 (2019).
55. Ishikawa, S. A., Zhukova, A., Iwasaki, W. & Gascuel, O. A fast likelihood method to reconstruct and visualise ancestral scenarios. *Mol. Biol. Evol.* **36**, 2069–2085 (2019).

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Author contributions

S.T.O. and M.M.E. designed the study. S.T.O., M.M.E., J.P.M., T.H., G.A.V., G.T., and M.B. acquired the study data. S.T.O., M.M.E., G.A.V. and P.J.G. were involved in project management and data visualisation. S.T.O., M.M.E., G.T. and M.B. drafted the manuscript. S.T.O., M.M.E., G.T. and M.B. carried out data analysis and interpretation. M.M.E., F.O.F., T.H., A.M.S., M.M.K. and J.P.M. provided resources, edited and reviewed the manuscript. AMS provided funding and facilitation of whole-genome sequencing.

Competing interests

The authors declare no competing interests.

Additional information

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