

Genomic analysis of the first cases of extensively drug-resistant, travel-related *Salmonella enterica* serovar Typhi in Oman

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ABSTRACT

Objectives: To highlight the importance of molecular testing in characterizing extensively drug-resistant (XDR) *Salmonella* Typhi (S. Typhi), and linking it to the current outbreak in Sindh, Pakistan.

Methods: Our study reports three travel-related typhoid fever cases caused by XDR S. Typhi that presented between January 2019 and August 2019. Antimicrobial susceptibility and genotyping with pulse-field gel electrophoresis (PFGE) were carried out. Whole-genome sequencing (WGS) was performed to characterize the genomic clonality in relation to the emerging outbreak of S. Typhi in Sindh, Pakistan, and to study the molecular resistance profiles.

Results: Laboratory testing revealed resistance to all first-line antibiotics (i.e. ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole), as well as to quinolones and third-generation cephalosporins, leading to a change in the patients' therapy to the use of carbapenems. Classical MLST (cMLST) revealed that the strains were of sequence type 1 (ST1) and the core genome sequence (cgWGS) analysis closely clustered our strains with internationally reported strains from Pakistan, India, and the UK. The strains were found to carry a *bla*_{CTX-15} gene-harboring IncY plasmid, which encodes resistance to ceftriaxone.

Conclusions: Our report alerts clinicians to the use of appropriate empirical treatments in such scenarios, and highlights the significance of the global spread of XDR S. Typhi.

1. Introduction

Salmonella enterica serovar Typhi (S. Typhi) is a Gram-negative bacterium that causes typhoid fever, which is a significant threat to public health in low- and middle-income countries. Antibiotic resistance is becoming increasingly common in S. Typhi (Gibani *et al.*, 2019).

Multidrug-resistant (MDR) S. Typhi, which is resistant to first-line antibiotics (ampicillin, chloramphenicol, and cotrimoxazole) was first detected in the late 1970s (Wain and Kidgell, 2004). MDR and quinolone-resistant S. Typhi is a major public health concern in Pakistan; sporadic cases of ceftriaxone-resistant strains were also reported by local surveillance efforts between 2009 and 2011 (Qamar *et al.*, 2014).

Extensively drug-resistant (XDR) S. Typhi, which is resistant to all first-line antibiotics, fluoroquinolones, and third-generation cephalosporins, was initially reported in the Sindh province of Pakistan in 2016 (Klemm *et al.*, 2018). XDR S. Typhi has since reached countries such as the UK (Nair *et al.*, 2021), the USA (Chatham-Stephens *et al.*, 2019), and Canada (Wong *et al.*, 2019). Between November 1,

2016 and December 9, 2018, 5274 XDR typhoid cases were reported by the Provincial Disease Surveillance and Response Unit (PDSRU) in the Sindh province of Pakistan, according to the World Health Organization (WHO, 2018).

XDR S. Typhi outbreak surveillance studies in Pakistan have provided a better understanding of the sources and factors involved in the global spread. The first reported cluster of 80 isolates showed genomic clonality with the possession of the same IncY plasmid (Klemm *et al.*, 2018).

In Oman, the national laboratory-based surveillance program tracks the antimicrobial susceptibility of all *Salmonella* species. Surveillance data obtained between 2014 and 2018 showed that the rate of occurrence of MDR S. Typhi was 21%, while the rate for ceftriaxone resistance was 2%, but no strains were identified as being XDR (OMASS, 2019). Approximately 8% of notifiable infections reported in Oman are related to travel. Typhoid accounted for 53.8% of travel-related infections from 1999 to 2013, which was 30.3% of all typhoid cases diagnosed in Oman during that period (Al-Abri *et al.*, 2015).

In this study, we reported the first three cases of XDR S. Typhi in Oman in relation to travel to Sindh, Pakistan — in which the outbreak

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Table 1
Laboratory results for the presented cases

Patients	Age (years)	Specimen	CBC	RFT	LFT	CRP (mg/L)	Outcome
1	9	Blood	Normal	Normal	ALP 159 U/L	44	Full recovery
2	19	Blood	Normal	Normal	ALT 351 U/L ALP 183 U/L	39	Full recovery
3	23	Stool	Low Hb Low WBC Recovered	Normal	ALT 400 U/L AST 500 U/L	37	Full recovery

CBC: complete blood count, Hb: hemoglobin, WBC: white blood cells, RFT: renal function test, LFT: liver function test, CRP: C-reactive protein, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase

is currently evolving — with the aim of determining the relatedness of local strains. In addition, we performed a whole-genome sequencing (WGS) analysis to find the global relatedness of the strains, characterize the resistance genes, and identify the plasmid types.

2. Case reports

2.1. Case 1

A previously healthy 9-year-old Pakistani boy presented to a health-care facility in January 2019 with a 1-week history of fever and abdominal pain. He had returned from Pakistan on the same day on which the onset of symptoms occurred. His blood culture revealed a strain of *S. Typhi* that was resistant to all first-line antibiotics, as well as to ciprofloxacin and ceftriaxone, and was sensitive to carbapenems and azithromycin. He was initially put on ceftriaxone (2 g once a day), which was changed the following day to meropenem (1 g three times a day) upon receipt of the preliminary antibiotic susceptibility results. He improved and was discharged after 2 weeks of meropenem treatment.

2.2. Case 2

A previously healthy 19-year-old Omani man presented to a district hospital in March 2019 with a 2-week history of fever and loose motion. He provided a history of travel to Pakistan, showing that he returned 1 day prior to the onset of symptoms after staying there for 1 week. His blood culture revealed XDR *S. Typhi* that was sensitive to carbapenems and azithromycin. He was put on meropenem (1 g three times a day) for 2 weeks and showed a good recovery.

2.3. Case 3

A 23-year-old Omani woman with no past medical history was admitted in August 2019 with a history of fever and rigors that started 2 days prior to returning from Pakistan, where she had stayed for 6 weeks to attend a wedding. Her stool revealed XDR *S. Typhi* that was sensitive to carbapenems and azithromycin, and her blood culture was negative. She was initially put on ceftazidime and metronidazole, which were later changed to ertapenem upon receipt of the preliminary antibiotic susceptibility results. She was discharged after 5 days of ertapenem treatment with no complications.

The results of the laboratory tests performed on the three patients and their clinical details are summarized in [Table 1](#). The common result among all three patients was a mild rise in liver enzymes, which was then normalized after treatment. C-reactive protein (CRP) levels increased mildly, while the white blood cell count was normal in two of the three patients.

3. Materials and methods

3.1. Samples

Pure cultures of the *S. Typhi* strains from patients' samples were sent to the Central Public Health Laboratories (CPHL), the reference labora-

tory for enteric pathogen typing and surveillance, for confirmation of the identification of the strains and susceptibility testing.

3.2. Laboratory tests

3.2.1. Identification

Initial phenotypic identification of the isolates was carried out with Vitek 2 using a GN card (bioMérieux, France).

3.2.2. Serotyping

Serotyping of the isolates was performed using a slide agglutination test with polyvalent and monovalent antisera (MAST, UK) against the somatic (O), flagellar (H), and (vi) antigens. Serotypes were classified according to the Kauffmann–White scheme [2022](#).

3.2.3. Susceptibility testing

Antibiotic susceptibility testing was performed with the Kirby–Bauer disk-diffusion method (Bauer and Kirby, 1966) and with Vitek 2 (bioMérieux, France), using an AST GN 215 card. In addition, the minimum inhibitory concentration (MIC) of ceftriaxone was determined with an E-test (predefined gradient concentrations; bioMérieux, France), and an ESBL test was carried out using the double-disk method (MAST, UK). Interpretation of the results was based on the Clinical and Laboratory Standards Institute's Performance Standards for Antimicrobial Susceptibility Testing guidelines, M100, 29th edition (CLSI; M100, [2019](#)).

3.3. Molecular tests

3.3.1. Pulse field gel electrophoresis (PFGE)

To assess the relatedness of the strains, PFGE was performed as per the standard operating procedure for PulseNet PFGE for *Escherichia coli* O157:H7, CDC protocol (PLN05) ([STEC 2021](#)) with a CHEF-DR-III (Bio-Rad). The PFGE conditions were optimized, and the run time was set to 17.6 hours. The PFGE conditions involved an initial switch time of 2.16 s and a final switch time of 63.8 s. The *Salmonella* Braenderup strain H9812 (kindly gifted by the CDC, Atlanta, USA) was used as a molecular weight marker. Gel images were taken using the Gel/ChemiDoc system (Bio-Rad Laboratories). Analyses and comparisons of the PFGE fingerprints were performed using BioNumerics Software (5.1 version, Applied Maths).

3.3.2. Whole-genome analysis

3.3.2.1. Genomic DNA extraction. Extraction of genomic DNA from the *S. Typhi* isolates was performed with a QIASymphony SP/AS automated nucleic acid extractor system (Qiagen) using a QIASymphony DSP Virus/Pathogen Mini Kit (ID 937036, Qiagen, Germany), according to the manufacturer's instructions. The gDNA was eluted in 100 µL of elution buffer. The quality and quantity of the gDNA were determined using a NanoDrop and Qubit (Thermo Scientific). The integrity of the DNA was also checked in 0.8% agarose gel.

3.3.2.2. Whole-genome sequencing (WGS). High-quality gDNA was sent to a reputed commercial third party (Macrogen, Korea) for whole-genome sequencing. The whole-genome sequencing was performed using TruSeq Nano DNA library preparation kit, followed by short-read sequencing on the Illumina HiSeq platform, using a 101-bp paired-end read. The raw read data were received as Fastq files.

3.3.2.3. Whole-genome sequence analysis. Analysis of whole-genome sequences was performed at the CPHL using tools and pipelines that were available in the public domain. Most of the analyses were performed using Enterobase (<http://enterobase.warwick.ac.uk>). The ResFinder and PlasmidFinder tools, which are available on the website of the Center for Genomic Epidemiology (CGE), were also used (<http://www.genomicepidemiology.org>).

In addition to the Enterobase and CGE tools, FastQC software (Galaxy version 0.72) was used to assess the quality of the raw data and to generate quality reports. *De novo* assembly was performed using a SPADes (v3.12.0) assembler (supplementary data) (Bankevich et al., 2012). The raw reads of all of the isolates were submitted to the Sequence Read Archive (SRA) (BioProject accession no. PRJNA665121).

3.3.3. Classical MLST and core-genome MLST comparison

To analyze the data for the whole-genome sequence, the *Salmonella* database tools in Enterobase were used. The Fastq files were uploaded to the Enterobase *Salmonella* database. Assemblies were generated using the Enterobase assembly pipeline. A classical seven-gene MLST (cMLST) analysis was performed with the Achtman seven-gene MLST scheme. Based on the results of the cMLST analysis, a comparison of our assemblies (three in number) with other similar *S. Typhi* assemblies that had the same cMLST profile in the database was carried out. Assemblies from 2012 onwards were selected for a core-genome MLST (cgMLST) comparison, which included all XDR assemblies from Pakistan that were available in the database. The cgMLST analysis was performed using the cgMLST V2+ HierCC V1 scheme. A grape tree (phylogenetic tree) was constructed using the MSTree V2 scheme. Further analysis was performed using the Microreact interface (<https://microreact.org>).

All three assemblies were also uploaded to Pathogenwatch (Pathogenwatch | A Global Platform for Genomic Surveillance) along with metadata. Genome analysis was performed automatically and a report was generated for every genome. Genotype number was generated using the Genotypi scheme (Wong et al., 2016). *S. Typhi* genomes from Oman were compared with existing *S. Typhi* genomes, and a collection of similar genomes (ST1) was created. A core genome tree (Pathogenwatch uses a scheme based on 3284 alleles) was constructed using selected genomes from the collection, including XDR strains from Pakistan, the UK, Italy, and the USA (from 2015 to 2021). The core genome tree was imported in iTOL and datasets were added.

A circular map of XDR *Salmonella* genomes was built to visualize the entire genome in comparison with the *S. Typhi* genome (accession no. LT882486) using BRIG (Alikhan et al., 2011).

3.3.4. Antimicrobial gene profiles

Antimicrobial resistance genes and their associated resistant phenotypes were defined using the online Resfinder. All of the genes were extracted in multi-FASTA format from the online pipeline of the Comprehensive Antibiotic Resistance Database (CARD) (<https://card.mcmaster.ca/home>).

4. Results

4.1. Confirmation of *S. Typhi*

The initial identification and serotyping confirmed the strains as *S. Typhi*. All of the strains were found to be resistant to all three first-line antibiotics tested, as well as to ciprofloxacin and ceftriaxone (Table 2). They were all confirmed as being extended-spectrum beta-lactamase

(ESBL)-producing *S. Typhi*, which rendered them resistant to many antimicrobial agents, including third-generation cephalosporins (e.g. ceftriaxone and ceftazidime) and aztreonam. They were susceptible to meropenem and azithromycin.

4.2. PFGE

Genotyping of the three strains with PFGE identified them as having the unique fingerprint type JPPX01.005, according to the PulseNet International/CDC PFGE fingerprint nomenclature (Figure 1).

4.3. Whole-genome analysis

The BLASTn search with the longest contigs showed a high similarity to the emerging Pakistani XDR *S. Typhi* strain 22420_110_pak6006_2016.

The complete genome of the Pakistani strain was accessible at NCBI with accession number LT882486. The raw reads of all of the isolates from Oman were submitted to the Sequence Read Archive (SRA), NCBI (BioProject accession no. PRJNA665121).

Classical MLST (cMLST) revealed that all of the isolates from Oman were of sequence type 1 (ST1) and had 100% similarity to the XDR reference isolate (LT882486) from Pakistan. Other XDR isolates from Pakistan, which represented the isolation years of 2017, 2018, and 2019, were also observed to have the ST1 cMLST profile. ST1 belongs to the cMLST clonal complex 13 (CC13) according to eBURST.

Since classical MLST is based on seven genes, and has comparatively low resolution in comparison with core genome MLST (cgMLST), our study compared cgMLST profiles of all ST1 genomes (since January 2015) on Enterobase and Pathogenwatch. The cgMLST results showed isolates from Oman and Pakistan in the same clade with 0–3 allele differences (Enterobase results are shown in a grape tree in Figure 2, while results from Pathogenwatch are shown in a rectangular tree in Figure 3). On Enterobase, XDR isolates from Oman and Pakistan were given same cgMLST type 83019. Similar results were obtained on Pathogenwatch (Pathogenwatch uses the Enterobase gMLST scheme). A common genotype no. 4.3.1.1. P1 was observed among XDR isolates that had a Pakistan XDR lineage (Genotypi scheme). The cMLST phylogeny tree, with the isolates selected in both Enterobase and Pathogenwatch from the years 2015 to 2021, clustered Omani *S. Typhi* isolates with isolates from Pakistan, the UK, Italy, and the USA with a difference of 0–3 alleles, which was suggestive of the same clone (Figures 2 and 3).

4.4. Antimicrobial gene profiles and plasmids

The resistance gene profiles of all three isolates from Oman were similar to each other. The genes for resistance against quinolone (*qnrS1*), trimethoprim (*dfrA7*), beta-lactam (*bla_{CTX-M-15}*, *bla_{TEM1B}*), phenicol (*catA1*), aminoglycoside (*aph(3')Ib* and *aph(6)IId*), and sulfonamide (*sul1* and *sul2*) were identified with ResFinder. Similar gene profiles were seen for the XDR assemblies from Pakistan (Table 3). The presence of the IncQ1 and IncY plasmids was shown with the PlasmidFinder tool in the isolates from Oman.

The graphical circular map in Figure 4 represents the three Omani *S. Typhi* assembled contigs in sequence alignment with the XDR *S. Typhi* complete genome (accession number LT882486). All Omani isolates were shown to be genetically related with the reference outbreak strain, with a minor gap. The outermost ring shows nine antibiotic-resistance genes extracted from the Resfinder tool, with the probable positions of genes scattered around the genome. These genes were also reported in the strains from the outbreak in Pakistan (Klemm et al., 2018).

5. Discussion

In Oman, typhoid is a reportable disease. In 2014, a laboratory-based *Salmonella* surveillance program was established, with all *Salmonella*

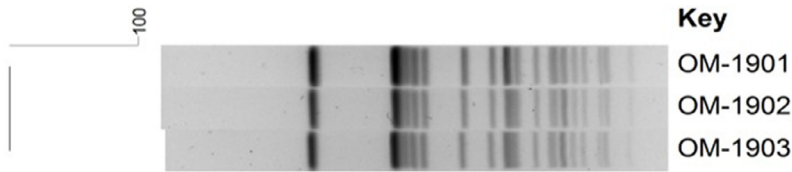


Figure 1. Comparison of PFGE fingerprints of three *S. Typhi* XDR strains from Oman. The whole genome was restricted using XbaI endonuclease, and a tree constructed using BioNumerics software version 5.01 (Applied Maths, Belgium).

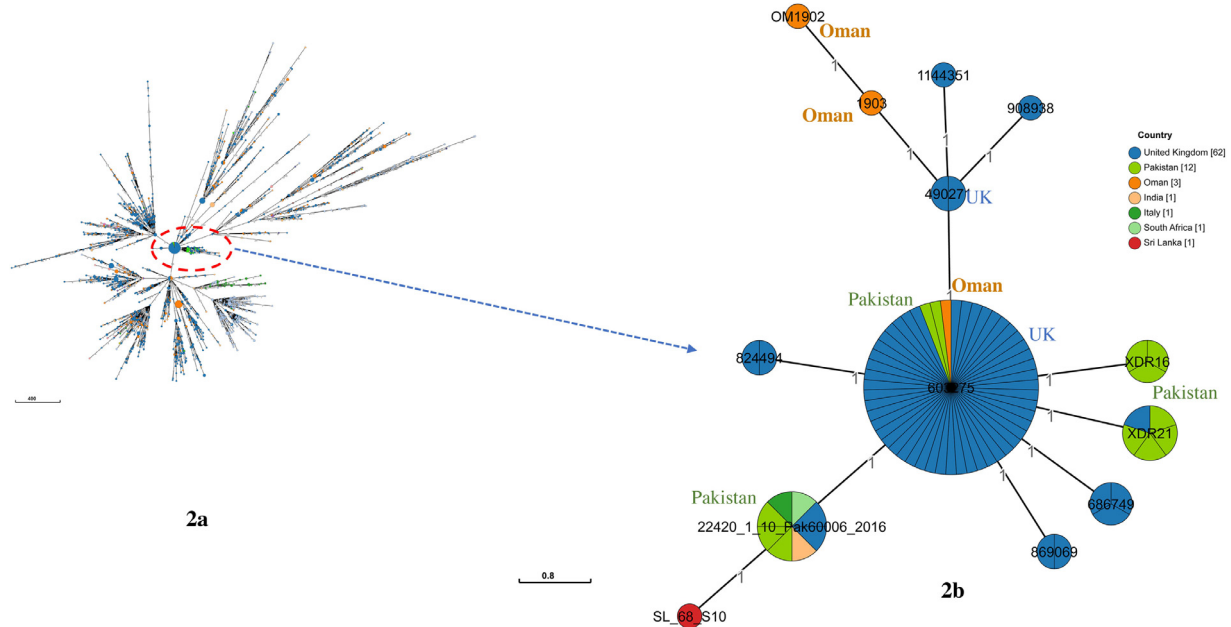


Figure 2. Phylogenetic grape tree of ST1 *S. Typhi* genomes on Enterobase (since 2015). (a) A comparison of all ST1 core genomes in Enterobase. (b) A selected subtree of the whole tree showing closest core genome matches to *S. Typhi* from Oman. XDR strains from Pakistan (2016 outbreak strain) display 0–3 allele variations (suggestive of the same clone). Strains in the UK, Italy, and India have also shown 0–3 allele variation compared with the Omani strains. These countries have also reported the emergence of XDR cases related to the 2016 XDR *S. Typhi* outbreak strain from Pakistan.

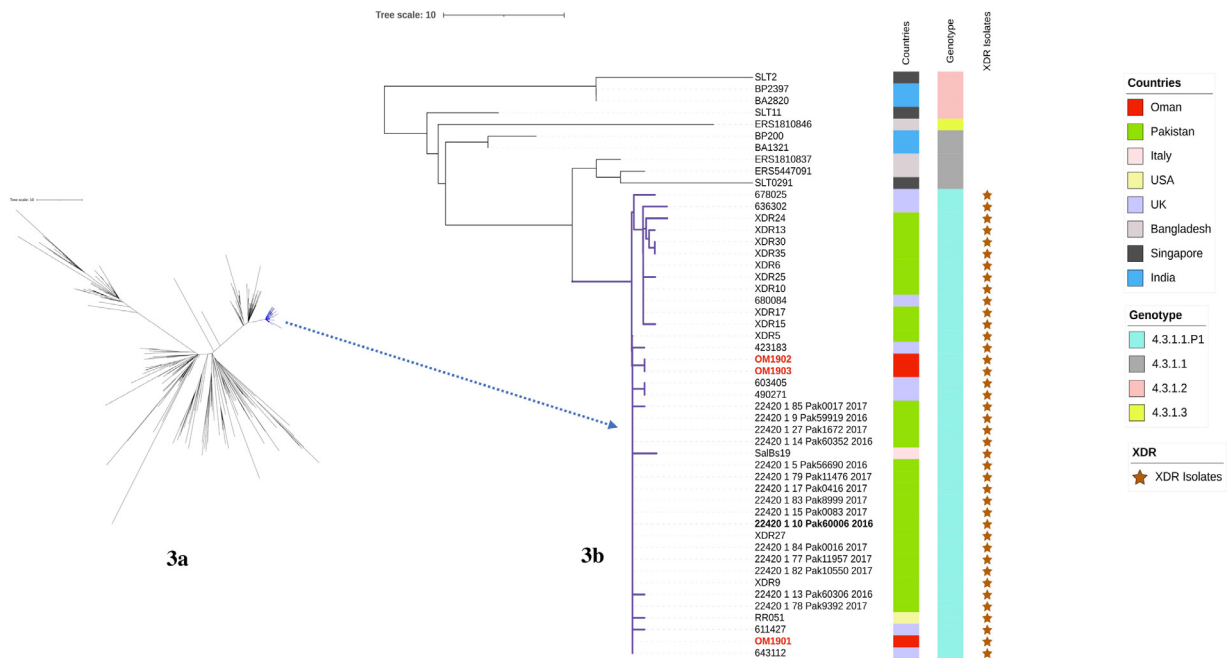


Figure 3. Phylogenetic trees for *S. Typhi* ST1 core genome comparisons on Pathogenwatch. (a) Unrooted tree of all the core genomes since 2015 (ST1). The blue clade represents all genomes that have the 4.3.1.1 P1 genotype and 0–3 allele variations. (b) Dendrogram of selected strains of genotype 4.3.1.1 P1 (XDR isolates) and strains of other genotypes. The tree was constructed using core genomes on Pathogenwatch and annotated on iTOL. The Omani strains have the closest matching (0–3 allele variations) with the 2016 XDR outbreak strains from Pakistan, and with XDR strains from the UK, the USA, and Italy (purple clade branch).

Table 2
Antimicrobial susceptibility testing (AMST) results

Sample name	AMST (disk diffusion)					MIC ($\mu\text{g/ml}$)		ESBL
	AMP	CHL	CIP	SXT	AZM	CRO ($\mu\text{g/ml}$)	MEM ($\mu\text{g/ml}$)	
OM1901	R	R	R	R	S	R (> 256)	S (≤ 1)	Positive
OM1902	R	R	R	R	S	R (> 256)	S (≤ 1)	Positive
OM1903	R	R	R	R	S	R (> 256)	S (≤ 1)	Positive

R: resistant, S: sensitive, AMP: ampicillin, CHL: chloramphenicol, CIP: ciprofloxacin, SXT: trimethoprim-sulfaxazole, AZM: azithromycin, CRO: ceftriaxone, MEM: meropenem, ESBL: extended-spectrum beta lactamase

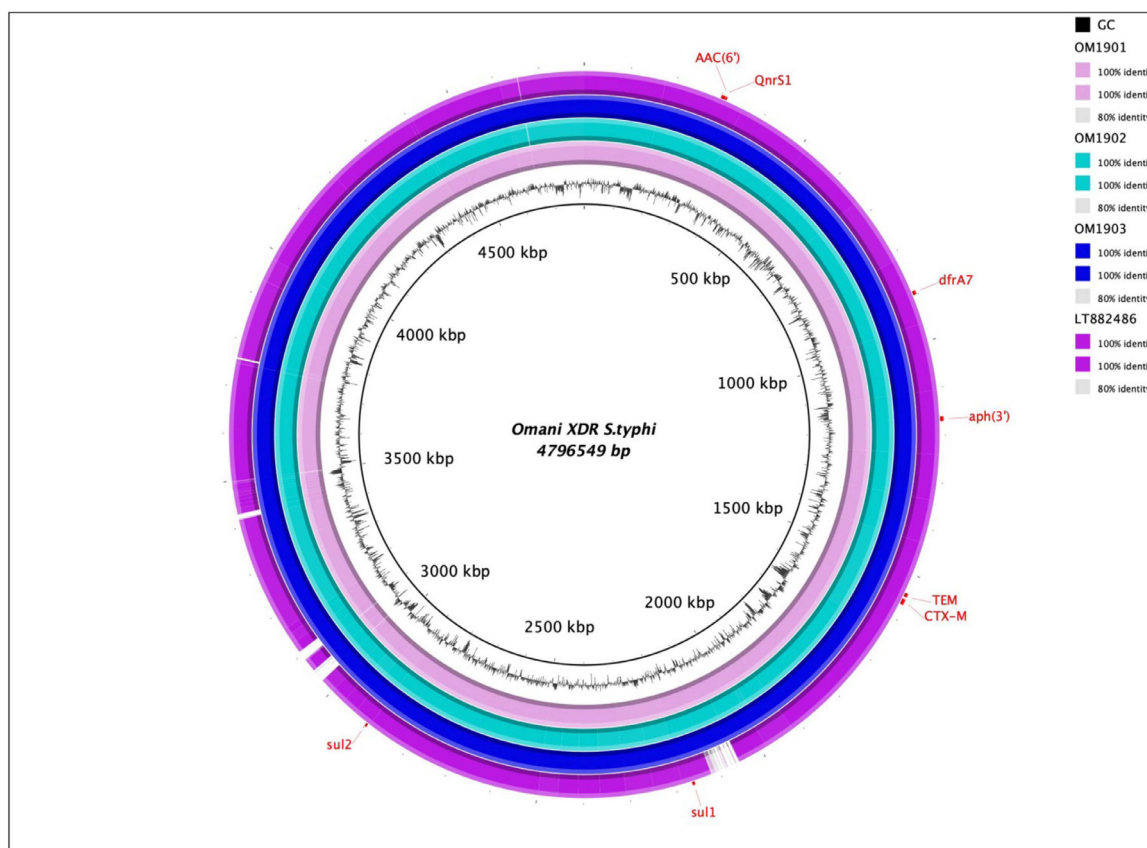


Figure 4. Circular genomic map representing comparisons of three Omani *Salmonella* Typhi XDR strains with the reference *S. Typhi* strain from the Pakistan outbreak (GenBank genome accession number LT882486). The innermost circle represents GC content. The three *S. Typhi* isolates were matched using BLASTn, giving a color-shade gradient from lightest (80% similarity) to darkest (100% similarity). The outer ring shows antibiotic resistance genes in red. The graph was created using the BLAST Ring Image Generator (Alikhan, 2011).

strains isolated from all sample types — enteric and non-enteric — sent to the CPHL from all over the country for serotyping and antibiotic susceptibility testing. According to the data from this surveillance program, resistance to first-line antibiotics and ciprofloxacin is increasing: in 2018, 39.3% and 81.7% of the *Salmonella* species and *S. Typhi* strains, respectively, were recorded as non-susceptible to ciprofloxacin (OMASS, 2019). All of the *S. Typhi* strains were susceptible to ceftriaxone in that year, while, between 2014 and 2017, 2.9% were recorded as non-susceptible and 21% of the isolates were MDR. The percentage of *S. Typhi* strains that were not susceptible to other antibiotics in 2018 was 27.3% each for ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (OMASS, 2019).

The same issue has been encountered in many countries worldwide, including neighbouring ones. In 2008, Rotimi *et al.* reported that 44% of *S. Typhi* isolates in Kuwait and the UAE were resistant to ciprofloxacin. With regard to all *Salmonella* species, the rate of resistance to ceftriaxone was 1.6% in each country, while 9.8% of Kuwait isolates were MDR, compared with 4.1% of UAE isolates (Rotimi *et al.*, 2008).

The situation has been similar in developed countries; for example, Public Health England (PHE, 2016) reported approximately 300 cases of laboratory-confirmed typhoid fever each year, of which > 90% were acquired abroad — the majority from Pakistan, India, and Bangladesh. The majority of *S. Typhi* isolates received by the Gastrointestinal Bacteria Reference Unit (GBRU) are of the globally epidemic and MDR clone H58 (Wong *et al.*, 2016).

The XDR *S. Typhi* outbreak in Pakistan started in 2016 in the province of Sindh, with the first case reported on November 30 (Yousafzai *et al.*, 2019). The cases were primarily from the cities of Hyderabad and Karachi (Klemm *et al.*, 2018).

This strain has now spread beyond Pakistan, and has been reported in several other countries, including the UK (Nair *et al.*, 2021), Canada (Wong *et al.*, 2019), and the USA (Chatham-Stephens *et al.*, 2019). In October 2019, according to the European Centre for Disease Prevention and Control, the Health Protection Surveillance Centre in Ireland reported three cases of XDR *S. Typhi* that were linked to histories of recent travel to Pakistan (European Center for Disease Prevention and Control

Table 3
Antimicrobial genes, plasmids, and MLST results

Sample ID	Year	Country	Resistance genes (ResFinder)	Plasmids (ResFinder)	cMLST (ST)	cgMLST (ST)
OM1901	2019	Oman	qnrS1, dfrA7, blaCTX-M15, blaTEM1B, catA1, aph(3)lb, aph(6)ld, sul1, sul2	IncQ1 (100%); IncY (98.8%)	ST 1	ST 83019
OM1902	2019	Oman	qnrS1, dfrA7, blaCTX-M15, blaTEM1B, catA1, aph(3)lb, aph(6)ld, sul1, sul2	IncQ1 (100%); IncY (98.8%)	ST 1	ST 83019
OM1903	2019	Oman	qnrS1, dfrA7, blaCTX-M15, blaTEM1B, catA1, aph(3)lb, aph(6)ld, sul1, sul2	IncQ1 (100%); IncY (98.8%)	ST 1	ST 83019
2018K-0753.Pak	2018	Pakistan	qnrS1, dfrA7, blaCTX-M15, blaTEM1B, catA1, aph(3)lb, aph(6)ld, sul1, sul2	IncQ1 (100%); IncY (98.8%)	ST 1	ST 83019
LT882486	2016	Pakistan	qnrS1, dfrA7, blaCTX-M15, blaTEM1B, catA1, aph(3)lb, aph(6)ld, sul1, sul2	IncQ1 (100%); IncY (98.8%)	ST1	ST 83019

2019). Other XDR *S. Typhi* cases linked to the Pakistani outbreak were reported in Australia and Denmark (Howard-Jones et al., 2019; Engsbro et al., 2019). Sequencing studies that were performed in some of these cases confirmed their similarity to the Pakistani clone. The first case of the spread of this strain in the Middle East was reported in January 2021 (Bharathan and Kurian, 2021).

Due to Pakistan’s geographical proximity to Oman, travel to and from the country is common, especially for Pakistani workers who reside in Oman and return home for holidays. This makes it challenging to avoid acquiring this spreading strain.

To our knowledge, this is the second report from the Middle East of XDR *S. Typhi* detected in relation to travel to Pakistan. In fact, while this report was being prepared, five more cases of this travel-related strain were announced (unpublished data).

XDR *S. Typhi* evolved from a highly resistant clone found in the countries of the Indian subcontinent and South Asia (Bharathan and Kurian, 2021). Genome sequencing analysis of the XDR *S. Typhi* strains from Pakistan showed that they were associated with the H58 haplotype, with numerous resistance determinants and a tendency for global spread (Klemm et al., 2018; Wong et al., 2015).

The relatedness of our strains to the Pakistani strains was clearly shown by the cMLST and cgMLST analyses. In addition, the cgMLST analysis showed similarities among the XDR isolates from Oman, Pakistan, the UK, and India, with a difference of 1–3 alleles, suggesting that they were the same clone. This clearly shows that this strain is spreading widely (Nair et al., 2018; Sah et al., 2020).

The resistance genes of the three strains detected by WGS analysis included *bla*_{TEM-1 B} (ampicillin), *qnrS1* (quinolones), *dhfrA7* (trimethoprim), *catA1* (chloramphenicol), *sul1,2* (sulfonamide), *aph(3)lb*, *aph(6)ld* (aminoglycosides), and *bla*_{CTX-M-15} (cephalosporins). These are carried in the IncY plasmid. These findings are similar to those for other XDR *S. Typhi* strains that have been linked to the emerging outbreak, and were reported earlier (Klemm et al., 2018). Moreover, another plasmid (IncQ1) was detected among our strains, showing a similarity to findings in isolates collected in Pakistan in 2016 and 2018 (Tagg et al., 2020). In fact, Nair et al. found that the IncY plasmid was absent in some of these strains, while there was evidence of a 4 kb ISEcpl-*bla*_{CTX-M-15}-tnp gene cassette insertion into the chromosome at one of three integration points. Further testing of resistance genes in our strains to check for chromosomal integration was not possible due to the limited testing capacity.

The spread of XDR *S. Typhi* has limited the treatment options to only azithromycin and carbapenems. Hence, eliciting a proper travel history from patients suspected to have typhoid fever is crucial in deciding on empirical therapies for such cases. In addition, this should trigger the application of strict infection control measures in the community and in healthcare institutions where these patients are admitted, in order to contain its spread promptly and effectively.

As a result of this surveillance program, these cases could be detected and linked with each other, despite the fact that they were scattered widely across the country. This supports the need to maintain strong surveillance systems for monitoring such organisms of public health concern.

6. Conclusion

The spread of XDR *S. Typhi* is a significant threat to healthcare systems. Prompt patient management and infection control measures are essential. Molecular characterization of such strains is important in linking them to closely related strains that are spreading globally. This should prompt the development of strong NGS programs, with more frequent analysis of such organisms. In addition, the surveillance of antimicrobial resistance (AMR) is essential in monitoring organisms of importance to public health.

Ethical approval

This work was performed as part of the national surveillance system. All work in relation to the cases was reported anonymously. Hence, ethical approval was not required.

Conflicts of interest

The authors have declared no conflicts of interest.

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