



Genome Note

Draft genome of a macrolide resistant XDR *Salmonella enterica* serovar Paratyphi A strain using a shotgun sequencing approachA. Khatoon^a, H.M.T. Malik^a, M. Aurongzeb^a, S.A. Raza^a, A. Karim^{a,b,*}^aJamil-ur-Rahman Center for Genome Research, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi, Pakistan^bAix Marseille Univ, CNRS, IGS, Structural and Genomic Information Laboratory (UMR7256), Mediterranean Institute of Microbiology (FR3479), Marseille, France

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ABSTRACT

Objectives: *Salmonella enterica* serovar Paratyphi A, the causative pathogen of enteric fever, is a major public-health concern affecting millions of people around the world. We conducted whole-genome sequencing and analysis of a novel macrolide-resistant *Salmonella* Paratyphi A strain isolated from Karachi, Pakistan.

Methods: Genomic DNA of *Salmonella* Paratyphi A strain JRCGR-AK14 was sequenced on a MiSeq platform. Read quality was evaluated and paired-end reads were assembled into contigs and scaffolds. The quality of contigs and scaffolds was evaluated and assembled contigs were annotated. Virulence genes, antimicrobial resistance genes (ARGs), tRNAs, rRNAs, coding sequences and clustered regularly interspaced short palindromic repeats (CRISPRs) were identified. ARGs and mutations in quinolone-resistance determining regions (QRDRs) were identified by Antimicrobial Resistance Identification By Assembly (ARIBA) and ResFinder. Known and unknown mutations in the QRDRs were predicted.

Results: The genome of *Salmonella* Paratyphi A was calculated at 4529866 bp with 4381 genes and 1088 hypothetical proteins. Several putative genes coding for multidrug efflux pumps were identified. In addition, gene mutations conferring resistance to nitrofurantoin (e.g. *marA*, *mdsC*, *Escherichia coli* *soxS*), pulvomycin (e.g. H-NS, *cpxA*, *E. coli* EF-Tu) and fosfomycin (CRP, *kdpE*, *E. coli* *glpT*) were also identified. Several ARGs along with the mobile genetic element transposon Tn10 were also identified. It is evident from the results that diverse redundant mechanisms are involved in regulation of drug resistance in this strain.

Conclusion: The current findings provide valuable data for understanding the multidrug resistance and pathogenic characteristics of clinical *Salmonella* Paratyphi A isolates.

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Salmonella enterica serovar Paratyphi A, the potent causative pathogen of typhoid fever, is a major public-health concern affecting millions of people around the world for decades. Poor hygiene conditions is one of the root causes of *Salmonella* Paratyphi A infection, which is transmitted exclusively in humans. According to the US Centers for Disease Control and Prevention (CDC), *Salmonella* causes approximately 1.2 million illnesses, 23 000 hospitalisations and 450 deaths each year in the USA [1].

In the Southeast Asia region, the incidence of typhoid fever is approximately 110 cases/100 000 population every year, rendering it a hotspot for *Salmonella* Paratyphi A-associated infections and making it the third highest incidence region for this infection [2]. Pakistan falls into this hotspot region. Moreover, extensive use of antibiotics such as trimethoprim/sulfamethoxazole, ampicillin and chloramphenicol has resulted in the appearance of multidrug resistance in *Salmonellae* since the late 1980s. Multidrug-resistant *Salmonella* Paratyphi A have markedly increased in Southeast Asia, Central Asia, South America and Africa since the 1990s [3]. *Salmonellae* are becoming resistant to many antibiotics, however the macrolide azithromycin remains active against extensively drug-resistant (XDR) typhoid [4]. However, increased resistance to macrolide–lincosamide–streptogramin B (MLS_B) antibiotics severely limits treatment options [5].

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Against the background of scarce genome-based data, whole-genome sequencing of an emerging novel macrolide-resistant *Salmonella* Paratyphi A strain isolated in Pakistan was performed in this study.

A presumptive *Salmonella* isolate was collected from the blood of an 18-year-old female in Karachi, Pakistan. The isolate was identified as *Salmonella* Paratyphi A based on biochemical and serological tests. Antimicrobial susceptibility was determined by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The characterised bacterial culture was grown overnight in tryptic soy agar at 37 °C. Genomic DNA for sequencing was extracted from the harvested bacterial cells using a QIAamp® DNA Mini Kit (QIAGEN). A paired-end library was constructed using a Nextera XT DNA Library Kit (Illumina Inc., San Diego, CA, USA) and sequencing was performed on a Solexa MiSeq platform (Illumina Inc.). Read quality was checked using FastQC, and KmerGenie v.1.0751 was used for the estimation of optimal k-mer length. Next, paired-end reads were quality trimmed and were de novo assembled into contigs and scaffolds using Unicycler v.3.0. The quality of contigs and scaffolds was evaluated by QUAST 5.02. software. Prokka v.1.12 [>500 bp; e-value cut-off default (10_6)] software for rapid annotation of prokaryotic genomes was used to annotate the assembled contigs.

tRNA genes were identified by tRNAscan-SE 2.0. rRNA genes were identified by RNAmmer 1.2, and coding sequences were discovered with Prokka v.1.12 with manual curation on ARTEMIS. Clustered regularly interspaced short palindromic repeats (CRISPRs) were

found with CRISPRCasFinder. Python script (<https://github.com/katholt/genotyphi/blob/master/README.md>) was used for identification of genotypes, and mutations in the quinolone resistance-determining regions (QRDRs) were identified by ResFinder 3.1. Characteristics of the 26 longest scaffolds of the *Salmonella* Paratyphi A strain (JRCGR-AK14) were plotted using Circos (Fig. 1). From the outer circle inwards; scaffolds; genes; gene count of 10 kb; average gene size of 10 kb; gene count of 20 kb; average gene size of 20 kb; GC skew; and GC content in a window = 1000 bp.

A total of 367710 paired-end reads (2 × 300-bp) were generated with 24 × coverage for strain JRCGR-AK14. Strain JRCGR-AK14 was identified by multilocus sequence typing (MLST) as *Salmonella* Paratyphi A ST129. The genome size of JRCGR-AK14 is 4529866 bp with a GC content of 52.17% and contains 4381 genes, 78 tRNAs, 1 tmRNA, 3 rRNAs, 2 CRISPRs and 1088 hypothetical proteins. Other results are given in Supplementary Table S1. No plasmids were detected in the genome. Phylogenetic analysis of the 16S rRNA gene was performed using MEGA-X software, multiple alignment was performed by ClustalW, and the phylogeny was constructed using the neighbour-joining method. FigTree v.1.4.4 was used for graphical visualisation (Supplementary Fig. S1).

The minimum inhibitory concentration (MIC) for azithromycin was found to be 2 µg/mL. Genome analysis of *Salmonella* Paratyphi A strain JRCGR-AK14 revealed various drug efflux systems, including major facilitator superfamily (MFS), SdiA quorum-sensing-mediated signalling, resistance-nodulation-cell division (RND) members (*acrA* and *acrB*), multidrug and toxin extrusion

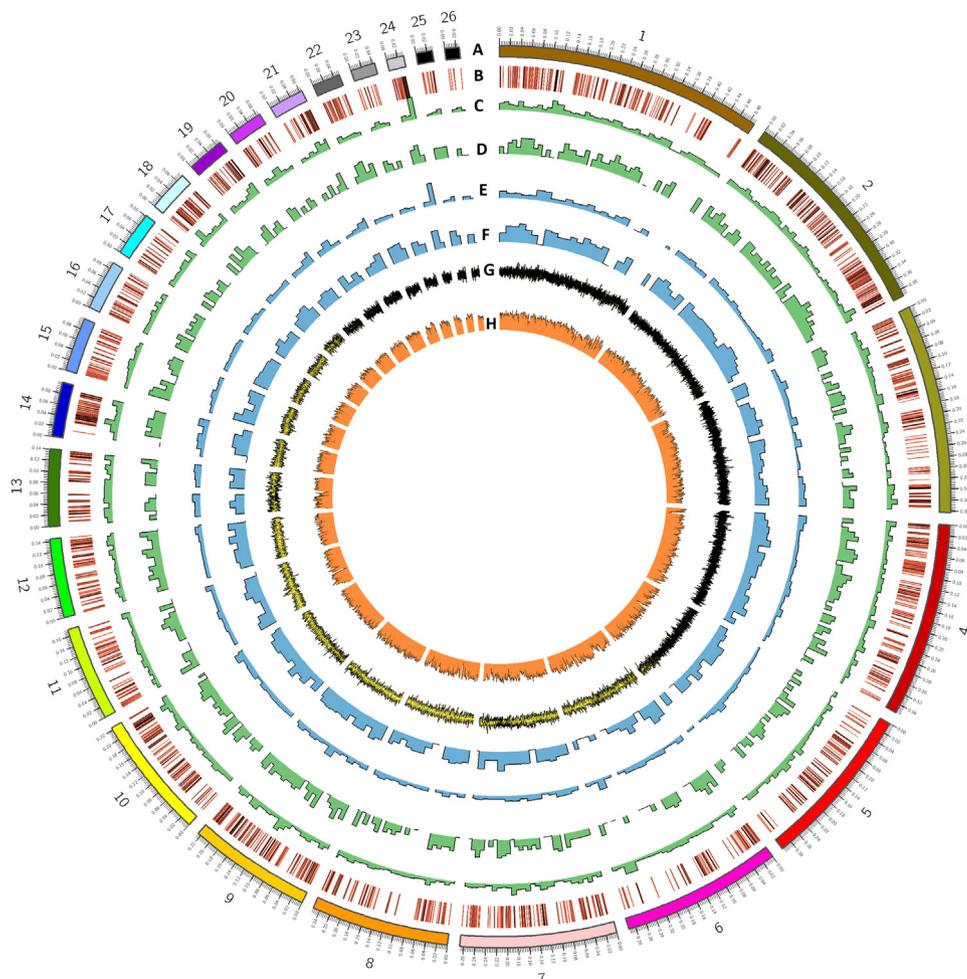


Fig. 1. Genome map of *Salmonella enterica* serovar Paratyphi A strain JRCGR-AK14: (A) scaffolds; (B) genes; (C) gene count of 10 kb; (D) average gene size of 10 kb; (E) gene count of 20 kb; (F) gene average size of 20 kb; (G) GC skew; and (H) GC content in a window = 1000 bp.

(MATE), multiple antibiotic resistance protein, ATP-binding cassette (ABC) and multidrug resistance transporter (EmrD). Moreover, resistance genes such as *marA*, *mdsC*, *E. coli soxS*, *bacA*, *mdtK*, *E. coli nfsA* with mutation involved in nitrofurantoin resistance, *h-ns*, *cpxA*, *E. coli EF-Tu* mutants involved in pulvomycin resistance, *CRP*, *kdpE*, *E. coli glpT* with mutation involved in fosfomycin resistance, *E. coli soxR* with mutation conferring antibiotic resistance, *msbA*, *pata*, *baeR*, *emrR*, *E. coli marR* mutant conferring antibiotic resistance, *pmrF*, *Haemophilus influenzae* PBP3 conferring resistance to β -lactam antibiotics, *aac(6')-Iaa*, *aph(3'')-Ib*, *bla_{TEM-1B}*, *catA1*, *sul2*, *sul1*, *dfrA7* and a mobile genetic element transposon Tn10 similar to several putative insertion sequence transposases were found. Known (*gyrA* and *parC*) as well as unknown (*parC*, *gyrB* and 16S_*rrsD*) mutations in the QRDRs were also detected. Details of the mutations are given in Supplementary Table S2. Moreover, six prophages were also observed in the sequence by PHAST. These findings reveal that complex and diverse redundant mechanisms involving various pathways regulate drug resistance in this strain.

To the best of our knowledge, this is the first reported draft genome sequence of a macrolide-resistant XDR *Salmonella* Paratyphi A strain. The occurrence of such multidrug-resistant pathogenic strains in the environment is linked to widespread use of antimicrobials, which favours the horizontal gene transfer of such genes to environmental strains and facilitates the rapid distribution of such genes, further worsening the scenario.

GenBank accession no

The draft genome sequence has been deposited in GenBank with the accession no. SUB5262432.

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Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jgar.2019.09.001>.

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