Genomic identification of the emerging multidrug-resistant Salmonella Virchow monophasic variant causing septic arthritis of the knee joint

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Abstract

In this study, a 4-year-old girl presented with a case of septic arthritis and fever. Salmonella was isolated from her knee effusion and feces; isolates were confirmed to be a novel S. Virchow monophasic variant (Salmonella 6,7,14:r:-) based on core genome MLST (cgMLST), CRISPR typing combined with analysis of the fljAB operon. Whole-genome sequencing analysis revealed that replacement of the fljAB operon by a 4.8 kb cassette from E. coli caused the nonexpression of phase-2 flagellar antigen in S. Virchow monophasic variant. Additionally, the acquisition of Salmonella genomic island 2 (SGI2) with an antimicrobial resistance gene cassette enabled the isolates to be multidrug-resistant (MDR) to chloramphenicol, tetracycline, trimethoprim, and sulfamethoxazole. The emerging of MDR S. Virchow monophasic variant causing human infection should be concerned in the national Salmonella surveillance system.

Short communication

Genomic identification of the emerging multidrug-resistant *Salmonella* Virchow monophasic variant causing septic arthritis of the knee joint

Short running title: Emerging of clinical MDR *S.* Virchow serovariant

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Summary

In this study, a 4-year-old girl presented with a case of septic arthritis and fever. *Salmonella* was isolated from her knee effusion and feces; isolates were confirmed to be a novel *S.*Virchow monophasic variant (*Salmonella* 6,7,*14* :r:-) based on core genome MLST (cgMLST), CRISPR typing combined with analysis of the *fljAB* operon. Whole-genome sequencing analysis revealed that replacement of the *fljAB* operon by a 4.8 kb cassette from*E. coli* caused the nonexpression of phase-2 flagellar antigen in*S.* Virchow monophasic variant. Additionally, the acquisition of*Salmonella* genomic island 2 (SGI2) with an antimicrobial resistance gene cassette enabled the isolates to be multidrug-resistant (MDR) to chloramphenicol, tetracycline, trimethoprim, and sulfamethoxazole. The emerging of MDR *S.* Virchow monophasic variant causing human infection should be concerned in the national*Salmonella* surveillance system.

Keywords:

Salmonella enterica serovar Virchow (*S.* Virchow);*Salmonella* 6,7,14:r:-; CRISPR typing; Core genome MLST (cgMLST);*Salmonella* genomic island 2 (SGI2)

Introduction

Salmonella enterica includes more than 2500 serovars and represents a major food-borne pathogen, which mainly causes gastroenteritis. However, focal suppurative infections of almost any organ may occur and produced different characteristic clinical syndromes (Lombardi et al., 2014; Galanakis et al., 2007). Meningitis is diagnosed in less than 1% of clinical salmonellosis; *Salmonella* infections account for 0.8% of all cases of osteomyelitis. *Salmonella*infection also causes abscesses, including intra-abdominal infections, spontaneous peritonitis, splenic abscess, and knee joint infections (Ispahani et al., 2000). Many non-typhoid *Salmonella* serotypes, including *S.* Enteritidis, *S.* Typhi, *S.* Typhimurium,*S.* Newport, *S.* Choleraesuis, *S.* OhiO, and *S.*Virchow, have been reported to be the causing factor in osteomyelitis or septic arthritis in humans (Salem, 2014; McAnearney et al., 2015; Kato et al., 2012; Weston et al., 2015; Katsoulis et al., 2004; Sy et al., 2013; Morgan et al., 1990). *S.* Virchow has also been connected with meningitis in adults (Lombardi et al., 2014). In this study, the first Chinese case of infection in the knee joint by *S.* Virchow monophasic variant (*Salmonella* 6,7,*14* :r:-) is reported; this novel serovariant without the *fljAB* operon was identified by whole-genome sequencing analysis.

2. Materials and methods

2.1 Sample collection and bacterial isolation

The case of a 4-year-old girl with typhoid fever and knee arthritis is reported in this study; she was hospitalized in Nantong, Jiangsu, China. The patient presented with 39.5 °C fever and swelling of the knee joint, detected using Nuclear Magnetic Resonance (NMR). The knee effusion, as well as stool and blood samples, were subjected to bacterial detection. Samples were rinsed in selenile broth medium and incubated at 37°C for 16-24 h. The broth culture was then subculture into MacConkey agar, Blood Agar, and XLT4 Agar. The suspected colonies were subjected to species identification using a VITEK-2® Compact (bioMerieux Inc., France). The study was performed following the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of the Chinese Centers for Disease Control and Prevention (CDC).

2.2 Antimicrobial susceptibility test

The antimicrobial susceptibility testing was performed using the agar dilution method to determine the minimum inhibitory concentrations (MICs) of the isolates. Antibiotics used in the study were listed in Table 1. The results of antimicrobial resistance were interpreted according to the clinical and laboratory standard institute (CLSI) 2018 guidelines (CLSI, 2018).

2.3 Whole Genome Sequencing Analysis

Genomic DNA of bacterial isolates was extracted using the DNeasy Blood & Tissue Kits (Qiagen, Germany) according to the manufacture's instruction. Qubit® 3.0 fluorometer (Invitrogen, USA) was used to measure the DNA concentration. A total amount of 0.2 μg DNA was then used as input material for the DNA library preparations using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA), and

subsequently sequenced using the 2 × 150 bp paired-end library on HiSeq 2500 sequencing system (Illumina). Following trimming and filtering by the NGS_QC Toolkit (v2.3.3), the raw reads were subjected to *de novo* assembly by SPAdes 3.6 (Bankevich et al., 2012). The subsequent annotation of the assembled genome was performed by Prokka version 1.12 (Seemann, 2014). Plasmid were identified using Plasmid Finder 2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/). The antimicrobial resistance genes and chromosomal mutations were analyzed using ResFinder 3.2 (Zankari et al., 2012). CRISPR typing of the isolates was performed as previously described (Li, 2021).

3. Results and discussion

According to the species identification by the VITEK-2® Compact, *Salmonella* was isolated from both the knee effusion and stool samples, although not from the blood. Serotype identification showed that the 2 strains (YZU1797 and YZU1798) displayed 6,7,*14* :r:- using the *Salmonella* serotype identification kit (SSI, Denmark). The phase-2 flagella antigen was not detected using the serotype identification kit repeated five times. It was expected that the strains would be identified as *S.* Infantis (6,7,*14* :r:1,5) due to its predominant infection in infants and children (Ranjbar et al., 2018). Nearly 80% of patients infected with*S.* Infantis were less than 12 years of age, and these isolates were recovered from stools, urine, blood or other biological fluids (Ranjbar et al., 2018).

Whole-genome sequencing analysis was subsequently performed to obtain the genome sequence of YZU1797 and YZU1798. The raw reads were then uploaded to the European Nucleotide Archive database under accession number PRJEB40529. The core genome MLST (cgMLST) was executed to reveal that both strains were *S.* Virchow (6,7,14:r:1,5). Since the*fljAB* operon (*fljA, fljB* , and *hin* genes) was involved in the synthesis of phase-2 flagella antigen (Lucarelli et al., 2012). Homology analysis was then carried out on the *fljAB* operon and its surrounding sequences in YZU1797 and YZU1798 with the published*S.* Virchow genomes (Figure 1). The results showed that both strains lost the fragment of *fljAB* operon, which was subsequently replaced by a ˜4.8 kb fragment obtained from *E. coli* . With nomenclature similar to the of the *S.* Typhimurium monophasic variant (*Salmonella* 4,[5],12:i:–) (Lucarelli et al., 2012), the serotype of both strains was named as *S.* Virchow monophasic variant (*Salmonella* 6,7,*14* :r:-).

Sixteen published genomes of *S.* Virchow were downloaded and indexed to construct the phylogenic tree using cgMLST analysis to reveal the genomic characteristics of both strains. Figure 2A demonstrates that the 18 strains were divided largely into 2 lineages. Two strains of ST197 belonged to lineage I; the other 16 strains, including YZU1797 and YZU1798, belonged to lineage II. Both strains demonstrated a particular relationship to strain BCW_2815 and BCW_2814 from Denmark and China, respectively (Figure 1, 2A). However, the BCW_2815 and BCW_2814 preserved the *fljAB* operon with a prophage Entero_P4 inserted at the left side of the *fljA* gene (Figure 1), indicating expression of H2 flagellar.

CRISPR typing was also performed to demonstrate the serotype of both strains and the phylogenic relationship of the 16 strains (Figure 2B). Twelve *S.* Virchow CRISPR types (VCTs) were identified amongst 18 strains, with VCT12 shared by both YZU1797 and YZU1798. The majority of spacers identified in the 2 strains were VirN and VirBN (Figure 2B); both strains were identified as *S.* Virchow as a consequence of this and their close relationship with the other *S.* Virchow strains of ST16 (Figure 2B), which is considered as the major MLST type for *S.* Virchow (Bachmann et al., 2014). Additionally, with similarities in the phylogenic tree based on cgMLST, CRISPR typing divided these 18 strains into 2 lineages (Figure 2B, Supplementary Figure S1). BCW_2818 and 82-1040 strain of ST197, located in a separate lineage I, had unique VCT1 and VCT2 types (Figure 2B, Supplementary Figure S1). In lineage II, both Chinese strains of VCT12 showed a relationship to BCW_2814 and BCW_2815 of VCT11 with difference in 2 spacers (Figure 2B). The perfect correspondence between CRISPR typing and cgMLST (Supplementary Figure S1) confirmed that CRISPR typing could be used as an efficient tool to analyze the phylogenic relationship of the isolates belonging to the same *Salmonella* serotype (Li et al., 2018).

The minimum inhibitory concentration (MIC) of 15 antibiotics for YZU1797 and YZU1798 showed that both strains were multidrug-resistant (MDR) to chloramphenicol, tetracycline, trimethoprim, sulfamethoxazole,

and nalidixic acid (Table 1). Genome sequencing analysis revealed that the presence of *cmlA9* , *sul1* , *drfA1* , *tetA(G)* , and*gyrA* (S83F) in both isolates were involved in the antimicrobial resistance phenotype. Identification of antimicrobial resistance genes in the other 16 *S.* Virchow genes confirmed that the BCW_2814 shared the same antimicrobial resistance genotype with YZU1797 and YZU1798 (Figure 2A). Considering genome sequencing results demonstrated that no plasmid existed in either strain, the antimicrobial resistance genes should be located in the chromosome. Further analysis of these genes revealed that both *S.* Virchow monophasic variants acquired a ˜43kb *Salmonella* genomic island 2 (SGI2), including the antimicrobial resistance gene cassette (*drfA1-cmlA9-tetR-tetA(G)-sul1*) (Figure 3). The SGI2 was first reported in 2005 an *S.* Emek isolate and was previously considered a variant from SGI1 (SGI1-J) (Levings et al., 2005). The SGI2 or SGI1-J was also detected in 3 clinical *S.* Virchow isolates from human blood in 1993 and 1994 in China (Doublet et al., 2009). Within the SGI2, the integron carrying the antimicrobial resistance gene cassette was inserted in the S023 reading frame flanked by a 5 bp target site duplication (TSD) (Figure 3), indicating that it was incorporated by transposition (Hall, 2010).

Conclusion

We report a novel clinical *S.* Virchow monophasic variant (*Salmonella* 6,7,*14* :r:-) resulting in an infection in the knee joint of a 4-year-old girl. Replacement of the *fljAB* operon by a cassette from *E. coli* leads to the lack of phase-2 flagellar antigens in *Salmonella* 6,7,14:r:-. CRISPR typing and cgMLST revealed that the *S.* Virchow monophasic variant isolates (YZU1797 and YZU1798) were closely related to the previously reported human isolate from China, *S.* Virchow BCW_2814. Compared with the 16 published *S.* Virchow genomes, both strains obtained a unique ˜43kb SGI-2 fragment, including *cmlA9* ,*sul1* , *drfA1* , and *tetA(G)* genes, providing multidrug resistance to chloramphenicol, tetracycline, trimethoprim, and sulfamethoxazole. Therefore, with the emerging of the MDR *S.*Virchow monophasic variant causing human salmonellosis, surveillance of*Salmonella* infections in human is recommended.

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Conflict of interest statement

The authors declare no conflict of interest.

Data Availability Statement

The data sets supporting the results of this article are included within the article and its additional files.

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Figure Legends

Figure 1. Sequence analysis of the *fljAB* operon in two*Salmonella* 6,7,*14* :r:- isolates. MAUVE software was used to compare sequence of the *fljAB* operon and its neighboring regions in *Salmonella* 6,7,*14* :r:- with the corresponding sequences in *S.* Virchow BCW_2814 and BCW_2815. The red rectangle represents the phage Entero_P4 inserted in the site close to the*fljAB* operon, while the blue rectangle depicts a fragment acquired from *E. coli* .

Figure 2. Phylogenic relationship of the two *Salmonella*6,7,*14* :r:- isolates with 16 reported *S.* Virchow strains.(A) The phylogenic tree of 2 *Salmonella* 6,7,*14* :r:- isolates and 16 *S.* Virchow strains based on the cgMLST analysis. The MLST type (ST), CRISPR type, and antimicrobial resistance genes are labelled at the right side with different colors and a black box, respectively. (B) CRISPR typing of 2 *Salmonella* 6,7,*14* :r:- isolates and 16 *S.* Virchow strains. The spacer names are shown in the upper side of the picture with Vir + NO (VirN) for CRISPR 1 and VirB + NO (VirBN) for CRISPR 2, respectively. The black box represents the strain carrying the spacer. The maximum parsimony tree was constructed using BioNumerics version 7.5 software (Applied Maths, France).

Figure 3. Homology analysis of SGI2 fragment between*Salmonella* 6,7,*14* :r:- and *S.* Emek. The red arrows represent the antimicrobial resistance genes, and the sky-blue arrows represent genes located in SGI2, while the dark blue arrows show the genes at both side of the SGI2. The sequence between 2 TSD sites depicts the integron inserted into the SGI2 site through transposition.

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`Table 1.docx` available at https://authorea.com/users/471193/articles/562823-genomic-identification-of-the-emerging-multidrug-resistant-salmonella-virchow-monophasic-variant-causing-septic-arthritis-of-the-knee-joint