

Tracing the source of blaCTX-M-15 producing ST34 *Salmonella typhimurium* isolated from feces and wound secretion

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Research

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Abstract

Background *Salmonella enterica* serovar typhimurium infection is common in food-borne diseases, isolating it from surgical incisions is rare. The aim of this study was to trace the transmission source of a surgical incision infected with *Salmonella typhimurium* in a Yunnan Province hospital patient and elucidate the underlying molecular mechanisms of antibiotic resistance.

Methods Primers were designed to amplify the drug resistant genes by polymerase chain reaction (PCR). Susceptibility to antibiotics was determined using Etest. The identical macrorestriction profiles were analyzed by pulsed field gel electrophoresis (PFGE) using XbaI. The two isolates were characterized using an agglutination test and multilocus sequence typing (MLST).

Results The MLST analysis revealed that isolates SM043 and SM080 belonged to the same genotype ST34, and the PFGE revealed that SM043 and SM080 had high similarity. The two *S. typhimurium* strains SM043 and SM080 were resistant to third generation cephalosporins. SM043 harbored blaCTX-M-15 , blaTEM-1 , qnrS-1 , qnrB , and acc-3 resistance genes, while blaCTX-M-15 , blaTEM-1 , blaCMY-2 , qnrS-1 , and acc-3 were detected in SM080.

Conclusions The source of the surgical incision infection by *S. typhimurium* may be a hospital-derived transmission. Thus, it is critical to strengthen hospital sanitation by addressing hand hygiene and sterilization of the overall operational environment to avoid outbreaks of *Salmonella* nosocomial infections.

1. Background

Salmonella is a member of the Enterobacteriaceae family, which was originally characterized by the ability of the bacteria to metabolize citrate as a sole carbon source [1]. Members of the genus *Salmonella* cause a well-characterized spectrum of disease in humans, which range from asymptomatic carriage to fatal typhoid fever. Third generation cephalosporins are commonly used as first-line antibiotics to treat invasive infections or enteric fevers caused by *Salmonella* spp. due to their pharmacodynamic properties and the low prevalence of resistance [2, 3]. Our previous study found that the drug resistance rate to third generation cephalosporins was low in the Yunnan Province [4]. Therefore, the resistance mechanism of *Salmonella* to third-generation cephalosporins in this region is still not available. However, outbreaks or cases of infection caused by *Salmonella* resistance to extended-spectrum cephalosporins have been reported around the world [5–7]. Recently, the growing antibiotic resistance of *Salmonella enterica* serovar typhimurium is creating new challenges for the control and prevention of potentially lethal infections [8].

Salmonella typhimurium was first isolated in cattle in 1988; the infections caused by it are more common in foodborne diseases. In China, *S. typhimurium* was reported as the most frequently isolated serotype of non-typhoidal *Salmonella* from foodborne illnesses [9, 10]. *Salmonella* infections involving sepsis, liver abscesses, surgical wounds, joint infections, and infected burn wounds have also been described in case reports from around world [9, 11]. In 2018, two *S. typhimurium* strains resistant to 3rd generation cephalosporins were isolated from a teaching hospital in Yunnan Province, China. (The name of the hospital has been concealed to maintain anonymity). One of the isolates was from a rare surgical incision secretion. In this study, we

aimed to determine the source of the *S. typhimurium* to prevent the salmonellosis from spreading or causing an outbreak. The antimicrobial resistance profiles of two *S. typhimurium* were analyzed simultaneously to elucidate the molecular type and drug resistance mechanisms.

2. Methods

2.1 Bacterial isolates

Two *S. typhimurium* strains, SM043 and SM080, resistant to third-generation cephalosporins were isolated from clinical specimens in 2018. SM043 was isolated from the feces of a 9-year-old patient with diarrhea and fever in October 2018. In November 2018, SM080 was isolated from an infected incision secretion from a 4-year-old child after surgery due to a car accident. The two isolates were identified by the VITEK 2 system (bioMérieux, Lyon, France). Serotypes were detected using the White-Kauffmann-Le Minor (WKL) scheme based on serological detection; the diagnostic serum of *Salmonella* (STATENS SERUM INSTITUTE, Denmark) was used to determine the serotype.

2.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by the disk diffusion method and the automated VITEK 2 Compact system with Gram-negative bacteria cards (bioMérieux, Lyon, France). The Etest method was employed to verify the drug sensitivity of 5 antibiotics, and the results were interpreted based on the CLSI guidelines [12] [13]. *Escherichia coli* (ATCC 25922) was used as the quality control strain for antimicrobial susceptibility testing.

2.3 Detection of drug-resistant genes

Bacterial chromosomal DNA was obtained with a TIANamp Bacterial DNA Kit according to the manufacturer's instructions (TIANGEN BIOTECH, Beijing, China). PCR and DNA sequence analysis were performed to confirm the presence of drug-resistant genes. The primers used in this study were described previously [14–20]. The β -lactamase genes included Ambler class A (bla_{CTX-M} , bla_{TEM} , bla_{SHV} , bla_{KPC} , and bla_{GES}), class B (bla_{NDM}), class C (bla_{CMY} , bla_{ACT} , and bla_{DHA}), and class D (bla_{OXA-48}). Moreover, genes related to quinolone activity included *qnrA*, *qnrB*, and *qnrS*, and the *aac* gene were also detected. All amplicon sequences were compared with those in the GenBank nucleotide database (www.ncbi.nlm.nih.gov/blast/).

2.4 Molecular typing

The SM043 and SM080 strains were genotyped using MLST and PFGE. Seven housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, *thrA*) were amplified according to the protocol described on the MLST website (<https://pubmlst.org>). Genotyping was carried out by referring to the molecular typing method of *Salmonella* serotype pulse field gel electrophoresis (PFGE) in PulsenetChina. *Salmonella* was entrapped with SeaKem Gold Agarose and digested with *Xba*I. The DNA fragment obtained was classified into PFGE by Chefmapper. Finally, Cluster analysis was performed with Bionumerics 7.6.

3. Results

3.1 Antimicrobial susceptibility testing

The drug-resistance profiles of SM043 and SM080 were consistent; the 3rd - and 4th - generation cephalosporins including ceftriaxone, cefotaxime, and cefepime showed high resistance. The MIC values of aminoglycoside and tetracycline were also high. The two isolates were sensitive to β -lactam compound drugs, carbapenems, macrolides, and quinolones. These results are summarized in Table 1.

Table 1
Characteristics of the two *Salmonella typhimurium* isolates

Strain	MIC(μ g/mL)					MLST	Resistance genes
	Imipenem	Ceftriaxone	Ampicillin	Ciprofloxacin	Gentamycin		
SM043	0.25	256	256	0.094	0.38	ST34	bla _{CTX-M-15} , bla _{TEM-1} , qnrB, qnrS-1, aac-3
SM080	0.25	256	256	0.094	0.38	ST34	bla _{CTX-M-15} , bla _{TEM-1} , bla _{CMY-2} , qnrS-1,

3.2 Drug-resistant genes

A total of 16 drug-resistant genes were amplified, and the two *S. typhimurium* isolates carried different drug resistance genes. β -lactamases resistance genes bla_{TEM-1} and bla_{CTXM-15}, quinolones resistance genes qnrB and qnrS-1, and aminoglycoside resistance gene Acc-3 were detected in SM043. β -lactamases resistance genes bla_{TEM-1}, bla_{CTXM-15}, bla_{CMY-2}, and qnrS-1 were detected in SM080. These results are summarized in Table 1.

3.3 Gene type

MLST analysis showed that SM043 and SM080 were defined as the single sequence type ST34. The two strains were successfully typed by PFGE and were classified into same PFGE cluster (Fig. 1.)

4. Discussion

While *Salmonella enterica* serovar typhimurium infection is common in food-borne diseases, isolating it from surgical incisions is rare. There were two *S. typhimurium* isolates from a hospital in the Yunnan Province in 2018, one from an outpatient and one from an inpatient. The first case, SM043, was isolated from fecal specimens from a pediatric outpatient with diarrhea in October 2018. The second case, SM080, was isolated from a child who developed a surgical wound infection after emergency trauma surgery in November 2018. PFGE analysis of the two *Salmonella typhimurium* isolates exhibited an identical macrorestriction profile. Further, MLST analysis defined the two *S. typhimurium* strains as the single sequence type ST34. Based on the genotype results of PFGE and MLST analysis, the genetic homology of the two isolates was high.

Environmental swabs from the bed linens, stethoscopes, doorknobs, and the hand swabs obtained from doctors and nurses, were collected and tested. However, *S. typhimurium* was not isolated from any of the environmental surfaces. According to the field investigation and analysis, we know that both the trauma surgery emergency department and pediatric outpatient department of the hospital were located in a temporary building due to construction in the hospital. Both children were confirmed patients in the temporary hospital building. Healthcare-associated infections (HAIs) are known to be key causes for morbidity and mortality in hospitalized patients [21]. Previous studies have suggested that environmental contamination plays a significant role in HAIs and in the unrecognized transmission of pathogens [22]. Cross-transmission of these pathogens can occur via the hands of healthcare workers, who become contaminated directly from contact with patients or indirectly by touching contaminated environmental surfaces. *S. typhimurium* has increased levels of resistance to stress conditions due to various survival mechanisms; this enables it to survive for a prolonged period of time in harsh environments [23]. Unfortunately, in this study, *S. typhimurium* could not be isolated from the hospital environments. In an earlier report of the Salmonella outbreak [24], epidemiological investigation revealed that the probable source of this outbreak was leakage of sewage water in the water supply system; however, no pathogens were isolated from the water samples. Salmonella typhimurium can be present in the environment for a long time, and several studies have shown that microbial contamination of the ward environment can be a source of nosocomial infection transmission. According to the field investigation, we know that due to partial renovation and construction, the hospital's the emergency department of trauma surgery and pediatric outpatient department were relocated to a temporary building. Closer investigation and understanding of the building structure indicate that the distribution of the clinic wards and the allocation of resources in the temporary location were not sufficient in terms of complying with the prevention measures during the transitional period. First and foremost, the lack of alcohol hand sanitizers, soaps and tissues in the ward has negatively impacted the basic hand hygiene practices of patients and their families, as well as the compliance of medical personnel in the wards. In this study, surgical incision infections occurred in children as young as 4 years old, who were primarily cared for by family members. Secondly, the building layout is not reasonable, especially toilet configuration is significantly insufficient. The number of toilets in this transition site, which is an old building, is low and the space is very limited, forcing medical staff, patients and patients' families to share toilets. The utilization rate of these toilets is extremely high. Due to the lack or absence of hand sanitizers in toilets, people cannot wash their hands after using toilets. Thirdly, we also found that the janitor team only regularly clean and disinfect the wards, ward grounds, but no beds in the wards, door handles, railings, light switches, window edges, toilet door handles, and other environmental surfaces were being effectively disinfected, which likely contributed to the spread of bacteria in the environment. The hands of health care workers are the main source of pathogen transmission in hospitals. It has been reported that 20–40% of nosocomial infections are caused by microorganisms present in the hospital environment that are transmitted directly or through the hands of healthcare workers (HCW). Therefore, in this research, we speculate that *S. typhimurium* exists in the hospital environments, which serve as potential reservoirs to pose a serious threat to patients. The surgical incision infection caused by *S. typhimurium* SM080 is most likely caused by person-to-person transmission. This may be due to deteriorating hand-hygiene, poor infection control measures, and crowded hospital wards. The nosocomial infection in the emergency trauma surgery was finally controlled by the following intervention measures. Highlighting as the most important intervention is the uninterrupted provision of hand-washing consumables and improved hand-washing facilities. Second, hand hygiene and nosocomial contact

prevention education for patients and staff. Third, regular trainings for the ward custodian team, strengthening the cleaning and disinfecting practices within the ward environment. As of present time, no other nosocomial infections caused by salmonella were reported.

In China, cephalosporins and fluoroquinolones are commonly used to treat salmonellosis. Resistance to third-generation cephalosporins is increasing in *Salmonella* spp. This is mainly due to the production of acquired AmpC β -lactamases and extended-spectrum β -lactamases (ESBLs) [25]. By harboring at least one ESBL or AmpC gene, both SM043 and SM080 were resistant to 3rd - and 4th - generation cephalosporins. It is important to continuously monitor the antimicrobial resistance and genetic status in *S. typhimurium* in order to detect emerging resistance trends. This is the first time that bla_{TEM-1} , $bla_{CTX-M-15}$, and bla_{CMY-2} have been detected in *S. typhimurium* in the Yunnan province.

5. Conclusions

In summary, contaminated hospital environments can serve as potential reservoirs for pathogens such as *S. typhimurium*. *S. typhimurium* is spread not only by the fecal-oral route, but also by contact with contaminated hospital environments. Our study can help people by increasing awareness regarding the potential risks of *S. typhimurium* infections, particularly by the strains resistant to 3rd -generation cephalosporins in hospital environments. Therefore, it is very important to monitor the occurrence of ST34 *S. typhimurium* in hospital environments and to take appropriate measures for controlling *Salmonella* spp. infections.

Abbreviations

PFGE

Pulsed Field Gel Electrophoresis; MLST:Multi-Locus Sequence Typing; WKL:White-Kauffmann-Le Minor; HAIs:Healthcare-Associated Infections; HCW:Health-Care Workers

Declarations

Conflicts of Interest:

The authors declare no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Author Contributions:

ZR designed the study; ZR review and edited the manuscript; QH collected the isolates and preformed the antimicrobial susceptibility test. QH drafted the first version of this manuscript; GY performed the PFGE and drafted the first version of this manuscript; QH, GY, and LY preformed the molecular biology experiments.

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Not applicable

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Figures

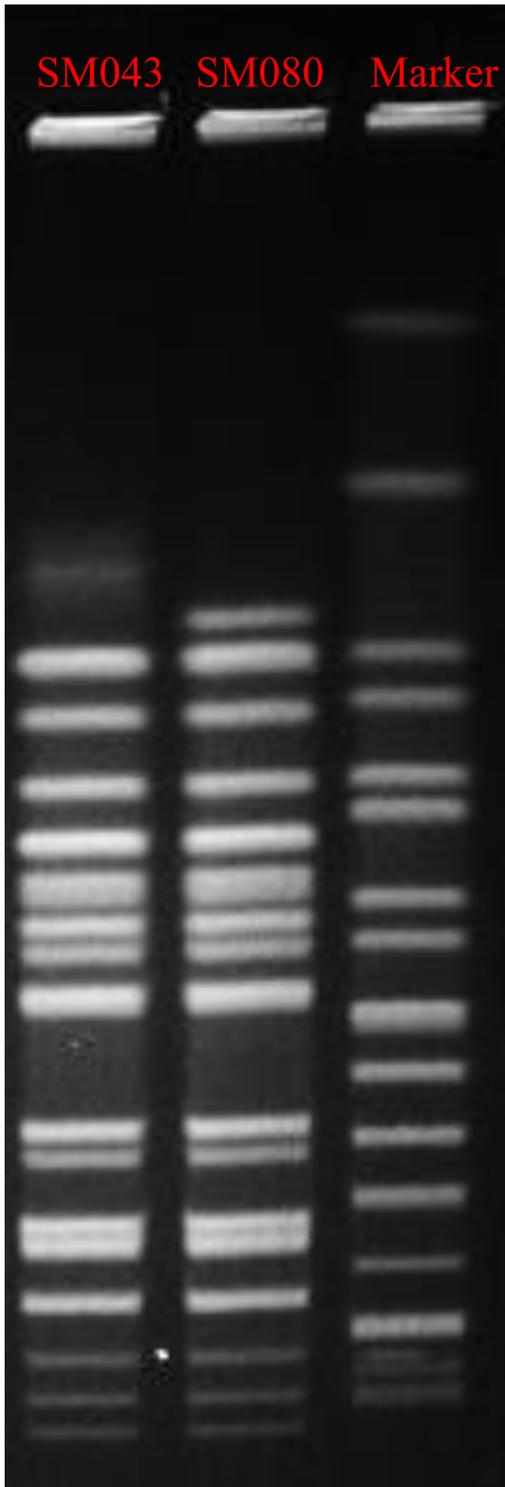


Figure 1

Pulsed-field gel electrophoresis patterns of XbaI-digested total DNA of two *Salmonella typhimurium* isolates.