Isolation of Salmonella enterica Serovar Kentucky Strain ST 198 and Its H2S-Negative Variant from a Patient: Implications for Diagnosis

M. John Albert, a Khaled Al Obaid, b Wadha Alfouzan, a Abdul Rashid Sheikh, b Edet Udo, a Hidemasa Izumiya, c Dieter M. Bulach, d Torsten Seemann b

Department of Microbiology, Faculty of Medicine, Kuwait University, Jabriya, Kuwait; bDepartment of Microbiology, Al-Amiri Hospital, Sharq, Kuwait; cNational Institute of Infectious Diseases, Tokyo, Japan; dVictorian Bioinformatics Consortium, Monash University, Clayton, Victoria, Australia

H2S-producing multiresistant Salmonella enterica serovar Kentucky strain sequence type (ST) 198 and its non-H2S-producing variant were isolated from a patient. Whole-genome comparison showed a base addition in the gene encoding molybdenum cofactor biosynthesis protein C, which could affect H2S production in the variant. Lack of H2S production has implications for diagnosis of salmonella.

CASE REPORT

A 66-year-old Kuwaiti woman was admitted to Al-Amiri Hospital, Kuwait, at the end of August 2012 because of a urinary tract infection and deteriorating renal failure. She had a 2-day history of dysuria, foul-smelling urine, abdominal pain, and vomiting. Medical history suggested that she suffered chronically from diabetes mellitus, hypertension, dyslipidemia, and advancing renal disease.

The day after admission, her urine microscopy showed white blood cells at 3+ and red blood cells at 2+ (on a scale of 0 to 3+). A semiquantitative culture of the urine sample was done using blood agar, cystine-, lactose-, and electrolyte-deficient (CLED) agar, and MacConkey agar (Oxoid, Basingstoke, Hampshire, United Kingdom) with incubation of the plates at 37°C for 24 to 48 h. Culture yielded 10^5 CFU/ml of an extended-spectrum-β-lactamase-producing Escherichia coli strain susceptible to meropenem by the Vitek II system (bioMérieux, Marcy l’Etoile, France). She was given meropenem intravenously (1 g every 12 h for 14 days). At the end of the therapy, her urinary tract infection resolved and she was discharged home.

Due to a wound in her sacral area, a swab specimen was taken on the same day as the urine sample. The swab was cultured for aerobic organisms on blood agar and MacConkey agar (Oxoid) at 37°C for 24 h and for anaerobic organisms on neomycin blood agar (Oxoid) at 37°C for 48 h.

There was a mixed growth of bacteria, including E. coli, Klebsiella pneumoniae, and a lactose nonfermenting organism on aerobic plates. Multiple colonies of the lactose nonfermenting organism were initially screened using Klöger’s iron agar (KIA) (Oxoid). After incubation at 37°C for 24 h, the slant was pink and the butt yellow without cracking of the medium. The reaction suggested that the organism did not ferment lactose but fermented glucose without production of gas, including H2S. The organism was further screened using API-20E and Vitek II systems (bioMérieux) according to the manufacturer’s instructions. It was identified as a salmonella organism in both systems (laboratory designation of the isolate as 915). As screening of multiple colonies showed the same reaction, it was assumed that the population of salmonella was homogeneous and negative for H2S production. No anaerobic organisms grew on neomycin blood agar.

Because a salmonella organism was cultured from her sacral wound, it was suspected that she could be an intestinal carrier of the organism and that the sacral wound might have been contaminated with fecal matter. A day after the sacral wound culture turned positive, her stool specimen was examined for salmonella organisms, although she did not have diarrhea. The specimen was cultured using MacConkey agar and Salmonella-Shigella agar (SSA; Oxoid) and enriched with selenite F broth (Oxoid) with subsequent subculture on Salmonella-Shigella agar. The plates were inoculated at 37°C for 24 h. MacConkey agar and SSA (Oxoid) grew lactose nonfermenting pale colonies, but the colonies on SSA had a black center. Multiple colonies were screened using KIA (Oxoid). The organism produced a pink slant and yellow butt with blackening and cracking of the medium. This indicated that it fermented glucose with H2S production. The organism was further screened using API-20E and Vitek II systems (bioMérieux), which identified it as a Salmonella species (laboratory designation of the isolate as 917). As screening of multiple colonies produced the same reaction, it was concluded that the population of salmonella in the stool was homogeneous and positive for H2S production. Wound and stool cultures were repeated a week later and salmonella was not isolated. Although no specific therapy was given against salmonella, because both the isolates were susceptible to meropenem (see below), this antibiotic may have eliminated them. Both the 915 and 917 isolates were identified as Salmonella enterica serotype Kentucky (antigens 8,20:i, z6) in an agglutination test according to the Kauffman-White scheme by using commercial antisera (Murex, Dartford, the United Kingdom; Statens Serum Institut, Copenhagen, Denmark; and Denka Seiken, Tokyo, Japan).

The clonal relationship between the two isolates was evaluated by pulsed-field gel electrophoresis (PFGE) using restriction enzyme XbaI as described previously (1). The two isolates appeared

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Address correspondence to M. John Albert, john@hsc.edu.kw. Copyright © 2014, American Society for Microbiology. All Rights Reserved. doi:10.1128/JCM.01775-14
very similar (Fig. 1), suggesting that they were clonal. Multilocus sequence typing (MLST) was performed on both isolates, and the data were submitted to the website (http://mlst.warwick.ac.uk/mlst/dbs/Senterica). Both isolates were classified as sequence type (ST) 198.

Antimicrobial susceptibility of the isolates was tested by Etest (AB Biodisk, Solna, Sweden) or the Vitek II system (bioMérieux) according to the manufacturers’ instructions and interpreted by Clinical Laboratory Science Institute (CLSI) guidelines (2). The MICs are given in Table 1. The antibiograms of the isolates were similar. They were multidrug resistant, including resistant to ciprofloxacin, but were susceptible to cephalosporins.

To determine if genomic changes could explain the H2S-negative variant, whole-genome sequencing of single colonies of isolates 915 and 917 was done at the Micromon sequencing facility, Monash University, Australia. Genomic DNA for each isolate was prepared using the Nextera XT DNA sample preparation kit (Illumina, San Diego, CA) using paired-end 300-bp reads according to the manufacturer’s instructions. Isolate 915 yielded 3.2 million reads totaling 977 Mbp, and isolate 917 yielded 2.2 million reads totaling 659 Mbp. Reads were filtered using Nesoni 0.118 to trim bases below Phred quality 10 and remove the Nextera transposome adaptor sequence (https://github.com/Victorian-Bioinformatics-Consortium/nesoni). After filtering, the final depth of coverage for 915 was 115×, and for 917 it was 77×.

Nesoni was used to align the reads for each isolate to the same reference genome (Salmonella enterica serovar Typhi CT18, GenBank accession number AL513382.1) and to report those positions differing only between isolates 915 and 917. Further analysis of the sequence polymorphisms was performed using Artemis v14.0 (Sanger Institute, Oxford, United Kingdom) by viewing the aligned short reads against the reference genome sequence; de novo assembly was not necessary. Comparison of the two S. Kentucky isolates showed mutations in three genes in isolate 915. These included insertion of five bases (TGCGC) before base 69 in codon 23 (leading to a codon frameshift) in the gene encoding the TetR family transcriptional regulator (tetR gene), insertion of a base G before base 426 of the codon 142 (leading to a codon frameshift) in the gene encoding molybdenum cofactor biosynthesis protein C (moaC gene), and deletion of a base A at base 670 of codon 224 (leading to a codon frameshift) in the gene encoding streptomycin phosphotransferase (sph gene). The single nucleotide polymorphism (SNP) coverage for isolate 915 was 47/11003, with no reads in disagreement. This depth of coverage is effectively 100% accurate for SNP calling for the Illumina sequencing platform (3, 4). Of these three mutations, the one in the moaC gene is significant (see below).

As the isolates were resistant to ciprofloxacin, the quinolone-resistant-determining region (QRDR) of the sequences was examined. Both isolates had identical changes in the gyrA gene and parC gene, resulting in amino acid substitutions Ser83Phe and Asp87Asn (in gyrA gene) and Ser80Ile (in parC gene).

In some salmonellae, a nonsense mutation in the phsA gene

![FIG 1 PFGE patterns of chromosomal DNAs of S. Kentucky strains 915 (lane 2) and 917 (lane 3) and standard S. enterica serovar Braenderup H9812 (lane 1) after digestion with XbaI enzyme.](image-url)

TABLE 1 MICs of antibiotics determined by Etest against S. Kentucky isolates 913 and 917 | MIC (µg/ml) (susceptibility or resistance) a against isolate: |  
| Antibiotic | 915 | 917 |  
| Gentamicin | 16 (R) | 16 (R) |  
| Kanamycin | 2 (S) | 2 (S) |  
| Spectinomycin | 512 (R) | 512 (R) |  
| Chloramphenicol | 4 (S) | 8 (S) |  
| Tetracycline | >256 (R) | >256 (R) |  
| Nalidixic acid | >256 (R) | >256 (R) |  
| Ciprofloxacin | >32 (R) | >32 (R) |  
| Levofloxacin | 8 (R) | 8 (R) |  
| Ampicillin | 32 (R) | 32 (R) |  
| Amoxicillin | >256 (R) | >256 (R) |  
| Piperacillin | 128 (R) | 128 (R) |  
| Cefpodoxime | 1 (S) | 2 (S) |  
| Cefotaxime | 1 (S) | 1 (S) |  
| Ceftazidime | 1 (S) | 1 (S) |  
| Cefepime | 1 (S) | 1 (S) |  
| Imipenem | 1 (S) | 1 (S) |  
| Meropenem | 1 (S) b | 1 (S) b |  
| Tigecycline | 8 (R) | 8 (R) |  
| Trimethoprim-sulfamethoxazole | 20 (S) | 20 (S) |  

a R, resistant; S, susceptible as per criteria (2).

b Rounded to the standard dilution scheme (2).
encoding the precursor of thiosulfate reductase is responsible for lack of \( H_2S \) production (5). We did not find any mutation in the \( phsA \) gene of isolate 915. This gene is essential for anaerobic reduction of thiosulfate to \( H_2S \). \( H_2S \) is toxic to mammalian cells, and the cecal mucosa detoxifies it by converting it to thiosulfate (6). Reactive oxygen radicals generated by luminal inflammation converts thiosulfate to tetrathionate. \( S. enterica \) utilizes tetrathionate as an electron acceptor in anaerobic respiration using TtrABC and TtrRS located on an SPI2 pathogenicity island (6). A non-\( H_2S \)-producing phenotype will generate more thiosulfate substrate for TtrABC/CRS anaerobic respiration based on tetrathionate. This will contribute to increased competitiveness of the organism in relation to other bacteria in the gut (7).

A cofactor for the activity of thiosulfate reductase is molybdopterin guanine dinucleotide molybdenum (MGD) (8). It consists of molybdenum covalently bound to two sulfur atoms of a tricyclic pterin moiety, molybdopterin (9). In isolate 915, we found an insertion of a G in the moaC gene causing a frameshift mutation. This protein is involved in the conversion of GTP to precursor Z, the first step in molybdenum cofactor synthesis (10, 11). We speculate that the frameshift mutation in moaC has affected the activity of the MGD cofactor of thiosulfate reductase, resulting in a lack of \( H_2S \) production. This seems to be a novel mechanism for the inability to produce \( H_2S \). Future studies could involve inserting the additional base causing the frameshift mutation in the gene and studying its effect on \( H_2S \) production. Mutations in the other two genes, tetR and sph, seem unlikely to affect \( H_2S \) production. tetR is a tetracycline resistance regulatory gene. Exposure of bacterial cells to tetracycline activates the gene, resulting in expression of resistance. Bacteria with defective tetR genes can, however, express resistance constitutively (12). The product of the sph gene modifies streptomycin, causing resistance to the antibiotic (13). Improved expression of resistance due to deletion in the gene has also been shown (14). However, studies need to be undertaken to rule out the polar effect of the altered tetR and sph genes on \( H_2S \) production by specifically altering the genes.

The ciprofloxacin-resistant \( S. \) Kentucky ST 198 clone is an international clone causing intestinal and sterile site infections in many parts of the world, including Europe, the United States, Canada, the Middle East, and Asia (15–18). \( S. \) Kentucky is normally found in chickens. It was hypothesized that the strain picked up resistance to ciprofloxacin in Egypt and spread worldwide via tourists and imported food (16). The current circulating clone is multiresistant, including resistant to ciprofloxacin and extended-spectrum cephalosporins, drugs recommended for treatment of salmonella infection (17). Our isolates were multiresistant and ciprofloxacin resistant but susceptible to cephalosporins. As resistance to ciprofloxacin is of therapeutic importance, we looked for the basis of resistance by examining the QRDR. As reported by others (15, 17), we also found genetic changes resulting in amino acid changes in the QRDR which explain the mechanism of resistance. In our patient, the \( H_2S \)-negative salmonella strain was isolated as part of a mixed flora from the wound, and even though the \( H_2S \)-positive salmonella was isolated from the stool, there was no diarrhea. Therefore, it appears that the organism was merely colonizing the patient. It is important to monitor cases to find out whether the \( H_2S \)-negative variant could be isolated from sterile sites and diarrheal cases to ascertain its pathogenicity.

An important test for identification of salmonella is the production of \( H_2S \) in media such as KIA and triple-sugar iron agar (TSA). In resource-poor developing countries (in Africa and Asia, where the strain has spread), many laboratories use a minimum number of media, such as KIA and TSA, for identification of enteric bacteria. Lack of \( H_2S \) production means this organism will be missed during screening in laboratories which do not have the resources to use a full range of identification methods. As explained earlier, the 915 mutant lost the ability to produce \( H_2S \) by a novel mechanism, and lack of \( H_2S \) production will result in increased fitness for survival and spread of the organism. It is not clear at which site mutation occurred—the intestine or wound. However, screening of multiple colonies from stool culture did not show the occurrence of the mutant. This suggested that mutation may have occurred in the sacral wound. Clinical microbiology laboratories should be aware of the potential of the \( S. \) Kentucky ST 198 clone to generate \( H_2S \)-negative mutants.

**Nucleotide sequence accession number.** The raw reads have been submitted to the Sequence Read Archive (SRA) under project accession number PRJEB6491.

**REFERENCES**


