Extremely Drug-Resistant *Salmonella enterica* Serovar Senftenberg Infections in Patients in Zambia

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Two cases of extremely drug-resistant *Salmonella enterica* serovar Senftenberg isolated from patients in Zambia were investigated by utilizing MIC determinations and whole-genome sequencing. The isolates were resistant to, and harbored genes toward, nine drug classes, including fluoroquinolones and extended-spectrum cephalosporins, contained two plasmid replicons, and differed by 93 single-nucleotide polymorphisms.

Infections with *Salmonella* that are resistant to multiple antimicrobials are associated with increased morbidity and mortality (1, 2), and the global emergence of such organisms is leaving clinicians with few, or no, treatment options (3). Recently, several studies have indicated the emergence and spread of multidrug-resistant *Salmonella* clones in Africa (4–7). Often, those clones have a different epidemiology than what is observed in developed countries, complicating control and prevention strategies (8). It is paramount to identify new multidrug-resistant clones as early as possible to hamper further dissemination (1).

Here, we describe two clinical cases of human salmonellosis in Zambia caused by extremely drug-resistant (EDR) *Salmonella enterica* serovar Senftenberg. The genomes of the isolates were sequenced to determine the multilocus sequence type (MLST) and to investigate the occurrence and genetic mechanisms of antimicrobial resistance, plasmid replicons, and genetic relatedness by single-nucleotide polymorphism (SNP) analysis.

On 18 January 2012, a 34-year-old male from Mazabuka, Zambia (72 km south of the capital, Lusaka), was admitted to the Mazabuka District Hospital. Based on medical examination, the patient was diagnosed with gastroenteritis and treated with ciprofloxacin and co-trimoxazole. Two days later, the patient was discharged, with continuing treatment on cephalixin and co-trimoxazole, but was readmitted with epistaxis and occipital pulsatile headache and treated with adrenaline and vitamin K. The patient was discharged 6 days later and scheduled to be reviewed. On 6 February the patient was referred to the renal unit of the University Teaching Hospital (UTH) in Lusaka, as he was pale, dehydrated, afebrile, tachycardic, with a scaphoid abdomen, and later he also developed uremic encephalopathy. This time, the patient was diagnosed with sepsis and chronic renal failure. Among other tests, which were all negative, a renal ultrasound was normal but the stool culture yielded *Salmonella* (isolate 588). Three days later, the patient was unable to eat and was fed through a nasogastric tube and intravenous fluids. The patient was transfused 4 days after admission, but no dialysis was initiated. The patient died the morning of 11 February 2012.

A second patient, a 30-year-old male from the Chibolya compound (2 km west of Lusaka and 74 km away from case 1), had spent most of his time in the compound. The patient was admitted to the UTH and diagnosed with gastroenteritis with tuberculosis (TB), after having been referred from a local clinic on 9 March

2012. Three months prior to admission the patient had been treated with antimicrobials due to sexually transmitted infections. Prior to admission on 9 March 2012, the patient complained of a headache, chills, fever, diarrhea, and general weakness. On 13 March, a stool sample was collected, and it yielded *Salmonella* (isolate 1028). The patient was reported to have consumed vegetables bought from the local market. Based on chest X ray, the patient was diagnosed with extrapulmonary TB and treated with rifampin, isoniazid, pyrazinamide, and ethambutol. On 16 March, the patient was also diagnosed with HIV and received emtricitabine, tenofovir, efavirenz, and co-trimoxazole.

The *Salmonella* isolates were shipped to the Technical University of Denmark (DTU) for further characterization. The isolates were serotyped, followed by MIC determinations as previously described, including the tigecycline MIC (9). Both isolates belonged to *Salmonella enterica* serovar Senftenberg and had an almost identical antimicrobial susceptibility pattern, conferring resistance to amoxicillin plus clavulanic acid (MIC, 16 µg/ml), ampicillin (MIC, ≥32 µg/ml), cefepime (MIC, ≥16 µg/ml), ceftaxime (MIC, ≥64 µg/ml), cefpodoxime (MIC, ≥32 µg/ml), ceftazidime (MIC, 128 µg/ml), ceftiofur (MIC, ≥8 µg/ml), ceftriaxone (MIC, ≥128 µg/ml), chloramphenicol (MIC, ≥64 µg/ml), ciprofloxacin (MIC, ≥4 µg/ml), gentamicin (MIC, ≥16 µg/ml), nalidixan (MIC, ≥64 µg/ml), neomycin (MIC, ≥32 µg/ml), spectinomycin (MIC, ≥256 µg/ml), streptomycin (MIC, ≥128 µg/ml), sulfamethoxazole (MIC, ≥1,024 µg/ml), tetracycline (MIC, ≥32 µg/ml), and trimethoprim (MIC, ≥32 µg/ml). In addition, isolate 588 was also resistant to florfenicol (MIC, ≥64 µg/ml). The isolates were susceptible to apramycin, ceftiofur, colistin, imipenem, meropenem, and tigecycline.

However, one could question if those antimicrobials would be used for treatment, since florfenicol and apramycin are only approved for animal usage, colistin is difficult to administer and has

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TABLE 1 Distributions of antimicrobial resistance genes in *Salmonella* Senftenberg isolates from Zambia

Antimicrobial agent(s)	Resistance gene, class, and/or mutation in isolate ID no.:	
	588	1028
Aminoglycoside	<i>aac(6')-IIC</i>	<i>aac(6')-IIC</i>
	<i>aac(6')-Iy</i>	<i>aac(6')-Iy</i>
	<i>aph(3')-Ic</i>	<i>aadA2</i>
	<i>aac(6')-Ib-cr</i>	<i>aac(6')-Ib-cr</i>
	<i>strA</i>	<i>strA</i>
	<i>strB</i>	<i>strB</i>
Beta-lactam	<i>bla</i> _{TEM-1}	<i>bla</i> _{TEM-1}
	<i>bla</i> _{CTX-M-15}	<i>bla</i> _{CTX-M-15}
	<i>bla</i> _{OXA-10}	<i>bla</i> _{OXA-30}
Fluoroquinolone		
Low-level resistance	<i>aac(6')-Ib-cr</i>	<i>aac(6')-Ib-cr</i>
High-level resistance	<i>gyr(A)</i> (S-83-Y and D-87-G)	<i>gyr(A)</i> (S-83-Y and D-87-G)
	<i>par(C)</i> (S-80-I)	<i>par(C)</i> (S-80-I)
Macrolide-lincosamide-streptogramin	<i>ere(A)</i>	<i>ere(A)</i>
Phenicol	<i>catB3</i>	<i>catB3</i>
	<i>catA2</i>	<i>catA2</i>
	<i>cmlA1</i>	
	<i>floR</i>	
Rifampin	<i>arr-2</i>	
Sulfonamide	<i>sul1</i>	<i>sul1</i>
	<i>sul2</i>	<i>sul2</i>
Tetracycline	<i>tet(A)</i>	<i>tet(D)</i>
	<i>tet(D)</i>	
Trimethoprim	<i>dfrA14</i>	<i>dfrA18</i>
	<i>dfrA18</i>	
	<i>dfrA23</i>	

renal toxicity (2), cefoxitin is grouped with extended-spectrum cephalosporins and would most likely not have any effect, due to the isolates already being resistant to broad-spectrum cephalosporins (10), and carbapenems are too expensive, considering that these patients were required to cover the hospital expenses themselves. Treatment with tigecycline may be effective toward nontyphoid *Salmonella*, but clinical trials need to be conducted to further investigate the full potential of its use for human treatment of infections caused by multidrug-resistant nontyphoid *Salmonella*.

The isolates were sequenced on the Ion Torrent PGM system (Life Technologies) following the manufacturer's protocols for 200-bp genomic DNA (gDNA) fragment library preparation (Ion Xpress Plus gDNA and Amplicon Library Preparation), template preparation (Ion OneTouch system), and sequencing (Ion PGM 200 sequencing kit). The sequence data were assembled and analyzed using the pipeline available on the Center for Genomic Epidemiology website (www.genomicepidemiology.org) (11, 12).

The isolates belonged to MLST ST14. The Resfinder tool detected the following resistance genes present either in both or in one of the isolates, as well as two mutations in *gyr(A)* and one

mutation in *par(C)* responsible for high-level fluoroquinolone resistance (Table 1). Both isolates contained an *incH12* plasmid replicon, and isolate 588 contained an *incA/C* plasmid replicon.

The genetic relatedness of the two isolates was examined and identified 93 high-quality SNPs (the informative SNPs were determined based on a minimum coverage of 20 times, base calling quality of 30, and a minimum distance of 10 bp between each SNP) between the two isolates, using the *S. Senftenberg* SS209 reference genome (Bio project number PRJEA82547) (13) and 530 and 521 SNPs between isolates 1028 and 588 and the reference genome. There are currently insufficient data on the nucleotide diversity between clonally related and unrelated *Salmonella* isolates to determine whether this was indicative of separate or clonally related strains. Whole-genome studies on *Salmonella* have indicated an accumulation rate of 1 to 2 SNPs per year (8). Thus, the 93 SNP differences observed here in combination with the differences in resistance profiles and genes may suggest that the isolates have an unrelated origin.

S. Senftenberg has also previously been reported as the cause of serious human infections (14–16). *S. Senftenberg* is well recognized as being common among poultry (17), but it has also been associated with infant formula (18), mussels (19), and vegetables (16, 20, 21). It is noteworthy that one of the patients claimed to have consumed vegetables prior to onset of symptoms. *S. Senftenberg* has the ability to adhere to plant leaves, perhaps contributing to infections in such cases (20). A similar case of one resistant *S. Senftenberg* isolate was recently reported for a traveler returning from Egypt, indicating the importance of this resistant serovar in Africa (22).

In conclusion, we have reported here cases from Zambia of extremely drug-resistant *S. Senftenberg* isolates that caused severe human infections and for which there were very few treatment options. We speculate that the clones are emerging and suggest that public health authorities become alert for any further dissemination.

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