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Extensive drug resistant *Salmonella enterica* serovar Senftenberg carrying *bla*_{NDM} encoding plasmid p5558 (IncA/C) from India

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ABSTRACT

Non-typhoidal *Salmonella* (NTS) are foodborne pathogens that are responsible for self-limiting gastroenteritis in humans. The present study aims at the molecular characterisation and comparative genomics of *Salmonella enterica* serovar Senftenberg strain P5558 isolated from the pus samples of a patient suffering from stump infection. The isolate was subjected to serotyping and antimicrobial susceptibility test to understand the phenotypical characteristics. Whole genome sequencing (WGS) was carried out and comparative genomics using computational tools showed the antimicrobial resistance and virulence gene profile of the isolates from the genome sequence data. Typing experiments confirmed that the isolate belong to *S. Senftenberg* with sequence type ST14. Resistance against β -lactams is associated with the presence of *bla*_{TEM-1}, *bla*_{OXA-9}, *bla*_{CMY-2} and *bla*_{NDM-1} genes. Similarly resistance to aminoglycoside was associated with five aminoglycoside modifying enzymes *aac*(6')-Ia, *aac*(6')-Ib, *aph*(3')-Ib, *aph*(6')-Ib and *ant*(3'')-Ia, sulfonamide with *sul*-1 and *sul*-2 and chloramphenicol with *florR* gene. Substitutions in *gyrA* (S83Y, D87G) and *parC* (S80I) genes found to be the reason for fluoroquinolone resistance. The plasmid profiling showed the isolate has four resistance plasmids in which plasmid p5558-NDM (IncA/C) harbours major resistance genes including *bla*_{NDM-1} and *bla*_{CMY-2}. Determination of virulence gene profile revealed that the genome carries all major *Salmonella* pathogenicity islands and virulence factors. From our findings it is clear that the isolate possess characteristic pathogenicity islands (SPI 1–6, 13, 14), major virulence factors and acquired resistance genes. Comparative analysis suggests the evolution and distribution of the MDR gene encoding plasmids in NTS.

KEYWORDS

Non typhoidal *Salmonella*; antibiotic resistance; virulence factors; *bla*_{NDM}; pathogenicity islands

Introduction

Non-typhoidal *Salmonella* (NTS) are foodborne pathogens that are responsible for self-limiting gastroenteritis in humans [1]. Although NTS mainly causes localized gut infections, the bacteria can even spread beyond the mucosa causing invasive infections in infants, the elderly or immunocompromised patients [2]. According to global estimates NTS cause 93 million infections with 155,000 deaths wherein 3.4 million illness and 681,316 fatalities per year on account of invasive non-typhoidal *Salmonella* (iNTS) [3]. *S. Senftenberg* isolates that were previously associated with colonization of hatcheries have recently emerged to cause severe infections [4]. Multidrug resistant isolates are commonly associated with animal sources but clinical isolates were also reported [5].

The emergence and spread of multidrug resistant (MDR) *Salmonella* strains are of major public health concern worldwide [6]. The increasing resistance to 'last resort' antibiotics such as carbapenems and other beta-lactams may have serious implications because of a few or no treatment options [7].

Resistance mechanism to carbapenems among *Salmonella* isolates is mainly due to the harbouring of mobile genetic elements encoding different classes of β -lactamases including KPC, TEM, NDM, OXA, IMP and VIM [8]. The first report of carbapenemase genes in *Salmonella* was the identification of *bla*_{KPC-2} in *S. Cubana* [9]. Further studies have identified *bla*_{IMP-4} from *S. Waycross* and *bla*_{VIM-2} in five clinical isolates of *S. Kentucky* [10]. The presence of *bla*_{NDM-1} in *Salmonella* was first reported in *S. Senftenberg* in 2011 [11]. Recent studies also suggest the carbapenemase genes are encoded by plasmids in *Salmonella* serovars and readily transferred between Enterobacterial species [12].

In this study we report the isolation of multidrug resistant *S. Senftenberg* and its molecular characterization. The WGS of the isolate was analyzed to explore the correlation of genotypial and phenotypical antibiotic resistance pattern and virulence determinants. We also assembled the *bla*_{NDM-1} gene carrying plasmid IncA/C which also possessed other drug resistant genes.

Materials and methods

Bacterial collection and characterisation

S. Senftenberg was isolated from a 22 year old male patient of Indian descent with stump infection after a lower limb amputation. The patient has developed amputation site infection (SSI) and hence referred to Christian Medical College, Vellore, India. Pus aspirated from the amputation site showed bacterial growth and the isolated Gram-negative rod was later confirmed as *S. Senftenberg*. Serogroup of the isolate was identified using standard microbiological techniques and commercial typing antiserum according to the manufacturer's instructions. The isolate was further serotyped according to the *Kauffmann-White* scheme [13]. Multilocus sequence typing (MLST) was carried out based on the seven housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA* and *thrA*) and sequence types were assigned using Enterobase database (<https://enterobase.warwick.ac.uk>).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed using agar disk diffusion (DD) method as well as automated susceptibility testing system. The isolate was tested against penicillins (ampicillin), cephalosporins (ceftriaxone, ceftazidime, cefepime), carbapenems (doripenem, imipenem, meropenem), quinolones (ciprofloxacin, levofloxacin), phenicols (chloramphenicol), sulfonamides (trimethoprim/sulfamethoxazole), and aminoglycosides (amikacin, gentamicin). Minimum inhibitory concentration (MIC) for ceftriaxone was further determined by the E-test strip (BioMérieux, France) and by broth micro dilution (BMD) method in accordance to the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines and interpretative criteria [14]. *Escherichia coli* ATCC 25922, *Enterococcus faecium* ATCC 29212, and *Pseudomonas aeruginosa* ATCC 27853 was used as quality control strain for antimicrobial susceptibility testing. Automated testing with the VITEK-2 GN281 card (Vitek 2 system, BioMérieux) was performed according to manufacturer's protocol.

Genomic DNA extraction and whole genome sequencing

Total genomic DNA of strain P5558 was extracted from an overnight culture (14–16 hrs) grown at 37°C on blood agar (In house) using the QIAamp DNA Mini Kit and the QIAcube instrument (Qiagen, Valencia, CA) according to the manufacturer's instructions. The extracted DNA was quantified and stored at –20°C until further use.

WGS was performed using Ion Torrent PGM platform (Life Technologies, Carlsbad, CA) using 400 bp read chemistry. Sequencing was performed as per the protocol

recommended by Life Technologies. Raw reads were assembled *de novo* using AssemblerSPAdes software v4.4.0.1 in Torrent suite server version 4.4.3. Sequencing generated 5,234,212 bp sequences with 188 contigs (≥ 500 bp) of 36 X coverage. The genome was annotated using the RAST server (Rapid Annotation using Subsystem Technology- <http://rast.nmpdr.org>) available from the PATRIC database (Pathosystems Resource Integration Centre -<https://www.patricbrc.org>) [15,16]. Multilocus sequence typing (MLST) of strain P5558 was determined using Enterobase database (<https://enterobase.warwick.ac.uk>) available in pubMLST (<https://pubmlst.org/mlst/>) based on concentrated sequences of seven loci (*aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*) [17]. Serotype of the isolate was further confirmed using the web based tool SeqSero (www.denglab.info/SeqSero) [18]. Molecular characterisation of the isolate was further established using CRISPR sequence type analysis (<http://crispr.i2bc.paris-saclay.fr/Server/>) based on Pasteur CRISPR database for *Salmonella*.

Genome analysis for resistance and virulence genes

The presence of antibiotic resistance genes (ARG) in the assembled whole genome were analyzed using CARD [19] and ARG-ANNOT [20]. Virulence genes present in the genome were identified by BLAST search against Virulence Factor Database [21]. The presence of phage insertions in the genome was detected using PHASTER [22] and *Salmonella* Pathogenicity Island (SPI) was identified using SPI-Finder available from CGE server (<https://cge.cbs.dtu.dk/services/>) and BLAST search against Pathogenicity island database [23].

Plasmid assembly and analysis

The presence of plasmid was analyzed using Plasmid finder 1.3 available from CGE server (<https://cge.cbs.dtu.dk/services/>). The incompatibility (Inc) type of the plasmid was analyzed using pMLST (<https://pubmlst.org/plasmid/>). The carbapenemase harbouring plasmid from the WGS of the isolate P5558 was assembled using plasmidSPAdes [24]. The plasmid was assembled further against a reference plasmid [12] using Bowtie2 [25] and plotted using CGview server [26].

Results

Characterisation of *S. Senftenberg*

The isolate was characterized by traditional serotyping and identified as *Salmonella enterica* serovar Senftenberg. *In silico* serotyping performed using 'SeqSero' web based tool correlated well with the traditional serotyping with confirmed antigenic formulae of 1,3,19: gst:- Based on the seven locus MLST

analysis the isolate was classified to ST14. MLST based eBurst analysis has further classified the isolate into eBurst groupings eBG55. CRISPR arrays were identified from the whole genome and the isolate found to harbour three confirmed CRISPR loci.

Antimicrobial susceptibility testing

The isolate P5558 was resistant to a range of antimicrobials and showed resistance against all β -lactams tested including oxyimino-cephalosporins, and carbapenems. Minimum inhibitory concentration (MIC) profile to antibiotics such as, ampicillin (≥ 32 $\mu\text{g/ml}$), ceftazidime (≥ 64 $\mu\text{g/ml}$), cefepime (≥ 64 $\mu\text{g/ml}$), ceftriaxone ($= 512$ $\mu\text{g/ml}$), doripenem (≥ 8 $\mu\text{g/ml}$), imipenem (≥ 8 $\mu\text{g/ml}$), meropenem (≥ 16 $\mu\text{g/ml}$), amikacin (≥ 64 $\mu\text{g/ml}$), gentamicin (≥ 16 $\mu\text{g/ml}$), ciprofloxacin (≥ 8 $\mu\text{g/ml}$), levofloxacin (≥ 8 $\mu\text{g/ml}$), Trimethoprim/Sulfamethoxazole (≥ 4 $\mu\text{g/ml}$) and chloramphenicol (≥ 32 $\mu\text{g/ml}$) were determined. The MIC values found to be above the cut-off values as per breakpoints adopted from CLSI M100-27 document [14] and hence the isolate is considered resistant to the tested antimicrobial agents.

Antimicrobial resistant genes

WGS analysis revealed the presence of the following antimicrobial resistant genes (ARGs) in *S. Senftenberg* isolate PP5558: Extended spectrum β -lactamases (*bla*_{TEM-1}), (*bla*_{OXA-9}) AmpC- β -lactamases (*bla*_{CMY-2}), metallo- β -lactamases (*bla*_{NDM-1}), aminoglycosides modifying enzymes *aac*(6')-Ia, *aac*(6')-Ib (*aacA4*), *aph*(3')-Ib, *aph*(6')-Ib, *ant*(3'')-Ia (*aadA1*), Sulfonamide resistance (*sul-1*, *sul-2*) chloramphenicol resistance (*florR*) and bleomycin resistance (*bleMBL*). Analysis of quinolone resistance- determining region (QRDR) identified two amino acid changes each in *gyrA* gene (S83Y, D87G) and *parC* gene (S80I) respectively. Correlation between the phenotypic and genotypic resistant pattern suggest the presence of respective genes for the phenotypic resistance in AST.

Plasmid characterization

The assembled genome showed the presence of four plasmids: a 146,017 bp IncA/C (designated as p5558-NDM), IncFIA, IncFIB and IncFII plasmid. p5558-NDM contained 187 putative ORFs with a GC content of 52%

Figure 1. Circular representation of, *bla*_{NDM-1} and *bla*_{CMY-2} encoding plasmid p5558-NDM. Starting from inside the first circle indicates the GC content and the second circle represents the GC skew (dark green, GC +, purple, GC -), the third circle represents the arrangement of contigs and the fourth circle indicates the reference genome pNDM-SAL. The fifth circle indicates the complete plasmid with CDS regions being the outermost circle. The AMR genes are denoted by red colour, Integrase/Transposase genes by light green colour and transfer apparatus using yellow colour.

with the first gene being the origin of replication (*repA*). The plasmid belongs to sequence type 1 (ST-1) of IncA/C plasmid group with typical IncA/C replicon and putative genes such as *parA*, *parB*, *rhcC* and *kfrA*. The characteristic *repA* gene (plasmid replication), *araCD* (conjugation master regulator) and transfer F plasmid transfer operon genes indicates similarity with other IncA/C plasmid in public sequence database [27]. The IncA/C encodes acquired resistance genes such as *bla*_{NDM-1}, *bla*_{CMY-2}, *aadA1*, *aacA4*, *sul-1* and *bleMBL* genes (Figure 1). The IncA/C type plasmid showed nearly 98% blast similarity to pNDM-SAL plasmid identified from *S. Senftenberg* strain BCH2406 isolated from Kolkata, India [12]. The presence of type IV secretion system (T4SS) conjugal transfer genes and integrons flanking the two resistance gene units suggests the transmission among other Enterobacteriaceae.

The resistance genes are arranged in two regions in which the first contains *sul1*, *aadA1*, *aacA4* and *qacE*. In this region three antimicrobial resistance genes are arranged between class 1 integron (*Int11*) and ISCR1 transposase with the conjugal transfer genes (*traGH*) conjugation master regulators (*acaDC*) upstream. The second region containing resistant genes *bla*_{NDM-1}, *bla*_{CMY-2}, *sugE* and *bleMBL* was flanked by *tnpA* transposase and surrounded by the T4SS cluster containing the conjugal transfer (*tra*) genes (Figure 1). IncFIA, IncFIB and IncFII plasmids neither confer any resistance genes or any virulence genes.

Salmonella pathogenicity islands and virulence genes

Comparative genomics of *Salmonella* pathogenicity Island (SPI) with the corresponding sequence of *S. Typhimurium* LT2 suggest the presence of SPI 1, 2, 3, 4, 5, 6 pathogenicity island. SPI-1 comprises of 39 genes with the same gene composition and arrangement as observed in the reference genome. Similarly SPI 2–5 consists of 31, 13, 10 and 8 genes with limited variability when compared with reference genome. The T6SS effector proteins coded by SPI-6 were found to be present in the genome however Vi-coding genes harboured by SPI-7 were absent. BLASTn analysis of whole genome of *S. Senftenberg* against Virulence Factor Database (VFDB) revealed the presence of virulence genes outside SPI. The *bcfC* (Bovine colonization factor: fimbria related) and the *fim* genes that mediates adherence to eukaryotic cells suggest the invasive potential of the isolate [28]. The presence of prophage insertions (Gifsy-1, Gifsy-2) were identified by PHASTER analysis while *rck* gene that provides protection against the complement-mediated immune response of the host was absent.

Discussion

Non typhoidal *Salmonella* (NTS) that often causes food-borne infections in the world are increasingly acquiring resistant genes with great frequency. Although resistance to carbapenems are still very rare in NTS, mobile genetic elements based transmission of resistance genes are in rise in the recent decade [29].

Based on the combination of phenotypic and genotypic serotyping the isolate was designated as *Salmonella enterica* serovar Senftenberg with antigenic formulae 1,3,19:g,s,t:-. MLST and *eBurst* grouping analysis further confirmed that the isolate belong to ST14 that clusters within *eBG55* which is common among isolates of serotype Senftenberg [30]. CRISPR1 and CRISPR2 allele spacers possess 40 and 20 spacers respectively and spacer regions were unique to *S. Senftenberg* [31]. A 16 years (2000–2015) retrospective review of serovar Senftenberg infections at Christian Medical College, Vellore, India showed only 6 isolates from a total of 1014 NTS isolates. *S. Senftenberg* was not frequently isolated from the clinical samples in this tertiary care hospital however, the prevalence of the serovar is on rise all over India since 1983 [32]. A report on the prevalence of *S. Senftenberg* in India states 571 strains of *S. Senftenberg* isolated from different parts of India in National *Salmonella* and *Escherichia* Center (NSEC) in Kasauli, India from 1969–1999 [32].

The phenotypic susceptibility for each antibiotic used has been compared with the absence/presence of specific AMR genes relevant to each antimicrobial type. Based on the 14 antimicrobials from six different groups of antibiotics tested, resistance against ampicillin associated with the presence of *bla*_{TEM-1} or *bla*_{OXA-9} genes [33]. Resistance to carbapenems (doripenem, imipenem and meropenem) and late generation cephalosporins (cefepime and ceftriaxone) associated with the carriage of *bla*_{NDM-1}. The presence of AmpC β -lactamase gene *bla*_{CMY-2} also confers resistance against cephamycins. Similarly resistance to aminoglycoside was associated with *aac(6')-Ia*, *aac(6')-Ib* (*aacA4*), *aph(3')-Ib*, *aph(6')-Ib* and *ant(3'')-Ia* (*aadA1*) genes, sulfonamide resistance with *sul-1* and *sul-2* and chloramphenicol resistance with *florR*. Amino acid substitutions in *gyrA* and *parC* genes associated with resistance to fluoroquinolones such as ciprofloxacin and levofloxacin (Table 1). The amino acid substitutions are characteristic mutation present in other quinolone resistant *S. Senftenberg* [34].

The presence of NDM harbouring plasmid p5558 indicates the frequent transmission of resistance determinants amongst Enterobacteriaceae. The p5558-NDM backbone of 146,017 bp shows 99% blast identity with that of the pNDM-SAL (GenBank accession number: KP742988) plasmid isolated from *S. Senftenberg* BCH02406 with a query cover of 99%. High homologies with NDM

Table 1. Correlation of phenotypic and genotypic resistant profiles.

Antimicrobial	Genotype (AMR genes)/Substitution	AST (MIC)
Beta lactams	<i>bla</i> TEM-1 <i>bla</i> OXA-9 <i>bla</i> CMY-2 <i>bla</i> NDM-1	Ampicillin (≥32 µg/ml) Ceftazidime (≥64 µg/ml) Cefepime (≥64 µg/ml) Ceftriaxone (=512 µg/ml) Doripenem (≥8 µg/ml) Imipenem (≥8 µg/ml) Meropenem (≥16 µg/ml) Amikacin (≥64 µg/ml), Gentamicin (≥16 µg/ml)
Aminoglycoside	<i>aac</i> (6')-Ia <i>aac</i> (6')-Ib (<i>aacA4</i>) <i>aph</i> (3')-Ib, <i>aph</i> (6')-Ib <i>ant</i> (3'')-Ia (<i>aadA1</i>)	
Fluoroquinolone	<i>gyrA</i> (S83Y, D87G) <i>parC</i> (S80I)	Ciprofloxacin (≥16 µg/ml), Levofloxacin (≥8 µg/ml)
Sulfonamide	<i>sul1</i> , <i>sul2</i>	Trimethoprim/Sulfamethoxazole (≥4 µg/ml)
Chloramphenicol	<i>florR</i>	Chloramphenicol (≥32 µg/ml)

carrying IncA/C plasmids among other Enterobacteriaceae such as *Proteus mirabilis* AR_0159 (CP021550), *E. coli* (CP021536) from different geographical location suggest the global circulation of the plasmid. Notably the *bla*_{NDM-1} gene was flanked by TN-3 like *tnpA* (transposase) gene. The presence of *tnpA* along with the T4SS may promote the transmission of *bla*_{NDM-1} amongst virulent Enterobacteriaceae [35]. The plasmid also contained Tn21 transposon (Tn21-*tnpA* and Tn21-*tnpR*) upstream to class 1 integron (*Int11*) carrying resistance genes *aacA4*, *aadA1*, *sul1* and quaternary ammonium compound-resistance gene (*qacE*) (Figure 1). Collectively the presence of several mobile genetic elements flanking the AMR genes, putative functional genes for replication (*repA*), stability (*kfrA*), partitioning system (*parA*, *parB*, *O53*), conjugative transfer (*tra*) and conjugation master regulators (*acaDC*) suggest high level of spread and transmission of p5558-NDM plasmid [36].

The genomic analysis of virulence determinants within the *Salmonella* pathogenicity islands of the isolate suggest SPIs are highly conserved across *Salmonella enterica*. Although *S. Senftenberg* demonstrated limited or moderate invasive capacity in previous studies, this is majorly associated with the lack of SPI-1. However recent studies suggest *S. Senftenberg* isolates can cause intestinal inflammatory diseases even without SPI-1 [37]. *S. Senftenberg* strain P5558 possesses SPI 1–5 that encodes essential virulence determinants. The type 3 secretion system (T3SS) encoded by SPI-1 and SPI-2 were arranged in a traditional genomic arrangement as observed in other virulent phenotypes [37]. Major virulence determinants of T3SS such as *avrA*, *ssaQ*, *mgtC*, *siid*, *sopB*, and *invA* in the isolate suggest the ability of the isolate to colonize in the liver of the host. Similarly SPI-3, SPI-4 and SPI-5 have traditional genomic arrangement and effector proteins harboured by SPI-5 for T3SS suggests the spleen colonizing ability of the isolate [38]. The presence of

variable prophage regions and fimbrial operons confirms the overall pathogenic potential of the isolate.

This study explains the resistome, plasmids and virulence factors of multidrug resistant NTS isolate *S. Senftenberg*. The AMR potential of the isolate was studied by a combination of phenotypic data (MIC) and genotypic data (WGS). The presence of specific AMR genes in the genome exhibited an upward trend of MIC values for β -lactams. Similarly the genotypic and phenotypic characteristics of β -lactams, aminoglycosides, fluoroquinolones, sulfonamides and chloramphenicol correlated significantly. The genome sequence information showed the presence of virulence factors such as T3SS and T6SS effector proteins, colonization and invasion factors, prophage insertions and fimbrial clusters which would otherwise be associated with the invasive apparatus of NTS. Although the determination of the invasive and non-invasive role of the isolate is challenging, the distribution of characteristic pathogenicity islands, other virulence factors and acquired resistance genes may aid the bacteria in potential propagation of AMR and virulence.

Genome sequence accession number

The raw sequence data of *S. Senftenberg* strain P5558 has been submitted to NCBI under BioProject ID PRJNA483094 with accession number QVOE00000001 - QVOE00000154.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

- [1] Eng SK, Pusparajah P, Ab Mutalib NS, et al. *Salmonella*: a review on pathogenesis, epidemiology and antibiotic resistance. *Front Life Sci.* **2015**;8(3):284–293.
- [2] Crump JA, Sjölund-Karlsson M, Gordon MA, et al. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clin Microbiol Rev.* **2015**;28(4):901–937.
- [3] Ao TT, Feasey NA, Gordon MA, et al. Global burden of invasive nontyphoidal *Salmonella* disease, 2010. *Emerging Infect Dis.* **2015**;21(6):941.
- [4] Boumart Z, Roche SM, Lalande F, et al. Heterogeneity of persistence of *Salmonella enterica* serotype Senftenberg strains could explain the emergence of this serotype in poultry flocks. *PLoS One.* **2012**;7(4):e35782.
- [5] Hendriksen RS, Joensen KG, Lukwesa-Musyani C, et al. Extremely drug-resistant *Salmonella enterica* serovar Senftenberg infections in patients in Zambia. *J Clin Microbiol.* **2013**;51(1):284–286.
- [6] Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. *Nat Med.* **2004**;10(12s):S122.
- [7] Meletis G. Carbapenem resistance: overview of the problem and future perspectives. *Ther Adv Infect Dis.* **2016**;3(1):15–21.
- [8] Sahuquillo-Arce JM, Hernández-Cabezas A, Yarad-Auad F, et al. Carbapenemases: a worldwide threat to antimicrobial therapy. *World J Pharmacol.* **2015**;4(1):75–95.
- [9] Miriagou V, Tzouveleakis LS, Rossiter S, et al. Imipenem resistance in a *Salmonella* clinical strain due to plasmid-mediated class A carbapenemase KPC-2. *Antimicrob Agents Chemother.* **2003**;47(4):1297–1300.
- [10] Le Hello S, Harrois D, Bouchrif B, et al. Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: a microbiological study. *Lancet Infect Dis.* **2013**;13(8):672–679.
- [11] Savard P, Gopinath R, Zhu W, et al. First NDM-positive *Salmonella* sp. strain identified in the United States. *Antimicrob Agents Chemother.* **2011**;55(12):5957–5958.
- [12] Sarkar A, Pazhani GP, Chowdhury G, et al. Attributes of carbapenemase encoding conjugative plasmid pNDM-SAL from an extensively drug-resistant *Salmonella enterica* Serovar Senftenberg. *Front Microbiol.* **2015**;6:969.
- [13] Popoff MY, Bockemühl J, Gheesling LL. Supplement 2002 (no. 46) to the Kauffmann–White scheme. *Res Microbiol.* **2004**;155(7):568–570.
- [14] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 23rd informational supplement. (CLSI document M100-S27). Wayne (PA): Clinical and Laboratory Standards Institute; 2017.
- [15] Aziz RK, Bartels D, Best AA, et al. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics.* **2008**;9(1):75.
- [16] Wattam AR, Abraham D, Dalay O, et al. PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res.* **2013**;42(D1):D581–D591.
- [17] Achtman M, Wain J, Weill FX, et al. Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathog.* **2012**;8(6):e1002776.
- [18] Zhang S, Yin Y, Jones MB, et al. *Salmonella* serotype determination utilizing high-throughput genome sequencing data. *J Clin Microbiol.* **2015**;53(5):1685–1692.
- [19] Jia B, Raphenya AR, Alcock B, et al. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res.* **2016**;45(D1):D566–D573.
- [20] Gupta SK, Padmanabhan BR, Diene SM, et al. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrob Agents Chemother.* **2014**;58(1):212–220.
- [21] Chen L, Yang J, Yu J, et al. VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res.* **2005**;33(suppl_1):D325–D328.
- [22] Arndt D, Grant JR, Marcu A, et al. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res.* **2016**;44(W1):W16–W21.
- [23] Yoon SH, Park YK, Kim JF. PAIDB v2. 0: exploration and analysis of pathogenicity and resistance islands. *Nucleic Acids Res.* **2014**;43(D1):D624–D630.
- [24] Antipov D, Hartwick N, Shen M, et al. plasmidSPAdes: assembling plasmids from whole genome sequencing data. *Bioinformatics.* **2016**;32(22):3380–3387.
- [25] Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods.* **2012**;9(4):357.
- [26] Grant JR, Stothard P. The CGView Server: a comparative genomics tool for circular genomes. *Nucleic Acids Res.* **2008**;36(suppl_2):W181–W184.
- [27] Hancock SJ, Phan MD, Peters KM, et al. Identification of IncA/C plasmid replication and maintenance genes and development of a plasmid multi-locus sequence-typing scheme. *Antimicrob Agents Chemother.* **2016**;61(2):e01740–16.
- [28] Thomas M, Fenske GJ, Antony L, et al. Whole genome sequencing-based detection of antimicrobial resistance and virulence in non-typhoidal *Salmonella enterica* isolated from wildlife. *Gut Pathog.* **2017**;9(1):66.
- [29] Baker S, Thomson N, Weill FX, et al. Genomic insights into the emergence and spread of antimicrobial-resistant bacterial pathogens. *Science.* **2018**;360(6390):733–738.
- [30] El Ghany MA, Shi X, Li Y, et al. Genomic and phenotypic analysis reveal the emergence of an atypical *Salmonella* Senftenberg variant in China. *J Clin Microbiol.* **2016**;54(8):2014–2022.
- [31] Fabre L, Zhang J, Guigon G, et al. CRISPR typing and subtyping for improved laboratory surveillance of *Salmonella* infections. *PLoS One.* **2012**;7(5):e36995.
- [32] Kumar S, John PC, Sharma NC, et al. *Salmonella* Senftenberg: epidemics in India and present status. *Ind Pediatr.* **1994**;31(12):1491–1495.
- [33] Jacoby GA, Munoz-Price LS. The new β -lactamases. *N Engl J Med.* **2005**;352(4):380–391.
- [34] Whichard JM, Gay K, Stevenson JE, et al. Human *Salmonella* and concurrent decreased susceptibility

- to quinolones and extended-spectrum cephalosporins. *Emerg Infect Dis.* [2007](#);13(11):16–81.
- [35] Huang J, Deng S, Ren J, et al. Characterization of a *bla*_{NDM-1}-harboring plasmid from a *Salmonella enterica* clinical isolate in China. *Mol Med Rep.* [2017](#);16(2):1087–1092.
- [36] Hu Q, Coburn B, Deng W, et al. *Salmonella enterica* serovar Senftenberg human clinical isolates lacking SPI-1. *J Clin Microbiol.* [2008](#) Apr 1;46(4):1330–1336.
- [37] Rychlik I, Karasova D, Sebkova A, et al. Virulence potential of five major pathogenicity islands (SPI-1 to SPI-5) of *Salmonella enterica* serovar Enteritidis for chickens. *BMC Microbiol.* [2009](#);9(1):268.
- [38] Chaudhuri RR, Morgan E, Peters SE, et al. Comprehensive assignment of roles for *Salmonella* Typhimurium genes in intestinal colonization of food-producing animals. *PLoS Genet.* [2013](#);9(4):e1003456.