Infection and Drug Resistance

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ORIGINAL RESEARCH

A Multidrug-resistant Monophasic *Salmonella* Typhimurium Co-harboring *mcr-1*, *fosA3*, *bla*$_{CTX-M-14}$ in a Transferable IncHI2 Plasmid from a Healthy Catering Worker in China

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Background: Polymyxins are currently regarded as a possible last-resort therapy to eradicate multidrug-resistant (MDR) gram-negative bacteria. Meanwhile, the old antimicrobial agent fosfomycin has recently been reintroduced into clinical use for the treatment of extended-spectrum β-lactamase (ESBL)-producing and carbapenem-resistant *Enterobacteriaceae*. This study investigated a multidrug-resistant *Salmonella* 4,[5],12:i:- strain from a food catering handler, which had the potential to act as a vehicle for transmitting MDR foodborne pathogens.

Methods: A *Salmonella* 4,[5],12:i:- YZU1189 strain was isolated from the fecal sample of a food catering worker according to the standard protocol of the *Salmonella* detection method from World Health Organization in 2003. Serotyping of YZU1189 was performed according to the Kauffmann–White scheme. The antimicrobial resistance phenotype of the strain was determined by the agar dilution method according to the instruction from Clinical and Laboratory Standards Institute (CLSI). Plasmid conjugation was performed between the donor strain *Salmonella* 4,[5],12:i:- YZU1189 and the recipient strain *Escherichia coli* C600. The genetic locations of *mcr-1*, *bla*$_{CTX-M-14}$ and *fosA3* genes were determined by the whole genome sequence analysis.

Results: *Salmonella* 4,[5],12:i:- YZU1189 was an ESBL-producing stain isolated from a healthy catering worker. The strain displayed resistance to aminoglycosides, beta-lactams, polymyxins, fosfomycins, phenicols, trimethoprims, sulfonamides, tetracyclines and fluoroquinolones. Whole genome sequence analysis and plasmid conjugation revealed that the strain had a transferable IncHI2 plasmid carrying the *mcr-1*, *bla*$_{CTX-M-14}$ and *fosA3* genes. Sequence homology analysis showed that this plasmid possessed high sequence similarity to previously reported *mcr-1*, *bla*$_{CTX-M-14}$ and *fosA3* positive plasmids in China.

Conclusion: This study reported a the multidrug-resistant *Salmonella* 4,[5],12:i:- isolate harboring *mcr-1*, *bla*$_{CTX-M-14}$ and *fosA3* from human for the first time in China. The occurrence of *mcr-1* and *fosA3* genes in the transferable IncHI2 plasmid pYZU1189 from the ESBL-producing *Salmonella* 4,[5],12:i:- isolate showed a potential threat to public health. Great concern should be taken for the spread of multidrug-resistant ESBL-producing *Salmonella* isolates from food catering workers to consumers.

Keywords: *Salmonella* 4,[5],12:i:-, whole genome sequencing analysis, multidrug resistant, ESBL, colistin resistance, *fosA3*

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Introduction

Salmonella enterica serovar Typhimurium is well known to be associated with foodborne diseases and outbreaks, as well as causing infectious diseases in animals

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and humans.[1] In recent year, the *Salmonella* Typhimurium monophasic variant (*Salmonella* 4,[5],12:i:-) was frequently reported from human salmonellosis in both Europe and the US.[2,3] *Salmonella* 4,[5],12:i:- is described as a variant of *Salmonella* Typhimurium lacking the second phase flagellum, which mostly belonged to the pandemic ST34 clone with an antibiotic resistance pattern of ASSuT (ampicillin, streptomycin, sulphonamides and tetracycline).[4] In addition, the *mcr-1* gene responsible for the colistin resistance has frequently occurred in ST34 *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:-.[4–7] In China, *Salmonella* 4,[5],12:i:- has increased to being one of the most predominant serovars causing human salmonellosis, while ST34 is the most common ST type of *Salmonella* 4,[5],12:i:-.[8] ESBL-producing *Salmonella* isolates have raised concern due to their resistance to cephalosporins, which have been widely applied for treating severe salmonellosis.[9] The ESBL-producing *Salmonella* strains showed a higher prevalence (8.58%) on retail chicken in China than in the US.[10,11] Meanwhile, fosfomycin has recently been reintroduced into clinical treatment for ESBL-producing *Enterobacteriaceae*. The occurrence of multidrug resistance in *Salmonella* 4,[5],12:i:- isolated from animal origin food products has increased the potential infection risk while treatment options are becoming limited.

In this study, we identified a MDR ST34 *Salmonella* 4,[5],12:i:- isolate carrying an IncHI2 plasmid with *mcr-1, fosA3,* $bla_{\text{CTX-M-14}}$ genes, which was from a healthy catering industry worker in Jiangsu province, China. To our best knowledge, this is the first report of the multidrug-resistant *Salmonella* 4,[5],12:i:- isolate harboring *mcr-1,* $bla_{\text{CTX-M-14}}$ and *fosA3* from a human in China.

Materials and Methods

Bacterial Isolate

A surveillance study for foodborne pathogens was performed toward food catering workers and diarrheal patients by Nantong CDC, Jiangsu, China.[12] The initial aim of this project was to evaluate the role of food handler as a vehicle for transmitting foodborne pathogens by screening human fecal samples. In order to selectively enrich *Salmonella*, samples were added to the selenite cysteine broth and incubated at 35°C for 24 h. The 10 μL of each incubated sample was inoculated onto xylose lysine deoxycholate (XLD, OXOID, England) and *Salmonella Shigella* (SS) agar plate, and cultured for 24 h at 37°C. Two or more presumptive *Salmonella* colonies were subcultured in the triple sugar-iron-agar medium. The serotyping of *Salmonella* was based on Kauffmann–White (KW) scheme. During the process, we identified *Salmonella* Typhimurium and its monophasic variant by multiplex-PCR for the *fliB-fliA* intergenic region and the *fljB* gene.[13]

Antimicrobial Susceptibility Testing

Minimum inhibitory concentrations (MICs) were determined by the agar dilution method. Antibiotics used in this study were listed in Table 1. MICs were interpreted by CLSI breakpoints,[14,15] except that florfenicol (>16 mg/L) was interpreted according to EUCAST epidemiological cutoff values (http://mic.eucast.org/Eucast2).

Plasmid Conjugation Experiments

Conjugation experiments were performed between donor *Salmonella* 4,[5],12:i:- and recipient streptomycin-resistant *E. coli* C600, and transconjugants were selected on the Luria–Bertani agar plate containing streptomycin (3000 mg/L) and colistin (2 mg/L). The *mcr-1* and *mdh* genes were used to distinguish donor, recipient and transconjugant based on the PCR analysis.[16,17]

Whole Genome Sequencing Analysis

To understand the genetic background of *Salmonella* 4,[5],12:i:- YZU1189, the strain YZU1189 was sequenced using the Hiseq 2500 platform. Raw sequencing reads were deposited in the European Nucleotide Archive database under the accession number PRJEB38934. The raw reads were trimmed and filtered by NGSQC toolkit (v2.3.3), and subjected to de novo assembly by SPAdes 3.6.[18] The assembled genome was annotated by Prokka version 1.12.[19] The acquired antimicrobial resistance genes and chromosomal mutations was identified by ResFinder 3.2.[20] The multilocus sequence type of the strain YZU1189 was obtained by MLST 2.0.[21] The virulence factors of strain YZU1189 was detected by BLASTn based on seven typical virulence genes in *Salmonella* Typhimurium, of which four genes located on prophages including *gipA* (encoding a Peyer's patch-specific virulence factor), *sspH1* (*Salmonella*-type III effector protein), *sodC1* (putative Cu/Zn superoxide dismutase) and *sopE1* (*Salmonella*-type III effector protein), and three genes (*spvC, pefA, rck*) in plasmids.[22]

Table 1 The Minimum Inhibitory Concentration (MIC) Values of the Donor (*Salmonella* 4,[5],12:i:- YZU1189), Transconjugant (*E. coli* C600-pYZU1189) and Recipient (*E. coli* C600)

Classes	Antibiotics	MIC (mg/L)			Antibiotic Resistance Genes (YZU1189)
		YZU1189 (*S.* 4,[5],12:i:-)	C600 (*E. coli*)	C600-pYZU1189 (*E. coli*)	
Aminoglycosides	Gentamicin Streptomycin Amikacin	16 (R) 4 (S) 0.5 (S)	0.25 (S) >256 (R) 1 (S)	64 (R) >256 (R) 2 (S)	*aac(3)-IV, aph(4)-Ia, aph(3')-Ia, aac(6')-Iaa*
Beta-lactams	Ampicillin Cefazolin Cefotaxime	>128 (R) >128 (R) 32 (R)	2 (S) 2 (S) 0.06 (S)	>128 (R) >128 (R) 4 (R)	$bla_{CTX\text{-}M\text{-}14}$
Carbapenems	Meropenem	0.015 (S)	0.03 (S)	0.03 (S)	
Polymyxins	Colistin	8 (R)	0.25 (S)	8 (R)	*mcr-1*
Fosfomycins	Fosfomycin	>512 (R)	32 (S)	>512 (R)	*fosA3*
Phenicols	Chloramphenicol Florfenicol	128 (R) >128 (R)	2 (S) 4 (S)	64 (R) 128 (R)	*floR*
Trimethoprims /sulfonamides Tetracyclines	Trimethoprim/ sulfamethoxazole Tetracycline	64 (R) 128 (R)	1 (S) 2 (S)	64 (R) 2 (S)	*sul2, sul3, drfA12* *tet(B)*
Fluoroquinolones	Ciprofloxacin Nalidixic acid	1 (S) >256 (R)	0.015 (S) 4 (S)	0.015 (S) 4 (S)	*gyrA*(D87N)

Abbreviations: MIC, minimum inhibitory concentration; R, resistant; S, susceptible.

Ethical Approval

The study protocol 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). Fresh feces of all healthy catering workers were sampled after obtaining written informed consents.

Results and Discussion

In the surveillance study, we observed a *Salmonella* 4,[5],12:i:- strain YZU1189 harboring *mcr-1, fosA3* and $bla_{CTX\text{-}M\text{-}14}$ from a healthy female catering worker. This MDR isolate was defined using the criteria established by Magiorakos et al.[23] MICs of 15 antibiotics for the strain YZU1189 showed that YZU1189 was resistant to antibiotics including aminoglycosides, beta-lactams, polymyxins, fosfomycins, phenicols, trimethoprims, sulfonamides, tetracyclines and fluoroquinolones (Table 1). Compared to the typical ASSuT resistance pattern in most of *Salmonella* 4,[5],12:i:- isolates, this strain lacked the phenotypic streptomycin resistance, while showed resistance to colistin and fosfomycin.

According to the MLST analysis, *Salmonella* 4,[5],12:i:- strain YZU1189 belonged to sequence type (ST) 34. Virulence factors analysis of seven genes showed that this isolate carried virulence factors *gipA* and *sodC1*, which were prevalent in *Salmonella* 4,[5],12:i:- isolates from foods in China.[8] Strain YZU1189 contained several antimicrobial resistance (AMR) genes including aminoglycosides (*aac(3)-IV, aph(4)-Ia, aph(3')-Ia, aac(6')-Iaa)*, β-lactamase ($bla_{CTX\text{-}M\text{-}14}$), polymyxins (*mcr-1*), fosfomycins (*fosA3*), phenicols (*floR*), sulphonamides (*sul2* and *sul3*), tetracycline (*tet(B))*, and trimethoprims (*drfA12*). In addition, the mutation of codon 87 (D87N) in the GyrA protein causing the quinolone resistance was also detected in this strain. The *mcr-1* gene was previously observed in ST34 *Salmonella* Typhimurium from pigs in China, which located mainly on IncHI2-like plasmids.[5] The PCR analysis showed that the transconjugant was positive for the *mcr-1* gene and negative for the *Salmonella* Typhimurium specific *mdh* gene, indicating the successful transmission of *mcr-1* positive plasmid into the recipient strain *E. coli* C600. The plasmid conjugation experiment

showed that the *mcr-1* gene in YZU1189 could be co-transferred with $bla_{CTX-M-14}$ and *fosA3* genes (Table 1).

By whole genome sequencing analysis, we confirmed several antimicrobial resistance genes located on an IncHI2 plasmid (246Kb), named pYZU1189 (accession number ERS4951189), which harbored *mcr-1, fosA3, floR, sul2, sul3, drfA12, aac(3)-IV, aph(4)-Ia, aph(3')-Ia,* $bla_{CTX-M-14}$ genes (Figure 1). The *mcr-1* gene was located in the common genetic background of IS*Apl1-mcr-1-orf*, which was also detected in pECHN-15–61(IncI2), pECFJ-B42-63(IncI2), and pECJS-B60-267(IncHI2) plasmids,[24] while the *fosA3* gene had the common genetic background of ΔIS*Ecp1-*$bla_{CTX-M-14}$-IS*903B-fosA3-orf* located in pYZU1189. IncHI2 plasmids harboring antimicrobial genes found in this study were also reported in *Enterobacteriaceae*, such as *E. coli* and *Salmonella spp*.[6,7,24] The sequence alignment showed that an IncHI2-type plasmid pHNSHP45-2 harboring a similar genetic background with *mcr-1,* $bla_{CTX-M-14}$ and *fosA3* in *E. coli* (Figure 1). Five *Salmonella* Typhimurium isolates carrying co-transferred *fosA3,* $bla_{CTX-M-14}$, *mcr-1, oqxAB* and *floR* genes from food animals between 2016 and 2017 in China have been previously reported.[6] More importantly, *mcr*-positive *Salmonella* 4,[5],12:i:- isolates with multiple antimicrobial resistance genes have been frequently reported from different sources in multiple countries worldwide,[25–32] indicating the increasing risk potential of this pathogen transmitting worldwide. Also, *mcr*-positive *Salmonella* 4,[5],12:i:- isolates from difference sources showed a potential dissemination between food animals and humans, which remains a significant threat to human health.

Conclusion

In conclusion, this study reported an ESBL-producing *Salmonella* 4,[5],12:i- isolate of human origin carrying a colistin resistance gene *mcr-1*, a fosfomycin resistance

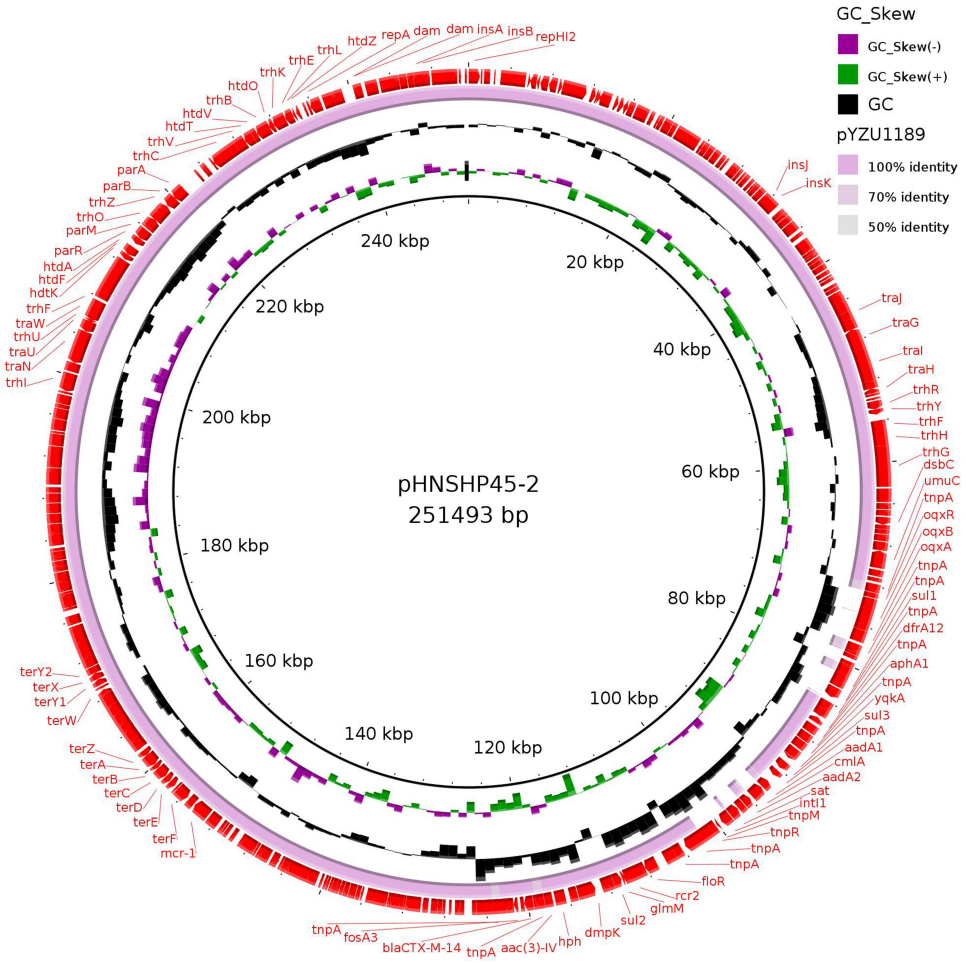

Figure 1 Sequence alignment of pYZU1189 and pHNSHP45-2 (GenBank no. KU341381). The pHNSHP45-2 was used as a reference to compare with *mcr-1*-bearing plasmid which possess the IncHI2 replicon in this study. Red arrows represent the plasmid pHNSHP45-2.

gene *fosA3* and a rare sulfonamide resistance gene *sul3*. The occurrence of *mcr-1* and *fosA3* genes in a transferable IncHI2 plasmid pYZU1189 from a ESBL-producing *Salmonella* 4,[5],12:i:- isolate brings great considerable public health threat. Great concern should be taken for the spread of multi-drug-resistant ESBL-producing *Salmonella* isolates from food catering workers to consumers. In the "One Health" aspect, quick and thorough action should be taken to reduce the use of colistin in food-producing animals in the farming industry. Interventions of restricting antibiotic use in food-producing animals should also be applied to reduce the prevalence of MDR bacteria.

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Disclosure

The authors report no conflicts of interest in this work.

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