LETTER TO THE EDITOR

Salmonella enterica serovar Typhimurium ST34 co-expressing bla<sub>NDM-5</sub> and bla<sub>CTX-M-55</sub> isolated in China

Lang Yang¹,*¹, Xiaofeng Hu¹,*¹, Xuebin Xu²,*¹, Chaojie Yang¹, Jing Xie¹, Rongzhang Hao¹, Xinying Du¹, Ligui Wang¹, Leili Jia¹, Peng Li¹, Shaofu Qiu¹ and Hongbin Song¹

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Dear Editor,

The bla<sub>NDM-5</sub> gene, which encodes New Delhi metallo-β-lactamase-5, was first reported in 2011 in Escherichia coli from a patient in the United Kingdom.¹ NDM-5 has been reported in many other countries. A variety of plasmid types have been reported to contribute to the widespread dissemination of β-lactam resistance genes. These NDM carriers are mainly identified in Enterobacteriaceae, frequently in Klebsiella pneumoniae and E. coli. Although NDM-1 has been found in Salmonella enterica, to our knowledge, no other variants of NDM have been identified in S. enterica. Here we characterize the first identification of Salmonella enterica serovar Typhimurium (S. Typhimurium) strain SSH006 carrying bla<sub>NDM-5</sub>, through routine surveillance in China. This is the first report of the bla<sub>NDM-5</sub>-harboring S. Typhimurium.

Strain SSH006 was recovered from a fecal sample of a 71-year-old male patient in September 2015 in the enteric clinic of the Minhang district, Shanghai, China. The strain SSH006 was identified as S. Typhimurium by amplification and sequencing of the 16S rRNA gene. The serotype was also determined by using slide agglutination S. Typhimurium. S. enterica serovar Typhimurium (v2.04) with 109-fold coverage. Multi-locus sequence typing analysis showed that SSH006 belongs to sequence-type 34 (ST34). In addition to bla<sub>NDM-5</sub>, multiple resistance genes were identified, including bla<sub>TEM-1</sub>, bla<sub>CTX-M-55</sub>, aadA1, sul1, sul2, sul3, aac(6')-Iaa, tet(A), floR and dfrA12. Interestingly, there were two bla<sub>TEM-1</sub>-I gene: one located on the chromosome and the other truncated and located adjacent to the bla<sub>CTX-M-55</sub> gene. A BLAST search indicated that the bla<sub>CTX-M-55</sub>-carrying contig covered 5 kb and consisted of a novel combination of K. pneumoniae plasmid pKP090853 and Shewanella sp. ANA-3 plasmid 1 (CP0000470, unpublished).

Antimicrobial susceptibility testing was performed with a Vitek 2 compact system, and the results were interpreted on the basis of the CLSI guidelines.² The results showed that strain SSH006 was resistant to most tested antibiotics, including imipenem, but was susceptible to aztreonam, amikacin, ciprofloxacin, levofloxacin and nitrofurantoin (Supplementary Table S1). Transferability of the bla<sub>NDM-5</sub> gene was assessed by conjugation experiments using the sodium azide-resistant E. coli strain J53 as the recipient. The transconjugants were selected on MacConkey agar plates with amoxicillin/clavulanic acid (Supplementary Table S1). Unexpectedly, resistance to cephalosporin antibiotics was also observed. Subsequent sequencing revealed that the cephalosporin resistance gene bla<sub>CTX-M-55</sub> was located on the large plasmid, which was simultaneously transferred to the transconjugants. The presence of the bla<sub>NDM-5</sub> and bla<sub>CTX-M-55</sub> genes in the transconjugants was demonstrated by PCR amplification.

The whole genome of S. Typhimurium SSH006 was sequenced by the Novogene Company (Beijing, China) on the HiSeq 2500 platform. Pair-end reads of 350 bp were assembled using SOAPdenovo (v2.04) with 109-fold coverage. Multi-locus sequence typing analysis showed that SSH006 belongs to sequence-type 34 (ST34). In addition to bla<sub>NDM-5</sub>, multiple resistance genes were identified, including bla<sub>TEM-1</sub>, bla<sub>CTX-M-55</sub>, aadA1, sul1, sul2, sul3, aac(6')-Iaa, tet(A), floR and dfrA12. Interestingly, there were two bla<sub>TEM-1</sub>-I gene: one located on the chromosome and the other truncated and located adjacent to the bla<sub>CTX-M-55</sub> gene. A BLAST search indicated that the bla<sub>CTX-M-55</sub>-carrying contig covered 5 kb and consisted of a novel combination of K. pneumoniae plasmid pKP090853 and Shewanella sp. ANA-3 plasmid 1 (CP0000470, unpublished).

The complete sequence of the bla<sub>NDM-5</sub>-carrying plasmid pNDM5-SSH006 is 46 253 bp in length and shares > 99% identity with the IncX3 plasmid pNDM5_MGR194 that was isolated in India,³ with 17 nucleotide changes. Twelve of the 17 nucleotide changes are located within the truncated ctuA1 gene, and one is located in the insertion sequence IS26 downstream of the bla<sub>NDM-5</sub> gene. In addition to these 13 nucleotide variations, the genetic context of bla<sub>NDM-5</sub> in the two plasmids is identical (ISSwil-IS3000-ΔISAb125-1SS-bla<sub>NDM-5</sub>-ble<sub>TRP3</sub>-tat-ΔctuA1-IS26-ΔumaD). The shotgun whole-genome sequence and complete sequence of plasmid pNDM5-SSH006 have been deposited in GenBank under accession number MTKV000000.

A variety of bla<sub>NDM-5</sub>-harboring plasmids have been identified and found to share similar sequences with plasmid pNDM5_MGR194, such as pEc1929,⁴ pNDM5_0215,⁵ pECNDM101,⁶ and pNDM5-IncX3,⁷

¹Institute of Disease Control and Prevention, Academy of Military Medical Sciences, Beijing 100071, China and ²Shanghai Center for Disease Control and Prevention, Shanghai 200236, China
*These authors contributed equally to this work.
Correspondence: Peng Li, SF Qiu; HB Song
E-mail: jiekenlee@126.com; qishf0613@hotmail.com; hongbinsong@263.net
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pNDM-QD28 and pNDM-QD29. Most of these have been isolated in China (Supplementary Table S2). All of the above plasmids had the same genetic context of NDM except pNDM_0215, which had an insertion of IS5 within the truncated insertion sequence IS3000 (Figure 1). Notably, the blaNDM-5-carrying IncN plasmid pTK1044 from Japan also shared a similar NDM genetic environment with a deletion of the truncated ISAba125 but was ~110 kb in length,10 thus suggesting a potential dissemination of NDM-5 via mobile genetic elements. Plasmids pJEG027 harboring blaNDM-411 and pKpN01-NDM7 harboring blaNDM-712 were also found to be almost identical to pNDM5-SSH006 except for the variation of the NDM gene. The blaNDM-7-carrying IncX3 plasmid pOM26-1 (KP776609, unpublished) is similar to pNDM5-SSH006, except that it has a deletion of ISAba125.

Given that blaNDM-5 and blaNDM-2 differ from blaNDM-4 by a single nucleotide change (G388A and G262T, respectively), the documentation of travel to India indicated that the blaNDM-5-carrying plasmid pTK1044 from Japan also shared a similar NDM genetic environment with a deletion of the truncated ISAba125 but was ~110 kb in length,10 thus suggesting a potential dissemination of NDM-5 via mobile genetic elements. Plasmids pJEG027 harboring blaNDM-411 and pKpN01-NDM7 harboring blaNDM-712 were also found to be almost identical to pNDM5-SSH006 except for the variation of the NDM gene. The blaNDM-7-carrying IncX3 plasmid pOM26-1 (KP776609, unpublished) is similar to pNDM5-SSH006, except that it has a deletion of ISAba125.

To the best of our knowledge, this is the first report of S. Typhimurium carrying the blaNDM-5 gene. The blaNDM-5-carrying plasmids have been reported in a narrow host range in E. coli, K. pneumoniae and recently P. mirabilis.13 Our work further expands the host range and provides additional evidence of the rapid dissemination of blaNDM-5 among different species of Enterobacteriaceae in China. Given that NDM-5 differs from NDM-1 by two amino-acid substitutions and confers increased resistance to expanded-spectrum cephalosporins and carbapenems,1 the ability of this blaNDM-5-carrying plasmid to transfer across species boundaries may pose a great threat to humans and is quite worrisome. S. Typhimurium ST34 clones have raised international concern regarding its rapid spread and multi-drug resistance. A previous study has revealed that S. Typhimurium ST34 clones experienced a rapid expansion in China and exhibit a low percentage susceptibility to cephalosporin antibiotics.14 However, S. Typhimurium ST34

SSI006 exhibited higher resistance to all tested cephalosporin antibiotics.

Here we report the first case of S. Typhimurium ST34 SSI006 harboring the blaNDM-5 gene. The co-existence of two transferable plasmids carrying blaCTX-M-55 and blaNDM-5 in S. Typhimurium highlights the urgent need for more extensive surveillance and effective action to control its further dissemination.

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Supplementary Information for this article can be found on the *Emerging Microbes & Infections* website (http://www.nature.com/emi)