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Letter to the Editor

Detection of *mcr-1*-mediated colistin resistance in *E. coli* isolate from imported chicken meat from Brazil

Sir

Carbapenem resistant *Enterobacteriaceae* (CRE) have emerged and disseminated, which are usually multidrug-resistant and effective antibiotics are limited, such as colistin and tigecyclin. In November in 2015, plasmid-mediated colistin resistance gene (*mcr-1*) was identified in *Escherichia coli* (*E. coli*) obtained from a pig in China [1]. This gene can transfer by conjugation. Many reports were published in a short period, indicating that *mcr-1* had been prevalent worldwide.

During the surveillance of ESBL/AmpC-producing bacteria from imported chicken meats collected at Kobe and Yokohama quarantine station in Japan, we isolated one *E. coli* strain (KT2378) harboring *mcr-1* from a chicken meat imported from Brazil in 2013. It exhibited resistance to piperacillin (PIPC) (MIC: 128 μg/mL), cefotaxime (CTX) (MIC: 32 μg/mL) and colistin (CL) (MIC: 8 μg/mL) and it was found to have TEM and CTX-M2 type genes by PCR. In addition, *mcr-1* was detected by PCR with specific primers (CLR5-F and CLR-5R) [1]. Whole ORF was amplified with specific primers (mcr-1/F: 5′-ATGATGCAGCATACT TCTGTGTGG-3′ and mcr-1/R: 5′-TCAGCGGAT-GAATGCGGTGCGGTC-3′) and DNA sequence was determined. It showed 100% homology with the published sequence of *mcr-1* in database (accession no. **NG_050417**). Sequence Type (ST) of KT2378 was revealed to be ST38. Transfer of antimicrobial resistance was attempted in liquid (4 h) and on a membrane filter (6 h). KT2378 and CSH55rif, a rifampicin resistant derivative of CSH55 (*Δ(lac, pro), supE, nal, thi*) were used as a donor and recipient, respectively. Although no transconjugant was obtained on selective plates containing colistin (4 mg/L) and rifampicin (40 mg/L) in liquid mating, transconjugants were obtained in membrane filter mating at the frequency of 2.6×10^{-2} per donor cell. Transfer of *mcr-1* to transconjugants was confirmed by PCR. Resistance to PIPC and CTX were not transferred in both matings, indicating that only *mcr-1* was located on a conjugative plasmid and TEM and CTX-M2 type genes were not located on the same plasmid. The total genomic DNA of a transconjugant, KT2387, was used for whole genome sequencing. The draft genome sequence of strain KT2387 was generated using Illumina MiSeq. After subtracting *E. coli* K-12 genome sequence, a plasmid of 33,304 bp in length was identified (accession no. **LC227558**). Forty-one ORFs were found and expected to encode proteins related to conjugation, plasmid replication and phosphoethanolamine transferase (MCR-1) conferring colistin resistance. As we expected from the result of conjugation, no gene encoding ESBL or AmpC was found. This plasmid was named pKT2378, and found identical to the IncX4-type *mcr-1* plasmid, pICBEC72Hmcr (accession no. **NZ_CP015977**) by BLAST search, except for two base pair substitutions at the position of 5425 (A to G) and 27559 (C to G) [2]. The former substitution resulted in an amino acid substitution at the position of 148 (N to G) in the putative gene encoding relaxase / mobilization nuclease domain protein, although the latter was not in a putative ORF. pICBEC72Hmcr is an IncX4-type *mcr-1*-bearing plasmid in *E. coli*, ICBEC72Hmcr (ST101), isolated from a patient with a diabetic foot infection in Brazil in 2016. The presence of almost identical plasmids in different host suggested that this *mcr-1*-bearing plasmid disseminated by conjugation. As we expected, IncX4-type *mcr-1*-bearing plasmids including pICBEC72Hmcr, isolated in different countries were reported to have very similar plasmid backbone sequence [2]. These plasmids were isolated from different bacterial species as well as in different countries. They suggested that this type of plasmids might play a big role in spread of *mcr-1* gene, and bacteria carrying this type of plasmid such as KT2378 had been transferred via meat from meat-production countries to other countries. Kawanishi et al. reported that 9306 *E. coli* strains were isolated from healthy animals between 2000 and 2014 in Japan and 39 of them were detected to carry *mcr-1* gene [3] Nine of *mcr-1* positive strains transferred colistin resistance, and were found to have a IncI2-type *mcr-1*-bearing plasmid. Recently, Ohsaki et al. reported the first detection of *E. coli* strain harboring the *mcr-1* gene located on IncI2 type plasmid in retail domestic chicken meat between 2015 and 2016 in Japan [4]. In 2017, IncX4 type *mcr-1*-bearing plasmid pRYU3223C-1 (accession no. **AP018411**) was detected from *Klebsiella pneumoniae* isolated from human first time in Japan [5]. pRYU3223C-1 is almost identical to pKT2378 like pICBEC72Hmcr. It is 6 bp longer than pKT2378 and there are 3 base pair substitutions. pKT2378 reported in this study was the first IncX4 type *mcr-1*-bearing plasmid detected in *E. coli* from meat in Japan. Although *mcr-1* had been disseminated in animals in Japan before 2013, new type of *mcr-1*-bearing plasmids were identified in imported chicken meat and human. Therefore, it is quite likely that resistant bacteria could transmit to human from meat, and the strict monitoring and surveillance of resistant bacteria among meat were strongly needed to prevent their dissemination to human.

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Competing interests

None declared.

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Ethical approval

Not required.

References

[1] Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 2016;16:161–8, doi:http://dx.doi.org/10.1016/S1473-3099(15)00424-7.

[2] Fernandes MR, McCulloch JA, Vianello MA, Moura Q, Pérez-Chaparro PJ, Esposito F, et al. First report of the globally disseminated IncX4 plasmid carrying the mcr-1 gene in a colistin-resistant *Escherichia coli* sequence type 101 isolate from a human infection in Brazil. Antimicrob Agents Chemother 2016;60:6415–7, doi:http://dx.doi.org/10.1128/AAC.01325-16.

[3] Kawanishi M, Abo H, Ozawa M, Uchiyama M, Shirakawa T, Suzuki S, et al. Prevalence of colistin resistance gene *mcr-1* and absence of mcr-2 in *Escherichia coli* isolated from healthy food-producing animals in Japan. Antimicrob Agents Chemother 2017;61:, doi:http://dx.doi.org/10.1128/AAC.02057-16.

[4] Ohsaki Y, Hayashi W, Saito S, Osaka S, Taniguchi Y, Koide S, et al. First detection of an *Escherichia coli* strain harboring the *mcr-1* gene in retail domestic chicken meat in Japan. Jpn J Infect Dis 2017;70:590–2, doi:http://dx.doi.org/10.7883/yoken.JJID.2016.572.

[5] Tada T, Uechi K, Nakasone I, Nakamatsu M, Satou K, Hirano T, et al. Emergence of IncX4 plasmids encoding *mcr-1* in a clinical isolate of *Klebsiella pneumoniae* in Japan. Int J Infect Dis 2018;75:98–100, doi:http://dx.doi.org/10.1016/j.ijid.2018.08.011.

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