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Clostridioides difficile: a potential source of NpmA in the clinical environment

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Sir,

Aminoglycosides are widely used to treat MDR Gram-negative bacterial infections with bactericidal activity mediated by binding the 16S rRNA aminoacyl-tRNA recognition site to prevent protein synthesis. Multiple aminoglycoside resistance mechanisms have been documented including 16S rRNA modification.[1] NpmA, an uncommon 16S rRNA methyltransferase originally identified in a clinical *Escherichia coli* isolate confers pan-aminoglycoside resistance.[2] In this study, routine WGS of hospital-acquired *Clostridioides* (*Clostridium*) *difficile* identified *npmA* in the genome of a clinical isolate (CD7814). *C. difficile* is a Gram-positive, spore-forming enteric pathogen and the cause of most hospital-acquired, antibiotic-associated diarrhoea. The epidemiology of *C. difficile* has changed in the past several decades with infections increasingly being reported outside of acute care settings.[3] The discovery of *npmA* in the genome of a clinical *C. difficile* isolate has implications for the spread of aminoglycoside resistance.

Clinical *C. difficile* isolates are routinely sequenced using Illumina NextSeq500 and Nextera libraries as part of an infection prevention initiative at our hospital.[4] Genomes are assembled using SPAdes, annotated with Prokka and characterized by searches of ResFinder and pubMLST databases (https://pubmlst.org/cdifficile/).[5–7] This analysis identified NpmA-coding sequences in *C. difficile* isolate CD7814 belonging to ST11. CD7814 carries two additional aminoglycoside resistance determinants: (i) *aph(3′)-III*, which encodes an aminoglycoside phosphotransferase; and (ii) *ant(6)-Ia*, which encodes an aminoglycoside nucleotidyltransferase.

Aminoglycoside susceptibility testing was performed by Etest on CD7814 and two additional ST11 isolates from our hospital that lack *npmA* (CD7861 and CD7786). Cell suspensions corresponding to 0.5 McFarland were prepared and plated onto Brucella blood agar plates supplemented with vitamin K_1 and haemin (Anaerobe Systems, Morgan Hill, CA, USA). Etest strips (bioMérieux, Durham, NC, USA) were applied and the plates were incubated anaerobically at 37°C for 48 h as previously described.[8] Etests were read according to the manufacturer's instructions. CD7814 demonstrated high-level resistance to gentamicin (>256 mg/L) relative to CD7861 (64 mg/L) and CD7786 (24 mg/L). High-level resistance to tobramycin and amikacin was observed in all ST11 *C. difficile* isolates tested. Although CLSI breakpoints for *C. difficile* are not defined for aminoglycosides, these data suggest that NpmA is expressed and associated with increased gentamicin resistance in CD7814.

Genomic analysis of the CD7814 assembly identified a large, presumably chromosomal contig of ∼150 kb containing the *npmA* gene. The majority of predicted ORFs surrounding *npmA* in CD7814 are hypothetical proteins whereas others encode proteins involved in recombination suggesting that *npmA* was acquired via horizontal gene transfer, which is consistent with the mosaic structure of the *C. difficile* chromosome (Figure 1a).[9] Five additional *C. difficile* genomes bearing *npmA* gene sequences that show 99% nucleotide identity to *npmA* from CD7814 and pARS3 were identified through BLAST and PubMed literature searches (Figure 1a). The DNA sequence flanking *npmA* in CD7814 shows little or no nucleotide identity to either *E. coli* pARS3 or the five *C. difficile npmA* flanking regions. The five *C. difficile* genomes are of human and animal origin and were collected from three different continents over a period of at least 10 years. Interestingly, these *C. difficile* isolates belong to three different STs but their genomes share 99% nucleotide identity across ∼3kb of the *npmA* region (Figure 1a). None of the five *C. difficile* genomes share any other sequence similarity to pARS3 outside of *npmA*. Together, these genomic data suggest that *npmA* is carried on a conserved element in the five *C. difficile* genomes and that the mechanism of *npmA* acquisition in CD7814 is different. In addition, all five *C. difficile* genomes encode a missense mutation in *npmA* resulting in a K131N substitution in NpmA relative to CD7814 and pARS3 sequences (Figure 1b). To maintain the established nomenclature for 16S rRNA methyltransferase genes, the CD7814 and, by default, the *E. coli* pARS3 *npmA* genes can be re-designated as *npmA1* while *npmA* sequences containing the K131N mutation can be designated *npmA2*.[10] CD7814 *npmA1* has been deposited in GenBank under accession number MH249957.

To the best of our knowledge, this is the first description of *npmA* and high-level aminoglycoside resistance in a hospital-acquired *C. difficile* isolate. Because of strict anaerobic growth

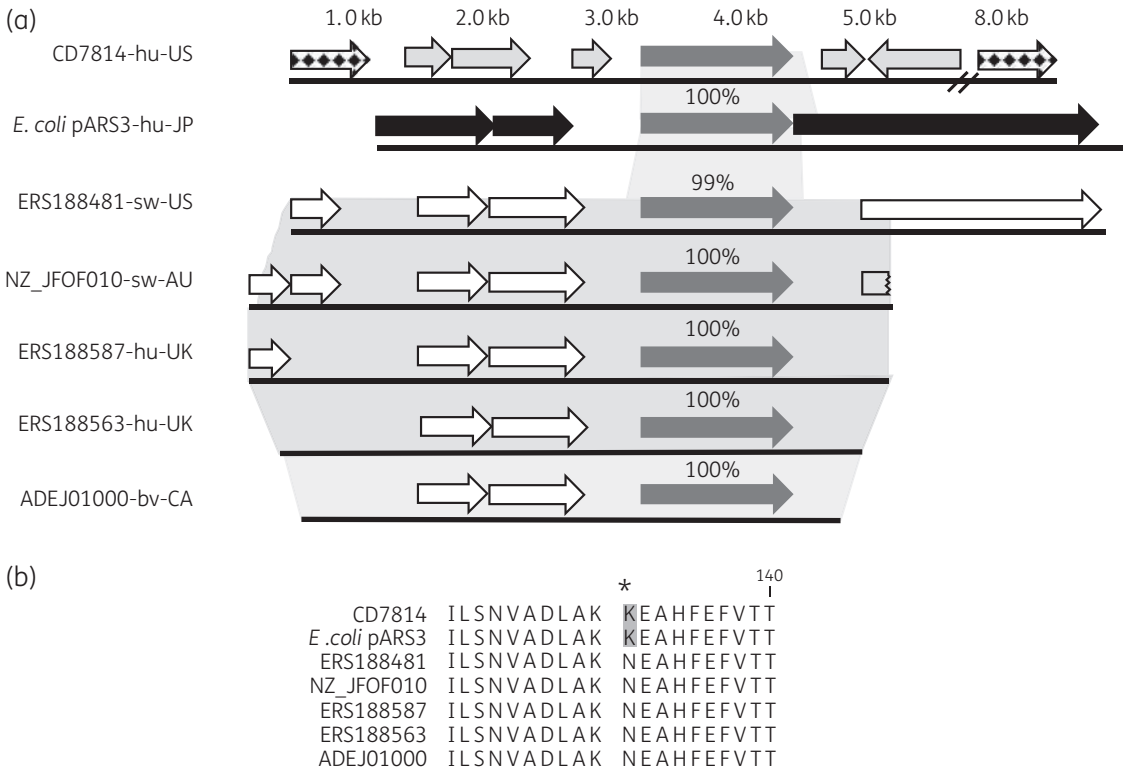

Figure 1. (a) Predicted ORFs surrounding the *npmA* gene (dark grey) in CD7814 (light grey), the *E. coli* pARS3 plasmid (black) and five *C. difficile* genomes of human and animal origin (white). Shaded areas highlight regions of sequence homology. Chequered arrows indicate ORFs encoding recombinases potentially associated with horizontal transfer of *npmA* into CD7814. hu, human; sw, swine; bv, bovine; US, USA, JP, Japan; AU, Australia; CA, Canada. (b) NpmA protein alignment depicting the K131N substitution in CD7814 and *E. coli* pARS3.

conditions, Etest is the most practical method to measure antibiotic susceptibility in *C. difficile*. As Etests for aramycin and neomycin are unavailable, a limitation of this study is our inability to demonstrate the specificity of NpmA methyltransferase activity for the N1-A1408 16S rRNA. However, the nucleotide identity between CD7814 *npmA* and the original *E. coli* sequence and the high-level aminoglycoside resistance observed support NpmA-mediated resistance in this clinical *C. difficile* isolate. To the best of our knowledge, no evidence to support high-level gentamicin resistance in the presence of *ant(6)-Ia* and *aph(3′)-III* has been reported.

In conclusion, this study demonstrates the presence of *npmA* and high-level aminoglycoside resistance in a clinical *C. difficile* isolate. The ability of *C. difficile* to persist in the environment as a spore former may facilitate acquisition of novel antibiotic resistance determinants. These data suggest that hospital-acquired *C. difficile* may be a reservoir for uncommon antibiotic resistance determinants such as *npmA*.

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Transparency declarations

None to declare.

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Wide dissemination of colistin-resistant *Escherichia coli* with the mobile resistance gene *mcr* in healthy residents in Vietnam

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Sir,

The importance of colistin in treating life-threatening infections caused by MDR bacteria is increasing. In this regard, dissemination of bacteria harbouring the mobile colistin resistance gene (*mcr*) in food animals has been highlighted, owing to the abundant usage of colistin in the agricultural sector in developing countries.[1] Although the prevalence of colistin-resistant bacteria in healthy human residents in these countries is unclear, a high prevalence of MDR bacteria in the faecal microbiota of residents in Vietnam and surrounding nations was recently revealed.[2] Therefore, here we examined the occurrence of dissemination of colistin-resistant bacteria harbouring *mcr* in the faecal microbiota of healthy residents of a representative Vietnamese village.

The study was conducted at Nguyen Xa village, Thai Binh province, Vietnam, from November 2017 to February 2018. The village, a representative rural community in Vietnam, had 7730 residents in 2008 households in 2015. A total of 98 healthy participants from 36 households were enrolled. The characteristics of the participants are summarized in Table 1.

One stool specimen was obtained from each participant using a transport swab with Cary–Blair transport medium (Eiken Chemical, Tokyo, Japan). Specimens were inoculated on selective agar medium (CHROMagar™ COL-APSE, CHROMagar, Paris, France) for isolation of colistin-resistant Gram-negative bacteria. The resulting Enterobacteriaceae-like colonies were isolated and characterized further for bacterial identification, antibiotic susceptibility, resistance genes and relevance between isolates, as described previously.[3–5]

The study was approved by the Ethics Committees of Osaka University (yakujin 29-8) and Thai Binh University of Medicine and Pharmacy (no. 773.1). All participants provided written informed consent. For any participant younger than 18 years, written informed consent was obtained from the respective parents.

As shown in Table 1, most of the stool specimens were culture positive on a selective-medium plate (CHROMagar™ COL-APSE). Among these colonies, colistin-resistant *Escherichia coli* (CR-E) that exhibited MICs of colistin between 8 and 16 mg/L were detected in 69 out of 98 specimens tested. The proportion of households that had members carrying CR-E was also quite high at 80.6% (29 positive, out of 36 households tested). Furthermore, almost all colistin-resistant isolates possessed *mcr-1* and/or *mcr-3*, except one that did not contain *mcr-1* to *-5*, as determined by PCR.

PFGE analysis of CR-E isolates showed that, within a household, the members of five households carried a similar strain, but between households there were no similar strains (Figure S1, available as Supplementary data at *JAC* Online).

Resistance profiles of CR-E to other antibiotics varied between 0 and 11, out of the 14 antibiotics tested (ampicillin, cefoxitin, cefotaxime, ceftazidime, meropenem, streptomycin, kanamycin, gentamicin, ciprofloxacin, nalidixic acid, tetracycline, chloramphenicol, fosfomycin and trimethoprim/sulfamethoxazole). The average number of antibiotics to which isolates were resistant was 5.6. The rate of MDR, defined as resistance to at least one antibiotic drug in three or more antibiotic classes,[6] of CR-E isolates was determined to be 92.8% (64/69). There were no carbapenem- or fosfomycin-resistant CR-E isolates (Table S1).

In contrast to the studies conducted on colistin-resistant bacteria in food animals, there have been only limited studies on the prevalence of colistin-resistant bacteria with *mcr* in healthy individuals. However, one recent study focused on *mcr-1*-carrying bacteria in the faecal samples of chicken farmers in Vietnam.[7] This study reported that 25% of farmers were colonized with *mcr-1*-carrying bacteria that grew on non-selective medium. However, this protocol may have the potential to lose CR-E strains owing to the abundant susceptible bacteria in stool specimens. As a result, it is difficult to obtain the true prevalence of colistin-resistant bacteria in stool specimens. In the current study, we utilized the selective medium, CHROMagar™ COL-APSE, for identifying colistin-