16S ribosomal RNA methylase RmtD produced by Klebsiella pneumoniae in Brazil

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Sir,

Aminoglycosides are bactericidal agents that are used in the treatment of life-threatening infections caused by Gram-negative pathogens. They exert their antibacterial property by blocking the peptide bond-forming translocation process through binding to the aminocetyl site (A-site) of the 16S ribosomal RNA (rRNA) that constitutes the bacterial 30S ribosome.1

The most common mechanism of resistance to aminoglycosides by Gram-negative pathogens is the production of aminoglycoside modification enzymes such as acetyltransferases, adenylyltransferases and phosphotransferases. Resistance to aminoglycosides due to production of 16S rRNA methylases has been reported for Gram-negative bacteria in recent years.2 They methylate residue G1405 in the A-site and confer high-level resistance to most injectable aminoglycosides including gentamicin, amikacin and tobramycin. Five such 16S rRNA methylases have been identified: RmtA, RmtB, RmtC, RmtD and ArmA. Most recently, NpmA, which encoded A1408 instead of G1405, was reported from Escherichia coli in Japan, further adding to the diversity of this class of enzymes.3 RmtD was initially found to be produced by a Pseudomonas aeruginosa clinical strain from Brazil. This particular strain also produced the metallo-β-lactamase SPM-1.4 It has been subsequently shown that RmtD is already widespread among certain clones of imipenem-resistant P. aeruginosa in Brazil.5 We report here the occurrence of RmtD in Klebsiella pneumoniae.

K. pneumoniae R2 was isolated at a hospital in Salvador, Brazil in 2004. The patient was a 69-year-old male who had been admitted for management of ischaemic stroke. Prior to the isolation of K. pneumoniae from a peri-gastrostomy infection site, he had been treated with ampicillin/sulbactam, piperacillin/tazobactam, levofloxacin and ciprofloxacin for management of pneumonia and urinary tract infection. No aminoglycosides had been given to the patient. The wound infection was subsequently treated with cefepime and polymyxin B, which led to resolution.

K. pneumoniae R2 was highly resistant (MICs > 256 mg/L) to all aminoglycosides tested including amikacin, tobramycin, gentamicin and arbekacin. It was also resistant to ampicillin, but was susceptible to cefotaxime, ceftazidime, cefepime, imipenem, meropenem and ciprofloxacin. The high-level aminoglycoside resistance trait of K. pneumoniae R2 was successfully transferred to E. coli XL-1 Blue NA1 (resistant to nalidixic acid) at a frequency of $0.6 \times 10^{-5}$ to $2 \times 10^{-4}$ cells per recipient cell by the standard filter mating method. Multiplex PCR analyses for the 16S rRNA methylase genes2 yielded an amplicon for rmtD in K. pneumoniae R2 as well as the transconjugant strain, which was confirmed by sequencing.

For cloning experiments, transformation of E. coli DH10B was performed with the plasmids of K. pneumoniae R2 by electroporation, which yielded amikacin-resistant transformants. Plasmid was extracted from one of the transformants and digested with EcoRI (New England Biolabs, Ipswich, MA, USA). The resultant fragments were ligated with plasmid vector pBC SK− (Stratagene, La Jolla, CA, USA) digested with the same restriction enzyme. Electrocompetent E. coli DH10B was prepared and transformed with these ligated products. Transformants were selected on LB agar plates containing 50 mg/L chloramphenicol and 50 mg/L amikacin. This yielded a recombinant plasmid with an ~8 kb EcoRI fragment containing rmtD (pRS1E2). E. coli DH10B (pRS1E2) exhibited high-level aminoglycoside resistance (MICs ≥ 256 mg/L for all aminoglycosides tested), which verified expression of rmtD. Sequencing of ~3 kb of the neighbouring regions of rmtD revealed the same genetic environment reported previously with rmtD from P. aeruginosa.4 rmtD was flanked by a putative tRNA ribosyltransferase gene upstream and a putative transposase gene downstream.

Plasmid electrophoresis revealed that K. pneumoniae R2 had plasmids of various sizes (Figure 1a). Southern hybridization analysis confirmed the location of rmtD on the largest and conjugative plasmid, pRS1 (~80 kb) (Figure 1b).

Among the other 16S RNA methylases, RmtA has only been isolated from P. aeruginosa in Japan.5 RmtB and C are confined to Enterobacteriaceae thus far, whereas ArmA is present in Enterobacteriaceae as well as Acinetobacter baumannii.7 RmtD was initially identified in P. aeruginosa from hospitals in the metropolitan areas of São Paulo, Brazil.4,5 Our findings, along with a recent report by Fritsche et al.,8 indicate that RmtD is the second 16S rRNA methylase produced by both Enterobacteriaceae and glucose non-fermenting species. The prevalence of the rmtD gene among imipenem-resistant P. aeruginosa clinical strains in São Paulo hospitals has been reported to be as high as 75%, largely due to dissemination of a single epidemic clone.5 Now that rmtD has made its way to K. pneumoniae, it has the potential to further spread to species belonging to Enterobacteriaceae by means of conjugal transfer. Although the RmtD-producing strain in the present study was relatively susceptible to other classes of antimicrobials, future
incorporation of the rmtD gene by multidrug resistance plasmids is a concern.

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Transparency declarations

None to declare.

References


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Detection of a single isolate of CTX-M-1-producing Escherichia coli from healthy pigs in Denmark

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Sir,

Extended-spectrum β-lactamase (ESBL)-mediated resistance is an increasing concern in human clinical settings. In Denmark, only a few cases of ESBL-producing Escherichia coli have been reported from food animals, however, there was no baseline study on ESBL prevalence among the healthy pig populations in Denmark. In this study, we investigated the prevalence of ESBL-mediated resistance in E. coli isolates obtained from faecal samples of healthy pigs in Denmark. Furthermore, ESBL-related genes and mutations were determined and cephalosporin consumption in pig farms associated with ESBL-mediated resistance was investigated.

As part of the DANMAP surveillance programme (The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme), a total of 137 faecal samples were randomly selected among the healthy pigs at farm level between November 2005 and March 2006. Faecal sample was enriched in MacConkey broth containing 2 mg/L cefotaxime and 3M™ Petrifilms™ Select E. coli Count Plates (SEC plates) with 2 mg/L cefotaxime were used to identify E. coli with reduced susceptibility to cefotaxime. E. coli appears on SEC plates as dark green to light-blue-green colonies and was subcultured on Mueller–Hinton II agar plates supplemented with 2 mg/L.