Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother 2021; **76**: 531–533
doi:10.1093/jac/dkaa436
Advance Access publication 11 November 2020

Co-occurrence of the bla_{VIM-1} and bla_{SHV-12} genes on an IncHI2 plasmid of an *Escherichia coli* isolate recovered from German livestock

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Sir,

The dissemination of carbapenemase-producing Enterobacterales (CPE) is an important public health issue. The number of human CPE isolates has been steadily increasing during recent years, worldwide. Despite the fact that carbapenems are not licensed for use in veterinary medicine, increasing numbers of CPE from the veterinary sector have been reported.[1] The transmission of CPE between pets/livestock and exposed humans as well as via food has been demonstrated.[2] In this study, a detailed characterization of a carbapenem-resistant porcine *Escherichia coli* co-harbouring bla_{VIM-1}, bla_{SHV-12} and bla_{ACC-1} genes, along with other resistance genes, is provided.

Within the German annual monitoring of ESBL/AmpC β-lactamase-producing *E. coli* from animals and food in 2017–18, the isolate 17-AB02384 was recovered from the caecal content of a fattening pig at slaughter. Based on EUCAST epidemiological cut-off values (https://www.eucast.org/mic_distributions_and_ecoffs/), the isolate showed a non-WT phenotype to carbapenems and other antimicrobials [Table S1 (Table S1 is available as Supplementary data at *JAC* Online)]. Molecular analysis revealed that *E. coli* 17-AB02384 belonged to the phylogenetic group A and the multilocus sequence type (MLST) 7593.[3] To our knowledge, this ST has not yet been described in *E. coli* from German livestock or food chain. However, ST7593 was reported for some NDM-5-producing isolates of retail meat samples in China.[4]

Initial S1-PFGE plasmid profiling and subsequent DNA–DNA hybridization[3] indicated that the bla_{VIM-1} gene was located on an approximately 300 kb IncHI2 plasmid, designated pEC17-AB02384, which was transferable into the *E. coli* J53 by conjugation at a transfer rate of 2×10^{-4}. For a detailed characterization, the whole-genome sequence of *E. coli* 17-AB02384 was determined by Illumina (CA, USA) short-read and PacBio (Menlo Park, USA) long-read sequencing, according to the manufacturers' recommendations. Hybrid assembly of the plasmid sequence was carried out using unicycler v.0.44. The resulting sequence of the plasmid pEC17-AB02384 was deposited at GenBank (NCBI) under the accession number MT163739. MLST, resistance and virulence genes were determined using online tools that were provided by the Danish Technical University (http://www.genomicepidemiology.org) . The annotation was carried out by RAST2 provided by PATRIC (www.patricbrc.org). Characteristics of the isolate and its plasmid are summarized in Table S2.

Besides bla_{VIM-1}, this plasmid harboured the ESBL gene bla_{SHV-12} and the AmpC β-lactamase gene bla_{ACC-1}. Overall, pEC17-AB02384 showed similarity to the VIM-1-encoding plasmids pSE15-SA01028 (90% identity, CP026661.1) and pRH-R178 (93% identity, HG530658.1) from *Salmonella enterica* subsp. *enterica* and *E. coli*, respectively, which were both recovered from German pigs (Figure 1a).[5] In contrast to these plasmids, pEC17-AB02384 harboured three additional resistance gene-carrying segments. The 9773 bp segment 1 comprised the resistance genes *sul1* and *qnrA1* and was flanked by 124 bp inverted repeats. It was inserted into the region upstream of the bla_{VIM-1}-carrying class 1 multiresistance integron in pEC17-AB02384 (Figure 1b). Downstream of the integrase gene *intI1* of the bla_{VIM-1}-carrying integron, the segment 2 was integrated. It carried the macrolide resistance operon *mph*(A)-*mrx*-*mphR*, the trimethoprim resistance gene *dfrA14* and another *intI1* gene. Immediately downstream of both *intI1* genes, 202 bp direct repeats were found (Figure 1b). It appears possible that a translocatable unit comprising the entire segment 2 was inserted into plasmid pEC17-AB02384 by recombination with the *intI1* gene of the bla_{VIM-1}-carrying integron and its adjacent repeat region. The 4979 bp segment 3 was inserted into segment 2 between the Δ*rec* and the *mph*(A) gene. It carried the ESBL gene bla_{SHV-12} and was flanked by IS*26* elements in opposite orientation. However, no direct repeats were detected at the immediate boundaries of segment 3, suggesting that the IS*26*-bounded segment 3 does not function as a transposon.

To our knowledge, the co-occurrence of bla_{VIM-1} and bla_{SHV-12} has not yet been described in plasmids from isolates of animal

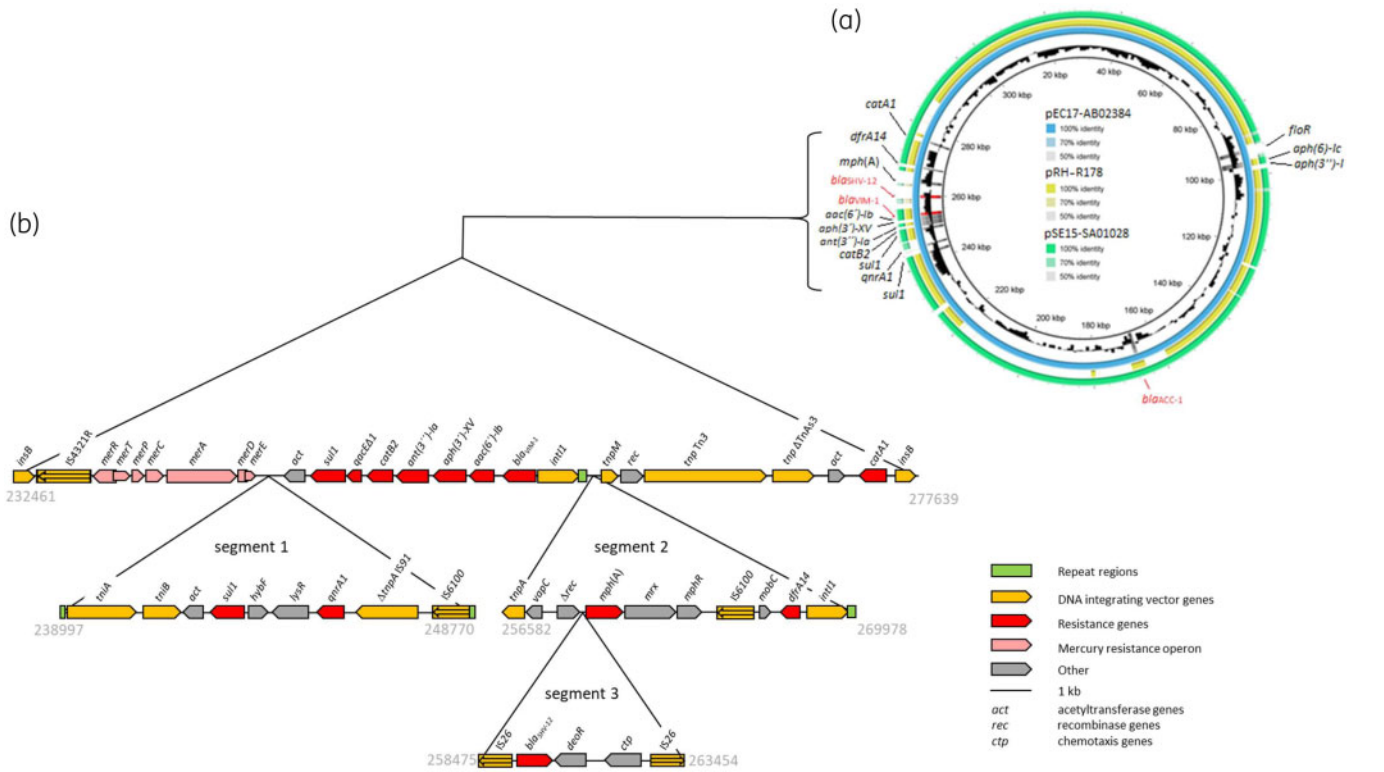

Figure 1. (a) Schematic illustrations of plasmids pRH-R178 and pSE15-SA01028 in comparison with plasmid pEC17-AB02384 described in this sudy. (b) Schematic illustration of the multidrug resistance region of plasmid pEC17-AB02384. The reading frames are displayed as arrows with the arrowhead showing the dirction of transcription. The numbers refer to the whole plasmid sequence of pEC17-AB02384, which is deposited in the GenBank database under accession no. MT163739. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

origin. Co-location of these genes was reported only for a single human clinical *Aeromonas caviae* isolate (KR869764) in 2014.[6] The corresponding plasmid belonged to the replicon type IncA/C and showed no substantial similarities to pEC17-AB02384. SHV-12 is an extended-spectrum β-lactamase that is commonly detected in isolates from poultry, but rarely from pigs.[7] Alonso *et al.*[7] provided further data on bla_{SHV-12}-carrying plasmids from human, animal and food sources. Among them, the *E. coli* plasmid pCAZ590 (LT669764) exhibits a similar SHV-12 region as the one identified in pEC17-AB02384. In general, bla_{SHV-12} seems to be associated with IS*26* elements, which might support its mobility. The ability of IS*26* to mobilize neighbouring genes might play an important role in the persistence of antimicrobial resistances.[8]

A comparison of the few known bla_{VIM-1}-carrying plasmids from German livestock revealed close relationships. This might indicate that a prototype-plasmid has adapted to bacteria in different animal populations and persists in an unknown reservoir. The characterization of CPE isolates and their plasmids will contribute to the further understanding of reservoirs, potential transmission pathways and persistence factors for plasmid maintenance in bacteria from animal husbandry.

Acknowledgements

We gratefully acknowledge the support of the regional laboratories and authorities in their general support during sampling and providing isolates in the framework of the monitoring. We also thank the laboratory team of the NRL-AR, especially Britta Lesniewsky and Silvia Schmoger, for excellent technical assistance.

Funding

This work was supported by the German Federal Institute for Risk Assessment (43-001), by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement number 773830: One Health European Joint Programme, and the German Federal Ministry of Education and Research (BMBF) under project number 01KI1727D as part of the Research Network Zoonotic Infectious Diseases.

Transparency declarations

We declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online.

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J Antimicrob Chemother 2021; **76**: 533–535
doi:10.1093/jac/dkaa450
Advance Access publication 5 November 2020

Distinct evolution of colistin resistance associated with experimental resistance evolution models in *Klebsiella pneumoniae*

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Sir,

Klebsiella pneumoniae ranks at the top of the global priority list of pathogens regarding demand for new treatment options.[1] Colistin represents a last-resort antibiotic for treatment of MDR *K. pneumoniae* infections.[2] Colistin resistance in *K. pneumoniae* is attributed to the accumulation of chromosomal mutations, mainly in *pmrAB, phoPQ, ccrAB* and *mgrB*.[3] In order to maintain the efficacy of such last-resort antibiotics, we need to design rational treatment strategies that consider resistance development associated with clinical antibiotic concentration–time profiles. *In vitro* experiments in the context of pharmacokinetic (PK) profiles represent a relevant approach to characterize the evolution of resistance. However, experimental evolution studies for colistin have mainly focused on approaches such as serial passaging that do not consider PK. The role of the experimental model and in particular the impact of clinical drug concentrations on evolution of resistance is poorly understood.

We aimed to study the impact of experimental evolution approaches for the development of resistance to colistin in *K. pneumoniae*. We performed experiments with a continuous culture chemostat replicating clinical PK profiles of colistin, a static clinical colistin concentration and standard serial passaging experiments, comparing the phenotypic and the genotypic changes.

Two colistin-susceptible clinical isolates of *K. pneumoniae* (KML9749 and KML9884) obtained from Leiden University Medical