Journal of Global Antimicrobial Resistance 16 (2019) 117–119

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Letter to the Editor

Complete nucleotide sequences of six *bla*$_{CTX-M-1}$-encoding plasmids from *Escherichia coli* isolated from urinary tract and wound infections in dogs

Sir,

In a recent study, we presented the characterisation of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae isolated from cats and dogs in Switzerland during 2012–2016 [1]. Six transmissible plasmids from *Escherichia coli* isolated from dogs randomly selected from this study were chosen for further analysis. Here we present the complete sequences of six *bla*$_{CTX-M-1}$-harbouring plasmids.

Plasmids were extracted using a QIAGEN® Large-Construct Kit (QIAGEN, Hombrechtikon, Switzerland) and were sequenced on a PacBio RS2 device (Pacific Biosciences, Menlo Park, CA) with a 10-kb size-selected insert library and P6/C4 chemistry. De novo assembly (HGAP3 algorithm) was performed using SMRT Analysis v.2.3.0 (Pacific Biosciences). Plasmid sequences were automatically annotated using the online Rapid Annotation using Subsystem Technology (RAST). Annotation was manually refined using the BLASTn and BLASTp programs (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Plasmids were typed by plasmid multilocus sequence typing (pMLST) using the PubMLST database (http://pubmlst.org/plasmid).

The complete nucleotide sequences of the six plasmids were deposited in the GenBank database under accession nos. **MG948330–MG948335**.

Five of the plasmids belonged to the IncI1 group, whereas the remaining plasmid belonged to IncHI1.

The largest of the IncI1 plasmids (p2305) belonged to the sequence type 3 (ST3) clonal complex (CC) 3 group, was 111 669 bp in size and showed 99% identity at nucleotide level with pH2291-112 (GenBank accession no. **KJ484629**), an R64-type IncI1 plasmid from an *E. coli* isolated from the faeces of a healthy human in 2010 in Switzerland [2]. As in plasmid pH2291-112, the *bla*$_{CTX-M-1}$ gene is present as an IS*Ecp1*–IS*5*–*bla*$_{CTX-M-1}$–*orf477*–*mrx* cluster located in the shufflon region between *pilV* and the shufflon-specific DNA recombinase gene *rci*. Other antimicrobial resistance genes included *aadA5*, *dfrA17* and *sul2* encoding aminoglycoside, trimethoprim and sulphonamide resistance, which are located in an accessory region highly similar to pH2291-112, except that in p2305 the associated *intI1* gene was truncated.

Three IncI1 plasmids (p2309, p2319 and p2411) were typed as ST35 and shared >99% nucleotide identity. Notably, p2309 carried the *bla*$_{TEM-1b}$ gene in a Tn*3*-family transposon (located upstream of the plasmid partitioning gene *parA*), whilst p2319 and p2411 carried the *bla*$_{TEM-210}$ gene at the same location. All three plasmids showed 99% identity at nucleotide level with pH1519-88 (GenBank accession no. **KJ484630**), an IncI1/ST145 plasmid from an *E. coli* isolated from the faeces of a healthy human in 2010 in Switzerland [2], although the ST3 subtype (allelic profile 2-1-4-1-2) is not closely related to the ST145 (1-4-3-10-1) subtype (http://pubmlst.org/plasmid). All three plasmids contained an IS*Ecp1*–*bla*$_{CTX-M-1}$–*orf477*–*mrx* module located between the *pilJ* and *pilI* genes of the type IV pilus biosynthesis *pil* operon. Both *bla*$_{CTX-M-1}$/IncI1/ST3 and ST35 plasmids have previously been reported in isolates from dogs [3].

The fifth IncI1 plasmid (p2454) was typed as ST63 and shared 94% nucleotide identity with pND11_107, an IncI1/ST69 plasmid from a porcine *E. coli* from a commercial pig farm in the USA (GenBank accession no. **HQ114281.1**). ST63 (allele nos. 1-4-5-4-7) differs by two alleles from ST69 (1-4-19-4-1). Plasmid pND11_107 does not harbour *bla*$_{CTX-M-1}$ but contains a 17 254-bp resistance-associated module with an unusual Tn*21*-like region encoding streptothricin, aminoglycoside and chloramphenicol resistance that is missing in p2454 [4]. In p2454, the IS*Ecp1*–*bla*$_{CTX-M-1}$–*orf477*–*mrx* element is located between the colicin Ib gene *cib* and the recombinase gene *resD*. As opposed to pND11_107, *cib* is disrupted and the colicin Ib immunity protein gene *cibi* is not present (Fig. 1A). Furthermore, a class 1 integron structure located between positions 15 440 bp and 16 453 bp identical to that of pND11_107 is present in p2454.

The IncHI1 plasmid (p3498) belonged to ST9 and contained the typical IncHI1 backbone encoding replication, maintenance and conjugative transfer. With 99% identity at nucleotide level, it is highly similar to pEQ1 (GenBank accession no. **KF362121.2**), a plasmid from an *E. coli* strain isolated from a healthy horse from an equine centre in the Czech Republic [5]. Both plasmids carry a 24-kb module containing a *fos* operon involved in short-chain fructooligosaccharide (scFOS) metabolism thought to play a key role in the adaptation of IncHI1 plasmids among equine *E. coli* [5]. The association of the *fos* operon with multidrug resistance plasmids is unusual and to our knowledge has not been observed in plasmids from canine *E. coli* isolates before. Furthermore, both plasmids contained a *mer* operon involved in mercury resistance between 125 610 bp and 129 303 bp as well as a set of antimicrobial resistance genes inserted in a complex multiple antibiotic resistance region (MARR). However, the MARR regions varied strongly, although both contained the *bla*$_{CTX-M-1}$ gene (Fig. 1B). p3498 contained novel structural features including a metal resistance determinant *chrA* encoding the chromate transport protein ChrA, and *qacE* encoding the quaternary ammonium compound-resistance protein QacE (Fig. 1B).

Overall, the similarities of plasmids p2454 and p3498 to plasmids of porcine and equine origin, respectively, point towards the possibility of a common gene pool and transmission events that may have taken place between food-producing and companion animals.

https://doi.org/10.1016/j.jgar.2018.12.006

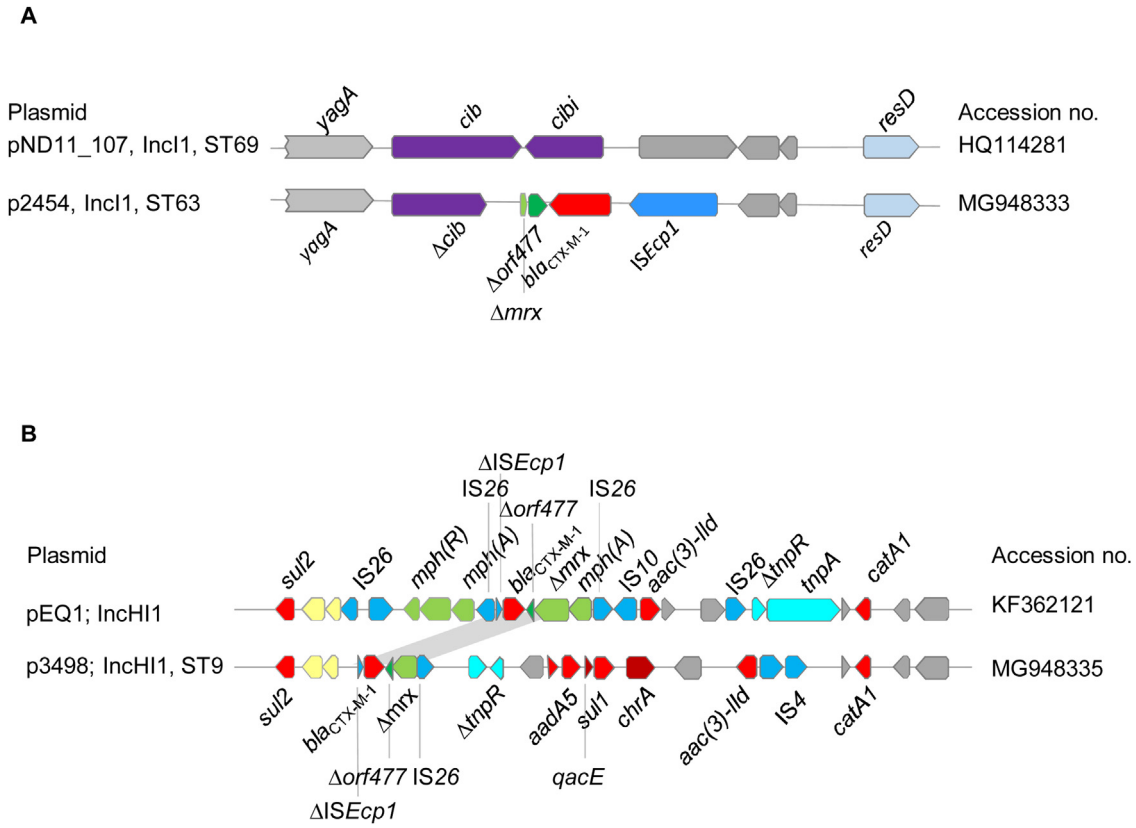

Fig. 1. (A) Structure of the $bla_{CTX\text{-}M\text{-}1}$ genetic environment in p2454 and comparison with pND11_107 of porcine origin. The red arrow indicates the antimicrobial resistance gene $bla_{CTX\text{-}M\text{-}1}$. Purple arrows indicate colicin genes (*cib*, colicin gene; *cibi*, gene encoding colicin immunity protein). The dark green arrow shows truncated *orf477*. The light green arrow shows the truncated macrolide resistance gene Δ*mrx*. The blue arrow indicates the IS*Ecp1* insertion sequence. Pale blue arrows denote resolvase genes *resD*. Grey arrows represent hypothetical proteins. (B) Structure of the $bla_{CTX\text{-}M\text{-}1}$ genetic environment in p3498 and comparison with equine pEQ1. Red arrows indicate antimicrobial resistance genes. Dark red arrows denote resistance genes for heavy metals and quaternary ammonium compound-resistance proteins. Light green arrows denote genes involved in macrolide resistance. Dark green arrows show truncated *orf477*. Blue arrows indicate insertion sequences. Turquoise arrows show transposase genes. Pale yellow arrows indicate regulatory protein genes *repA* and *repC*. Grey arrows represent hypothetical proteins. The area in grey indicates a homologous region.

Acknowledgments

The authors are grateful to the staff of the Functional Genomics Center Zürich, Switzerland, in particular to Simon Grüter and Andrea Patrignani for technical support and bioinformatics support.

Funding

This work was supported by funding from the University of Z ürich (Zürich, Switzerland).

Competing interests

None declared.

Ethical approval

Not required.

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Received 13 October 2018

Available online 21 December 2018