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Short Communication

INCX3 PLASMID CARRYING *BLA*$_{SHV-12}$ AND *QNRS1* IN A JAPANESE RACEHORSE-ORIGIN *ESCHERICHIA COLI* ISOLATE

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Abstract

Plasmids play an important role in the spread of antimicrobial resistance genes (ARGs) in bacteria. This study reports the complete sequence of the IncX3 plasmid identified in *Escherichia coli* isolated from faeces of a Japanese racehorse. Sequence analysis revealed that this plasmid harbours the *bla*$_{SHV-12}$ gene, which encodes an extended spectrum β-lactamase, and the quinolone resistance gene *qnr*S1. The IncX3 plasmids carrying ARGs have been previously identified in bacteria isolated from humans, animals and a variety of environments. This is the first report of the complete sequence of the IncX3 plasmid carrying *bla*$_{SHV-12}$ and *qnr*S1 genes from a Japanese racehorse, which provides insights into understanding the spread and mechanism of antimicrobial resistance particularly in Japanese racehorses.

Key Words: *bla*$_{SHV-12}$, IncX3 plasmid, *qnrS1*

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INTRODUCTION

Antimicrobial resistance genes (ARGs) can be transmitted not only vertically but also horizontally to other species (Li et al., 2022). In the horizontal gene transfer (HGT), the transmission of ARGs can be facilitated by mobile genetic elements (MGEs) such as plasmids, transposons, integron-containing gene cassettes and insertion sequences (Partridge et al., 2018). Conjugation, transformation, and transduction are the main mechanisms of HGT and are involved in bacterial adaptation to new environments (Emamalipour et al., 2020).

Some antibiotics, such as third-generation cephalosporins (3GCs) and fluoroquinolones, are of critical importance in modern treatments of particular infections (https://www.who.int/). Resistance to 3GCs can occur by extended spectrum β-lactamases (ESBLs). ESBLs are a type of β-lactamase that can destroy β-lactam antibiotics including 3GCs. ESBL genes such as bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ can be easily transmitted and experience rapid mutation (Tseng et al., 2023). Resistance to fluoroquinolones is mediated mainly by plasmid-mediated quinolone resistance (PMQR) genes, such as *qnrA, qnrB, qnrC, qnrD, qnrS and qnrVC* (Jacoby et al., 2014). Furthermore, ESBL genes are commonly co-located with qnr genes in a resistant plasmid (Salah et al., 2019). In this study, we report identification of a resistance plasmid responsible for ESBL and quinolone resistance from a commensal *Escherichia coli* isolated from a Japanese racehorse.

MATERIALS AND METHODS

A cefotaxime-resistant *E. coli* strain JRT83AEC, isolated from a faecal sample of a healthy racehorse collected at the Japan Racing Association Ritto Training Centre in 2018, was obtained from the Laboratory of Animal Health, University of Miyazaki collection. Phenotypically, this isolate was resistant to cefotaxime (minimum inhibitory concentration (MIC): 128 μg/ml). In this study, we used a whole-genome sequencing approach to investigate possible mechanisms and transmission patterns of the cefotaxime resistance.

The genomic DNA was extracted using Maxwell®Cell DNA purification kit (Promega, Madison, WI, USA) from the bacterium cultured using LB broth containing 2 μg/ml cefotaxime (Duchefa Biochemie B.V. Haarlem, North Holland, the Netherlands) with shaking incubation at 37°C for 18-20 hr. A sequence library was prepared using Nextera DNA Flex Library Preparation Kit according to the manufacturer's protocol (Illumina) and sequenced on the MiSeq platform using MiSeq Reagent kit v3 (Illumina) to obtain 2 × 300 bp paired-end reads. Long-read sequence data were obtained using MinION with Rapid Sequencing Kit and R9.4 MinION flowcell (Oxford Nanopore). A hybrid genome assembly using the short-reads and long-reads was conducted by Unicycler v0.4.8. Resistome, plasmidome, virulome, multilocus sequence type (ST), and serotype were identified using PlasmidFinder 2.1, ResFinder-4.1, VirulenceFinder

2.0, MLST 2.0 with scheme 1, SerotypeFinder 2.0 tools at the Center for Genomic Epidemiology (https://www.genomicepidemiology.org/). Mobile genetic elements were identified using mobileOG-db (beatrix-1.6) tools at Proksee (https://proksee.ca). A plasmid carrying resistant gene was manually curated using BlastP (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Plasmid comparison was performed using Easyfig v2.2.2 (https://mjsull.github.io/Easyfig/). The nucleotide sequences of *E. coli* strain JRT83AEC was deposited at the EMBL-EBI European Nucleotide Archive [https://www.ebi.ac.uk/ena/browser/home] under Biosample SAMEA114261757.

RESULTS AND DISCUSSION

The total length of the resulting *E. coli* strain JRT83AEC assembly was 4,770,644 bp consisting of four linear contigs and eight circular contigs. Gene prediction and annotation revealed that the strain JRT83AEC belongs to the sequence type ST1494 and serotype O9:H31. PlasmidFinder identified four plasmids in the assembly: IncY/IncFIB(pB171), IncFIB(K), IncX3 and Col440I. We found the 43,705 bp circular IncX3 plasmid, designated as pJRT83AEC, contained bla_{SHV-12} and *qnrS1* genes. The genome statistics of *E. coli* JRT83AEC are summarised in Table 1.

Table 1. Characteristic of JRT83AEC

Attribute	
Species	*E. coli*
Sequence type (ST)	ST1494
Serotype	O9:H31
Assembled genome length (bp)	4,770,644
N_{50} (bp)	4,413,084
No. of contigs	12
No. of CDS	4651
GC content (%)	50.83
Resistance genes	bla_{SHV-12}, *qnrS1*
Plasmid types	*IncY/IncFIB(pB171), IncFIB(K), IncX3, Col440I*
Virulence genes	*csgA, fimH, gad, hha, lpfA, nlpI, terC, yehA, yehB, yehC, yehD*

The ESBL-producing bacteria caused by bla_{SHV-12} gene have been widely distributed in Japanese human patients, livestock, food-producing animals and vegetables (Usui et al., 2019). The *qnrS1* gene has been reported in bacteria isolated from humans, cattle and poultry in Japan (Asai et al., 2010). Furthermore, bla_{SHV-12} + *qnrB* and $bla_{CTX-M-3}$

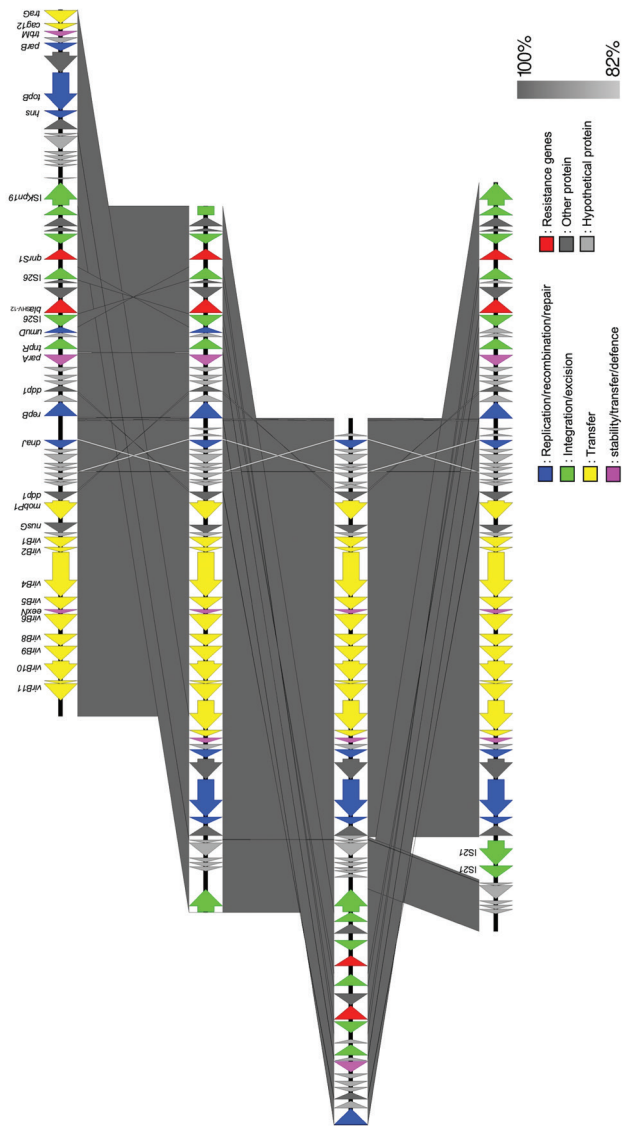

Figure 1. Sequence comparison of the plasmid pJRT83AEC with the IncX3 plasmids from *C. freundii* (CP099292.1), *C. sakazakii* (MT759838.1) and *E. coli* (CP100549.1). Vertical blocks between sequences indicate regions of shared similarity shaded according to BLASTn sequence identity (>95% nucleotide similarity).

+ *qnrS* have also been reported to be carried by cefotaxime-resistant *Enterobacter cloacae* isolated from companion animals in Japan (Harada et al., 2017). In this study, we found bla_{SHV-12} was co-localized with *qnrS1* on the IncX3 plasmid pJRT83AEC.

The IncX3 plasmids have a narrow bacterial host range and are mainly found in Enterobacteriaceae (Guo et al., 2022). Nevertheless, this plasmid has been reported from variable sources and geographic regions (Liakopoulos et al., 2018). The pJRT83AEC plasmid showed the highest similarity to the IncX3 plasmid carried by an environmental strain of *Citrobacter freundii* and an infant strain of *Cronobacter sakazakii* isolated in the UK and Poland, respectively (Figure 1). Recently, IncX3 plasmids carrying bla_{SHV-12} and *qnrS1* have emerged among Enterobacterales isolated from broilers in Switzerland (Figure 1). In Japanese racehorses, the administration of 3GCs might be not commonly used for treatment due to cefazoline, a first-generation cephalosporin antibiotic, still showing good susceptibility profiles (Sato et al., 2020). In specific conditions though, fluoroquinolone antibiotic can be administered to prevent diseases such as shipping fever, caused by long-distance transportation (Endo et al., 2017), and our findings suggested that bla_{SHV-12} that is located in the same plasmid as *qnrS1* could be maintained in the healthy horse intestine when the quinolone derivates are administered with subtherapeutic dose.

In the IncX3 plasmid pJRT83AEC, bla_{SHV-12} is flanked on both sides by the IS26 transposable elements (Figure 1). The IS*26*-bla_{SHV-12}-IS*26* region has also been reported in the IncI1 plasmid pCAZ590 isolated from a chicken in Spain (Alonso et al., 2017) and has been reported as a global dissemination element (Wyrsch et al., 2022). Considering the wide distribution of bla_{SHV-12} and/or *qnrS1* associated with IS*26*, it is important to continuously monitor the resistance profile, especially in Japanese horses.

CONCLUSION

In conclusion, this study provides insight into the resistance mechanism and transmission of ARGs. The resistance determinants found in the plasmid, bla_{SHV-12} and *qnrS1*, are plausibly spread to pathogens and the environment in the equine community via faecal contamination.

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Authors' contributions

The study was designed by ES and TK. Laboratory work was performed by ES and IN. Data analysis and interpretation were performed by ES. The bioinformatics

analysis was supervised by TK. ES prepared the initial manuscript draft and all authors contributed to the manuscript revision and approved the final version.

Competing interests

The authors declare that they have no competing interests.

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PRISUSTVO PLAZMIDA INCX3 SA GENIMA REZISTENCIJE *BLA*$_{SHV-12}$ I *QNRS1* U IZOLATU *ESCHERICHIA COLI* POREKLOM OD TRKAČKOG KONJA U JAPANU

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Kratak sadržaj

Plazmidi imaju veoma značajnu ulogu u prenošenju gena rezistencije na antibiotike među bakterijama. Naša studija se zasniva na analizi kompletne genetske sekvence plazmida IncX3 identifikovanog u izolatu *Escherichia coli* poreklom iz fecesa trkačkog konja u Japanu. U okviru nukleotidne sekvence navedenog plazmida utvrđeno je prisustvo *bla*$_{SHV-12}$ gena odgovornog za sintezu β-laktamaze širokog spektra kao i gena rezistencije na fluorohinolone *qnrS1*. Plazmidi IncX3 koji sadrže gene rezistencije se neretko detektuju ispitivanjem bakterija izolovanih kako iz spoljašnje sredine, tako i iz materijala humanog i animalnog porekla. Međutim, u našem ispitivanju prikazan je prvi nalaz izolata E. coli poreklom od trkačkog konja sa teritorije Japana kod kojeg je analizirana kompletna sekvenca plazmida IncX3 sa prisutnim *bla*$_{SHV-12}$ i *qnrS1* genima. Rezultati ovog istraživanja predstavljaju značajan doprinos razumevanju mehanizama prenošenja gena rezistencije u populaciji trkačkih konja u Japanu.

Ključne reči: *bla*$_{SHV-12}$, plazmid IncX3, *qnrS1*