Molecular characteristics of commensal *Escherichia coli* producing extended-spectrum beta-lactamases from pregnant women from Ibadan, Nigeria

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Introduction and Objectives

The aim of this study was to investigate the molecular characteristics of commensal *Escherichia coli* producing extended spectrum beta lactamases (ESBLs) or showing fluoroquinolone resistance, isolated from pregnant women from Ibadan, Nigeria. Plasmid Mediated Quinolone Resistance (PMQR) determinants have been previously described as prevalent in commensal strains from healthy animals in Nigeria. High variability of genes, plasmids and strains suggested wide circulation of resistance determinants among the faecal flora of healthy food-producing animals in this country. The dissemination of these determinants was mostly due to the transmission of successful plasmids by horizontal exchange rather than to the spread of specific bacterial clones. (Fortini D, *et al.* Plasmid-mediated quinolone resistance and β-lactamases in *Escherichia coli* from healthy animals from Nigeria. *J Antimicrob Chemother.* 2011; 66:1269-72).

In this study we characterized PMQR determinants, beta-lactamases, plasmids and strains among commensal *Escherichia coli* isolated from pregnant women in the city of Ibadan, Nigeria

Methods

Faecal samples from 34 pregnant women were plated on Eosin-methylene blue agar containing 8 µg/mL cefotaxime at the day of admission to the hospital (one strain for each patient was included in the study). Clonal relatedness of the strains was determined by ERIC-PCR. PMQR genes, bla_{CTX}, bla_{TEM}, bla_{SHV} and bla_{OXA-1} were screened by PCR and DNA sequencing.

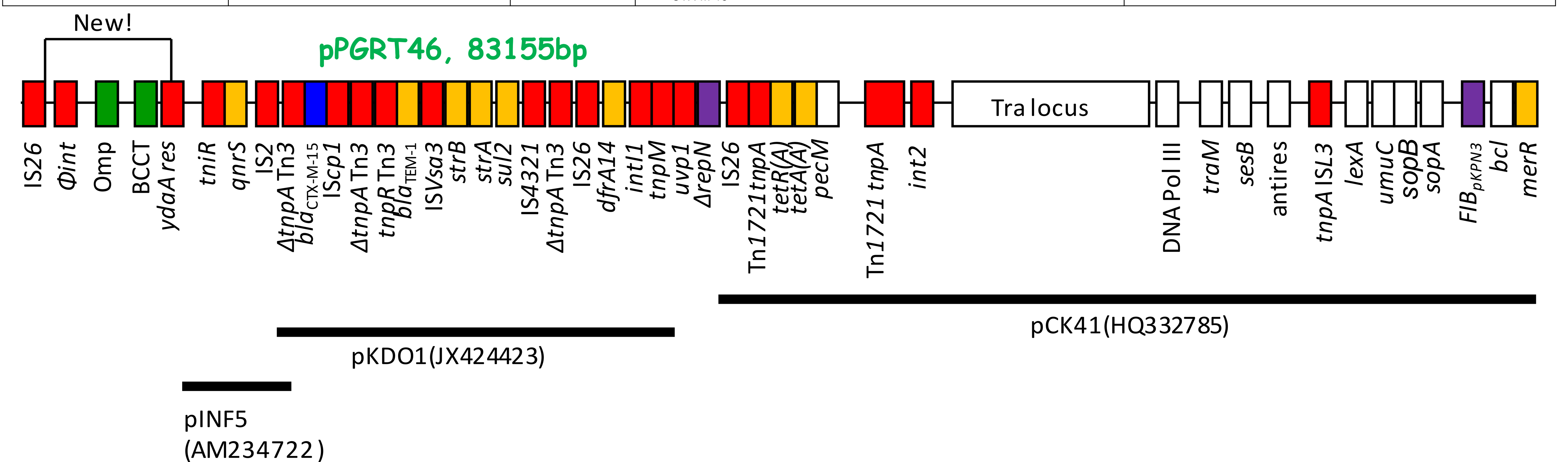
Plasmid typing was performed by PCR-Based Replicon Typing for 25 replicons (PBRT-Kit, DIATHEVA, Fano IT). Prototypic plasmids were transferred by transformation to Top10 *E. coli* competent cells.

The entire plasmid content of strain PgR46 was determined by applying the 454-Genome Sequencer FLX procedure (Roche Diagnostic, Monza, Milan). Contigs were generated by the gsAssembler software v.2.6. Gene prediction was performed for the complete plasmid sequence with Artemis Version 8 (Sanger Institute). Pairwise alignment was performed by a BLASTN and BLASTP homology search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). PCR-based gap closure was performed to obtained the complete circular plasmid sequence

Results and Discussion

- The majority of the strains (20/34) belonged to two major groups (A and B ERIC-types) (Strains Table)
- The *aac(6')-Ib-cr*, *qnrS1*, *qepA1*, *qnrB1* genes were identified in 17, 7, 3, and 2 strains, respectively.
- The $bla_{CTX-M-15}$ gene was identified in 19 strains (Table), in 9 strains it was located on IncF plasmids. The full sequence of one plasmid carrying both $bla_{CTX-M-15}$ and *qnrS1* was obtained (pPGRT46)
- In strain pX53, *qnrS1* was identified on the very rare IncX2 plasmid, previously identified only in *E. coli* from poultry from Nigeria suggesting a potential animal reservoir of PMQR genes in food-animals in this country.

STRAINS	RESISTANCE	ERIC type	Beta-lactamase/PMQR	Plasmid replicon
PX19	CTX CP GEN TET	A	$bla_{CTX-M-15}$, *qepA1*	FIA,FIB,FII
PX1	CTX CP CHL GEN STR TET	A	$bla_{CTX-M-15}$	FIA,FIB,FII
PX63	CTX CP CHL GEN STR TET	A	$bla_{CTX-M-15}$; *qepA1;aac6-1r_cr*	FIA,FIB,FII
PX53	CTX GEN STR TET	A	$bla_{CTX-M-15}$; bla_{TEM-1};*qnrS1;aac6-1r_cr*	X2
PX66	CTX CP CHL GEN STR TET	A	$bla_{CTX-M-15}$; bla_{OXA-1};*qnrS1;qnrB1,aac6-1r_cr*	HI2;Y
PX 14	CTX CP CHL GEN TET	A	$bla_{CTX-M-15}$, bla_{TEM-1};*qnrS1;aac6-1r_cr*	Not determined
PX7	CTX CP CHL GEN STR TET	A2	$bla_{CTX-M-15}$; bla_{CMY-2}	Not determined
PX 40	CTX CP GEN STR TET	B	$bla_{CTX-M-15}$	FIA,FIB,FII
PgR46	CTX STR TET	B	$bla_{CTX-M-15}$; bla_{TEM-1}; *qnrS1;aac6-1r_cr*	Y,FIBKpn *(PLASMID SEQUENCED IN THIS STUDY)*
PgR 61	CTX STR TET	B	$bla_{CTX-M-15}$; bla_{TEM-1};*qnrS1;aac6-1r_cr*	Y,FII, FIBKpn
PX61	CTX CP CHL GEN TET	B	$bla_{CTX-M-15}$; bla_{TEM-1};*qnrS1;aac6-1r_cr*	Not determined
PX58	CTX GEN STR TET	B	$bla_{CTX-M-15}$; bla_{OXA-1}; bla_{CMY-2}; bla_{TEM-1}; *aac6-1r_cr*	Not determined
PX 4	CTX CP	B1	$bla_{CTX-M-15}$; bla_{CMY-2}; *qnrS1*	Not typable
PX 68	CTX CP CHL TET	C1	$bla_{CTX-M-15}$; *qepA1*	Not typable
PX48	CTX CP CHL GEN STR TET	C1	$bla_{CTX-M-15}$; bla_{OXA-1}; bla_{CMY-2}; bla_{TEM-1}; *aac6-1r_cr*	FIBKpn
PX 52	CTX CHL STR TET	G	$bla_{CTX-M-15}$; bla_{OXA-1}; bla_{TEM-1}; *aac6-1r_cr, qnrS1*	I2
PgR10	CTX CP CHL GEN STR TET	G	$bla_{CTX-M-15}$; bla_{OXA-1}; *qnrB1*	HI2
PX 10	CTX CHL GEN STR TET	M	$bla_{CTX-M-15}$; *aac6-1r_cr*	FIBKpn
PX 42	CTX CHL GEN STR TET	M	$bla_{CTX-M-15}$	FIBKpn



Plasmid pRG46 features. The scaffold of pPGRT46 was similar to that of plasmid pCK41 identified in *Edwardsiella tarda* from fishes in South Korea. It harbours two replicons: one functional of the *FIBkpn* type, previously identified in other plasmids mostly from *Klebiella pneumoniae* and one not functional of the IncN type, deleted by the integration of an IS26 element. The variable region also included the $bla_{CTX-M-15}$ gene, flanked by the IS*Ecp1* inserted into a Tn3 *tnpA* transposase gene, *strA*, *strB*, *sul2*, and *dfrA14* genes, in the same configuration previously described for plasmid pKDO1, identified in *K. pneumoniae* isolated from pediatric patients in Brno, Czech Republic (Dolejska *et al.*, 2013). pPGRT46 also carried *qnrS1*, which showed the same environment previously described in plasmid pINF5. A completely novel region that did not match with any nucleotide sequence in GenBank was identified in pPGRT46. In this region a novel BCCT transporter, of the betaine/carnitine/choline transporter family, was identified by BlastP and is flanked by the *omp* gene, encoding for a putative outer membrane protein. These features can contribute to the virulence and adaptation of bacterial to the human hosts or in certain environments.

CONCLUSIONS: This study describes novel and rare plasmids at the origin of the dissemination of common resistance determinants, such as $bla_{CTX-M-15}$ and *qnrS1* in *E. coli* from healthy women from Nigeria and suggests plasmid exchange with other bacteria isolated from animals.