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Presence of plasmid-mediated quinolone resistance gene *qnrS* is associated with extended spectrum β-lactamases production in clinical enterobacterial isolates from an intensive care unit from Bucharest

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BACKGROUND:

The aim of this study was to investigate the **plasmid-mediated quinolone resistance (PMQR)** in clinical *Enterobacteriaceae* isolates, as there is virtually no data concerning genetic support of quinolone resistance in microbial strains from Romania.

MATERIALS / METHODS:

A total of 84 *Enterobacteriaceae* (45 *Klebsiella pneumoniae*, 26 *Escherichia coli*, 4 *Enterobacter cloacae*, 3 *Serratia marcescens*, 3 *Proteus vulgaris*, 2 *E. aerogenes* and 1 *Citrobacter freundii*) **with decreased quinolone susceptibility** were selected during July-August 2015. Bacterial identification and antibiotic susceptibility testing were performed by Vitek II method. Screening of **PMQR genes (*qnrA*, *qnrB* and *qnrS*)**, **extended-spectrum β-lactamases (ESBL) genes (*bla*$_{TEM}$ and *bla*$_{CTX-M}$)** and **carbapenemases genes (*bla*$_{NDM}$ and *bla*$_{OXA-48}$)** were performed by PCR.

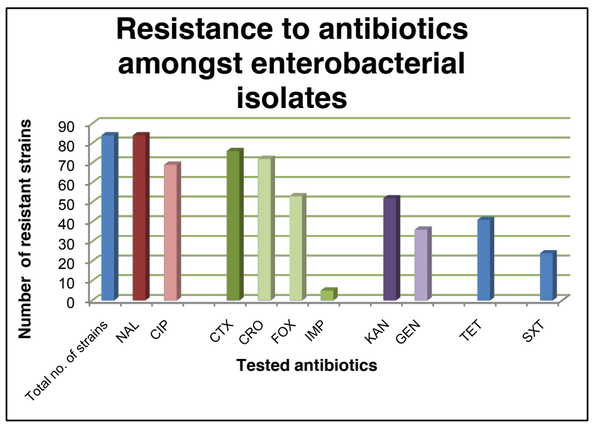

Fig. 1 Overall resistance to antibiotics in analysed strains

RESULTS AND DISCUSSIONS

ANTIBIOTIC SUSCEPTIBILITY AMONGST ENTEROBACTERIAL ISOLATES WITH DECREASED QUINOLONE SUSCEPTIBILITY

Klebsiella pneumoniae isolates

84% of the *K. pneumoniae* isolates were multi-drug resistant (MDR) and 91% exhibited the ESBL phenotype, being resistant to nalidixic acid (100%), ciprofloxacin (91%), to β-lactams: cefotaxime (91%) ceftriaxone (84%), cefoxitin (53%) and imipenem (11%), to aminoglycosides: kanamycin (82%), gentamicin (55%), tetracycline (82%) and trimethoprim-sulphametoxazole (9%) (Fig. 1, Table 1).

Species	No. of strains	Sample	Phenotype	Antibiotic resistance profile	β-lactamase gene	Carbapenem-ase gene	PMQR gene
K. pneumoniae	3	tracheal secretion / blood/ urine	ESBL / (MDR)	NAL, CIP, CTX, CRO, (FOX), (IMP), KAN, (GEN), TET, (SXT)	*bla*$_{CTX-M-like}$	-	*qnrS*
K. pneumoniae	1	urine	ESBL / MDR	NAL, CIP, CTX, CRO, FOX, IMP, KAN, GEN, TET, SXT	*bla*$_{CTX-M-like}$	NDM	*qnrS*
K. pneumoniae	5	wound / urine/ stool	ESBL	NAL, CIP, CTX, CRO, (FOX), (IMP), KAN, (GEN), TET, (SXT)	*bla*$_{TEM-like}$	-	*qnrS*
K. pneumoniae	1	wound	ESBL	NAL, CIP, CTX, (CRO), (FOX), KAN, (GEN), TET, (SXT)	-	-	*qnrS*

Table 1. Characterisation of *K. pneumoniae* strains carrying *qnrS* gene
(parantheses indicates variability amongst isolates)

Other Enterobacteria isolates

67% of the *E. coli* and the other enterobacterial species (39 strains) were MDR and 69% of the strains exhibited ESBL phenotype, being resistant to nalidixic acid (100%) and ciprofloxacin (72%), to β-lactams (cefotaxime 90%, ceftriaxone 87%, cefoxitin 74%), aminoglycosides (kanamycin 38%, gentamicin 28%), tetracycline (10%) and trimethoprim-sulfametoxazole (51%) (Fig. 1, Table 2).

Species	No. of strains	Sample	Phenotype	Antibiotic resistance profile	β-lactamase gene	PMQR gene
E. coli	3	urine	ESBL / (MDR)	NAL, CIP, CTX, (CRO), (FOX), (IMP), KAN, (GEN), TET, (SXT)	*bla*$_{CTX-M-like}$	*qnrS*
S. marcescens	1	sputum	ESBL	NAL, CIP, CTX, (CRO), (FOX), KAN, (GEN), TET, SXT	*bla*$_{CTX-M-like}$	*qnrS*
E. coli	3	stool	ESBL	NAL, CIP, CTX, (CRO), (FOX), KAN, (GEN), (TET), (SXT)	-	*qnrS*

Table 2. Characterisation of the other Enterobacteria strains carrying *qnrS* gene
(parantheses indicates variability amongst isolates)

GENETIC SUPPORT OF ANTIBIOTIC RESISTANCE IN K. PNEUMONIAE

Genetic support of β-lactam resistance was determined by ***bla*$_{CTX-M-like}$ genes in 56%** and by ***bla*$_{TEM-like}$ in 29%** of the strains. Carbapenem resistance was mediated by ***bla*$_{OXA-48-like}$ genes in 6%** and by ***bla*$_{NDM-like}$ genes in 2%** of the strains. PMQR gene ***qnrS* was identified in 22%** of the strains, who also carried *bla*$_{CTX-M-like}$ genes and exhibited phenotypic resistance to ciprofloxacin (Table 1).

GENETIC SUPPORT OF ANTIBIOTIC RESISTANCE IN OTHER ENTEROBACTERIA ISOLATES

***bla*$_{CTX-M-like}$ gene was identified in 59%** of the isolates, while ***bla*$_{TEM-like}$ was encountered in 5%. *qnrS* gene was identified in 18%** of the isolates (6 *E. coli* and 1 *S. marcescens*), always associated with *bla*$_{CTX-M-like}$ gene and the ESBL phenotype (Table 2).

Statistical analysis of the **correlation of *qnrS* gene and *bla*$_{CTX-M-like}$ or *bla*$_{TEM-like}$** (main responsibles for the ESBL phenotype in our study) between ***K. pneumoniae versus* other Enterobacteria groups** using **one tailed Chi-squared test** revealed a **significant value of 0.0206,** supporting our observation that the **presence of PMQR gene *qnrS* is associated with ESBL production** in *K. pneumoniae* (Fig. 2)**.** However, due to small number of samples, this conclusion could not be extrapolated for the other Enterobacteria group.

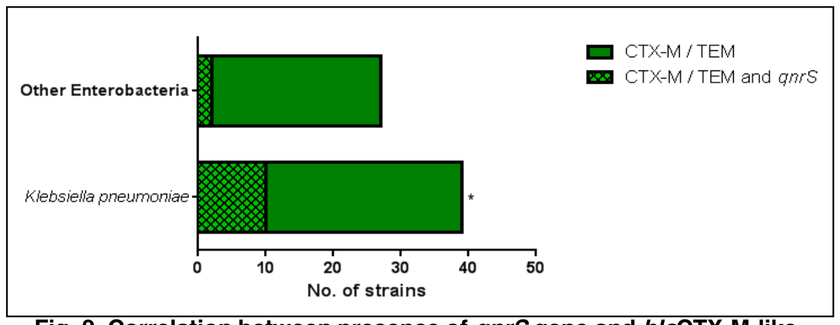

Fig. 2. Correlation between presence of *qnrS* gene and *bla*CTX-M-like or *bla*TEM-like

Conclusion: Our study highlights the association of PMQR *qnrS* gene with the *bla*$_{CTX-M-like}$ genes mainly in *Klebsiella pneumoniae* strains, isolated from patients from an ICU in Bucharest, which suggests a common platform of dissemination.

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Abbreviations: NAL - nalidixic acid; CIP - ciprofloxacin; CTX - cefotaxime; CRO - ceftriaxone; FOX - cefoxitin; IMP - imipenem; KAN - kanamycin; GEN - gentamicin; TET - tetracycline; SXT - trimethoprim + sulfamethoxazole