

# PREVALENCE OF BROAD-SPECTRUM CEPHALOSPORIN RESISTANCE IN COMMENSAL *ESCHERICHIA COLI* STRAINS COLLECTED FROM HEALTHY CHILDREN IN TUNISIA

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## INTRODUCTION

An important reservoir of antibiotic resistance genes is found in commensal bacteria, which are further capable of facilitating the spread of such genes to pathogenic strains. Several studies have reported that children tend to be the most exposed to antibiotics and have the highest risk of carrying resistant commensal bacteria [1].

The aim of this study was to evaluate the prevalence of broad-spectrum cephalosporins resistance in commensal *E. coli* carried by healthy schoolchildren, characterize their encoding genes and analyze the distribution of phylogenetic groups.

## Materials and Methods

### ❖ Sample collection

- This study was conducted during 2012-2013 school year in three elementary schools of different regions of Manouba (Tunisia). The population study, was represented by healthy children. Criteria of exclusion were: previous hospitalization, presence of gastrointestinal symptoms and antibiotics use during the four months prior the sample collection. At least 105 rectal swabs were collected from 105 evaluable voluntary students.

### ❖ Isolates and antimicrobial susceptibility testing

- Strain screening:** Samples were inoculated on desoxycholate lactose agar plates supplemented with cefotaxime (2µg/ml), selective screening agar for the isolation of *E. coli* resistant to 3<sup>rd</sup> generation cephalosporins.
- Strain identification :** Standard procedures
- Antibiotic susceptibility testing:** Disk diffusion method, according to Clinical Laboratory and Standards Institute guidelines (CLSI)
- Extended-spectrum β-lactamase (ESBLs) production:** Double-disk synergy-test (DDST) between clavulanic acid and ceftazidime, cefotaxime or cefepime

### ❖ β-lactamase genes detection

Isolates with positive DDST were screened for *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub> and *bla*<sub>TEM</sub> genes, those with AmpC phenotype were screened for plasmid-mediated cephalosporinases genes (*bla*<sub>MOX</sub>, *bla*<sub>CIT</sub>, *bla*<sub>DHA</sub>, *bla*<sub>ACC</sub>, *bla*<sub>EBC</sub> and *bla*<sub>FOX</sub>) [2, 3].

- Molecular typing:** Phylogenetic groups were determined by PCR [4].

## RESULTS

- Six cefotaxime resistant (6.6%) *E. coli* strains were found.
- They were resistant to gentamicin (n=4), tobramycin (n=3), amikacin (n=2), nalidixic acid (n=3), ciprofloxacin (n=2) tetracycline (n=3), chloramphenicol (n=1) and cotrimoxazole (n=4).
- bla*<sub>CTX</sub> genes were detected in 5 strains; *bla*<sub>CTX-M-15</sub> in 2 strains (belonging to B1 and D groups) and *bla*<sub>CTX-M-1</sub> in 3 strains (belonging to B1 and A groups).
- All *bla*<sub>CTX</sub> genes were associated with *bla*<sub>TEM-1</sub> gene.
- The remained isolate harbored *bla*<sub>CMY-2</sub> and belonged to D group.

### ➤ Phylogenetic analysis

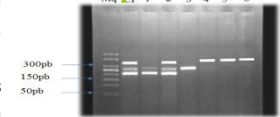


FIG 1: Triplex PCR profiles specific for *E. coli* phylogenetic groups: B1 (278 pb), A (211 pb) et D (152 pb). Lane 1: Wash buffer (300pb); Lane 2: Positive control; Lane 3-6: *E. coli* strains (1: B1 group, 2: B2 group, A group, 4, 5 and 6 D group)

Table 1: Characteristics of commensal cefotaxime resistant *E. coli* isolates

Strains	Epidemiologic data			<i>bla</i> genes	Associated resistance phenotypes	Phylogenetic groups
	Age (years)	sex	origin			
2M18	10	Male	Rural	<i>bla</i> <sub>CTX-M-15</sub> <i>bla</i> <sub>TEM-1</sub>	Gen-Tm	D
Th11	10	Female	Urban	<i>bla</i> <sub>CTX-M-1</sub> <i>bla</i> <sub>TEM-1</sub>	Gen-Tm	A
Th12	11	Male	Urban	<i>bla</i> <sub>CTX-M-15</sub> <i>bla</i> <sub>TEM-1</sub>	Te-Mno-Na-C-Sxt	B1
Th25	13	Female	Urban	<i>bla</i> <sub>CMY-2</sub>	Fox-Sxt	D
Th44	12	Male	Urban	<i>bla</i> <sub>CTX-M-1</sub> <i>bla</i> <sub>TEM-1</sub>	Gen-Tm- Am-Te-Mno-Na-Cip-sxt	B1
Th45	10	Male	Urban	<i>bla</i> <sub>CTX-M-1</sub> <i>bla</i> <sub>TEM-1</sub>	Gen-Tm-Am- Te-Mno-Na-Cip-sxt	B1

Am: amikacin; Cip:ciprofloxacin; C: chloramphenicol; Fox: cefoxitine; Gen:gentamicin; Mno: minocycline; Na:nalidixic acid;Sxt: cotrimoxazole; Te: tetracycline;Tm: tobramycin.

## Conclusions

- Our results indicate the diversity of phylogroups among cefotaxime resistant *E. coli* strains .
- The intestinal tract of healthy children in Tunisia seems to be a reservoir of ESBL and PMBL encoding genes.
- Thus, it would be important to develop a strategy to prevent the emergence and the diffusion of resistant *E. coli* from intestinal flora.

## REFERENCES

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