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Study of Efflux Genes in *E. coli* Isolated from Community Acquired Urinary Tract Infections

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A B S T R A C T

The present study mainly aimed to study the prevalence of antibiotics resistance among *E. coli* isolated from community acquired urinary tract infections (UTIs) and to determine the prevalence of *AcrA/B* and *tolC* efflux genes among the isolated *E. coli*. The study was a cross-sectional study that included 100 E. coli strains isolated from patients with community acquired UTIs. Antibiotics susceptibility to *E. coli* was determined by disc diffusion method. Efflux genes *AcrA/B* and *tolC* were determined by polymerase chain reaction (PCR). The prevalence of efflux genes in the isolated *E. coli* were 71% for each of *acrB* and *tolC* genes and 55% for *acA* gene. The antibiotics resistance of *E. coli* was significantly associated with the presence of *acrA/B* and *TolC* genes among different antibiotics such as amikacin, ampicillin and cefuroxime (P=0.0001). While, resistance to nalidixic acid, trimethoprim-sulfamethoxazole, levofloxacin and gentamicin had non-significant association with the presence of the efflux genes. The studied efflux genes show that the genes were significantly associated with each other, P=0.0001. *AcroA* gene was present in combination with *acroB* and *tolC* in 51 isolates and *acrB* with *tolC* in 61 isolates only 16 isolates had one gene either *acrB* or *tolC*. The results of the present study suggest increase in the prevalence of antibiotics resistance in *E. coli* associated with community acquired urinary tract infections. This resistance may be attributed to irrational use of the antibiotics and the presence of efflux genes that result in antibiotics resistance. The acrA/B and tolC genes had high prevalence in *E. coli*.

Introduction

Urinary tract infections (UTIs) is a common community acquired infections that affects large proportion of population and accounts for the increase antibiotics use in the community (1). The most frequent bacterial pathogen associated with this infection is *Escherichia coli* (*E. coli*) that accounts for up to 80% of UTIs (2). Therefore, the recommended empirical antibiotics therapy for these infections is mainly trimethoprim-sulfamethoxazole (cotrimoxazole), nitrofurantoin, fosfomycin, or pivmecillinam in UTIs in low prevalence of antibiotics resistance. Other antibiotics such as fluoroquinolones or cephalosporins are prescribed if there is antibiotics resistance in the community acquired infections that exceeds 20% (3).

The data concerning antibiotics susceptibility in community acquired UTIs is essential for the treatment of urinary tract infections especially for the common bacterial pathogens.

There is a concern nowadays that *E. coli* develops antibiotics resistance to multiple types of antibiotics through genetics elements that transfer through plasmids from other members of *Enterobacteriaceae* (4). This alarming sign of multiple antibiotics resistance emergence leads to withdraw attention for careful prescription of antibiotics in the community acquired UTIs and to increase the efforts for the prevention of these infections. These basic measures are essential to prevent the emergence of antibiotics resistant mutant strains in community acquired infections that leads to resistant and recurrent urinary tract infections that may be complicated by renal failure (5, 6).

The development of antibiotics resistance rely on several molecular mechanisms among these mechanisms is the efflux pumps mechanism. This mechanism increases the minimum inhibitory concentration and leads to the emergence of mutant bacteria with reduced antibiotics concentration inside the bacterial cells. There are various genes in *E. coli* that leads to the resistance through efflux mechanism. These genes include tripartite drug efflux complexes such as *AcrAB-TolC* (6, 7). Also, AcrB an RND member of multidrug efflux system is found in *E. coli* and results in the pump out amphiphilic and lipophilic antibiotics through the *tolC* outer membrane channel. *AcrA*, which is an elongated protein, brings both the inner and the outer membrane closer together. This protein acts as a trimmer and is in contrast to *acrB* (8).

There are few studies in Egyptian patients about the antibiotic's resistance of *E. coli* in community acquired UTIs and the prevalence of efflux genes among these isolates (9, 10).

Therefore, the aims of the present study were to study the prevalence of antibiotics resistance among *E. coli* isolated from community acquired UTIs and to determine the prevalence of *AcrA/B* and *tolC* efflux genes among the isolated *E. coli.*

Materials and Methods

The study was a cross-sectional study. The study included patients with community acquired urinary tract infections from out patients' clinics of Mansoura University hospital from January 2018 till June 2018. The patients were adults above 18 years with urinary tract infections. Patients with previous antibiotics therapy or autoimmune diseases were excluded from the study. The study was approved by Mansoura Ethical Committee and approval consents were obtained from the patients.

The patient was subjected to medical history taking and clinical examination. Mid-stream urine sample was obtained from each patient

in sterile container and transported to the laboratory rapidly. In the laboratory, urine samples were subjected to full urine examination for the presence of pus cells. Urine samples with white blood cells counts above 10/HPF were subjected to microbiological culture at MacConkey's agar by standard calibrated loop at 37°C for 24 hours. The positive culture with colony counts $>10^5$cfu/ml were identified by Gram stain, biochemical reaction and subjected to antibiotics susceptibility by discs diffusion method.

Antibiotics susceptibility by disc diffusion

The antibiotics sensitivity for isolated *E. coli* was determined by discs diffusion method according to clinical laboratory standard institute guideline (CLSI). The used antibiotics in this study included: amikacin (30µg), amoxicillin/clavulanic acid (30µg), ampicillin (10µg), ampicillin/sulbactam (10/10µg), cefixime (5µg), cefotaxime (30µg), ceftazidime (30µg), cefuroxime (30µg), ciprofloxacin (5µg), cotrimoxazole (25µg), gentamicin (10µg), levofloxacin (5µg), nalidixic acid (30µg), nitrofurantion (300µg), norfloxacin (10µg) and tetracycline (30µg) (Oxoid -UK).

Polymerase Chain Reaction (PCR) for acrA/B and tolC in *E. coli*

DNA Extraction from Isolated E. coli

DNA was extracted from pure isolated *E. coli* colonies by boiling method as described previously (12).

PCR for *acrA/B* and *tolC*

The sequences of the used primers were listed in table 1. Multiplex PCR was used for the amplification of *acrA/B*. The amplification was carried out by the use of Qiagen amplification mixture (Qiagen-Germany) with total volume 25 µl containing 5 microns of DNA, 25 mM $MgCl_2$ and 2.5 mM each of the four dNTP(s), and 1unit of Taq DNA polymerase. The programs for the thermocycler was as the following: 40 cycles consisting of 94ºC for 1 min; 52ºC for 1 min; 72ºC for 2 min. The last cycle was followed by 7 min at 72ºC. and the extension time at 72ºC was 3.5 min. Negative controls were performed by the use of distilled sterile water and *E. coli* K12 was used as a positive control. The products were analyzed by agarose gel electrophoresis on 1.2% (12).

For the amplification of *tolC* gene the following PCR procedures were carried out 4 min at 94°C; (ii) 30 cycles, with 1 cycle consisting of 30 s at 94°C, 30 s at 65°C, and 30 s at 72°C; (iii) a final extension step of 5 min at 72°C (13).

Statistical analysis

The data was analyzed by SPPS 22. The data will be expressed as percentages and comparison will be by Chi-Square and P will be significant when <0.05.

Results and Discussion

The study included 100 strains of *E. coli* isolated from patients with community acquired UTIs. The resistance of isolated *E. coli* for antibiotics show high frequency of resistance to ampicillin, cotrimoxazole, ampicillin/sulbactam and cefotaxime (74%, 73%,70% and 69% respectively), The lower frequency of resistance of *E. coli* was to nalidixic acid, norfloxacin and levofloxacin (18%, 21% and 29% respectively). Moderate resistance prevalence rates were found to gentamicin, amikacin and ciprofloxacin (31%, 38% and 42% respectively), table 2.

The prevalence of efflux genes in the isolated *E. coli* were 71% for each of *acrB* and *tolC* genes and 55% for *acA* gene, table 3

Table.1 The used primers sequences for efflux genes.

Gene	Primers Sequences	bp
acrA	5′-GGTCGTTCTGATGCTCTCA-′3 5′-GGCTTGCTGGTTATTATCAG-′3	1078
acrB	5′-CGTCTAACAGTGACTCCACGG-′3 5′-TTCAATCAGACCTTTACCTTC-′3	2730
tolC	5′-ATGCAAATGAAGAAA-′3 5′-TTAATGACGGAACGGATT-′3	100

Table.2 Antibiotics resistance in *E. coli*

Antibiotics	No.	%
Amikacin	38	38%
amoxicillin/clavulanic acid	62	62%
ampicillin/sulbactam	70	70%
ampicillin	73	73%
cefixime	63	63%
cefotaxime	69	69%
ceftazidime	53	53%
cefuroxime	53	53%
ciprofloxacin	42	42%
cotrimoxazole	74	74%
gentamicin	31	31%
levofloxacin	29	29%
nalidixic acid	18	18%
nitrofurantoin	65	65%
norfloxacin	21	21%
tetracycline	65	65%

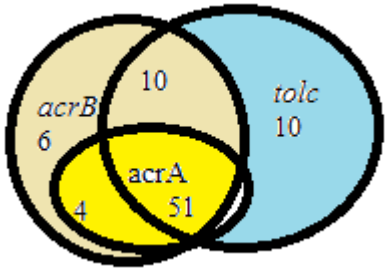
Table.3 The prevalence of efflux genes *acA*/B and *tolC* in *E. coli*

Gene	No.	%
acA	55	55%
acrB	71	71%
tolC	71	71%

Table.4 Association of antibiotics resistance and the presence of efflux genes in E. coli

	acrA **(n=55)** **No. %**	***acrB*** **(n=71)** **No. %**	***tolC*** **(n=71)** **No. %**
Amikacin P	28 0.003	32 0.03	32 0.05
amoxicillin/clavulanic acid P	40 0.0001	48 0.01	48 0.01
ampicillin/sulbactam P	46 0.002	56 0.006	57 0.005
Ampicillin P	55 0.0001	54 0.0001	58 0.0001
Cefixime P	43 0.001	52 0.02	51 0.03
Cefotaxime P	46 0.0001	54 0.02	57 0.0001
Ceftazidime P	36 0.01	43 0.03	41 0.2
Cefuroxime P	41 0.0001	47 0.0001	47 0.0001
ciprofloxacin	26 0.2	29 0.4	32 0.3
Cotrimoxazole P	49 0.0001	59 0.0001	61 0.0001
Gentamicin P	16 0.6	22 0.7	21 0.7
Levofloxacin P	18 0.2	22 0.3	22 0.3
nalidixic acid P	12 0.3	17 0.1	16 0.3
Nitrofurantoin P	48 0.0001	54 0.0001	59 0.0001
Norfloxacin P	14 0.3	19 0.02	19 0.5
Tetracycline P	44 0.001	53 0.004	52 0.03

Figure.1 Combination of the studied efflux genes



The antibiotics resistance of *E. coli* was significantly associated with the presence of *acrA/B* and *TolC* genes among different antibiotics such as amikacin, ampicillin and cefuroxime (P=0.0001). While, resistance to nalidixic acid, levofloxacin and gentamicin had non-significant association with the presence of the efflux genes (Table 4).

The studied efflux genes show that the genes were significantly associated with each other, P=0.0001. *AcroA* gene was present in combination with *acroB* and *tolC* in 51 isolates and *acrB* with *tolC* in 61 isolates only 16 isolates have one gene either *acrB* or *tolC*, figure 1.

Community acquired urinary tracts infections are common infection with *E. coli* the most frequent associated pathogen. Moreover, *E. coli* usually have multiple drug resistance mechanisms. (14). Therefore, the accurate clinical treatment needs monitoring of the resistance mechanism (15).

In the present study, there was high prevalence of resistant *E. coli* for cotrimoxazole73%. This finding was online with previous studies in other developing countries (16-20). However, lower resistance to cotrimoxazole was reported in other study (21). This difference reflects the policy of the antibiotics use between the developed and the developing countries.

There were high rates of resistance of *E. coli* to ampicillin, ampicillin/sulbactam and cefotaxime (74%,70% and 69% respectively). This finding was online with previous studies (22, 23). On the other hand, the resistance rates to amoxicillin or ampicillin/β-lactamase inhibitors were approximately 40% and rates of resistance to fluoroquinolones and trimethoprim-sulfamethoxazole approached 30% (15). These findings highlight the importance of specific algorithm for antibiotics treatment in each geographical locality according to the study of antibiotics susceptibility results.

The lower frequency of resistance of *E. coli* was to nalidixic acid, norfloxacin and levofloxacin. The findings were online with previous studies for out clinics patients (15, 24-27).

Thus, the use of nalidixic acid and nitrofurantoin is an appropriate choice for treatment of cystitis and levofloxacin is an appropriate choice for empirical therapy to treat complicated urinary tract infections (27, 28).

The findings of high frequency of resistance in *E. coli* draw attention to the increase resistance to antibiotics in the community acquired infections. The approach for designing new antibiotics drugs requires accurate identification of the intrinsic mechanisms of resistance such as efflux pumps. The prevalence of efflux genes *acrA*, *acrB* and *tolC* were 71% and 55%. This finding was similar to previous study (10, 29).

The studied efflux genes, *acrA*, *acrB* and *tolC* were significantly associated with each other,

P=0.0001. Previous study, indicates that there was significant association between the three genes and this association lead to the interactions between the inner and outer membrane components of the tri-partite multidrug efflux pump *AcrA/B- TolC* and stabilize the interactions between AcrAB-*TolC*, and the antibiotics within the complex (30).

The antibiotics resistance of *E. coli* was significantly associated with the presence of *acrA/B* and *TolC* genes among different antibiotics such as amikacin, ampicillin and cefuroxime. While, resistance to nalidixic acid, levofloxacin and gentamicin had non-significant association with the presence of the efflux genes. This finding can be explained by the presence of multiple mechanisms for antibiotics resistances. Therefore, antibiotics resistance to amikacin, ampicillin and cefuroxime may be due to the presence of efflux genes in *E. coli*.

The results of the present study suggest increase in the prevalence of antibiotics resistance in *E. coli* associated with community acquired urinary tract infections. This resistance may be attributed to irrational use of the antibiotics and the presence of efflux genes that result in antibiotics resistance. The acrA/B and tolC genes had high prevalence in *E. coli*.

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