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Detection of *oqxA* and *oqxB* efflux pump genes among nosocomial coliform bacilli: An observational cross-sectional study

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ABSTRACT

Objectives: To identify and test the antibiotic susceptibility of nosocomial coliform bacilli and investigate the presence of *oqxA* and *oqxB* genes among the multidrug-resistant (MDR) phenotypes.

Methods: One hundred and twenty different healthcare-associated infection samples were collected. Coliform bacilli were isolated, identified by conventional methods, and then antibiotic susceptibility tests were done using the VITEK2 system and disk diffusion methods. OqxAB operon was identified using a conventional PCR-based technique. *oqxA* and *oqxB* genes were compared between MDR *Klebsiella pneumonia* (*K. pneumonia*) phenotypes and MDR *Escherichia coli* (*E. coli*) phenotypes. Besides, oqxAB operons were compared between phenotypes of *K. pneumonia* and *E. coli isolates*.

Results: Seventy coliform bacilli were isolated with the predominance of *K. pneumonia* and *E. coli*. Besides, 82.1% of *K. pneumonia* strains and 53.3% of *E. coli isolates* were MDR phenotypes. Significant more *oqxB* genes alone were found in MDR *E. coli* than that in MDR *K. pneumoniae* phenotypes (χ^2=10.160, *P*=0.003). OqxAB operon was significantly more in MDR phenotypes of *E. coli* than that in the susceptible phenotypes (*P*<0.001). There was significantly less of this operon in susceptible *E. coli* isolates than that in susceptible *K. pneumoniae* isolates (*P*<0.001). OqxAB positive isolates that were also resistant to fluoroquinolones, tetracycline, trimethoprim, and chloramphenicol, most probably suggested functional pumps.

Conclusions: MDR coliform bacilli are strongly implicated in healthcare-associated infection. Attention should be paid to the presence of oqxAB pump, as an important mechanism in the development of resistance against many antimicrobials because it contributes to co-resistance with other categories; therefore, this pump could be a good target for efflux pump inhibitors.

KEYWORDS: Healthcare-associated infection; coliform bacilli; Multidrug-resistant efflux pump; *oqxA* gene; *oqxB* gene

1. Introduction

Coliform bacilli are among the most commonly reported Gram-negative pathogens from healthcare-associated infection (HAI)[1]. The olaquindox/quinolone AB (oqxAB) efflux pump is one of the resistant mechanisms that contribute to intrinsic, acquired, and phenotypic resistance by lowering intracellular antibiotic concentration and promoting site mutation accumulation. This pump contributes to resistance to multiple agents like detergents, disinfectants, and certain antibiotics as quinoxalines, quinolones, fluoroquinolones (FQs), trimethoprim, tetracycline, and chloramphenicol[2,3].

This study aims to identify the nosocomial coliform bacilli from Tanta University Emergency Hospital, test their antimicrobial susceptibility patterns, and investigate the presence of *oqxA* and

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oqxB genes among resistant phenotypes.

2. Materials and methods

2.1. Ethical approval

This study was reviewed and approved by the Ethics and Research Committee of Quality Assurance Unit, Faculty of Medicine, Tanta University, Egypt (No. 32634/10/18).

2.2. Inclusion criteria

This observational cross-sectional study was carried out on 120 different nosocomial isolates from patients who acquired HAI after admitted to Tanta University Emergency Hospital from December 2018 to December 2019. All patients whether taking antibiotics or not were subjected to thorough history taking and clinical examination to demonstrate any evidence of the commonest types of HAIs *e.g.* catheter-associated urinary tract infection, ventilator-associated pneumonia, central line-associated bloodstream infection, and surgical site infection according to classifications of Centers for Disease Control and Prevention[4].

2.3. Exclusion criteria

Patients incubating any infection at the time of hospital admission or revealed any isolated bacteria other than coliform bacilli were excluded.

2.4. Collection and handling of specimens

Specimens were collected, transported then stored under complete aseptic precautions[5]. Processing of samples was carried out in the Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University.

2.5. Identification of coliform bacilli isolates

Specimens were subjected to the routine protocol for identification of coliform bacilli as follows: Gram-stained smear, cultivation on Nutrient and MacConkey's agar plates then aerobic incubation at 37 ℃ for 24-48 h. Moreover, urine samples were cultivated on cystine-lactose-electrolyte-deficient agar plates to determine significant bacteriuria (bacterial count exceeding 10^5 organisms/mL was considered significant). After overnight incubation, colonies were identified by colony morphology, Gram staining, and different biochemicals[6].

2.6. Antimicrobial susceptibility testing of coliform bacilli isolates

In the present study, two antimicrobial susceptibility testing methods were used: Kirby-Bauer disk diffusion and VITEK 2 compact system. In the disk diffusion method, the used antibiotics were the commercially available quinolones and FQs that were not included in the used VITEK card. Other antimicrobial categories associated with the oqxAB efflux pump *e.g.* tetracycline, chloramphenicol, and trimethoprim were also tested. The results were interpreted by measuring the diameters of inhibition zones[7].

Concerning VITEK 2 Compact system method, Gram-negative antimicrobial susceptibility testing-N233 cards for Gran-negative bacteria (BioMérieux, France) were used. All procedures were performed according to the manufacturer's recommendations. The results interpretation was done by the VITEK 2 advanced expert system™ software.

2.7. Detection of oqxA and oqxB genes

This step was initially done in the Central Lab of Tanta University and continued in the Molecular Unit of Bacteriology, Tor Vergata University, Rome. Specific primers for each gene were prepared (Thermo Fisher Scientific co., USA) according to Kim and his colleges[8] as shown in Table 1. DNA was extracted from a fresh pure culture of coliform isolates by DNA extraction kit (QIAGEN) then amplification of the target genes by conventional end-point PCR and detection of the amplified genes by agarose gel electrophoresis[9].

2.8. Statistical analysis

Data were analyzed using Microsoft Excel 2007 and IBM SPSS software package version 20.0. Qualitative data were described by frequency and percentages. Comparisons between different groups regarding categorical variables were tested using the *Chi*-square test. The significant level of this study was set at α=0.05.

3. Results

In the present study, 70 coliform isolates were identified, accounting for 58.3% of the total samples. The most predominant

Table 1. Primers of *oqxA* and *oqxB* genes were used in this study.

Genes	Primers	Sequence (5'→ 3')	Size of the amplified product (bp)
oqxA	Forward	CTCGGCGCGATGATGCT	392
	Reverse	CCACTCTTCACGGGAGACGA	
o*qxB*	Forward	TTCTCCCCCGGCGGGAAGTAC	512
	Reverse	CTCGGCCATTTTGGCGCGTA	

Table 2. Distribution of *oqxA* and *oqxB* genes among multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli*.

Resistant coliform isolates	MDR *Klebsiella pneumoniae* (*n*=32)	MDR *Escherichia coli* (*n*=16)	χ^2	*P*
Both *oqxA* and *oqxB* genes	15 (46.8%)	7 (43.7%)	0.042	0.979
oqxA gene alone	16 (50.0%)	3 (18.7%)	4.356	0.113
oqxB gene alone	0 (0%)	5 (31.2%)	10.160	0.003*
Non *oqxA* and *oqxB* genes	1 (3.1%)	1 (6.2%)	0.719	0.999

MDR: Multidrug-resistant. *: Significantly different.

coliforms were *K. pneumoniae* and *E. coli* isolates which accounted for 32.5% and 25%, respectively. Only one isolate of *Citrobacter freundii* was identified while *Enterobacter* spp. were not detected in any of the collected samples.

Regarding resistance pattern, 82.1% of *K. pneumonia* strains and 53.3% of *E. coli* isolates were multidrug-resistant (MDR) (The isolate is non-susceptible to at least one agent in ⩾3 antimicrobial categories) phenotypes. The results show significantly more MDR phenotypes than susceptible phenotypes (The isolate is susceptible to all tested antimicrobial categories or non-susceptible to <3 antimicrobial categories) both in *K. pneumoniae* and *E. coli* isolates (χ^2=11.703, *P*=0.008).

It was found that 45.8% of total MDR coliform isolates had both *oqxA* and *oqxB* genes, and no significant difference was found between MDR *K. pneumoniae* and MDR *E. coli* isolates (χ^2=0.042, *P*=0.979). The present findings revealed significantly more *oqxB* genes alone in MDR *E. coli* than that in MDR *K. pneumoniae* isolates (χ^2=10.160, *P*=0.003). On the other hand, there was no significant difference in the *oqxA* gene alone between MDR *K. pneumoniae* and MDR *E. coli* isolates (χ^2=4.356, *P*=0.113) (Table 2).

Significantly more oqxAB operons were found in susceptible phenotypes of *K. pneumoniae* isolates [7(100%)] than that in *E. coli* isolates [0(0%)] (*P*<0.001). Besides, *E. coli* isolates showed statistically significant more oqxAB operons in MDR phenotypes [15(93.7%)] than that in susceptible phenotypes [0(0%)] (*P*<0.001). Moreover, there was no significant difference in the presence of the operon among MDR *K. pneumonia* [31(96.8%)] and *E. coli* phenotypes [15(93.7%)] (*P*>0.999). Figures of agarose gel electrophoresis showing *oqxA* and *oqxB* genes in MDR *K. pneumoniae* and *E. coli* isolates. Size of the amplified product of *oqxA* genes is at 392 bp and 512 bp for *oqxB* genes (Figure 1).

In the trial to investigate the functionality of the oqxAB efflux pump, we correlated the presence of oqxAB operon and the phenotypic resistance to all antimicrobial agents associated with this pump together (FQs, trimethoprim, tetracycline, and chloramphenicol). We found that 90.3% of oqxAB positive *K. pneumoniae* and 46.6% of oqxAB positive *E. coli* isolates were resistant to all four associated classes of antibiotics with a statistically significant higher rate in *K. pneumoniae* isolates (*P*=0.002).

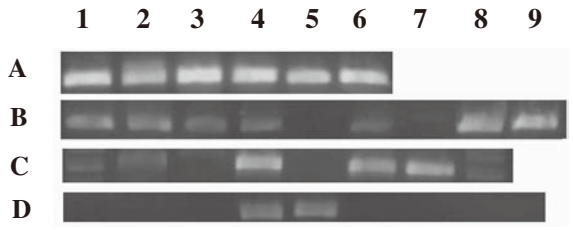

Figure 1. Agarose gel electrophoresis showing *oqxA* and *oqxB* genes in multidrug-resistant *Klebsiella pneumonia* and *Escherichia coli* isolates. A: Agarose gel electrophoresis shows *oqxA* genes in multidrug resistant *Klebsiella pneumoniae* isolates (392 bp); B: Agarose gel electrophoresis shows *oqxB* genes in multidrug resistant *Klebsiella pneumoniae* isolates (512 bp); C: Agarose gel electrophoresis shows *oqxA* genes in multidrug resistant *Escherichia coli* isolates (392 bp); D: Agarose gel electrophoresis shows *oqxB* genes in multidrug resistant *Escherichia coli* isolates (512 bp). Lane 6 in (A) and (B), lane 4 in (C) and lane 5 in (D) are positive control for *oqxA* or *oqxB*; Lane 5 in (B) and (C), and lane 6 in (D) are negative control. The original gel photograph is shown in Appendix 1.

4. Discussion

Progressive increase of MDR Gram-negative pathogens in hospitals augments the burden of HAIs globally. Thus, it is necessary to determine different resistance patterns properly and understand their mechanisms to fight this threat.

In the present study, coliform bacilli isolates accounted for 58.3% of the total clinically suspected HAIs. *K. pneumoniae* and *E. coli* were the predominant isolates. This could be explained by that they are members of our microbiome and can easily cause infections in hospitalized patients as an endogenous source especially in the absence of infection control measures. Similarly, other studies found that *Klebsiella* spp. were the most frequently isolated pathogens from HAIs[10,11] while other studies done by Fakhr *et al.* found that *Pseudomonas* spp. predominated[12].

Regarding the resistance pattern of coliform bacilli isolates, more MDR phenotypes could be related to many factors including long duration of antibiotic therapy in hospitals, prolonged length of hospital stay, and frequent exposure to invasive procedures with the absence of effective infection control policies and procedures. Similarly, other studies detected a high rate of MDR phenotypes among *K. pneumoniae* and *E. coli* isolates from HAIs[13,14].

It was also found that 45.8% of these MDR coliform isolates had both *oqxA* and *oqxB* genes in our study. The high presence of oqxAB operon might reflect the physiological role of these pumps in bacterial life. Another explanation of the overexpression of these

genes is the inappropriate use of antimicrobial and/or disinfectant agents related to this pump. Moreover, close contact with domestic animals as dogs and cats could be a possible source for dissemination of *oqxAB* genes to humans as supported by another study by Zhao *et al.*[15].

Concerning *K. pneumoniae* isolates, both *oqxA* and *oqxB* genes were detected in 46.8% of MDR phenotypes. Interestingly, the operon was also present in 100% of susceptible *K. pneumoniae* isolates. This could be explained by being mainly chromosomally located in both resistant and sensitive phenotypes, perhaps as a reservoir for these genes, but the phenotypic difference occurs according to the level of gene expression. If oqxAB efflux pump genes were expressed at a basal level, it would produce proteins associated with important physiological functions in bacterial life whereas, the overexpression of these genes is more important in the development of resistance. This overexpression can be achieved either constitutive (by mutations) or transient (under specific conditions as exposure to substrates or antibiotics related to the efflux pump)[16]. This supports the idea that the more usage of antibiotics related to each efflux pump, induces overexpression of this pump.

Previous studies reported a similar higher incidence of oqxAB operon among *K. pneumoniae* isolates who found that the incidence was 100%, 92.5%, respectively[17,18], while other studies reported a lower incidence of 74%[8].

On the other hand, only one resistant *K. pneumoniae* isolate had neither *oqxA* nor *oqxB* genes. This could be attributed to the actual absence of this efflux pump, which represents one of many other endogenous efflux systems in Gram-negative bacteria.

Concerning *E. coli* isolates, both *oqxA* and *oqxB* genes were detected in 43.7% of MDR *E. coli* isolates. Similarly, Liu *et al.* found that 42% of clinical resistant *E. coli* isolates were positive for both genes[19]. In contrary, other studies reported lower incidence of 6.05% and 18.07%, respectively[20,21].

Furthermore, there were significantly more oqxAB operons among MDR over susceptible *E. coli* phenotypes (P<0.001). This could be explained by the that the oqxAB operon in *E. coli* is most probably carried on plasmids[2]. Those transmissible plasmids usually carry many other genes of bacterial resistance producing MDR phenotypes and play an important role in the dissemination of resistance by horizontal transfer in-between bacteria, especially within hospitals.

Previously, Cheng *et al.* established that the oqxAB efflux system is constitutively expressed when located in bacterial mobile elements like plasmids, so the expression level of the plasmid-borne oqxAB operon was higher than that of the chromosomal genes[22].

Moreover, we observed higher non-significant existence of the *oqxA* gene among *K. pneumonia* isolates with or without the *oqxB* gene. There were also significantly more *oqxB* genes alone among MDR *E. coli* than that in MDR *K. pneumoniae* isolates (P=0.003). This agreed with other studies[9-23].

Despite the presence of the two genes is necessary to form the oqxAB pump, no definite explanation of the detection of only one gene without the other one until now. This could be explained by the presence of different mutants that might not be detected by the used primers[17-22]. Extra studies are needed to determine the significance of each gene alone. However, the presence of any of them is considered a potential reservoir for the spread of these genes.

Interestingly, Wong *et al.* revealed that there was a major difference between chromosomally located oqxAB operon and any other chromosomal efflux pump genes, as it can become plasmid-borne *via* transposition events, during which the *oqxAB* genes become overexpressed, most probably because of loss of inhibition by the oqxR repressor. Moreover, they cleared that the expression level of the plasmid-borne oqxAB operon was >80 fold of the chromosomal genes[24].

In this study, a trial was done to investigate the functionality of this pump by correlating between the presence of *oqxAB* pump genes and resistance to certain antimicrobial agents associated with the oqxAB pump (FQs, tetracycline, chloramphenicol, and trimethoprim). It was found that 90.3% of *K. pneumoniae* and 46.6% of *E. coli* isolates containing oqxAB operon were resistant to all the four tested classes of antibiotics. We suggest that oqxAB positive isolates together with resistance to the four tested antibiotics is most probably an indication of the functioning pump, and those isolates are good targets to be tested with efflux pump inhibitors in a trial to be used as an antimicrobial adjuvant or alternative. This opinion was supported by other studies[15-19].

Still, there were some limitations to this study, for instance, we could not determine whether oqxAB operon was located on plasmids or chromosomes, as more profound molecular techniques are needed to analyze the locations of these oqxAB operons.

5. Conclusions

In conclusion, MDR coliform bacilli particularly *K. pneumoniae* and *E. coli* are seriously involved in various types of HAIs. Although MDR oqxAB efflux pumps are mainly chromosomally located in *Klebsiella spp*, they are increasingly disseminating in-between nosocomial MDR coliform bacilli. This efflux pump mediates co-resistance with other antimicrobial categories *e.g.* FQs, tetracycline, chloramphenicol, and trimethoprim. For practical guidance, the presence of oqxAB operon and phenotypic resistance to other associated antibiotics can be used as good evidence of the expression of these genes and the functionality of this pump. Further studies are needed, as trials, to use oqxAB efflux pump inhibitors that can be promising in the treatment of MDR pathogens as an adjuvant or an alternative to antimicrobial agents.

Conflict of interest statement

The authors report no conflict of interest.

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Authors' contributions

All authors contributed equally to this article.

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