ORIGINAL ARTICLE

Emergence of Fluoroquinolone resistance and carbapenemase plasmids in *Enterobacter cloacae* isolated from Egyptian Pediatric Hospital

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Key words: *Enterobacter cloacae*, PMQR, Carbapenemase, co-existence, Conjugation

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**ABSTRACT**

**Background:** Enterobacter cloacae cause opportunistic infections that are frequently associated with multidrug resistance (MDR). Co-carriage of plasmid mediated quinolone resistance (PMQR) genes and carbapenemases worsened the problem of resistance. **Objectives:** The current work aimed to detect the frequency of PMQR in *E. cloacae* as well as the co-carriage of carbapenemase resistance determinants in Egypt. **Methodology:** Fourteen *E. cloacae* isolates were collected from children suffered from gastroenteritis admitted at Pediatric Hospital at Assiut University. Identification and antimicrobial susceptibility were done by Vitek-2® system. Detection of PMQR and carbapenemases was done by PCR. Conjugation experiment was performed to test the transmissibility of the resistance determinants. **Results:** PMQR genes were detected in 3/14 (21.4%) of *E. cloacae* isolates. *qnrB* and *qnrS* were detected in 2/14 (14.3%) and 3/14 (21.4%) of the isolates, respectively. Two *E. cloacae* isolates co-harbored *qnrB* and *qnrS*. Neither *qnrA*, *qnrD* nor *aac (6’)-Ib* was detected. Carbapenemase genes were detected in 7/14 (50%) *E. cloacae* isolates; *blaNDM-1*, *blaOXA-48* and *blaVIM-2* were detected in 6/14 (42.9%), 2/14 (14.3%) and 1/14 (7.1%) of *E. cloacae* isolates respectively. Only one isolate (7.1%) co-harbored *blaNDM-1*, *blaVIM-2* and *blaOXA-48* genes. **Conclusion:** PMQR and carbapenemases determinants are common among *E. cloacae* isolates in Egypt with the co-existence of multiple resistance determinants in the same isolate. All transmitted determinants suggest their presence on transmissible plasmids.

**INTRODUCTION**

Enterobacter is a group of gram-negative facultative anaerobic bacilli that can be found widely all over the world. ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) is the world's global cause of nosocomial infections. Opportunistic infections caused by *E. cloacae* and *E. aerogenes* are usually associated with antibiotic resistance (MDR) classically treated with carbapenems and fluoroquinolones (FQs). Unfortunately, the upswing in antibiotic resistance among *Enterobacteriaceae* has made choosing effective treatment regimens more difficult. Consequently, surveillance and resistance investigations became a high priority.

Fluoroquinolone resistance (FQR) are usually due to mutations in genes coding for topoisomerase IV and DNA gyrase as well as the acquisition of plasmid mediated quinolone resistance (PMQR) genes like *qnr*, *qepA*, and *aac (6’)-Ib-cr*. Astonishingly, PMQR has been emerged worldwide, sometimes even in the absence of FQ therapy. On the other side, carbapenem resistance can be acquired by a variety of mechanisms; the most important is plasmid-mediated carbapenemases, including *bla-NDM*.

Numerous studies have stated co-carriage of PMQR and *bla-NDM*, which further worsened the problem of resistance. With increased carbapenem resistance and
simultaneous fluoroquinolone resistance in NDM-1-positive Enterobacteriaceae, a detailed analysis of this relationship is required. Moreover, the wide distribution and ease of dissemination of plasmids through horizontal transmission serves as a global medical problem. Although MDR Enterobacter was stated in many geographical places, the prevalence of PMQR determinants in Enterobacter sp. in Egypt remains unknown. Accordingly, the current work aimed to evaluate the frequency of PMQR in E. cloacae isolated from children suffered from gastroenteritis admitted at Pediatric Hospital at Assiut university and also co-carriage of carbapenemase resistance determinants.

METHODOLOGY

Ethical statement

The Ethical Committee of Faculty of Medicine at Assiut University, Egypt, approved this research in accord with the World Medical Association's code of ethics (Declaration of Helsinki) with IRB local approval number:17300623 dated 11/8/2021.

Isolation and identification of Enterobacter cloacae:

This cross-sectional research was conducted on 168 stool swabs collected from children referred to the Pediatric Hospital at Assiut University with gastroenteritis. Swabs were sent to the Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University and inoculated into Luria-Bertani broth (LB) broth (Himedia, India) then subcultured on Macconkey agar. GN-ID cards of Vitek-2® system (biomerieux, France) were used to identify E. cloacae isolates. The isolates were stored in LB broth and 30% glycerol then kept frozen at -20°C till use.

Susceptibility testing for antimicrobials:

The antimicrobial susceptibility profile of all E. cloacae isolates was determined using AST-GN204 cards of Vitek-2® system (biomerieux, France). Tested antimicrobials were: Ampicillin, piperacillin/tazobactam, cefotaxime, imipenem, meropenem, amikacin, fosfomycin, nitrofurantoin, trimethoprim/sulfamethoxazole, norfloxacin and ciprofloxacin.

Detection of PMQR and carbapenemase determinants by PCR:

The qnr genes as qnrA, qnrB, qnrS, qnrD and aac(6')-Ib and carbapenemases genes as blaNDM-1, blaVIM-2 and blaOXA-48 were detected by means of conventional polymerase chain reactions (PCR) with specific primers listed in table (1).

Prior to DNA extraction, Enterobacter isolates were cultured on LB agar supplemented with nalidixic acid (20µg/ml for PMQR enrichment) and with imipenem (1µg/ml for plasmid-mediated carbapenemase enrichment). DNA was extracted by heating bacterial suspensions for 10 minutes at 95°C followed by centrifugation. PCR was performed at a 20 µl volume using PCR master mix (Promega, USA).

Amplification was performed in a thermal cycler (T100 gradient system (Bio-Rad, Hercules, CA) using the following protocol; initial denaturation for 5 min at 95°C, 30 cycles of denaturation at 94°C for 30 seconds, annealing for 30 seconds then extension for 20-40 seconds at 72°C, and a final extension step at 72°C for 7 minutes.

After one hour of electrophoresis at 100 volts on a 2% agarose gel with added ethidium bromide, amplification products were seen under UV light. A 100-bp DNA ladder (Promega, USA) was used as a marker.

Table 1: Primers sequences encoding PMQR and carbapenemases genes.

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequence</th>
<th>Tm</th>
<th>Size band</th>
<th>Reference</th>
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<td>5’- GAT CGGCAA AGG TTA GGT CA-3’</td>
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<tr>
<td>qnrB</td>
<td>5’- GAT CGT GAA AGC CAG AAA GG-3’</td>
<td>55°C</td>
<td>469 bp</td>
<td>(16)</td>
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<tr>
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<td>5’- ACG ATG CCT GGT AGT TGT CC-3’</td>
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<td>qnrS</td>
<td>5’- CAA TCA TAC ATA TCG GCA CC-3’</td>
<td>53°C</td>
<td>641 bp</td>
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<td></td>
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<tr>
<td>qnrD</td>
<td>5’- CGA GAT CAA TTT ACG GGG AAT A-3’</td>
<td>58°C</td>
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<tr>
<td>acc (6’)-Ib</td>
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<td>blaVIM-2</td>
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<td>52°C</td>
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<td>blaOXA-48</td>
<td>5’- GCG TGG TTA AGG ATG AAC AC-3’</td>
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<td>438 bp</td>
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<td>5’- CAT CAA GTT CAA CC CAAC CG-3’</td>
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Conjugation experiment:

A conjugation experiment was performed on 3 Enterobacter cloacae isolates harbouring both PMQR and carbapenemases to test the transmissibility of the resistance determinants. The isolates were cultured on LB broth with E. coli J53 as the recipient. Transconjugants were selected from colonies on LB agar plates supplemented with sodium azide (200 μg/ml) for counter selection, and imipenem (0.5 μg/ml) or ciprofloxacin (2 μg/ml). PCR was used to detect carbapenemases and PMQR in transconjugants.

RESULTS

Enterobacter cloacae and their antimicrobial susceptibility profile:

The Vitek-2 system detected 14 E. cloacae isolated from the 168 samples. Two of E. cloacae isolates (14.3%) were MDR, while 7/14 (50%) were determined to be extensively drug resistant (XDR).

Prevalence of PMQR and carbapenemase genes among E. cloacae isolates:

PMQR genes were detected in 3/14 (21.4%) of E. cloacae isolates; qnrB was detected in 2/14 (14.3%) of the isolates while qnrS was detected in 3/14 (21.4%) of the isolates. Two E. cloacae isolates co-harboured qnrB and qnrS (Figure 1). Neither qnrA, qnrD nor aac (6 ′)-Ib was detected.

Carbapenemase genes were detected in 7/14 (50%) E. cloacae isolates; blaNDM-1 was detected in 6/14(42.9%) of E. cloacae isolates, blaOXA-48 was detected in two isolates (14.3%) while blaVIM-2 was detected in one isolate (7.1%). One isolate (7.1%) co-harboured blaNDM-1, blaVIM-2 and blaOXA-48 genes (Figure 2).

Fig. 1: Agarose gel electrophoresis of qnrS and qnrB genes in E. cloacae isolates. Lane M = 100 bp DNA ladder; Lane 1= negative control; Lane 2 = positive isolate for qnrS (641 bp); Lane 3 = positive isolate for qnrB (469 bp); Lane 4 = negative isolate.
Fig 2: Agarose gel electrophoresis of \( \text{bla}_{\text{OXA-48}}, \text{bla}_{\text{NDM-1}}, \) and \( \text{bla}_{\text{VIM-2}} \) genes in \( E. \) cloacae isolates. Lane M = 100 bp DNA ladder; Lane 1= negative control; Lane 2 = \( \text{bla}_{\text{OXA-48}} \) (438 bP), Lane 3= \( \text{bla}_{\text{NDM-1}} \) (621 bp); Lane 4= \( \text{bla}_{\text{VIM-2}} \) (801 bp).

Co-existence of PMQR and carbapenemases determinants in \( E. \) cloacae isolates:
Two (14.3%) \( E. \) cloacae isolates harboured both \( qnrS \) and \( \text{bla}_{\text{NDM-1}} \), while only one (7.1%) \( E. \) cloacae isolate harboured both \( qnrB \) and \( \text{bla}_{\text{NDM-1}} \) (Table 2).

<table>
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<tr>
<th>SN</th>
<th>Antimicrobial Phenotype</th>
<th>Ciprofloxacin R/I/S</th>
<th>Imipenem R/I/S</th>
<th>( qnrB )</th>
<th>( qnrS )</th>
<th>( \text{bla}_{\text{NDM-1}} )</th>
<th>( \text{bla}_{\text{VIM-2}} )</th>
<th>( \text{bla}_{\text{OXA-48}} )</th>
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<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: SN, Serial number; R, Resistant; I, Intermediate; S, Sensitive; MIC, Minimal inhibitory concentration.  
Keys: A=Ampicillin=Piperacillin/Tazobactam; C=Cefotaxime; I= Imipenem; M=Meropenem; AK= Amikacin; F = Fosfomycin; NT=Nitrofurantoin; T=Trimethoprim/Sulfamethoxazole; NF=Norfloxacin; CP=Ciprofloxacin.
Transfer of resistance genes by conjugation:

PMQR determinants (qnrB and qnrS) as well as carbapenemases (blaNDM-1) were all successfully transmitted from E. cloacae isolates to the recipient (E. coli J53) via conjugation.

DISCUSSION

Multi-drug resistant microorganisms spread in many countries, with fluoroquinolones and/or carbapenems are often used for treatment. Increased resistance to both antimicrobials is contributing to treatment failure and restricting therapeutic choices. Our previous studies of FQ-MDR in Enterobacteriaceae showed dominance of FQR and carbapenem resistance both in E. coli and K. pneumoniae with PMQR and NDM-1 high carriage prevalence. The present study aimed to detect the frequency of PMQR in E. cloacae isolated from children suffered from gastroenteritis admitted at Pediatric Hospital at Assiut University and also the co-carriage of carbapenemase resistance determinants. This is the first study to our expertise about the existence of PMQR and carbapenem determinants among E. cloacae isolates in Egypt. Regarding E. cloacae strains antimicrobial susceptibility, most strains have been shown to be multdrug resistant. This is in harmony with many studies.

Our findings demonstrated 21.4% as a prevalence of PMQR genes among E. cloacae isolates on the molecular level. qnrS was the most prevalent (21.4%) followed by qnrB (14.3%), neither qnrA, qnrD nor aac (6 )-Ib was detected while in a study in France showed that the qnrB was the most prevalent. In 14.3% of our E. cloacae isolates qnrS co-occurred with qnrB. In agreement with our results, a previous study reported high prevalence of PMQR genes among E. cloacae isolates. In addition, in Enterobacteriaceae and gram-negative bacteria, quinolone resistance was stated in most parts of the world.

Fluoroquinolone resistance was detected in 8/14 (57.2 %) of the E. cloacae isolates, with the presence of qnrB and qnrS only in 3 of these FQR isolates. Although PMQR presence per se confers only diminished susceptibility to FQ, but the existence of the first step of susceptibility reduction promptly enhances further adaptive mutations and resistance development. Although 21.4% of the qnr-positive isolates in this study exhibited quinolone resistance, but the rest of the FQR isolates did not harbour any of the PMQRs, thus FQ resistance might be attributed to chromosomal mutations in gyrA and parC. This was in line with a study suggested that the presence of PMQR might confer low-level resistance by inducing at least one quinolone resistance determining region (QRDR) substitution.

Carbapenem resistance caused by carbapenemase production is currently spreading throughout the Enterobacteriaceae. Another Egyptian study conducted in Tanta University Hospitals in Egypt have found 62.7 % as a high prevalence of carbapenemases among Enterobacteriaceae.

In this study, carbapenem resistant isolates isolated from children suffered from gastroenteritis were received. The coexistence of carbapenemase and PMQR presence, conjugation experiment was done to test the co-transmissibility of resistance determinants. All determinants were transmitted suggesting their presence on transmissible plasmids. The coexistence of resistance genes in the same isolate and their transmission commonly through mobile genetic elements often was the principal causes of the appearance of MDR or even XDR E. cloacae strains.

In an attempt to understand the relationship between carbapenemases and PMQR presence, conjugation experiment was done to test the co-transmissibility of resistance determinants. All determinants were transmitted suggesting their presence on transmissible plasmids. The coexistence of resistance genes in the same isolate and their transmission commonly through mobile genetic elements often was the principal causes of the appearance of MDR or even XDR E. cloacae strains.

CONCLUSION

This study results revealed the presence of PMQR and carbapenemases determinants among E. cloacae isolates in Egypt. In addition, there was co-existence of multiple resistance determinants in the same isolate. All transmitted determinants suggest their presence on transmissible plasmids.

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Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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