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# Detection Of Plasmid Mediated Quinolone Resistance Among Isolates From Cirrhotic Patients With Spontaneous Bacterial Peritonitis In A University Hospital

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***Abstract***
***Introduction:Spontaneous bacterial peritonitis in advanced liver cirrhosis and its treatment have a global burden in healthcare management especially in our locality. Fluoroquinolones are widely used as prophylaxis in that group of patients. Data concerning the prevalence of PMQR determinants in SBP are quite limited.***
***Purpose: To study prevalence of PMQR determinants in isolates from ascetic fluid of cirrhoticpatients with SBP and the relation to ESBL production.***
***Patients and Methods:Ascetic fluid culture was performed; Identification and antimicrobial susceptibility of isolates weredone by conventional methods. FQ resistant isolate are subjected to double disc synergy test for phenotypic detection of ESBL and Polymerase chain reaction for detection of PMQR determinants.***
***Results:Among total of145 Ecoli, 21 Klebsiella pneumonia and 31 gram positive cocci,we found that 70 Ecoli (48.2%), 10 K pneumonia (47.6%) and 10 (32.2%) Gram positive isolates were FQ-resistant. All tested FQ-Resistant Gram negative isolates (100%) harbored at least one of studied PMQR genes.acc6`-Ib-cr is most frequent (87.5%) followed by qnrS(81.2%), while qnrB frequency was 12.5% and qepAwas detected in 10% of gram negative bacilli. In Gram positive cocci 6 isolates showed PMQR (acc6`-Ib-cr&qnrSonly). A statistically significant association was found between ESBL producers and acc6`-Ib-cr gene, an association was also found with qnrS gene.***
***Conclusion:PMQR determinants and ESBLs prevalence is high among FQ-resistant isolates from acetic fluid of cirrhotic patients diagnosed with spontaneous bacterial peritonitis. The PMQR genes and their association with ESBL-producing plasmids have a great effect on multidrug resistance spread, thusmight lead to serious problems. Judicious use of quinolones as prophylaxis in SBP patients is essential.***
***Keywords:Spontaneous bacterial peritonitis, Fluoroquinolone resistance, PMQR determinants, extended spectrum beta lactamases.***

## INTRODUCTION:

Spontaneous bacterial peritonitis is frequent and life threatening complicationin cases have advanced liver cirrhosis with hospital mortality that reaches up to 80% [1].Intestinal bacterial translocation through gut wall is the cause, so decreasing the burden of intestinal pathogenic bacteria and consequently its translocation is effective in preventing SBP [2]Secondary antibiotic prophylaxis

foravoidance of recurrence in cirrhotic caseswith ascites and primary antibiotic prophylaxis with elevated-risk profile is recommended by recent guidlines[3].
Quinolones are widely used as a prophylactic therapy in that patient group; unfortunately quinolone prophylaxis had beenrelated to overgrowing of intestinal gram positive bacteria as Staphylococcus aureus and Enterococcus spp., and also related to increased prevalence ofquinolone-resistant gram negative bacteria (QR-GNB) [4]Resistance to quinolones has emerged over the last years due to extensive use of quinolones in human and veterinary medicines; They have been prescribed to treat UTIsinfections as well as respiratory tract, intra-abdominal and skin infections[5, 6].
Plasmid mediated quinolone resistance genes (PMQR) changed quinolone resistance pattern. PMQR was first described in 1990s [7,8]First PMQR, qnrA, was described in 1998 **[9]** and, to date, about 100 Qnr variants have been identified, being classified to 6 families as: QnrA, B, S, C, D and VC. The Qnr protein belong to pentapeptide-repeat protein and confer quinolone resistancesthroughout physical protection of DNA gyrase and topoisomerase IV from inhibition by quinolones[10].PMQRhave been mainly detected in transposons and/or integrons, meanwhile; these genes have also been reported with a certain frequency in chromosomal locations, reflecting the plasticity of PMQR-related mobile genetic elements [11].
Horizontal transmission of these mobile elements represents a major clinical problem due to easy dissemination with wide geographical distribution[12]; In addition, co-location of PMQR with multiple resistance determinants (frequently extended spectrum b-lactamases, carbapenemaseand Amp-C-type b lactamase genes) in MDR plasmids represents an alarm [13].Actual prevalence of these determinants is not precisely known. Incidences varying<1% to <50% have been reported, depending on the bacterial species and resistance mechanism[14].Due to lacking data especially in our locality, we decided to investigate PMQR prevalence and their association with ESBL production in isolates from ascetic fluid in patients suffering from spontaneous bacterial peritonitis who have a high rate in Egypt. Extensive use of quinolones as prophylaxis among these patients had paid attention for that emerging problem.

**Methods:**
Cross sectional investigationconducted for 35months (January 2017_November 2019) in Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University. Specimens were collected from ICU of Tropical Medicine Department, Zagazig University Hospitals.

**Bacterial Isolates**
Isolates were collected from ascetic fluid of cirrhotic patients diagnosed clinically with spontaneous bacterial peritonitis with PML countof ascetic fluid≥ 250 cells/ mm3, who had not yet received antibiotics,A sterile syringe was used to withdraw 10 mlfromascitic fluid and inoculated immediately in the blood culture bottles (Inoculated at bedside).Foil cap of blood culture bottle was held cut and then the rubber cap was wiped using an ethanol swab, then perforated with the syringe containing the collected ascitic fluid, the top of culture bottle was wiped again with ethanol swab and foil cap was replaced, the sample was mixed with the broth without delay, bottles were labeled with the name, the number of case and the date of collection.Blood culture bottles incubated on 37°C and subculture was done after the first night incubation on blood agar and MacConkey agar plates and then were aerobically incubated on 37°C / 24 hours.The plates were examined after the incubation period for growth, Negative blood culture bottles were checked and sub cultured every other day, If there was no evidence of microbial growth after 10 days of incubation, the culture was considered negative [15,17].Identification up to species level was done by conventional methods.

**Phenotypic detection of FQ Resistance**
CollectedEnterobacteriacae and Gram positive isolates subjected to phenotypic FQ resistance detections by disk diffusion using ciprofloxacin and levofloxacin antibiotic discs.

**Disc Diffusion Method**
Antimicrobial susceptibility was done by disc diffusion method (Modified Kirby–Bauer technique) using Muller Hinton agar according to Clinical Laboratory Standards Institute.[12] For

Enterobacteriacae, in addition to FQ discs (ciprofloxacin (CIP) 5 µg and levofloxacin (LVX) 5 µg), we used antimicrobial discs including carbapenems (Imipenem (IMP) 10 µg, Meropenem (MEM) 10 µg), aminoglycosides (Amikacin (AK) 30 µg, gentamicin (GEN) 10 µg), penicillin/β-lactamase inhibitor (amoxicillin/clavulanic acid (AMC) 20/10 µg), monobactam (Aztreonam (ATM) 30 µg), broad-spectrum cephalosporins (cefoxitin (FOX) 30 µg, ceftazidime (CAZ) 30 µg, cefepime (FEP) 30 µg, cefotaxime (CTX) 30 µg and ceftriaxone (CRO) 30 µg), trimethoprim-sulfamethoxazole (SXT) 25 µg.
Gram positive isolates were subjected to FQ discs (ciprofloxacin (CIP) 5 µg and levofloxacin (LVX) 5 µg), in addition; we usedpenicillin (oxacillin (OX) 1µg, fifth generation cephalosporin (ceftaroline (CPT) 30µg), aminoglycosides (gentamycin (CN) 10µg), carbapenems (imipenem (IPM) 10µg, meropenem (MEM) 10µg), clindamycin (CLI) 2µg, doxycycline (DO) 5µg, trimethoprim-sulfamethoxazole (SXT) 25 µg, quinopristin-dalfopistin (QDA) 15µg, linezolid (LZD) 30µg, vancomycin (VA) (E test strips).

**Phenotypic Detection of ESβL**
*Enterobacteriaceae*were subjected to initial screening for ESBL production by inhibitionzones produced by ceftazidime (30 µg), ceftriaxone (30 µg), and cefotaxime (30 µg). Breakpoints indicative of ESBL suspicion were ≤22 mm forceftazidime, ≤25 mm for ceftriaxone, and ≤27 mm forcefotaxime. Double-disk synergy test was usedfpr phenotypicESBL detection, Antibiotic disks were ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), and amoxicillin/clavulanic acid (20/10 µg) [17] 4 antibiotic discs were placedat 20 mm edge to edge from amoxicillin/clavulanic acid disk which was placed in the middle.After one day incubation, if enhanced zone of inhibition between cephalosporin antibiotics and the amoxicillin/clavulanic acid disk occurred, test would considered as ESBL positive.Multi-drug resistant bacteria was defined as resistane to at least three usually-active drugs from different classes, vancomycin-resistant enterococci, methicillin-resistant staphylococci, extendespectrum beta lactamase produced by Gram negative bacteria[18].

**Genotypic Detection of FQ Resistance Determinants**
Isolates found resistant to FQ were subjectdforPCR(Polymerase Chain Reaction) amplification of PMQR.
**Plasmid Extraction**

For Enterobacteriaceae, extraction of bacterial plasmid was performed using QIAprep® Spin Miniprep Kit (Qiagen GmbH, Hilden, Germany).

**DNA Extraction**
DNA extraction was performed usingQIAamp® DNA Mini kit (Qiagen GmbH, Hilden, Germany).

**Simple PCR to Detect PMQR**
5 PMQR genes detection was performed usingsimple PCR, for QnrA, B and S, aac(6′)-Ib-cr and qepA genes. Amplification was performed usingTaq PCR Master Mix (Qiagen GmbH, Hilden, Germany) and primerslisted on Tab. 1.Following components were added for every 20 µL reaction:10µL of PCR Master Mix (2X), 2 µM of forward primer, 2 µM of reverse primer,**(Table1)** 2 µg of template DNA, 4 µL of water (nuclease free). The Amplification was carried out under the following conditions: 30 sec at 96°C for denaturation, then annealing for 30sec with temperature according to each primer as shown in **table1** and elongation at 72° for 60 sec **[19]** DNA fragments were analysed by electrophoresis in 2% agarose gel.
Our investigation was done in accordance with Declaration of Helsinki and approved by institutional review board (IRB) – Faculty of Medicine, Zagazig University.

**Table I: Primers Sets Used for Detection of PMQR Genes**[20, 21, 22]

| Gene | Primer sequence (5′ to 3′) | Size of PCR product (bp) | Annealing temperature(°C) |
|---|---|---|---|
| **qnrA-F** | ATTTCTCACGCCAGGATTTG | 516 | 53 |
| **qnrA-R** | GATCGGCAAAGGTTAGGTCA | | |
| **qnrB-F** | ATGACGCCATTACTGTATAA | 469 | 53 |
| **qnrB-R** | GATCGCAATGTGTGAAGTTT | | |
| **qnrS-F** | ACG ACA TTC GTC AAC TGC AA | 417 | 53 |
| **qnrS-R** | TAA ATT GGC ACC CTG TAG GC | | |
| **aac(6')Ib-cr-F** | TTGCGATGCTCTATGAGTGGCTA | 482 | 54 |
| **aac(6')Ib-cr-R** | CTCGAATGCCTGGCGTGTTT | | |
| **qepA-F** | GCA GGT CCA GCA GCG GGT AG | 199 | 60 |
| **qepA-R** | CTT CCT GCC CGA GTA TCG TG | | |

**Statistical analysis**

Data was analyzed throughout using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA).Qualitative data was expressed as numbers & percentage.Chi-square test or Fisher exact test were used to compare percent of categorical variables. Probability values (p) < 0.05 were considered statistically significant (S).

**Results**

During the study period, 145 Ecoli, 21 Klebsiella pneumonia and 31 gram positive cocci were collected from hospitalized cirrhotic patients diagnosed with SBP from ICU of Tropical medicine department, Zagazig University Hospitals. The Gram positive cocci included (16 staph aures, 9 Enterococci, 3 CoNS and 3 Viridans streptococci). Among the isolates, 70Ecoli (48.2%), 10K pneumonia (47.6%) and 10 (32.2%) Gram positive isolateswere FQ-resistant (resistant to ciprofloxacin and levofloxacin) are wereincluded in investigation.

Antibiotic susceptibility results among FQ-resistant Ecoli isolates showen in **Tab. 1**. Out of 70 FQ-resistant Ecoli isolates, 55 (78.5%) expressed MDR phenotype;the highest rates of resistance were detected to cephalosporins (90% -100%), Aztreonam (87.1%), piperacillintozabactam (82.8%) and amoxacillinclavulinate (68.5%). Meropenem and Imipenem showed good action with least resistance rates (5.7% and 7.1% respectively). Antibiotic susceptibility pattern among FQ-resistant K pneumonia are showed in **Table 2**. MDR phenotype was expressed in 6 (60%) of K pneumonia isolates. The highest resistance rates were also detected for cephalosporins (90-100%), Aztreonam (100%), amoxacillinclavulinate (70%) and piperacillintozabactam (70%). Meanwhile, all isolates except one were sensitive toimipenem (90%) and meropenem (90%). ESβL phenotype was detected in 55(68.7%) out of 80 FQ-resistant Enterobacteriacaeincluding 50 isolates Ecoli and 5isolates K pneumoniae. Regarding FQ resistant Gram positive cocci, **Table 3** summarized their antibiotic susceptibility pattern. All*S. aureus*isolates were sensitive to ceftaroline, imipenem, vancomycin and linezolid; oneMRSA was detected and was thus considered MDR. None of the gram positive isolates was vancomycin or linezolid resistant.

PCR for five PMQR determinants was performed, **Table 4** illustrated PMQR detection among 80 FQ-resistantEnterobacteriacae isolates. All tested FQ-Resistant Gram negative isolates (100%) harbored at least one of the tested PMQR genes. We detected *acc6`-Ib-cr* gene with highest frequency (70/80–87.5%) followed by*qnrS*(65/80 - 81.2%) **(Figure 3,4)**.Lower rates of*qnrB* (10/80 – 12.5%) and *qepA*(8/80 – 10%) were found while we found no *qnrA* gene. In Gram positive isolates, PMQR were detected in 6 out of 10 FQ-resistant isolates as summarized in **Table 5**. *qnrS* (6/10- 60%) and *acc6`-Ib-cr* (5/10- 50%) were the only found in Gram positive isolates, None of qnrA, qnrB or qepA were found in Gram positive isolates.

Concerning association between ESβL production and PMRQ genes, **Table 5** demonstrated statistically significant association between production of ESβL&*qnrS* genecarriagep<0.001, Significantly association was alsoreportedamong*acc6`-Ib-cr* gene detections and ESβL productionsp=0.035.

**DISCUSSION**

Spontaneous bacterial peritonitis in advanced liver cirrhosis and its treatment have a global burden in healthcare management especially in our locality. Fluoroquinolonesconstitutes an important line in the prophylaxis from SBP recurrence or in cirrhotic patients with high-risk profiles, These patients may take prophylaxis for entire life or till liver transplantation[3]; however, extensive use of FQ has led to and increased prevalence ofquinolone-resistant strains especially in developing countries [23].

FQ resistances rates were found to be 48.2% of E coli isolates,47.6% of K pneumonia isolates but was lower in Gram positive isolates (32.2%). This comes in consistent with a recent studyin our locality that detected FQ resistance among 46.8% of uropathogenicE coli [24].A systemic review detected moderately high rate of quinolone resistance in Arab countries up to 31%- 38% in Tunisia and Lebanon respectively[25]. Globally, quinoloneresistancerates varies from 35% to 57% inseveral geographical areas[26]**.**Higher rate obseved in Iran (45.3–61.9%) and in Pakistan (84.2%) [27,28]. Egyptian studies had been previously reported high rates of quinolone resistance in isolates from SBP[29,30]nearly similar rates also detected byArdolino E et al [31]. Lower rates of resistance among SBP patientwere detected by other studies in Germany [32, 33].Adequate antibiotic prescription policy followed in developed countries could be the cause of these variations.

Cirrhoticcaseswere frequently exposedto health care structure, and carrying higher probabilitiesof colonization by multidrug resistant germs. Fluoroquinolone exposure had led to higher rates of resistant microorganisms in enteric microbiota[34].We detected MDR phenotype in 78.5% of FQ-resistantEcoli and 60% of FQ-resistant klebsiella isolates; this was in accordance with a systematic review byFoire et al.,who reported an overall proportion of MDR bacteria in SBP from 22% to 73% of cases across several studies[35]. Piano et al found less prevalence of MDR (35%)[36]. While in Germany, no MDR organisms were isolated from SBP patients in a study by Lutz et al [32].

Several studies on FQ-resistant strains from urinary tract infection reported nearly similar results [24, 37, 38, 39], whichwas attributed to FQ resistance genes coexistence along with genetic determinants of other antimicrobialson mobile genetic elements.

The isolated FQ-resistant *E.coli*unfortunately give higher rates of resistance to cephalosporins (90%-100%), where are used as first line empirical treatment of SBP, There was also high resistance rates to amoxicillin clavulinic acid and piperacillin/tazobactam. *K.pneumonia* isolates also showed high resistance to cephalosporins, amoxicillin/clavulinic acid, piperacillin/tazobactam but fortunately, carbapenems, amikacin and gentamycin showed good action against gram negative isolates that was in accordance to the new guidelines by European Associated for the Study of Liver(EASL)that recommended carpabenems for nosocomial SBP especially in localities with high frequency of ESBL[3]. Meanwhile, lower resistance levels among SBP patients were reported in Germany by Lutz et al.whodetected 31% resistance to 3$^{rd}$ generation cephalosporin in, 35% to quinolones and 23% to piperacillintozabactam[32] while in Spain resistance was only 9% to 3$^{rd}$ generation cephalosporins[40].In addition, In addition, our result comes similar to the findings of Majlesi et al who found that FQ-resistant Enterobacteriaceae isolates showed resistance to other antimicrobial agents like cefotaxime, ceftazidime, cefoxitin, cefepime, aztreonam, amoxicillin-clavulanic acid, tetracyclines, rifampicin, and trimethoprim-sulfamethoxazole, but remain susceptible to carbapenem antibiotics [41].

As Regard to gram positive cocciin our study, 25% (4/16) of staph aures, 66.6% (2/3) CoNS, 22.2% (2/9) of Enterococci and33.3% (1/3) of streptococci were resistant to FQs, which was inconsistent to results of aprevious study [42].One MRSA was detected, that isolate was sensitive to carbapenems, vancomycin, linezolid and ceftaroline (the only fifth generation (anti MRSA) cephalosporin). All other staphylococcus isolates were sensitive to oxacillin**;** several studies reported isolation of MRSA in SBP [42,43].Quinolone use was reprted to be associated with a risk ratio of 3 of acquiring methicillin-resistant Staphylococcus aureus (MRSA) infection, the highest across different class of

antibiotics, with relevant consequences in infections other than SBP.[44] We found no vancomycin resistant cocci.

The Previous discrepancy in antimicrobial susceptibility pattern and frequency of MDR phenotype detection could be due to different study populations, different samples and variable antibiotic selective pressures so regional epidemiologicalstudies are essential in order to design hospital antimicrobial stewardship programs.

Regarding ESBL production, we detected ESBL production in (55/80)68.7% of FQ-resistant Enterobacteriacae, including 50Ecoli isolates and 5 Klebsiella isolates. Nearly consistent results were found in FQ-resistant Enterobcteriacae isolated from patients with UTI [45],whileother studies reported lower rates [46,47]The co-resistance to ciprofloxacin and ESBL production demonstrated enormous impacts of antibiotic pressure and selection in intestine post susceptible bacteria had killed by many antibiotics[34].

Plasmid mediated quinolone resistance genes (PMQR) have changed the pattern of quinolone resistance.Patient group in our study are frequently exposed to quinolones as it is used as prophylaxis in those patients after a previous attack of SBP or GI bleeding. Regarding PMQR genes, All tested Enterobacteriacaeisolates (100%) were found carrying at least one of the PMQR with*acc(6`)-Ib-cr*being the most prevalent (87.5%) followed by *qnrS* gene (81.2%)and less frequently *qnrB* gene *(12.5%)*and*qepA (10%). No qnrA* gene was detected.

The incidence and distribution of PMQR genes shows a great discrepancy. A study on *klebsiella*in Egypt by Hammad et al.detected PMQR genes in all isolates but the highest prevalence of *qnrS* (81.3%) and lower percentage of *acc(6`)-Ib-cr* (23.3%) than our results [48]while In accordance to our study, a recent study in our locality found*Acc(6`)-Ib-cr* the most prevalent gene (80.1%) and less frequently *qnrS, qnrB and qepA*[24]Several other studies also reported nearly similar rates of PMQR detection [ 30,45 , 49].

In accordance to our results; no *qnrA* was detected in Egyptian study and inIran [24,49]while Thai, Mexican and Korean studies showed no detection of *qepA*gene [50, 51,52]. *qepA* was documented in Japan, France andChina [54].Harboring more than one PMQR genes was frequently found in 58 isolates 72.5% carried both *acc(6`)-Ib-cr&qnrS*while 7 isolatescarried *acc(6`)-Ib-cr&qnrS&qnrB* genes at the same time. Co-existence of acc(6`)-Ib-cr&qnr were reported in other studies [24, 54].

In contrast, lower prevalence for PMQR than our finding (<1%) were observed[55, 56, 57, 58]. Probablygeographical changes, different pattern of antibiotic resistance and infection control policies are causes of this discrepancy in the PMQR distribution.

Concerning association between ESBL production and PMQR genes detection, a study carried out by Khalil et al,found a prevalence of PMQR genes 85.7% among ESBLs producing Enterobacteriaceae clinical isolates [59]. In addition, HigherPMQR prevalence determinants was 93.1% [60].Interestingly we found all ESBL positive Enterobacteriacaeisolates harbored PMQR, Statistically significant association was found between *qnrS*carriage and ESBL ($p<0.001$). Additionally, there was significant association between *acc(6`)-Ib-cr*andESBLpositive isolates, that was consistent with previous studiesin IranandKorea [49, 61].Perhaps that is due to co-carriage of PMQR genes with other resistance determinants frequently ESBL, AmpC-type β-lactamase and carbapenemase genes on a plasmid [12].

Additionally, PMQR genes were sought in the gram positive isolates. Interestingly 6of tested gram positive isolates that showed phenotypic resistance to quinolones harbored PMQR. Previous studies reported PMQR genes *(qnr& acc6`-Ib-cr)* in gram positive bacteria carried on chromosomes [62].

Association between PMQRs and ESBLs is clinically important since treatments option for these isolates are limited and led to therapy failure; so more cautious use of quinolones as a prophylaxis in SBP should be considered with proper selection of cases and re-evaluation of antibiotic prophylaxis in case of selection of quinolone resistance [17].

The limitations in our study was not performing molecular epidemiology and typing, also PMQR in gram positive isolates need further investigations with larger number of isolates.The high prevalence of MDR, ESBL and quinolone resistance among patients with SBP should direct us to the correct and judicious use of fluoroquinolone.

## CONCLUSION

PMQR determinants and ESBLs prevalence is high among FQ-resistant isolates from cirrhotic patients diagnosed with spontaneous bacterial peritonitis. The PMQR genes and their association with ESBL-producing plasmids have a great effect on spread of multidrug resistance and may lead to serious problems for treatment. This necessitates detection of PMQR determinants and ESBL genes among non-susceptible fluoroquinoloneEnterobacteriaceae for appropriate empirical treatment and infection control.

**Disclosure**

No conflicts of interest in this work.

**Conflict of Interest**: none
**Financial Disclosures**: none

**Table (1): Antibiotic Susceptibility Pattern of FQ Resistant *Ecoli*.**

| **Antibiotic Disks** | **Ecoli Isolates (No=70)** | | | | | |
|---|---|---|---|---|---|---|
| | Sensitive | | Intermediate | | Resistant | |
| | No | % | No | % | No | % |
| **Ciprofloxacin** | - | - | - | - | 70 | 100% |
| **Levofloxacin** | - | - | - | - | 70 | 100% |
| **Amox./clav.** | 22 | 31.4% | - | - | 48 | 68.5% |
| **Piperacilintazobactam** | 5 | 7.1% | 7 | 10% | 58 | 82.8% |
| **Cefoxitin** | 3 | 4.2% | 17 | 24.2% | 50 | 71.4% |
| **Cefotaxime** | - | - | - | - | 70 | 100% |
| **Ceftriaxone** | - | - | - | - | 70 | 100% |
| **Ceftazidime** | - | - | - | - | 70 | 100% |
| **Cefepime** | 2 | 2.8% | 5 | 7.1% | 63 | 90% |
| **Aztreonam** | 2 | 2.8% | 7 | 10% | 61 | 87.1% |
| **Meropenem** | 58 | 82.8% | 8 | 11.4% | 4 | 5.7% |
| **Imipenem** | 55 | 78.5% | 10 | 14.2% | 5 | 7.1% |
| **Gentamycin** | 49 | 70% | 8 | 11.4% | 13 | 18.5% |
| **Amikacin** | 50 | 71.4% | 6 | 8.5% | 14 | 20% |
| **Trimethoprim sulpha.** | 24 | 34.2% | 18 | 25.7% | 28 | 40% |
| **MDR** | - | - | - | - | 55 | 78.5% |
| **ESBL production** | - | - | - | - | 50 | 71.4% |

**Table (2): Antibiotic Susceptibility Pattern of FQ Resistant *K. pneumoniae* isolates.**

| **Antibiotic Disks** | ***K. Pneumoniae* Isolates (No=10)** | | | | | |
|---|---|---|---|---|---|---|
| | Sensitive | | Intermediate | | Resistant | |
| | No | % | No | % | No | % |
| **Ciprofloxacin** | - | - | - | - | 10 | 100% |
| **Levofloxacin** | - | - | - | - | 10 | 100% |
| **Amox./clav.** | 1 | 10% | 2 | 20% | 7 | 70% |
| **Piperacilintazobactam** | 2 | 20% | 1 | 10% | 7 | 70% |
| **Cefoxitin** | - | - | - | - | 10 | 100% |
| **Cefotaxime** | - | - | - | - | 10 | 100% |
| **Ceftriaxone** | - | - | 1 | 10% | 9 | 90% |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Ceftazidime** | - | - | - | - | 10 | 100% |
| **Cefepime** | - | - | - | - | 10 | 100% |
| **Aztreonam** | - | - | - | - | 10 | 100% |
| **Meropenem** | 9 | 90% | - | - | 1 | 10% |
| **Imipenem** | 9 | 90% | 1 | 10% | - | - |
| **Gentamycin** | 5 | 50% | 3 | 30% | 2 | 20% |
| **Amikacin** | 5 | 50% | - | - | 5 | 50% |
| **Trimethoprim sulpha.** | 5 | 50% | 1 | 10% | 4 | 40% |
| **MDR** | - | - | - | - | 6 | 60% |
| **ESBL production** | - | - | - | - | 5 | 50% |

**Table (3): Antibiotic Resistance Pattern among FQ Resistant Gram positive isolates**

| **Antibiotic Disks** | *S. aureus* | | CoNS | | Enterococci | | v. streptococci | |
|---|---|---|---|---|---|---|---|---|
| | No=4 | (%) | No=2 | (%) | No=3 | (%) | No=1 | (%) |
| **Ciprofloxacin** | 4 | 100% | 2 | 100% | 3 | 100% | 1 | 100% |
| **Levofloxacin** | 4 | 100% | 2 | 100% | 3 | 100% | 1 | 100% |
| **Oxacillin** | 1 | 25% | 2 | 100% | 3 | 100% | 1 | 100% |
| **Ceftaroline** | 0 | 0 | 0 | 0 | - | - | - | - |
| **Quinopristin/dalfopristin** | 3 | 75% | 2 | 100% | 2 | 66.6% | 0 | 0 |
| **Meropenem** | 1 | 25% | 0 | 0 | - | - | 0 | 0 |
| **Imipenem** | 0 | 0 | 0 | 0 | - | - | 0 | 0 |
| **Gentamycin** | 1 | 25% | 2 | 100% | 3 | 100% | 1 | 100% |
| **Doxycycline** | 1 | 25% | 1 | 50% | 3 | 100% | 0 | 0 |
| **Clindamycin** | 2 | 50% | 1 | 50% | - | - | 1 | 100% |
| **Trimethoprim sulpha** | 2 | 50% | 2 | 100% | - | - | 1 | 100% |
| **Vancomycin** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Linezolid** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

CoNS=coagulase negative staphylococci

**(No=80)**

| *PMRQ gene* / *Isolate(n)* | *acc6`-Ib-cr* | *qnrS* | *qnrB* | *qepA* | *qnrA* |
|---|---|---|---|---|---|
| ***E.coli*(70)** | 60 | 57 | 6 | 6 | 0 |
| ***K pneumonia* (10)** | 10 | 8 | 4 | 2 | 0 |
| **Total (%)** | 70 (87.5%) | 65 (81.2%) | 10 (12.5%) | 8 (10%) | 0 |

**Table (5)PMRQ determinants detected in PCR tested Gram positive isolates (No=10)**

| *PMRQ gene* / *Isolate(n)* | *acc6`-Ib-cr* | *qnrA* | *qnrB* | *qnrS* | *qepA* |
|---|---|---|---|---|---|
| ***S aureus* (4)** | 1 | 0 | 0 | 1 | 0 |
| **CoNS (2)** | 1 | 0 | 0 | 1 | 0 |
| **Viridansstrept (1)** | 1 | 0 | 0 | 1 | 0 |
| **Enterococci (3)** | 2 | 0 | 0 | 3 | 0 |
| **Total (%)** | 5 (50%) | 0 | 0 | 6 (60%) | 0 |

**Table(6):** Relation between ESBL and PMRQ gene among tested *Enterobacteriaceae.*

| | **ESBL (No=55)** | | **Total number** | ***p** |
|---|---|---|---|---|
| | Present(No=55) | Absent(No=25) | | |

| | No | % | No | % | No=80 | |
|---|---|---|---|---|---|---|
| *qnr S* | | | | | | |
| **Present** | 55 | 100% | 10 | 40% | 65 | < 0.001(s) |
| **Absent** | 0 | 0% | 15 | 60% | 15 | |
| ***acc(6`)-Ib-cr*** | | | | | | |
| **Present** | 45 | | 25 | 100% | 70 | 0.035(s) |
| **Absent** | 10 | | 0 | 0% | 10 | |
| *qnr B* | | | | | | |
| **Present** | 5 | 9% | 5 | 20% | 10 | 0.31 |
| **Absent** | 50 | 90.9% | 20 | 80% | 70 | |
| *qep A* | | | | | | |
| **Present** | 5 | 9% | 3 | 12% | 8 | 0.99 |
| **Absent** | 50 | 90.9% | 22 | 88% | 72 | |

*Fisher exact test S= significant

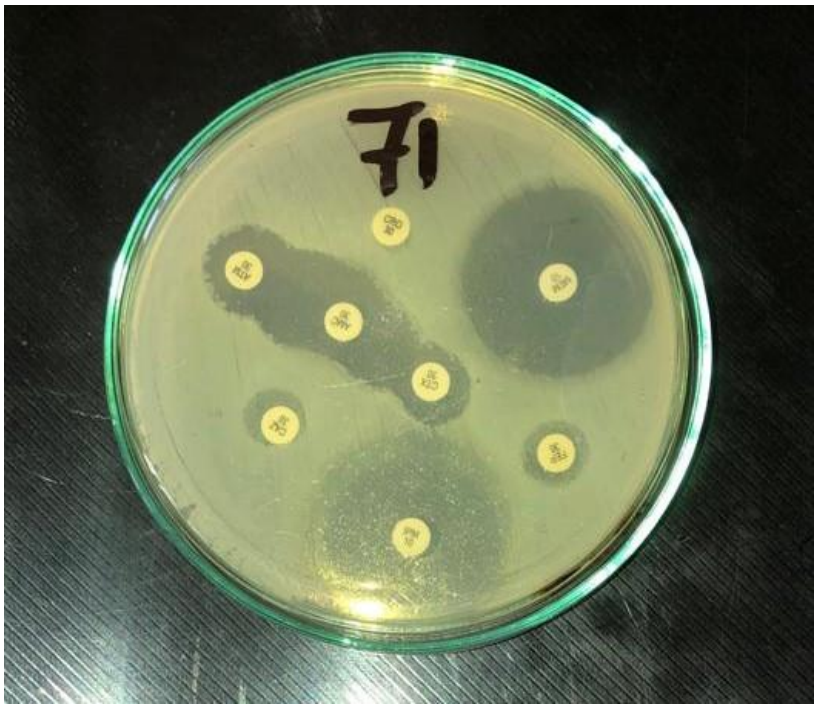



**Figure (1) Double disk synergy test for 2 gram negative isolates showing positiveESβL (Key hole appearance) with enhanced zone of inhibition between AMC, ATM and CTX discs**

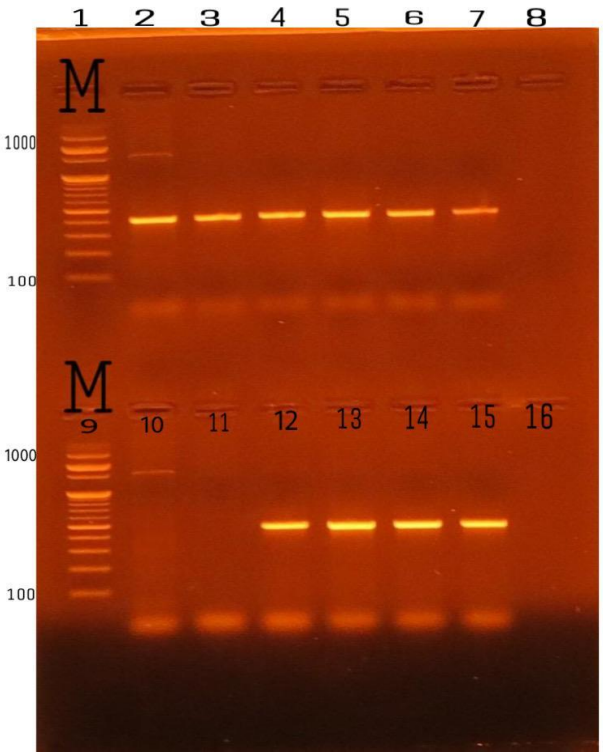
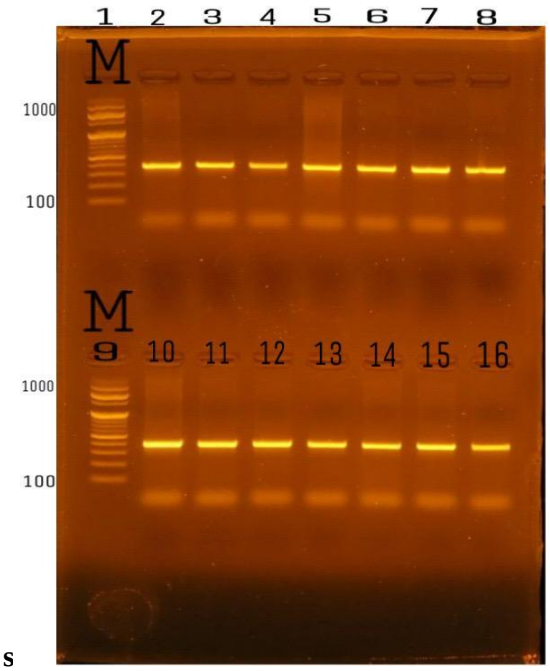



**Figure (2): Agarose gel electrophoresis for *acc(6`)-Ib-cr* gene. Positive samples showed bands at 482 bp.**

Lane (1,9) showed Molecular weight marker from 100 to 1000 bp
Lanes (2, 3, 4, 5, 6, 7, 12, 13, 14, 15, 16) showed positive *acc(6`)-Ib-cr*gene




**S**

**Figure (3): Agarose gel electrophoresis for *qnrS* gene. Positive samples showed bands at 417 bp**

Lane (1,9) showed Molecular weight marker from 100 to 1000 bp
Lanes (2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 16) showed positive *qnrS*gene

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