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Prevalence of CTX M Extended-Spectrum Beta-Lactamases in Clinical Gram-Negative Bacteria

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Background: Research reports about molecular characterization of CTX M extended spectrum β-lactamases are fragmentary from Bahrain.

Objective: To characterize Gram-negative bacterial isolates for the prevalence of specific Geno-groups and Geno-types of CTX M extended-spectrum β-lactamases.

Design: A Prospective Point Prevalence Study.

Setting: Pathology Department, King Hamad University Hospital, Bahrain.

Method: Forty-seven Gram-negative bacterial isolates resistant to third (3GC) and/or fourth-generation cephalosporins (4GC) and 16 Gram-negative isolates susceptible to these classes of cephalosporins were studied. The bacterial identification and antibiotics sensitivity was performed by automated systems. Isolates were further characterized for CTX M geno-groups and types by multiplex PCR, monoplex PCR and sequencing of the representative isolates.

Result: The majority of the isolates were found to be multiple-drug resistant showing concomitant resistance of cephalosporins with other classes, such as fluoroquinolones and aminoglycosides. However, this collection of bacterial isolates was persistently sensitive to carbapenems such as imipenem and meropenem. In addition, few isolates demonstrated resistance to tigecycline.

Sixty-three isolates were studied; 47 (74.6%) showed resistance to 3GC and/or 4GC. Multiplex PCR demonstrated the presence of blaCTX M in 45 (95.7%) isolates. Further confirmation with multiplex and monoplex PCR revealed 41 (91.1%) and 4 (8.9%) bacterial isolates harboring blaCTX M Geno-group 1 and blaCTX M Geno-group 9, respectively. Out of 16 3GC/4GC sensitive isolates, 3 (18.8%) had CTX M genes, all were Geno-group 1. Sequencing revealed the presence of CTX-M 15 type ESBL from Geno-group 1 positive isolates; however, Group 9 isolates did not reveal any CTX-M type, rather they were non-specific amplifications.

Conclusion: Bahraini G-ve bacterial population demonstrated multi-drug resistance to antibiotics. CTX M 15 type of ESBL is prevalent in Bahrain.

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CTX M type extended-spectrum β-lactamases (ESBLs) are class A ESBLs showing resistance to third and fourth-generation cephalosporins and to aztreonam[1,2]. Since the first discovery of CTX M 1 type ESBL, numerous variants of this ESBL have been reported. CTX M ESBLs are prevalent ESBLs worldwide and currently approaching 160[3]. Increasing prevalence of such ESBLs has put a pressure on clinical settings for increased usage of carbapenems; as a result carbapenem-resistance has also appeared[4-7]. Although ESBL phenotypes have been reported from Bahrain, the reports pertaining to molecular characterization of such an important class of ESBLs are fragmentary.

The aim of this pilot study is to characterize the Gram-negative bacterial isolates for the prevalence of specific Geno-groups and Geno-types of CTX M enzymes.

METHOD

Sixty-three bacterial isolates were randomly collected during March to April 2013. Forty-seven were G-ve isolates resistant to third generation cephalosporins (3GC) and/or fourth-generation cephalosporins (4GC) and 16 isolates susceptible to these classes of cephalosporins.

The bacterial identification and antibiotics susceptibility was performed by BD Phoenix-100 automated system.

Isolates were molecularly characterized for CTX M Geno-groups and types by multiplex and monoplex PCRs according to the method described previously[8]. The sets of primers used in this study were targeting blaCTX M group 1, blaCTX M group 2, blaCTX M group 8, blaCTX M group 9 and blaCTX M group 25.

Eighteen (40%), out of which 16 (35.5%) were from group 1 and two (4.5%) were from group 9, were sequenced to confirm the exact type of CTX-M ESBL. The sequencing was performed by Genoscreen. The sequencing results were analyzed by using the Chromas software and BLAST search engine (The Basic Local Alignment Search Tool). Further alignment of the obtained sequences was performed against the specific CTX M types by using the Clustal W software.

Primers used in the PCR reactions and sequencing are shown in Table 1.

Table 1: List of Primers Used for Multiplex and Monoplex-PCRs and Sequencing

Primer used for respective CTX M Groups	Primer sequences used	Expected Amplicon size
CTX M gp1 Forward	5'-AAA AAT CAC TGC GCC AGT TC-3'	415 bp
CTX M gp1 Reverse	5'-AGC TTA TTC ATC GCC ACG TT-3'	
CTX M gp2 Forward	5'-CGA CGC TAC CCC TGC TAT T-3'	552 bp
CTX M gp2 Reverse	5'-CCA GCG TCA GAT TTT TCA GG-3'	
CTX M gp9 Forward	5'-CAA AGA GAG TGC AAC GGA TG-3'	205 bp
CTX M gp9 Reverse	5'-ATT GGA AAG CGT TCA TCA CC-3'	
CTX M gp8 Forward	5'-TCG CGT TAA GCG GAT GAT GC-3'	666 bp
CTX M gp25 Forward	5'-GCA CGA TGA CAT TCG GG-3'	327 bp
*CTX M gp8/25R	5'-AAC CCA CGA TGT GGG TAG C-3'	
CTX M gp1 Sequencing	5'-AAA AAT CAC TGC GCC AGT TC-3'	-
CTX M gp9 Sequencing	5'-CAA AGA GAG TGC AAC GGA TG-3'	-

*The same primer was used as a reverse primer for Geno-group 8 and Geno-group 25

RESULT

Sixty-three G-ve bacterial isolates were randomly collected from the clinical specimens. The patient-cohort comprised of 20 males and 43 females. The age of the patients ranged from 36 days to 85 years.

Forty-seven were resistant to third generation (3GC) and/or fourth-generation cephalosporin (4GC); 16 G-ve isolates were susceptible to the cephalosporins. These 3GC and/or 4GC resistant and sensitive isolates were further grouped as cephalosporin resistant (CR) and cephalosporin sensitive (CS) groups, for the purpose of analyzing antibiotics susceptibility and molecular results.

Forty-seven (74.6%) clinical specimens, which grew CR isolates, were as follow: 35 (74.5%) urine, 6 (12.8%) pus swab, 4 (8.5%) soft tissue, 1 (2.1%) placental swab and 1 (2.1%) bone tissue. The G-ve isolates obtained from these samples were 41 (87.2%) Escherichia coli, Klebsiella pneumoniae 5 (10.6%) and 1 (2.1%) Proteus vulgaris.

Sixteen clinical specimens, which grew CS isolates were as follow: 11 (68.7%) urine, 4 (25%) pus swab and 1 (6.3%) aspirate. The GNB isolates obtained from these samples were 12 (75%) Escherichia coli, 2 (12.5%) Klebsiella pneumoniae, 1 (6.2%) Enterobacter hermanii and 1 (6.2%) Proteus mirabilis.

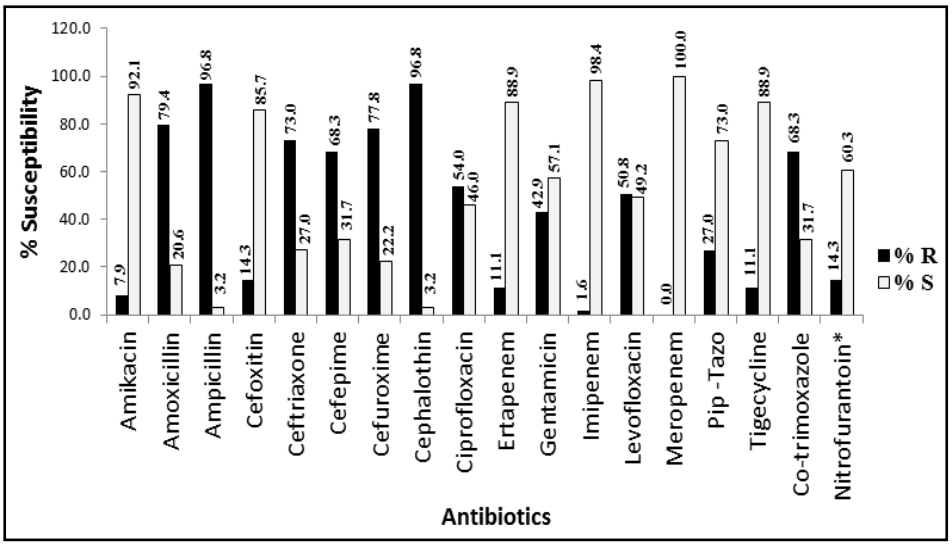
The majority of isolates were multiple-drug resistant to cephalosporins fluoroquinolones and aminoglycosides. Maximum resistance was noticed for cephalothin and ampicillin in 61 (96.8%) each, followed by amoxicillin, cefuroxime and ceftriaxone in 50 (79.4%), 49 (77.8%) and 46 (73%) isolates, respectively.

Among aminoglycosides, resistance to gentamicin was noticed in 27 (42.9%) isolates, whereas, only 5 (7.9%) isolates were resistant to amikacin. Among the fluoroquinolones, resistance to levofloxacin was noticed in 32 (50.8%) isolates whereas, the resistance to ciprofloxacin was noticed in 34 (54%) isolates. Cotrimoxazole resistance was 43 (68.3%) in the current collection of G-ve isolates.

All the 63 isolates were sensitive to meropenem; however, resistance to imipenem was noticed in only 1 (1.6%) isolate. Resistance to tigecycline was found in 7 (11.1%) isolates of the present collection.

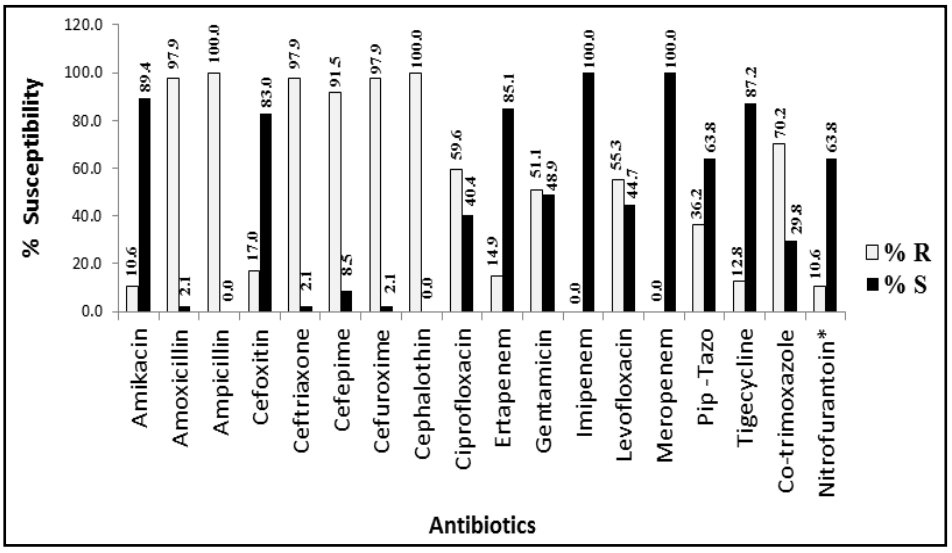
Significant concomitant resistance to co-trimoxazole 33 (70.2%), ciprofloxacin 28 (59.6%), levofloxacin 26 (55.3%), gentamicin 24 (51.1%) and piperacillin-tazobactam 17 (36.2%) was revealed. The resistance to amikacin in this group was significantly low, 5 (10.6%), and all the isolates of CR group were found sensitive to imipenem and meropenem.

CS group showed significantly high resistance to ampicillin and cephalothin, 14 (87.5%) each. Resistance to amoxicillin and cefuroxime was found in 4 (25%) and 3 (18.6%) isolates. Among fluoroquinolones, 6 (37.5%) isolates showed resistance to ciprofloxacin and levofloxacin. Ten (62.5%) were resistant to co-trimoxazole. All the isolates of this group were sensitive to amikacin, meropenem, ertapenem and piperacillin-tazobactam. One (6.3%) isolate of this group showed resistance to imipenem and tigecycline, see figures 1, 2 and 3.



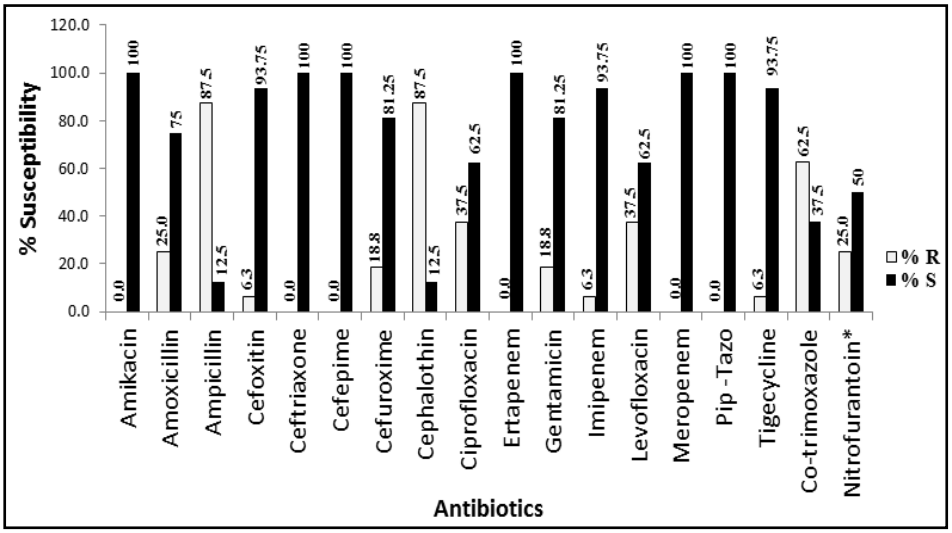
*Nitrofurantoin was tested only in 47 isolates

Figure 1: Antibiotic Susceptibility Pattern of the Clinical Isolates Studied (N=63).



*Nitrofurantoin was tested only in 37 CR isolates

Figure 2: Concomitant Resistance Pattern in 3GC and/or 4GC Resistant Isolates (N=47)



*Nitrofurantoin was tested only in 12 CS isolates

Figure 3: Concomitant Resistance Pattern in 3GC and/or 4GC Sensitive Isolates (N=16)

Multiplex PCR demonstrated the presence of blaCTX M in 95.7% (45/47) isolates from CR group. Further confirmation with multiplex and monoplex-PCR revealed 91.1% (41/45) and 8.9% (4/45) of blaCTX M Geno-group 1 and blaCTX M Geno-group 9, respectively. Out of 16 3GC/4GC sensitive isolates (CS group), 3 (18.8%) had CTX M genes; all were Geno-group 1. Figure 4 and 5 are representative of molecular results. Sequencing revealed the presence of CTX M 15 type ESBL from Geno-group 1 positive isolates. Group 9 isolates did not reveal any CTX M type, rather they were non-specific amplifications, see figure 6.

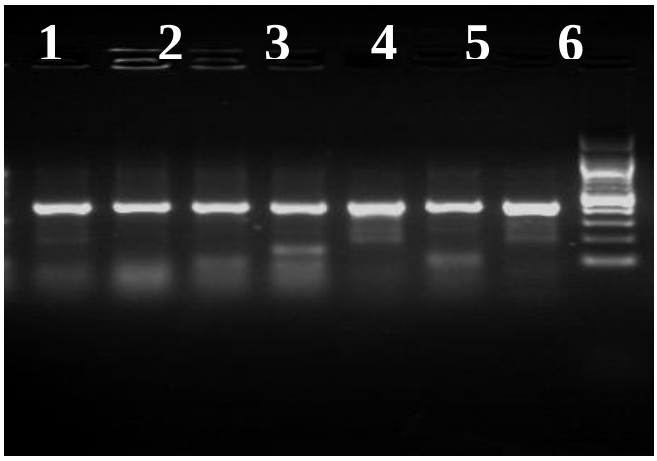


Figure 4: Representative Gel Showing the Targeted Amplicons of 415 Bp Corresponding to CTX M Group-1 (Lanes 1-7). Lane M Demonstrates 100 Bp Ladder.

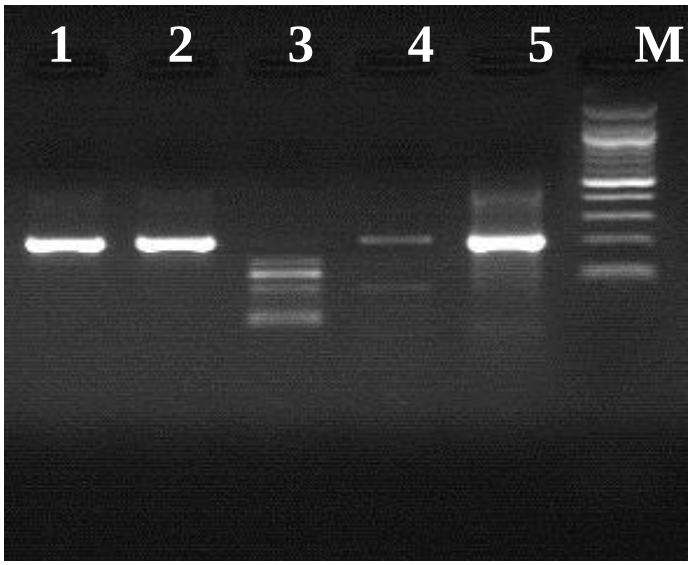


Figure 5: Representative Gel Showing the 205 Bp Amplicons of CTX M Group 9 (Lanes 1, 2 And 5). Lanes 3 and 4 Show non-Specific PCR Amplification. Lane M Demonstrate 100 Bp Ladders

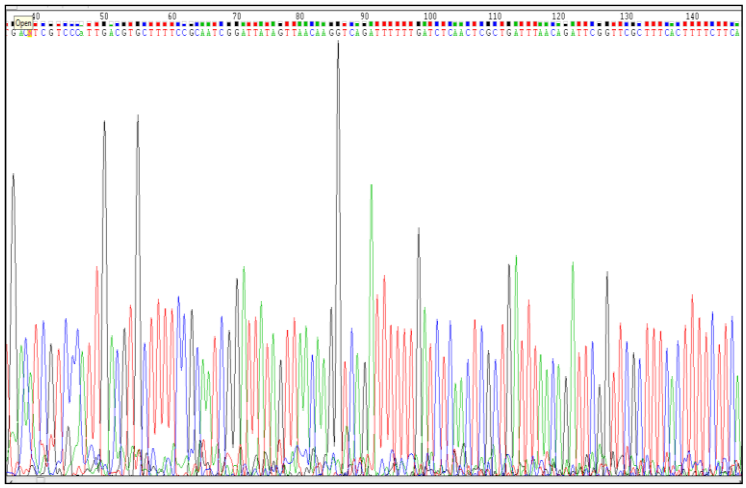


Figure 6: Sequencing Chromatogram of One of the Representative CTX M Group 1 Positive Isolates (Strain No. M57). Analyses of the Sequencing Results Demonstrate Presence of CTX M-15 Type ESBL

DISCUSSION

Acquisition of antibiotics bla genes and conferring resistance to respective classes of antibiotics is a major concern globally these days. Cephalosporins are a widely prescribed antibiotic class, which has shown an increasing resistance globally[1].

Extended-spectrum β-lactamases confer resistance to broad-spectrum cephalosporins and aztreonam. CTX M (Class A) ESBLs are important and extensively studied ESBLs in different parts of the world including Middle East; however, the data from Bahrain regarding this important type of ESBL are fragmentary[9-12].

In this pilot study, we noticed that antibiotics resistance in our bacterial population is noteworthy and should not be disregarded. The majority of our isolates were multi-drug resistant.

Our isolates demonstrated significantly high resistance to penicillins/cephalosporins, except cefoxitin, which only 14.3% of isolates demonstrated resistance. Piperacillin-tazobactam showed the resistance in 27% of isolates. Fluoroquinolones showed high resistance rates; levofloxacin showed comparatively lower resistance rate than ciprofloxacin, see figure 1. Among aminoglycosides, amikacin was found to be a better empiric therapeutic option as it showed less resistance (7.9%) in our bacterial population.

These isolates demonstrated consistent sensitivity to carbapenems (imipenem and meropenem), all the isolates were sensitive to meropenem. However, it is alarming to see an emergence of resistance to tigecycline in our bacterial population.

Molecular characterization of the isolates demonstrated that CTX M type of EBLs are highly prevalent (74.6%) in our bacterial population. CTX M 15 was the only CTX M type noticed in this sample. The isolates demonstrating the CTX M group 9 (based on PCR) were non-specific amplifications and thus suggest that we should not infer the results solely based on PCR data, rather the results should be confirmed by sequencing before inferring a final conclusion.

CONCLUSION

The resistance to cephalosporins is quite high in our bacterial population. The isolates are multi-drug resistant (MDR) and have concomitant resistance with other classes of antibiotics such as fluoroquinolones and aminoglycosides. However, the isolates were consistently sensitive to carbapenems (meropenem in particular). Tigecycline resistance has appeared in Bahraini Gram-negative bacterial population. CTX M 15 is the prevalent CTX M type of ESBL in Bahrain.

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