The Antimicrobial Resistance Patterns & Distribution of Antibiotic Resistance Genes among Carbapenem Resistant Escherichia Coli Isolated from Diabetic Foot Infection

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Abstract

Background: Diabetic foot infection (DFI) is an infection in soft tissue or bone in patients with diabetes mellitus, treatment of DFIs requires appropriate antibiotic selection, continuous updates of the microorganisms that are responsible for infection and their resistance pattern, the aim of this study to determine the frequency of antimicrobial resistance patterns, ESBLs, and MBLs produced carbapenem resistant Escherichia coli isolated from patients with DFI.

Methods: About 111 swabs specimen were collected from patients suspected with DFI during the period of study, determine the antibiotic susceptibility of E. coli isolates by using Kirby-Bauer disk diffusion method to 23 antimicrobial agents. ESBL production was screened by two tests initial method by the disk diffusion method of ceftazidime, ceftriaxone, cefotaxime, and aztreonam (30μg/disk each), according to the CLSI (2021) and confirmatory method by Double disk diffusion test, MBL production by the disk diffusion method of imipenem and meropenem. Molecular detection for ESBL genes by monoplex PCR and MBL genes by multiplex PCR.

Results: From 111 swabs specimen E. coli isolates had represented in 19 (27.5%) among Gram-negative bacteria. Antibiotic susceptibility test revealed that 10 carbapenem resistant E. coli isolates, and the highest resistant to β-lactam class and cephalosporin, results indicated also 100% of isolates were MDR and 63.1 XDR. PCR assay to ESBL genes revealed that blaCTX-M was found in 3 (30%) and blaOXA was noticed in 4 (40%). The PCR data of MBL genes revealed that, the frequency of MBL genes among isolates as following blaVIM1 (50%), blaNDM2 (100%), and blaSIM 2 (100%).

Conclusion: The study show high frequency of antibiotic resistant pattern and the presence of ESBL and MBL genes among carbapenem resistant E. coli isolated from DFI.

Keywords: Diabetic Foot Infection, TEM, SHV.

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INTRODUCTION

Diabetic foot infection (DFI) is an infection in soft tissue or bone in people with diabetes, and has grown to be a major reason for non-traumatic amputation. Contamination of bacteria among diabetic foot can cause foot ulceration and this can lead to development of gangrene and then amputation, according to some studies about 15-34% of diabetic patients are at risk for developing foot ulcers during their lives. The characterization of Escherichia coli isolates from skin and soft tissue infections have been previously published. However, this description still poor from DFIs, while E. coli is a major pathogenic Gram-negative bacteria isolated from these ulcers. Treatment of DFIs requires appropriate antibiotic selection, continuous updates of the microorganisms that are responsible for infection and their resistance pattern. Antibiotic resistance rates vary by country and geographical region. Rising levels of resistance to main antibiotics were initial caused by Extended-Spectrum-Beta-Lactamase (ESBL) producing Gram-negative bacteria in the mid-2000’s, and more lately by carbapenemase-producers. The emergence of carbapenem as a therapy for bacteria that are resistant to β-lactamase is a significant advance in clinical practice.
Gram-negative bacteria (GNB). However, the therapeutic use of carbapenem is in threat with the development of carbapenemases, mainly metallo-β-lactamase (MBL) 

Extended spectrum β-lactamases is an enzyme produced by Gram negative bacteria Enterobacteriaceae, which can hydrolyze penicillin but also third generation cephalosporin and monobactam and other antibiotics by inactivation of penicillin and cephalosporin by using plasmid-mediated ESBLs such as the TEM, SHV, or CTX-M groups. This study purposed to determine the frequency of antimicrobial resistance patterns for Carbapenem resistant Escherichia coli isolated from patients with diabetic foot infection, and investigated the presence of ESBLs and MBL using conventional and multiplex PCR technique.

MATERIAL AND METHODS

Sample collection and Identification of Bacteria Isolates

This cross section study was conducted in Al-Sader Medical City in the Najaf province during the period from December 2021 to March 2022. The study population consisted of 111 swabs specimen from patients clinically suspected by the physician to have DFI, and the specimens immediately transported to the microbiology laboratory. All Gram-negative isolates from swab in the current study had been identified by depending on Morphological characteristic, Microscopically examination (gram-stain), and Biochemical tests according to standard method described by MacFaddin and Hart. E.coli isolates were also identified by the VITEK-2 compact system.

Antimicrobial Sensitivity Testing

Antimicrobial sensitivity testing of E. coli isolates was performed by using disk diffusion methods (Kirby-Baur method). The selection of antibiotic disks (table 1) were performed according to the guidelines recommended by the CLSI. All susceptibility results were interpreted according to the standard values performed by CLSI (2021).

Phenotypic detection for the Production of (ESBLs) Initial test

All the E.coli isolates screened for susceptibility to cefotaxime, ceftazidime and ceftriaxone (30mg/disk each) using disc diffusion sensitivity testing. The isolates were classified as potential ESBL producers if the zone diameter for cefotaxime was ≤26 mm, ≤21 mm for ceftazidime and ≤23 mm for ceftriaxone.

Confirmatory test using the double disc diffusion test (DDT)

The DDT was carried out by using a standard diffusion assay on Mueller Hinton agar. Disks containing ceftriaxone, cefotaxime, ceftazidime, and aztreonam (30 mg) were placed at different distances (20—30 mm from center to center, depending on the species) around a disk containing amoxicillin-clavulanic acid (30 mg). Extension of the inhibition zone to the amoxicillin-clavulanic acid disk was indicated suggestive of ESBL production.

Phenotypic detection for carbapenem resistance

In the present study, carbapenem resistance was defined as resistant to at least one of the two-carbapenem antibiotics tested: imipenem, and meropenem based on the CLSI (2021) guidelines. Isolate that showed as zone of inhibition ≤19 mm in diameter for imipenem and/or meropenem was considered as carbapenem resistant.

Molecular Detection of Antimicrobials Resistance Genes

ESBLs genes

All carbapenem resistant E.coli were screening for detection the presence ESBLs genes by monoplex PCR, the primers sequence and PCR condition illustrated in previous references. Amplicons were separated by agarose gel electrophoresis in 1.5 % (w/v) agarose gel, stained with ethidium bromide. The positive results were detection when the DNA band base pairs of sample equal to the target product size. The PCR were prepared in total volume 25μl PCR mixture including 12.5μl Promega Master mix, 2μl forward primer(10μM), 2μl reverse primer (10μM), 5μl DNA template (10-250 ng), and 3.5μl nuclease free water, prepared according protocol kit of the manufacturing company (Favorgen). PCR conditions had performed in T3000 thermocycler (Biometra).

MBLs genes

All carbapenem resistant E.coli were screening for detection the presence MBLs genes by multiplex PCR, the primers sequence and PCR conditions were published elsewhere. Amplicons were separated by agarose gel electrophoresis in 1.5 % (w/v) agarose gel, stained with ethidium bromide. The positive results were detection when the DNA band base pairs of sample equal to the target product size. The PCR were prepared in total volume 50μl PCR mixture including 25μl Promega Master mix, 1.5μl forward primer(10μM), 1.5μl reverse primer (10μM), 2μl DNA template (10-250 ng), and 8μl nuclease free water, prepared according protocol kit of the manufacturing company (Favorgen). PCR conditions had performed in T3000 thermocycler (Biometra).

RESULTS

The results indicated that out of 111 swabs were cultured, there 93 (83.7%) of specimens were positive for the presence of growth bacterial, while 18 (16.2%) had non-growth bacterial. Out of 93 of bacteria isolates were
bacterial growth and the rate of Gram-negative bacteria was 69(74.1%) and 34 (36.5%) was Gram positive bacteria, 19 (27.5%) were identified as *E. coli* based upon colonial characteristics and conventional biochemical tests and VITEK 2 compact system test.

Antibiotic susceptibility test of *E. coli* isolates
The resistance profile to 23 antibiotics disks belonged to 12 classes of antibiotics as showed in table (1). The highest resistance rate to ampicillin (100%), piperacillin (95%), ceftriaxone (95%),and followed by cefotaxime, cefepime, cefixime, ciprofloxacin, tetracycline (89.4%),respectively, and followed by tobramycin (84.2%), norfloxacin (84.2%), ceftazidime (79%), Aztreonam (79%), levofloxacin (79%), and nitrofurantoin (79%), respectively. While highest sensitivity of the *E. coli* has been to the colistin (95%), imipenem (63.1%) and amoxicillin-clavulanic acid (63.1%). According to the resistance profile (100%) were MDR resistant to at least one antimicrobial for three different categories, and (63.1%) were XDR the isolates were resistant to at least one antibiotic in all but two or fewer antimicrobial categories.

<table>
<thead>
<tr>
<th>Antibiotic class</th>
<th>Antimicrobial agent</th>
<th>No.(%) of isolates exhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Ampicillin</td>
<td>19(100%)</td>
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<tr>
<td></td>
<td>PIPERACILLIN-ACETIC</td>
<td>18(95%)</td>
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<tr>
<td>Beta-lactam</td>
<td>AMOXICILLIN-CLAVULANATE ACID</td>
<td>7(37%)</td>
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<td></td>
<td>PIPERACILLIN TAMBOACTAM</td>
<td>9(47.3%)</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>CEFOTAXIME</td>
<td>17(89.4%)</td>
</tr>
<tr>
<td></td>
<td>CEFTRIAXONE</td>
<td>18(95%)</td>
</tr>
<tr>
<td></td>
<td>CEFOTAXIME</td>
<td>17(89.4%)</td>
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<tr>
<td></td>
<td>CEFEPIME</td>
<td>17(89.4%)</td>
</tr>
<tr>
<td></td>
<td>CEFIXIME</td>
<td>17(89.4%)</td>
</tr>
<tr>
<td>Monobactams</td>
<td>AZTREONAM</td>
<td>15(79%)</td>
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<tr>
<td>Carbapenem</td>
<td>IMIPENEM</td>
<td>6(32%)</td>
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<tr>
<td></td>
<td>MEROPENEM</td>
<td>10(53%)</td>
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<tr>
<td>Aminoglycosides</td>
<td>AMIKACIN</td>
<td>10(53%)</td>
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<tr>
<td></td>
<td>GENTAMICIN</td>
<td>12(63.1%)</td>
</tr>
<tr>
<td></td>
<td>TOBRAMYCIN</td>
<td>16(84.2%)</td>
</tr>
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<td>Quinolones</td>
<td>CIPROFLOXACIN</td>
<td>17(89.4%)</td>
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<tr>
<td></td>
<td>LEVOFLOXACIN</td>
<td>15(79%)</td>
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<tr>
<td></td>
<td>NORFLOXACIN</td>
<td>16(84.2%)</td>
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<td>Folate pathway</td>
<td>TRIMETHOPRIM-SULPHAMETHAZOLE</td>
<td>13(68.4%)</td>
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<tr>
<td>Nitrofurans</td>
<td>NITROFURANTOIN</td>
<td>15(79%)</td>
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<tr>
<td>Phenicol</td>
<td>CHLORAMPHENICOL</td>
<td>10(53%)</td>
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<tr>
<td>Tetracyclines</td>
<td>TETRACYCLINE</td>
<td>17(89.4%)</td>
</tr>
<tr>
<td>Lipopeptides</td>
<td>COLISTIN</td>
<td>15(79%)</td>
</tr>
</tbody>
</table>

Detection of ESBLs production
The presence of ESBLs was investigated in all *E.coli* isolates using phenotypic tests as follows:

Phenotypic screening for the Production of (ESBLs)
The *E.coli* isolates tested by initial screening test, 100% showed resistance to at least one of the third generation cephalosporins (potential ESBL producers). A positive result was noted with 89.41% for cefotaxime, 95% for ceftriaxone, 79% for ceftazidime and aztreonam respectively. All isolates that expressed antibiotic resistance to any of the third generation cephalosporins and aztreonam tested were subjected to confirmatory methods by DDT. However, based on the double disc diffusion test, no isolate was found to be ESBL producer.

Molecular Detection and Distribution of ESBLs genes
All 10 carbapenem resistant *E.coli* isolates were screening by conventional PCR for the occurrence of ESBL genes, the result revealed that *bla*OXA was found to be the most prevalent gene among isolates, which are noticed in 4 (40%) isolates Figure (1), followed by *bla*CTX-M that was found in 3 (30%) isolates Figure(2), while *bla*TEM and *bla*SHV were not found in any isolate.

Figure 1: Agarose gel electrophoresis of image that show monoplex PCR amplified product from extract DNA of E.coli isolates with blaOXA genes primers, Lane (1,2,3,4) exhibit positive results with blaOXA gene (564 bp). DNA ladder with size\bp (100-1500bp).

Figure 2: Agarose gel electrophoresis of image that show monoplex PCR amplified product from extract DNA of E.coli isolates with blaCTX-M genes primers, Lane (1,2,3) exhibit positive results with blaCTX-M gene (554 bp). DNA ladder with size\bp (100-1500bp).

Figure 3: Agarose gel electrophoresis of image that show multiplex PCR amplified product from extract DNA of E.coli isolates with blaNDM, blaVIM, blaIMP, blaSIM, blaSPM genes primers, Lane (1) show positive results with blaNDM gene (621 bp), blaSIM gene (570 bp), and blaVIM gene (390 bp). Lane (2) show positive results with blaNDM gene (621 bp) and blaSIM gene (570 bp). DNA ladder with size\bp (100-1500bp).

Molecular Detection and Distribution of MBLs genes
All 10 carbapenem resistant E.coli isolates were examined by multiplex PCR for the occurrence gene determinants encoding MBL genes (blaIMP, blaVIM, blaNDM, blaSPM, and blaSIM). The results indicated that presence only two E.coli isolate carry the MBL genes (blaVIM, blaNDM, and blaSIM). The frequency of MBL genes among isolates as following blaVIM1 (50%), blaNDM2 (100%), and blaSIM 2 (100%) as shown in figure (3). While blaSPM and blaIMP were not found in any isolate.

DISCUSSION
Diabetic foot (DF) is refersto the alterations and abnormalities that occur either separately or together in the feet and legs of diabetic patients. These reasons are less likely to penetrate antimicrobial therapies in DFI, making it more challenging to deliver effective antimicrobial activity to the target location and accelerating the emergence of bacterial resistance. In the current study the prevalence of E.coli was 19 (27.5%) among isolates from DFI, similar results have been found previous studies done in Iraq and India with frequency 30% and 25.5% respectively. Additionally, other studies conducted in different regions around world with the prevalence of E.coli were 19.9%, 14.4%, and 13% done in France, Romanian, and UK respectively. The other findings the prevalence of E.coli was low compared with present study among specimens diabetic foot patients such as study done in Malaysia, Pakistan, and China, which reported 3.8%, 7.2%, and 2.4% respectively.
The main purpose of this study is to determine the frequency of antimicrobial resistance patterns of E.coli isolated from patients with DFI. The results of current study indicated that the highest resistance rate to ampicillin (100%), piperacillin (95%), ceftriaxone (95%), and followed by cefotaxime, cefepime, cefixime, ciprofloxacin, tetracycline (89.4%), respectively, this results are in agreement with previous studies done in Iraq, India, and Ethiopia. While other findings revealed low resistance to this antibiotics such studies conducted in different regions around the world. In contrast, the highest sensitivity of the E. coli has been to the colistin (95%), imipenem (63.1%) and amoxicillin-clavulanic acid (63.1%). This results are consistent with previous studies performed in South Korea and India that indicated highest sensitive to colistin (100%) and another study performed in Iraq found the sensitive to Amoxicillin-clavulanic acid was 82%. The current study demonstrated a high sensitive to imipenem (63.1%), the results is consistent with a study done in Kurdistan Region of Iraq indicated that high sensitive to imipenem 82%. In contrast, a study performed in India revealed that sensitivity rate to imipenem was 33%. In current study, multi resistant isolates to E. coli were divided into MDR, XDR and possible PDR according to criterion proposed by Magiorakos et al. (2012). The results indicate that prevalence MDR (100%), XDR (63.1%). Moreover, studies worldwide, including those in Najaf and Egypt which indicated that MDR is the most common among E. coli was 83.1% and 93% respectively. The MDR isolates are frequent correlated with hospital acquired infections and their extended is associated with raised mortality, morbidity, and healthcare costs. Among other microorganisms, Enterobacteriales family indicated the biggest danger because of the presence of ESBLs.

In the present study the prevalence to ESBL genes among carbapenem-resistance Escherichia coli isolates, which reported that blaOXA was 40% and blaCTX-M was 30%, this results similar to study in Nepal carried out on E.coli indicated that blaCTX-M and blaOXA genes were detected in 15% and 37.4% of ESBL isolates, respectively. In contrast another studies performed in Iraq that found ESBL genes among isolates of Escherichia coli and indicated high prevalence compared with the present study where reported the prevalence of blaCTX-M was 95% and blaOXA was 83.6% among isolates of Escherichia coli. Besides, many studies in different sites around world indicated that blaCTX-M genes more incidence among E.coli in clinical isolates such as study done in USA, India, Korea, and Nigeria who reported the prevalence of blaCTX-M genes were 16%, 72%, 25%, and 50% respectively.

In this study the occurrence of only two carbapenem-resistance E.coli isolates and the percentage of this genes were blaVIM 50% and 100% to both blaNDM and blaSIM respectively, this result similar to result of study conducted in India by Tewari et al., (2022) revealed that occurrence of MBL genes as following blaVIM (n=4; 22%), blaSIM (n=4; 22%), and blaNDM (n=2; 11%) among E.coli isolates. In contrast, the other finding in Pakistan who reported the high occurrence of blaVIM (n= 30; 25%) and blaNDM (n=43; 36%)).

CONCLUSIONS

In this study revealed the high frequency to antimicrobial resistant pattern among E.coliisolates from patients with DFI, the results showed that 100% of isolates were MDR, and the results also indicated that the highest resistance rate toward beta-lactam class and the generation of cephalosporin, while highest sensitivity of the E. coli has been to the colistinand imipenem, and also there is the presence of MBL genes (blaVIM, blaNDM, and blaSIM ) and ESBL genes (blaOXA and blaCTX) among carbapenem resistant E.coli isolates.

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