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The correlation between antimicrobial resistance patterns and ESβL-encoding genes in gram-negative bacteria from patients with diabetic foot ulcers

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Diabetic foot ulcer (DFU) is an important consequence of diabetes mellitus that may lead to serious infecteion and lower-limb amputation. These infections are often polymicrobial, with increasing rates of multidrug resistance (MDR) from gram-negative bacteria making treatment difficult. The present study aims to investigate gram-negative bacteria isolated from individuals with DFUs, their antibiotic resistance patterns, and confirm the presence of some important resistance genes using molecular techniques. This cross-sectional study comprised 280 individuals with infected diabetic foot ulcers. Bacterial isolates were obtained from swab and tissue specimens utilizing conventional microbiological techniques. The isolates were evaluated for the generation of ESβLs. Antimicrobial susceptibility was assessed using the disk diffusion technique (MIC). A PCR test was conducted to detect ESβL genes (*blaTEM*, *blaSHV*, *blaCTX-M*) in resistant strains. Out of 280 positive cultures, gram-negative bacteria accounted for 64.6% (n = 181) of infections. Among 117 selected major gram-negative isolates, 92 (78.6%) were ESβL producers. Phenotypically, *E. coli* 34 (37%), *P. aeruginosa* 31 (33.7%), and *K. pneumoniae* 27 (29.3%) were the dominant ESβL-positive species. Significant resistance was found against β-lactams, fluoroquinolones, and cephalosporins, whereas carbapenems, polymyxin B, and amikacin retained high efficacy. Molecular analysis revealed the *blaCTX-M* gene as the most prevalent (68.5%), followed by *blaTEM* (54.3%) and *blaSHV* (39.1%). Gram-negative bacteria were the main cause of DFUs, showing a high level of resistance to multiple drugs and a high number of ESBL-producing organisms. These results highlight the need for strict antibiotic stewardship and culture-directed therapy to enhance treatment outcomes and lower amputation risk.

Keywords: diabetic foot ulcer; amputation; multiplex PCR; ESβL production; carbapenem resistance; *Pseudomonas aeruginosa*; *Escherichia coli*.

Introduction

Diabetes mellitus is a worldwide epidemic, affecting more than 537 million people globally (McDermott et al., 2023), and leading to a myriad of long-term effects that severely impair patients' quality and duration of life (Moya-Salazar et al., 2023). Among the most common dreaded complications is the diabetic foot ulcer (DFU), which occurs in a lifetime in 19% to 34% of persons with diabetes; the five-year mortality rate after a DFU diagnosis is roughly 30%, rising to nearly 70% for those who need major amputation (Armstrong et al., 2023).

Bacterial infections and diabetes have a symbiotic relationship; diabetes makes the individual more susceptible to bacterial infection and complications (Gramberg et al., 2023). Diabetes predisposes individuals to serious trophic complications and wounds that are often referred to as "unhealable wounds". When diabetic wounds are left untreated, they favour the growth of bacteria that may eventually adapt to the treatments available, leading to the necessity for amputation. A smear of bacteria from diabetic wounds shows a notable history of multi-drug resistance, resulting in the possibility of unfavourable patient outcomes and soaring fund costs (Tsaffo et al., 2024). DFIs are classically polymicrobial and comprise several different species of bacteria, such as aerobes, anaerobes, gram-positives and gram-negatives (Barshes et al., 2022; Sande et al., 2023).

Staphylococcus aureus has hitherto been named as the commonest of all offending pathogens; however, more recent evidence has begun to point towards a change in the paradigm in which gram-negative bacilli are more often implicated as predominant pathogens, particularly in chronic, previously treated, or severe ulcers (Matheson et al., 2021).

The tendency is suggested to be one that is stronger in warmer climates and for lower- and middle-income countries (Macdonald et al., 2021). Genera that are now advocated for being isolated from DFIs include *Pseudomonas*, *Escherichia*, *Klebsiella*, and *Proteus* (Idrees et al., 2024). The greatest hurdle in dealing with DFIs is the worldwide emergence of antimicrobial resistance (AMR) (Wada et al., 2023). Overexposure and misuse of antibiotics has facilitated the selection of multidrug-resistant (MDR) organisms such that many empirical antibiotic regimens prove ineffective. The rise of extended-spectrum β-lactamase (ESβL)-producing Enterobacteriaceae and carbapenem-resistant organisms (CROs) in DFIs significantly limits potential choices of therapy and is correlated with a poorer clinical outcome, including more frequent amputations (Saleem et al., 2025).

Timely and rapid identification of pathogens and susceptibility testing is thus paramount for effective management. Conventional culture based identification is the gold standard, but is slow and often cannot identify fastidious or anaerobic microorganisms. The molecular method (PCR techniques) remains a rapid, sensitive alternative for detection of pathogens and specific resistance genes, which is useful for guiding targeted therapy (Nazer et al., 2024).

This study was aimed at providing an overview of gram-negative bacteria responsible for DFIs. The main objectives were to estimate the number of gram-negative bacteria obtained from DFU patients, characterize their phenotypic antimicrobial resistance patterns, and detect the prevalence of ESβL-encoding genes via molecular detection using previously characterized ESβL phenotypes.

Matherials and methods

A cross-sectional study was performed from August 2024 to July 2025 at the diabetes and endocrinology unit of various hospitals in

Baghdad, Iraq. This study comprised 280 patients with a clinical diagnosis of diabetes mellitus and an infected diabetic foot ulcer (DFU) who were diagnosed by an experienced physician by the presence of at least two traditional indications of inflammation (erythema, pain, purulence, warmth, induration, and tenderness). Demographic and clinical data were gathered, which include age, gender, place of residence, type of diabetes, culture type, and antibiotic use. Ulcers were graded by using the Wagner classification system. Patients of any age and gender who had ulcers of Wagner grade 1 or higher were enrolled subsequently providing informed consent. Patients who had received antibiotic medication during the previous two weeks were excluded to minimize confounding effects on microbiological results.

After debridement, the ulcer surface was cleansed by washing with sterile saline. Superficial swabs were avoided so as not to inoculate the ulcer with the colonizing flora. Depending on the ulcer depth, deep tissue specimens (at least 0.5 cm^3) or swab samples were aseptically obtained from the base of the ulcer. In purulent wounds, pus was collected with a sterile needle and syringe. Swabbing was done with two swabs in each ulcer (one for culture and Gram staining and one for PCR assay); aseptic precautions were maintained in all procedures, the samples placed in defined sterile containers (or transport medium), and sent without delay on the day of receipt for examination in the microbiology laboratory.

Inclusion criteria included patients with clinically infected DFUs graded 1 to 5 according to Wagner's classification. Wounds from non-diabetic causes were excluded.

Swabs were streaked onto plates of blood agar, MacConkey agar, and mannitol salt agar and incubated for 24–48 h. at 37°C aerobically. Cultured organisms were described with respect to the colony morphology, Gram characteristic, and standard biochemical tests (Atlaw et al., 2022). Only gram-negative isolates were studied further. They were described according to lactose fermentation, appearance on MacConkey's agar, color/texture, Gram stain, microscopic morphology, oxidase, TSI, and citrate tests. The automated Vitek 2 compact system was utilized for final identification and confirmation, following the manufacturer's guidelines.

Antimicrobial susceptibility testing for all isolates was conducted via the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar. The panel of antibiotics tested for major gram-negative isolates belonging to the following groups of antibiotics included β-lactam (ampicillin 10 μg/mL, amoxicillin-clavulanate 20/10 μg/mL, piperacillin/tazobactam 100/10 μg/mL), aminoglycosides (amikacin 30 μg/mL and gentamicin 10 μg/mL), carbapenems (meropenem 10 μg/mL and imipenem 10 μg/mL), fluoroquinolones (levofloxacin 5 μg/mL and ciprofloxacin 5 μg/mL), cephalosporins (cefotaxime 30 μg/mL and ceftazidime 30 μg/mL), sulfonamides (trimethoprim-sulfamethoxazole 1.25/23.75 μg/mL), and polypeptides (polymyxin B 300 U/mL and colistin 10 μg/mL). The isolates exhibiting resistance to three or more antibiotic types were classified as multi-drug resistant (MDR) based on the inhibited zone diameters. All recovered microorganisms were evaluated for MDR characteristics, and those meeting the criteria were appropriately identified and labeled as MDR strains (Khan et al., 2023).

Double-disk synergy testing (DDST) was applied to determine the antibiotic synergy against the microorganisms producing ESβL. Bacterial colonies, from MacConkey agar, at a concentration of 0.5 McFarland, were cultivated on Mueller-Hinton agar medium. The associated antibiotic discs were the third-generation cephalosporins cefotaxime and ceftazidime (both at 30 μg/mL), with amoxicillin/clavulanate solution concentrated to 20/10 μg/mL of amoxicillin. The culture plates were incubated at 35 °C for 24 h. The inhibitory zone between either of the cephalosporins and the amoxicillin-clavulanate disk extends into a D-shape (keyhole shape); as phenotypically explored through the combination disk test per CLSI 2026 (Clinical and Laboratory Standards Institute, 2026), it confirms that the organism produces extended-spectrum beta-lactamases (ESβLs).

In compliance with CLSI (2026) guidelines, the colistin minimum inhibitory concentrations (MICs) were determined in all selected major gram-negative bacterial isolates using both agar dilution and broth microdilution methods.

This molecular approach is used for polymicrobial or difficult-to-identify cultures and to confirm the identities of key isolates and to detect pathogens that may not grow in conventional cultures. We used a commercial DNA extraction kit for bacterial DNA extraction from multidrug-resistant (MDR) isolates of *E. coli*, *P. aeruginosa*, and *K. pneumoniae* following the instructions of the manufacturer. The use of multiplex PCR allowed the detection of the presence of the main ESβL-encoding genes (*blaTEM*, *blaSHV*, and *blaCTX-M*). Briefly, bacterial colonies were lysed with enzymatic and buffer solutions, and DNA was purified via silica-membrane binding, washing, and elution steps to yield high-purity genomic DNA. The master mix consisted of 10 μL PCR buffer, 2 μL dNTPs (10 mM), 1 μL of each primer (10 μM), Taq DNA polymerase 0.5 μL (5 U/μL), DNA template 2 μL, and nuclease-free water up to the total volume of 50 μL; after that, an agarose gel (1.5%) was stained with ethidium bromide to reveal the PCR amplification products. The electrophoresis was run for 45 min at 100 V and was subsequently visualized by a gel documentation system after exposure to UV light. Positive samples exhibited bands at 446 bp (*blaTEM*), 212 bp (*blaSHV*), and 551 bp (*blaCTX-M*).

The specific sequences of the primer used in this study are presented in Table 1, based on Islam et al. (2022).

Table 1
Primer sets and PCR steps

Genes	Primer sequence	Size of product, bp	PCR conditions
blaTEM	Forward: 5′- TCGTGTCGCCCTTATTCCCTTTTT-3′ Reverse: 5′- GCGGTTAGCTCCTCCGGTCCTC-3′	446	95℃ for 15 min. 94℃ for 30 sec.
blaSHV	Forward: 5′-GTGGATGCCGGTGACGAACAGC -3′ Reverse: 5′-TGGCGCAAAAAGGCAGTCAATCCT -3′	212	60℃ for 30 sec. 72℃ for 2 min.
blaCTX-M	Forward: 5′-CGCTTTGCGATGTGCAG-3′ Reverse: 5′-ACCGCGATATCGTTGGT -3′	551	72ºC for 10 min.

SPSS version 24 (IBM, Armonk) was used for analyzing all clinical and microbiological data. Descriptive statistics (mean ± SD, frequencies) were utilized to describe patient characteristics and the type of microbiological data: bacterial prevalence and resistance rates. Chi-square (χ^2) tests were used to test associations between categorical variables. Statistical significance was referred to as a P-value <0.05.

Results

Table 2 provides clinical and demographic details of the study population. The prevalence of male patients was 62.5% to 37.5% females ($\chi^2 = 18.73$; $P < 0.001$). The majority of patients were aged 50–65 years (52.9%), followed by those older than 65 years (28.2%) and younger than 50 years (18.9%) ($\chi^2 = 39.25$, $P < 0.001$). Most participants resided in urban areas (77.1%), with significantly fewer from rural regions (22.9%) ($\chi^2 = 74.31$, $P < 0.001$). Type II diabetes mellitus was considerably more prevalent than Type I diabetes (66.4% vs. 33.6%; $\chi^2 = 29.52$, $P < 0.001$). In terms of severity, the most common were Wagner Grade 3 (43.6%), Grade 4 (34.6%) and Grade 2 (18.6%). Grade 1 (2.1%) and Grade 5 (1.1%) were rare and showed highly significant distribution ($\chi^2 = 192.40$, $P < 0.001$). In contrast, microbiological culture patterns were almost equally distributed between monomicrobial (50.4%) and polymicrobial (49.6%) infections ($P = 0.821$).

The gender distribution of patients with infected diabetic foot ulcers is shown in Table 3. There were were 175 male patients (62.5%) and 105 female patients (37.5%) of the study population. These results show a high male-predominance in people with diabetic foot

ulcers, which may mean that men are more likely to have severe diabetic foot infections in the population studied than women.

Table 2
Clinical and demographic characteristics of patients with infected diabetic foot ulcers (n = 280)

Characteristic	Category	Number, n (%)	Chi-square (χ^2)	P-value
Gender	male	175 (62.5)	18.73	< 0.001
	female	105 (37.5)		
Age group, years	< 50	53 (18.9)	39.25	< 0.001
	50–65	148 (52.9)		
	> 65	79 (28.2)		
Place of residence	urban	216 (77.1)	74.31	< 0.001
	rural	64 (22.9)		
Type of diabetes	type-I	94 (33.6)	29.52	< 0.001
	type-II	186 (66.4)		
Wagner ulcer grade	grade 1	6 (2.1)	192.40	< 0.001
	grade 2	52 (18.6)		
	grade 3	122 (43.6)		
	grade 4	97 (34.6)		
	grade 5	3 (1.1)		
Culture type	monomicrobial	141(50.4)	0.05	0.821
	polymicrobial	139 (49.6)		

Table 3
Gender distribution of patients with infected diabetic foot ulcers

Gender	Number	Percentage
Male	175	62.5
Female	105	37.5
Total	280	100.0

Table 4 shows the distribution of the severity of diabetic foot ulcer per Wagner classification system. The most common were Grade 3 ulcers (122 patients, 43.6%), followed by Grade 4 ulcers (97 patients, 34.6%) and Grade 2 ulcers (52 patients, 18.6%). On the other hand, there were only 6 (2.1%) cases of early-stage Grade 1 ulcers and 3 (1.1%) cases of advanced Grade 5 ulcers. These results suggest that most of the patients were diagnosed with advanced diabetic foot lesions (DFL) (DFL Grade 3 and 4), indicating that the patients were not clinically presented early and in the study population there was a high burden of severe ulceration.

Table 4
Distribution of Wagner ulcer grades among patients with infected diabetic foot ulcers

Wagner ulcer grade	Number	Percentage
Grade 1	6	2.1
Grade 2	52	18.6
Grade 3	122	43.6
Grade 4	97	34.6
Grade 5	3	1.1
Total	280	100.0

The distribution of the major Gram-negative bacterial isolates from the infected DFU is summarized in Table 5. The most prevalent organism was *E. coli* (42 isolates or 23.2%) followed by *P. aeruginosa* (39 isolates or 21.5%) and *K. pneumoniae* (36 isolates or 19.9%). *Proteus mirabilis* and *Proteus vulgaris* were common (respectively 13.3% and 11.6% of the isolates), while the presence of *Acinetobacter baumannii* (7.2%) and *Morganella morganii* (3.3%) was less frequent. In conclusion, the results show that Enterobacterales (*E. coli*, *K. pneumoniae*) and *P. aeruginosa* were the most common Gram-negative bacteria in DFU infections in this study.

Table 5
Distribution of major gram-negative bacterial isolates recovered from infected diabetic foot ulcers

Bacterial isolate	Number	Percentage
Escherichia coli	42	23.2
Pseudomonas aeruginosa	39	21.5
Klebsiella pneumoniae	36	19.9
Proteus mirabilis	24	13.3
Proteus vulgaris	21	11.6
Acinetobacter baumannii	13	7.2
Morganella morganii	6	3.3
Total	181	100.0

Table 6 shows the distribution of the gram-negative bacteria isolates recovered from the diabetic foot ulcers infected with major ESβL producing bacteria. The most common ESBL-producing organism was *E. coli* (34 isolates, 37.0%) followed by *P. aeruginosa* (31 isolates, 33.7%) and *K. pneumoniae* (27 isolates, 29.3%). All three pathogens had similar distribution, suggesting they are all important determinants of the ESβL associated diabetic foot infection, with *E. coli* being the most common multidrug resistant pathogen in the present study.

Table 6
Distribution of ESβL-producing major gram-negative bacterial isolates recovered from infected diabetic foot ulcers

Bacterial type	Number	Percentage
E. coli	34	37.0
P. aeruginosa	31	33.7
K. pneumoniae	27	29.3
Total	92	100.0

The antimicrobial resistance and susceptibility data of the important Gram-negative ESβL producers are summarized in Table 7. In general, all three bacterial species were very resistant to β-lactam antibiotics: resistance levels were very high or even 100% in several isolates for ampicillin, amoxicillin-clavulanate and ceftazidime. Limited effectiveness of these commonly prescribed agents was seen with a substantial level of resistance to fluoroquinolones (ciprofloxacin and levofloxacin) and trimethoprim-sulfamethoxazole. Carbapenems, however, showed good antimicrobial activity with susceptibility rates in excess of 85% for all isolates, in contrast. Similarly, amikacin was highly effective, and polymyxin B and colistin were the most effective agents with susceptibilities of more than 92% in all bacterial species. The results underscore the high level of multidrug resistance in ESβL-producing pathogens responsible for DFI and the importance of carbapenems, aminoglycosides and polymyxins for the treatment of severe infections.

Table 7
Antimicrobial resistance and susceptibility profiles of ESβL-producing gram-negative bacterial isolates

Antibiotics	*E. coli* (n = 34)		*P. aeruginosa* (n = 31)		*K. pneumoniae* (n = 27)	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Ampicillin	32 (94.1)	2 (5.9)	31 (100)	0	27 (100.0)	0
Amoxicillin-clavulanate	33 (97.1)	1 (2.9)	30 (96.8)	1 (3.2)	18 (66.7)	9 (33.3)
Piperacillin/tazobactam	29 (85.3)	5 (14.7)	10 (32.3)	21 (67.7)	19 (70.4)	8 (29.6)
Amikacin	5 (14.7)	29 (85.3)	4 (12.9)	27 (87.1)	5 (18.5)	22 (81.5)
Gentamicin	15 (44.1)	19 (55.9)	10 (32.3)	21 (67.7)	11 (40.7)	16 (59.3)
Meropenem	3 (8.8)	31 (91.2)	4 (12.9)	27 (87.1)	3 (11.1)	24 (88.9)
Imipenem	3 (8.8)	31 (91.2)	3 (9.7)	28 (90.3)	4 (14.8)	23 (85.2)
Ciprofloxacin	23 (67.6)	11 (32.4)	19 (61.3)	12 (38.7)	16 (59.3)	11 (40.7)
Levofloxacin	22 (64.7)	12 (35.3)	18 (58.1)	13 (41.9)	16 (59.3)	11 (40.7)
Cefotaxime	24 (70.6)	10 (29.4)	26 (83.9)	5 (16.1)	20 (74.1)	7 (25.9)
Ceftazidime	31 (91.2)	3 (8.8)	27 (87.1)	4 (12.9)	24 (88.9)	3 (11.1)
Trimethoprim-sulfamethoxazole	29 (85.3)	5 (14.7)	27 (87.1)	4 (12.9)	22 (81.5)	5 (18.5)
Polymyxin B	1 (2.9)	33 (97.1)	1 (3.2)	30 (96.8)	2 (7.4)	25 (92.6)
Colistin	1 (2.9)	33 (97.1)	1 (3.2)	30 (96.8)	2 (7.4)	25 (92.6)

The distribution of ESβL-encoding resistance genes among the major gram-negative bacterial isolates is summarised in Table 8. The gene *blaCTX-M* was most common and found in 63 of 92 (68.5%) isolates, especially more often in *Escherichia coli* (76.5%) and *Pseudomonas aeruginosa* (74.2%) than in *Klebsiella pneumoniae* (51.9%) ($\chi^2 = 6.91$, P = 0.032). The blaTEM gene was detected in 54.3% of the isolates and was more common in *K. pneumoniae* (66.7%) and *P. aeruginosa* (61.3%) and showed a statistically significant association with bacterial species ($\chi^2 = 6.14$, P = 0.046). The *blaSHV* gene was not as commonly found and was present in 39.1% of the isolates with no significant difference in its distribution among the three bacterial species ($\chi^2 = 5.07$, P = 0.079). The results suggest that *blaCTX-M* is the most prevalent ESβL-associated resistance determinant in the isolates from DFUs, and that *blaTEM* is the second most common determinant, while *blaSHV* is less prominent in the resistance profile of the pathogens analyzed.

Table 8
Distribution of ESβL-encoding resistance genes among major gram-negative bacterial isolates

Resistant gene	*E. coli* (n = 34)	*P. aeruginosa* (n = 31)	*K. pneumoniae* (n = 27)	Total (n = 92)	Chi-square (χ^2)	P-value	Significance
*bla*CTX-M	26 (76.5%)	23 (74.2%)	14 (51.9%)	63 (68.5%)	6.91	0.032	significant
*bla*TEM	13 (38.2%)	19 (61.3%)	18 (66.7%)	50 (54.3%)	6.14	0.046	significant
*bla*SHV	10 (29.4%)	16 (51.6%)	10 (37.1%)	36 (39.1%)	5.07	0.079	not significant

Discussion

Our findings support the multifactorial nature of the gender disparity in DFUs; males have both biological susceptibility (neuropathy, vascular disease) and behavioral or occupational exposures (occupational patterns, delayed care-seeking and self-care behaviors, smoking, manual labor) that elevate their risk. Also, regarding the type of diabetes, most patients had type II diabetes (66.4%), whereas 33.6% had type I diabetes. Subclinical hypothyroidism (SCH) influences the potential of developing insulin resistance, prediabetes, and type 2 diabetes in non-diabetic Iraqi patients. Early recognition of visible signs and symptoms of SCH and its care may help in averting the development of T2DM (Al-Bayaty et al., 2025).

In the Babylon governorate in Iraq, 63% of cases of DFU were in males, to some degree a result of manual labor and standing for protracted periods wearing ill-fitting shoes (Fadheel & Al-Sultani, 2024). Males are more likely to smoke than females, a major risk factor for PAD and poor healing of wounds, likely accounting for a higher incidence rate of DFUs in males and more cases of lower limb amputations as a result. Several reviews observed that men delay seeking treatment for lesions of the foot and have a lower adherence to foot-care practices, which leads to more severe ulceration by the time they present (Jarl et al., 2019). A study in China that included a meta-analysis of 4,399 patients with type 2 diabetes found that male sex increased DFU risk by 74% (OR = 1.74; 95% CI: 1.55–1.96, $P < 0.0001$); also identified as risks were smoking, hypertension, renal failure, and hyperglycemia ($HbA1c \geq 7$) (Hu et al., 2024).

Also, the results of our study revealed that gram-negative bacteria were the predominant pathogens, and among all gram-negatives the predominant one was *E. coli*. Our results agree with the most recent findings but contrast with some earlier studies. Our results were comparable with the studies from Turkey by Coşkun et al. (2024), which also revealed 57.6% prevalence for gram-negatives, also highlighting *E. coli* and *P. aeruginosa* as leading pathogens. A meta-analysis of studies conducted in sub-Saharan Africa also revealed a predominance of gram-negative pathogens. An Ethiopian study by Atlaw et al. (2022), showed higher prevalence for gram-negatives (68%), but the hierarchy was slightly different – *Pseudomonas* spp. (18.9%) being more common than *E. coli* (16.5%). In an even more recent study in Oman, 75% of isolates were gram-negative, *P. aeruginosa* (17%) and *E. coli* (16%) again featuring in the major positions of major isolates (Sannathimmappa et al., 2021).

This is different from some of the other studies conducted on high income countries that still showed that gram-positive organisms such as *S. aureus* were more prevalent, suggesting geographic, environmental and healthcare practice factors may also play a role in the microbiology of DFI. However, apart from regional variation in the absolute frequencies of individual pathogens, the present study confirms an international agreement and reports that gram-negative aerobes are predominant pathogens in DFIs. Thus, there is an evident need for empirical treatment, which is based on local antimicrobial surveillance of *E. coli*, *P. aeruginosa*, *K. pneumoniae* (Macdonald et al., 2021). The study elucidates that *E. coli* continues to be the most prevalent ESβL-producing pathogen found in DFUs, which probably indicates broad colonization potential and habitation of chronic environments. The presence of *P. aeruginosa* and *K. pneumoniae* also suggests the level of multidrug resistance in nosocomial and opportunistic infection in diabetic patients. The distribution is broadly in line with DFU studies conducted all over the world, although the pathogens found are different from the recent studies detailed above (Leibovitch et al., 2021).

A retrospective cohort study from a tertiary center (2014–2018) showed that the ESβL positive rate was also significant at 24.5%, where ESβL infections were associated with more advanced ulcer grades and greater risk of major amputations (Leibovitch et al., 2021). A study from Turkey found ESβL production in 66.7% of *E. coli* isolates with similar resistance rates in *P. aeruginosa* and *Klebsiella* spp., corroborating emergent resistance to common oral agents and an increasing incidence of carbapenem resistance. The majority of isolates from DFUs in Ethiopia were ESβL positive (53.9%), where both *K. pneumoniae* and *Pseudomonas* were significant contributors and *E. coli* was also significant indicating a burdensome ESBL burden in sub-Saharan Africa (Woldeteklie et al., 2022).

Additionally, antimicrobial resistance is perhaps the most concerning finding of this study. The substantial presence of widespread resistance may reflect a strong exertion of selective pressure due to overuse of antibiotics both in the hospital and in the community. The findings of the studies from Peru and Nigeria, which also report high resistance rates, are consistent with this finding. The widespread resistance of gram-negative isolates to ampicillin, first-generation cephalosporins, and fluoroquinolones (ciprofloxacin) is a particular concern since these are heavily used agents in empirical protocols (Shahi & Kumar, 2016). The presence of these resistance mechanisms makes many β-lactam antibiotics such as third generation cephalosporins ineffective, so clinicians have to use last resort drugs like carbapenems. However, the rise of carbapenem resistant isolates as shown in our and other studies presents an imminent threat and they need to monitored closely (Shahi & Kumar, 2016).

Moreover, the present study revealed that the correlation of ulcer severity (Wagner grade) and antimicrobial resistance patterns provides quantitative support to the clinical notion that more chronic and severe wounds harbor more resistant organisms, due to prior exposure to antibiotic therapy, persisting biofilms that enable horizontal gene transfer, and the complex ecology of a chronic wound. Polymicrobial infections make therapy more difficult, requiring broad-spectrum regimens (Kwon & Armstrong, 2018; Buch et al., 2019).

Overall, *blaCTX-M* has the highest prevalence of all antibiotic resistance genes tested; however, it is closely followed by the moderately common antibiotic resistance gene (*blaTEM*). The antibiotic resistance gene with the least prevalence across all tested bacterial strains was *blaSHV*. These results align with the other study reported by Alves et al. (2016), and Hagel et al. (2019), who found the dominance of the *blaCTX-M* gene among ESβL producers, as this gene family has become the most widespread type of ESβLs worldwide. Additionally, Pérez et al. (2019), demonstrated that the *blaCTX-M* gene accounts for 83.2% of isolates obtained from individuals upon admission. Furthermore, the *blaCTX-M* gene was found to be present in 96.6% of the community isolates (Hazirolan et al., 2018).

Among the ESβL-producing gram-negative bacteria detected in this study, some were found to carry more than one gene associated with antimicrobial resistance. The existence of *blaTEM*, *blaSHV*, and *blaCTX-M* in ESβL-producing gram-negative bacteria indicates the genetic variability seen in isolates may be associated with a horizontal gene transfer across several bacterial species or genera. The result was in line with the results of Zhao et al. (2015) and that of Bajpai et al. (2017).

Conclusions

The current study shows the presence of a considerable number of diabetic foot ulcers infected with gram-negative bacteria with high levels of multidrug resistance and ESβL production. The infections are primarily caused by *E. coli*, *P. aeruginosa*, and *K. pneumoniae*, which have become increasingly resistant to extended-spectrum penicillins along with several other antibiotics often used against them, including polymyxin B and colistin. They are a beneficial option for ESβL-producing gram-negative bacteria. Most importantly, this study highlights the failure of current empirical antibiotic regimens and, thus, the need for robust antimicrobial stewardship. With timely initiation of targeted therapy known to improve clinical outcomes and reduce the volume of amputation, routine microbiological culture and susceptibility testing are essential. Furthermore, surveillance for local resistance patterns and the molecular epidemiology of resistance mechanisms is paramount if we are to gain control of the emerging challenge of AMR in this high-risk patient population. The appropriate use of antibiotics and monitoring for antimicrobial resistance should be promoted to prevent the further spread and dissemination of ESβL gram-negative bacteria in Iraq.

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