Original Article

Detection of ESBLs and carbapenemases among Enterobacteriaceae isolated from diabetic foot infections in Ouargla, Algeria

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Abstract
Introduction: The emergence and rapid spread of Enterobacteriaceae carrying extended spectrum beta-lactamases (ESBLs) and carbapenemases represent a great threat to clinical treatment due to their multi-drug resistance. This study investigated ESBLs and carbapenemases encoding genes in Enterobacteriaceae collected from diabetic foot infections (DFIs) in Ouargla, southern Algeria.
Methodology: A total of 70 Enterobacteriaceae strains were recovered from 76 patients with DFI between February 2017 and April 2018. Antimicrobial susceptibility testing was performed using the disc diffusion method, and the presence of *bla* genes was detected using polymerase chain reaction (PCR) and DNA sequencing. The genetic transfer of the plasmids was carried out by conjugation using the broth mating method.
Results: The most common isolate was *Proteus mirabilis,* followed by *Escherichia coli*, *Morganella morganii* and *Klebsiella pneumoniae.* The prevalence of ESBL and carbapenemase-producing Enterobacteriaceae was 11.42% and 2.85 % respectively. Plasmid-mediated *AmpC* was detected in 5.71% isolates. Conjugation experiments showed the transferability of $bla_{CTX-M-2}$.
Conclusions: Our findings support the view that various pathogens found in DFIs differ from one part of the country to another. This study reports the first description of metallo-β-lactamase NDM-5 producing *Klebsiella pneumoniae* clinical isolate in Algeria.

Key words: Enterobacteriaceae; NDM-5; ESBL; Diabetic Foot Infections; Ouargla.

Introduction

Diabetes mellitus is associated with several complications and diabetic foot infections (DFIs) are one of the most feared complications. These infections are one of the leading causes of morbidity [1,2]. Several epidemiological studies from subtropical countries have demonstrated that the most common organisms isolated from DFIs were aerobic Gram negative bacilli (GNB) including Enterobacteriaceae [3]. Antibiotic resistance especially extended-spectrum beta-lactamase producers (ESBLPs) constitute a major problem associated with treatment failure of DFIs [4,5]. Genes encoding ESBLs are common among Enterobacteriaceae [5]. In addition, there is an emergence of carbapenemases encoding genes worldwide. Metallo β-lactamases (MBLs) can hydrolyze almost all β-lactams, including extended-spectrum cephalosporins and carbapenems [6].

There is a paucity of data on ESBL and carbapenemase-producing Enterobacteriaceae carrying *bla* genes from DFIs in Algeria. In addition, no study on antibiotic-resistant strains in DFIs in the southern part of the country has been reported. Therefore, this study was conducted to describe ESBLs and carbapenemases producing Enterobacteriaceae isolated from DFIs in Ouargla, southern Algeria.

Methodology

Sample size and bacterial isolates

A total of 103 clinical samples were collected from patients with DFIs from February 2017 to April 2018 at the Mohamed Boudiaf and Maison du diabète hospitals in Ouargla, southern Algeria.

All Enterobacteriaceae species were identified both by conventional techniques and by using the API 20E Gallery (BioMerieux, Marcy l'Etoile, France) and the Vitek2 automated system (BioMérieux, Marcy l'Etoile, France).

Antimicrobial drug susceptibility testing and ESBL detection

Antimicrobial drug susceptibility was determined by a disc-diffusion method on Mueller–Hinton (MH) agar plates (Bio-Rad, Marnes-la-Coquette, France), according to the recommendations of the Antibiogram Committee of the French Society for Microbiology [7]. The following antimicrobial agents were tested: amoxicillin (AML 20 µg), amoxicillin /clavulanic acid (AMC 20/10 µg), cephalexin (CL 30 µg), cefoxitin (FOX 30 µg), cefotaxime (CTX 30 µg), ceftazidime (CAZ 30 µg), cefepime (FEP 30 µg), aztreonam (ATM 30 µg), ertapenem (ETP 10 µg), imipenem (IPM 10 µg), nalidixic acid (NA 30 µg), gentamicin (CN 10 µg), ciprofloxacin (CIP 5 µg), trimethoprim-sulfamethoxazole association (SXT) trimethoprim (1.25µg), sulfamethoxazole (23.75µg). ESBL production was determined by a synergetic test between amoxicillin/clavulanic acid and at least one of the following antibiotics: ceftazidime, aztreonam and cefepime. *Escherichia coli* ATCC 25922 was used as quality controls for antimicrobial susceptibility and ESBL screening tests. The minimal inhibitory concentrations (MICs) were performed by the E-test method (AES, AB Biodisk, Solna, Sweden).

Preparation of DNA template for PCR

DNA templates for polymerase chain reaction (PCR) were prepared by suspending five colonies of an overnight growth of Enterobacteriaceae isolates in Luria–Bertani agar (Bio-Rad, Marnes-la-Coquette, France) in 500µL DNase and RNase-free water (Invitrogen, Paisley, UK). The suspension was boiled at 100 °C for 10 min in a thermal block (Polystat 5; Bioblock Scientific, Cedex, France) and was then centrifuged at 19,000 × g for 5 min. An aliquot of the supernatant was used as a DNA template for PCR. This DNA was stored at -20 °C until use.

Detection of β-lactamase-encoding genes

The molecular study was performed at the Molecular Bacteriology Laboratory at the Pasteur Institute of Casablanca in Morocco.

Enterobacteriaceae isolates were screened by PCR amplification of the following β-lactamase-encoding genes: bla_{CTX-M} phylogenetic lineage groups 1, 2 and 9, bla_{TEM}, bla_{SHV}, bla_{OXA-1}, bla_{PER}, bla_{VEB}, bla_{AmpC}, and the carbapenem-hydrolyzing β-lactamase genes (bla_{VIM}, bla_{IMP}, bla_{KPC}, bla_{GES}, bla_{NDM}, and bla_{OXA-48}) as described previously [8,9].

The β-lactamase producing strains *E.coli* U2A1790 (CTX-M-1), *E. coli* U2A1799 (CTX-M-9), *Salmonella* sp. U2A2145 (CTX-M-2), *Salmonella* sp. U2A1446 (TEM-1 and SHV-12), *Pseudomonas aeruginosa* U2A1125 (PER), *Acinetobacter baumannii* U2A2026 (VEB) and *E. coli* U2A2446 (OXA-1) were used for all β-lactamase detection methods as positive controls. *E.coli* K12J5 strain was used as the negative control.

Sequencing of resistance genes

All the amplified products were sequenced to validate their identities. Both strands of the purified amplicons were sequenced with a Genetic Analyzer 3130×1 sequencer (Applied Biosystems, Foster City, CA, USA), with the same primers that were used for PCR amplification. The nucleotide and deduced protein sequences were analyzed with the tools available at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

Conjugation experiments and plasmid analysis

Conjugation assays were performed by a broth mating method using the azide-resistant (AzR) mutant of *E. coli* $K_{12}J_5$ as the recipient strain. Transconjugants were selected on MH agar containing azide (250 mg/L) and ceftazidime (2 mg/L) (Bio-Rad, Marnes-la-Coquette, France) and incubated for 18–24 h at 37 °C. The putative transconjugants were tested for susceptibility to all antibiotics, as indicated previously, to identify transferable antibiotic resistance determinants. Minimal inhibitory concentrations of β-lactams (ceftazidime) were determined by E-test for ESBL carrying strains and their transconjugants. Plasmid DNA was extracted from both donor and transconjugants using a Plasmid Mini-Prep Kit (Qiagen Ltd, West Sussex, UK) according to the manufacturer's instructions. The sizes of the plasmids were estimated by electrophoresis on 0.7% agarose using the plasmids from *E.coli* V517 as the standard markers.

Results
Study population

Out of 76 patients, 56 (74%) were male and 20 (26%) were female. The majority had type 2 diabetes and the age was 30-81 years.

Bacterial isolates

A total of 112 bacterial isolates were obtained from 82 positive cultures; GNB (n = 74; 66%) were isolated from diabetic foot infections more frequently than Gram-positive cocci (GPC) (n = 38; 34%).

In addition, 70 Enterobacteriaceae strains were isolated from DFIs and were identified as *Proteus mirabilis* (n = 31), *Escherichia coli* (n = 13),

Morganella morganii (n = 11), *Klebsiella pneumoniae* (n = 9), *Proteus vulgaris* (n = 3), *Enterobacter cloacae* (n = 2) and *Citrobacter freundii* (n = 1).

Antimicrobial drug susceptibility patterns

Enterobacteriaceae isolates were screened for the production of ESBL. Among the 70 strains, 11.42% were ESBL producers (n = 8) including *Klebsiella pneumoniae* (50%, n = 4), *Escherichia coli* (37.5%, n = 3), and *Enterobacter cloacae* (12.5%, n = 1).

The results of antibiotic susceptibility indicated that all isolates that were ESBL producers were resistant to amoxicillin, amoxicillin-clavulanic acid, cephalexin, cefotaxime, ceftazidime, cefepime and aztreonam. 62.5 % of the isolates exhibited resistance to cefoxitin. Two *Klebsiella pneumoniae* were carbapenem-resistant (ertapenem and imipenem).

Resistance to non-β-lactam including trimethoprim-sulfamethoxazole, gentamicin, nalidixic acid and ciprofloxacin was also recorded at 100%, 87.5%, 75%, and 75% respectively.

Molecular detection of bla-genes and DNA sequencing

Ten isolates of Enterobacteriaceae were positive for *bla* genes. The strains contained a diversity of β-lactamases namely CTX-M-1, CTX-M-2, SHV and TEM enzymes. However, $bla_{CTX-M-9}$, bla_{OXA-1}, bla_{PER} and bla_{VEB} were not detected. The combination $bla_{CTX-M-1}$ + bla_{TEM} was the most common (5/10) and the genotype bla_{TEM} + bla_{SHV} was identified in 4 strains. The bla_{VIM} and bla_{NDM} genes were amplified from two *Klebsiella pneumoniae* isolates. These two strains were found to coproduce CTX-M-1 and CTX-M-2 ESBLs in combination with other beta-lactamases (TEM and SHV). bla_{IMP}, bla_{KPC}, bla_{GES}, and bla_{OXA-48} were not detected in any isolate. Plasmid-mediated *AmpC* gene bla_{DHA} was detected in three isolates (1 *Proteus mirabilis*, 1 *Morganella morganii* and 1 *E.coli*) and bla_{CIT} was found in two *E.coli*.

DNA sequencing analysis demonstrated that one isolate carried the CTX-M-28 subgroup. The subgroup CTX-M-15 and CTX-M-55 were carried by two and one isolate respectively, bla_{TEM} and bla_{SHV} sequences indicated that the isolates harboured the TEM-1 (n = 1), SHV-9 (n = 1). The subgroup for bla_{DHA} was DHA-13 (one isolate); sequencing revealed the presence of bla_{NDM-5} in *Klebsiella pneumoniae*. All the combinations of *bla* genes that were identified are listed in Table 1.

Results of conjugation experiments

Conjugation experiments were carried out for two isolates (S36 and S61). Transfer of an ESBL phenotype was successful in only one isolate (TC61), the transconjugant was resistant to amoxicillin, amoxicillin/clavulanic acid, cephalexin, ceftazidime, cefepime, aztreonam, and gentamicin; the minimal inhibitory concentration (MIC) of ceftazidime was 4 µg /mL. Donor strain S61 transferred the $bla_{CTX-M-2}$ gene to the recipient strain on a 7.2 kb plasmid.

Discussion

Our study was carried out over a period of 14 months, from February 2017 to April 2018, on 76 patients with DFI.

Table 1. Phenotypic and genotypic features of BL-PE isolates collected from DFIs.

Code	Isolate	Ward	Gender / age (years)	Resistance phenotype	β-Lactamases genes	Carbapenems resistance gene
S7	*Enterobater cloacae*	General surgery	M/46	AML.AMC.CL.FEP.CTX.FOX.CAZ.ATM.CN.SXT.NA	$bla_{CTX-M-2}$	-
S10	*Proteus mirabilis*	General surgey	M/50	AML.AMC.CL.FOX.AN	bla_{DHA-13}	-
S22	*Escherichia coli*	General surgey	M/46	AML.AMC.CL.FEP.CTX.CAZ.ATM.SXT.NA.CIP	$bla_{CTX-M-1}$, $bla_{CTX-M-2}$, bla_{TEM}	-
S25	*Escherichia coli*	General surgery	M/46	AML.AMC.CL.FEP.CTX.FOX.CAZ.ATM.CN.SXT.NA.CIP	$bla_{CTX-M-2}$, bla_{CIT}	-
S36	*Klebsiella pneumoniae*	MD	M/46	AML.AMC.CL.FEP.CTX.FOX.CAZ.ATM.ERT.IMP.CN.SXT.NA.CIP	$bla_{CTX-M-28}$, $bla_{CTX-M-2}$, bla_{SHV}, bla_{TEM}	bla_{VIM}, bla_{NDM}
S42	*Morganella morganii*	MD	M/61	AML.AMC.CL.FOX.SXT	bla_{DHA}	-
S51	*Klebsiella pneumoniae*	MD	M/73	AML.AMC.CL.FEP.CTX.CAZ.ATM.CN.SXT.NA.CIP	$bla_{CTX-M-15}$, bla_{SHV-9}, bla_{TEM}	-
S55	*Escherichia coli*	ICU	M/80	AML.AMC.CL.FEP.CTX.FOX.CAZ.ATM.CN.SXT	bla_{TEM-1}, bla_{DHA}	-
S59	*Klebsiella pneumoniae*	MD	M/66	AML.AMC.CL.FEP.CTX.CAZ.ATM.CN.SXT.CIP	$bla_{CTX-M55}$, bla_{SHV}, bla_{TEM}	-
S61	*Klebsiella pneumoniae*	MD	M/66	AML.AMC.CL.FEP.CTX.FOX.CAZ.ATM.ERT.IMP.CN.SXT.NA.CIP	$bla_{CTX-M-15}$, $bla_{CTX-M-2}$, bla_{SHV}, bla_{TEM}	bla_{VIM}, bla_{NDM-5}

BL-PE: bêta-lactamase-producing Enterobacteriaceae; M: male; MD: Maison du diabète; ICU: intensive care unit.

Our results report that DFIs were higher in males (74%) than in females (26%). The male predominance could be attributed to factors such as strenuous outdoor work and poor compliance to foot care practices [10–13].

The majority of the patients were elderly, although the age range was 30-81 years. Thus, predominance of elderly patients can be explained based on the fact that diabetic foot ulcer is a chronic complication of diabetes [10] and diabetic complications directly correspond to the span of diabetes [14]. The majority of the patients suffered from type 2 diabetes, which is similar to reports by other studies [10,12,13].

In our study, GNB were more prevalent (66%), and Enterobacteriaceae dominated among all isolates. These findings correlated with those of Djahmi *et al.* and Bouharkat *et al*. [13,15]. Studies in warm countries [Middle-East and North Africa (MENA) and south-east Asia] have found a higher prevalence of aerobic GNB [10,16–18]. In contrast, studies from the western countries reported GPB to be the most frequently isolated pathogens [19,20].This discrepancy in the prevalence of GNB in DFIs between eastern and western countries remains largely unknown. Self-treatment with antimicrobials, sanitary habits leading to suboptimal hand hygiene and foot sweating caused by hot climate are proposed to be responsible for increased prevalence of GNB in the developing countries compared to the west [21].

Predominance of GNB, especially Enterobacteriaceae in our study could be attributed to late presentation of the patient with DFI, walking barefoot, use of inappropriate footwear and delay in medical care.

A total of 70 Enterobacteriaceae strains were isolated from DFIs, *Proteus mirabilis* was the most predominant (44.28%) in our study. Among Enterobacteriaceae, this predominance is observed in several studies [11,22], including a study in Algeria [15]. Other studies reported that *E.coli* was the most commonly isolated among Enterobacteriaceae [13,23]. Hence, bacterial predominance in DFIs is not conclusive and different from region to region. There are several reports of bacterial predominance in the literature.

Over the last few years, the emergence of antibiotic-resistant bacterial strains poses a major risk of therapeutic inadequacy leading to therapeutic failure of DFI [24]. Enterobacteriaceae have been responsible for several types of infections in communities and hospitals. The spread of ESBL-producing bacteria restrains the activity of a wide number of antibiotics, leading to serious therapeutic difficulties to treat both community and hospital infections [22]. Furthermore, DFIs are the most frequent complication requiring hospitalization[25].

Extensive studies on infection with ESBL and carbapenemase-producing organisms in patients with DFI in Algeria are scarce. In this study, we focused for the first time on Enterobacteriaceae isolated from DFIs in the region of Ouargla (Southern Algeria). 11.42% of Enterobacteriaceae were ESBL producers, and two isolates (2.85%) were carbapenemase producers. These findings are similar to the results reported by Jouhar *et al.* and Belefquih *et al.* [18,26].

Recently, studies reported the presence of ESBL-producing Enterobacteriaceae to be 33% and 27% respectively [10,27], and the rates of ESBL-producing microorganisms have been rising steadily over the last few years. In our study, out of eight ESBL-producing isolates, 50% were identified as *Klebsiella pneumoniae* and 37.5 % as *E.coli* strains that were isolated from DFIs. Saltoglu *et al*. have reported 38% of *E.coli* and *Klebsiella* isolates to be ESBL producers in DFIs [28]. Indeed, ESBL-producing *E. coli* and *Klebsiella* were reported to have increased [29]. Another study by Saseedharan *et al.* reported that among the Enterobacteriaceae, 43% were ESBL producers (33% of *E.coli* isolates and 61% of *K. pneumoniae*) [30].

Infections due to ESBL-producing bacteria increased the hospitalization rate of DFI patients [31]. Hence, routine screening of ESBL-producing Enterobacteriaceae should be emphasised.

In the case of the non-β-lactam drugs, ESBL-producing Enterobacteriaceae strains were found to be 100% resistant to trimethoprim-sulfamethoxazole, while resistance to gentamicin, nalidixic acid and ciprofloxacin were 87.5%, 75% and 75% respectively. This could be attributed to the unregulated use of these antibiotics. Additionally, the different frequencies of antibiotics prescriptions could explain the different rates of antibiotics resistance in bacteria isolated from DFIs [32].

The dissemination of ESBL–producing Enterobacteriaceae worldwide is due to the increased use of third-generation cephalosporins [3]. Our results revealed that bla_{CTXM} was the most common gene (87.5%), followed by bla_{TEM} (75 %) and finally bla_{SHV} (50%). These results agree with those reported by previous studies in Egypt and India [33,34]. In addition, CTX-M type β-lactamase has been reported in earlier studies, which also supports our findings [35]. There are reports of increased prevalence of bla_{CTX-M} among ESBLs producers in neighbouring countries such as

Morocco (80%) [36], Tunisia (83%) [37] and Lybia (85.9%) [38]. In fact, in the last decade, bla_{CTX-M} has explosively disseminated and become the most common resistance gene in β-lactams around the world, indeed CTX-M is endemic [39].

Among the isolates, 4 (5.71%) were identified as AmpC producers, two cefoxitin-resistant ESBL-producing *E.coli* and two cefoxitin-resistant non ESBL producing *Proteus mirabilis* and *Morganella morganii* carried plasmid-mediated *AmpC* (bla_{CIT} and bla_{DHA}). The prevalence of isolates harboured plasmid-mediated *AmpC* from hospitalized patients in Algiers and Tlemcen were 2.4% and 1% respectively [40,41]. In Algeria CMY-2 and DHA-1 were previously reported in clinical isolates of Enterobacteriaceae by Messai *et al.*, Iabadene *et al.* and Nedjai *et al.* [40,42,43]. In addition, CMY-4 was detected in clinical isolates in Algeria [44]. However, we have detected for the first time bla_{CIT} in clinical isolates and DHA-13 enzyme in Algeria; CTX-M-2 was co-produced with CIT, and DHA cephalosporinase was found associated with TEM-1 in two *E.coli* strains recovered from two hospitalized patients with DFI.

The emergence of carbapenemases among Enterobacteriaceae is a major public health problem globally [45]. This situation has become apparent as a threat to public health in Algeria [46]; few studies have reported carbapenemase producers among clinical Enterobacteriaceae isolated in Algeria [47,48], and one study from southern Algeria reported faecal carriage in hospitalized patients [49]. Our study reports the detection of MBL-producing *K. pneumoniae* isolates in southern Algeria; the two *K. pneumoniae* co-harboured bla_{NDM}, bla_{VIM} and ESBL genes. The authors have previously reported that the Indian subcontinent, the Balkans regions, and the Middle East constitute the main reservoir for NDM-producing isolates [50,51]. Robin *et al.* described the first CPE in Algeria; the VIM-19 variant was reported in five Enterobacteriaceae strains (*E. coli* [n = 2], *K.pneumoniae* [n = 2], and *Providencia stuartii* [n = 1]) [52] and during the same study period two strains *E.coli* and *Klebsiella pneumoniae* producing VIM-19 were recovered from an Algerian patient [53].

Here, we report an NDM-5 producing *Klebsiella pneumoniae* isolated from a patient with DFI. The NDM-5 is a variant of NDM-1; the first description of NDM-5 producing *E.coli* in the United Kingdom was from a patient returning from a recent hospitalization in India [54]. The NDM-5 enzyme confers high resistance to carbapenems and an expanded-spectrum cephalosporins relative to NDM-1 [54]. Several studies from India have reported NDM-producing Enterobacteriaceae in patients with diabetic foot ulcers [30,55]. Recently, the Middle East has been considered an additional and important reservoir for bla_{NDM} [47]. Numerous recent studies from this area reported NDM-5 producing *Escherichia coli* and *Klebsiella pneumoniae* [48,56].

We have reported the first NDM-5-producing *K. pneumoniae* clinical isolate in Algeria, from DFI. The bla_{NDM-5} gene was associated with $bla_{CTX-M-15}$, $bla_{CTX-M-2}$, bla_{TEM}, bla_{SHV} and bla_{VIM}. This strain was also resistant to trimethoprim-sulfamethoxazole, nalidixic acid, ciprofloxacin and gentamicin. This strain was isolated from a patient who had a history of recent travel to hajj and acquired diabetic foot ulcer there. The present data and other reports [56,57] show that the screening for such isolates may be effective in limiting the spread of these resistant microorganisms in the hospital setting, as well as in the community. The development of rapid, cheap and easy-to-handle diagnostic techniques is therefore, essential for the identification of carbapenemase producers [58].

In the present study, drug susceptibility testing showed that the transconjugant strain was resistant to β-lactams (amoxicillin, amoxicillin/clavulanic acid, cephalexin, ceftazidime, cefepime and aztreonam). In addition, the transconjugant exhibited co-resistance to gentamicin. Conjugation experiments and plasmid analysis demonstrated that plasmid-encoded ESBL gene $bla_{CTX-M-2}$ was transferred to the recipient strain. Resistance genes such as aminoglycosides, tetracyclines, sulfonamides and trimethoprim were often carried by plasmids carrying the bla_{CTX-M} genes [36]. The spread of ESBL genes is usually due to transferable plasmids which constitute one of the most important mechanisms driving the emergence of antibiotic resistance.

Conclusions

In conclusion, the microbiological profile of DFI should be taken into consideration when choosing empiric antimicrobial treatment. Our findings established that the pattern of resistance in the south of Algeria has a different molecular profile in comparison with other regions of the country and it is necessary to prevent further dissemination of ESBLs and MBLs producing strains. Thus preventive strategies to control the dissemination of such strains are urgently required. This study can help physicians to better define the empiric treatment of DFIs caused by Enterobacteriaceae. However, more studies from other regions of the country are needed. In addition, it is

important to spread awareness among patients with diabetes to seek timely medical care.

References

1. Lipsky BA, Senneville É, Abbas ZG, Aragón-Sánchez J, Diggle M, Embil JM, Kono S, Lavery LA, Malone M, Van Asten SA, Urbančič-Rovan V(2020) Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update). Diabetes Metab Res Rev 36: e3280.
2. Kwon KT, Armstrong DG (2018) Microbiology and antimicrobial therapy for diabetic foot infections. Infect Chemother 50: 11–20.
3. Uçkay I, Gariani K, Pataky Z, Lipsky BA (2014) Diabetic foot infections: state-of-the-art. Diabetes Obes Metab 16: 305-316.
4. Lipsky BA (2007) Empirical therapy for diabetic foot infections: are there clinical clues to guide antibiotic selection? Clin Microbiol Infect 13: 351-353.
5. Poole K (2004) Resistance to β-lactam antibiotics. Cell Mol Life Sci 61: 2200-2223.
6. Walsh TR, Toleman MA, Poirel L, Nordmann P (2005) Metallo-β-lactamases: the quiet before the storm? Clin Microbiol Rev 18: 306-325.
7. Société Française de Microbiologie (2018). CASFM/EUCAST Société Française Microbiol Ed 1: 125.
8. Barguigua A, Otmani F El, Talmi M, Bourjilat F, Haouzane F, Zeroulai K, Timinouni M(2011) Characterization of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from the community in Morocco. J Med Microbiol 60: 1344–1352.
9. Pérez-Pérez FJ, Hanson ND (2002) Detection of plasmid-mediated *AmpC* β-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40: 2153-2162.
10. Sekhar MS, Unnikrishnan MK, Rodrigues GS, Vyas N, Mukhopadhyay C (2018) Antimicrobial susceptibility pattern of aerobes in diabetic foot ulcers in a South-Indian tertiary care hospital. Foot 37: 95–100.
11. Hamid MH, Arbab AH, Yousef BA (2020) Bacteriological profile and antibiotic susceptibility of diabetic foot infections at Ribat University hospital; a retrospective study from Sudan. J Diabetes Metab Disord 19: 1397–1406.
12. Goh TC, Goh TC, Bajuri MY, C. Nadarajah S, Abdul Rashid AH, Baharuddin S, Zemri KS (2020) Clinical and bacteriological profile of diabetic foot infections in a tertiary care. J Foot Ankle Res 13: 1-8.
13. Bouharkat B, Tir Touil A, Mullié C, Chelli N, Meddah B (2020) Bacterial ecology and antibiotic resistance mechanisms of isolated resistant strains from diabetic foot infections in the north west of Algeria. J Diabetes Metab Disord 19: 1261–1271.
14. Khanolkar MP, Bain SC, Stephens JW (2008) The diabetic foot. QJM 101: 685–695.
15. Djahmi N, Messad N, Nedjai S, Moussaoui A, Mazouz D, Richard JL, Sotto A, Lavigne JP (2013) Molecular epidemiology of *Staphylococcus aureus* strains isolated from inpatients with infected diabetic foot ulcers in an Algerian University Hospital. Clin Microbiol Infect 19: 398-404.
16. Ahmadishooli A, Davoodian P, Shoja S, Ahmadishooli B, Dadvand H, Hamadiyan H, Shahariarirad R(2020) Frequency and antimicrobial susceptibility patterns of diabetic foot infection of patients from Bandar Abbas District, Southern Iran. J Pathog 2020: 1–10.
17. Benwan K Al, Mulla A Al, Rotimi VO (2012) A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. J Infect Public Health 5: 1–8.
18. Jouhar L, Jaafar RF, Nasreddine R, Itani O, Haddad F, Rizk N, Hoballah JJ (2020) Microbiological profile and antimicrobial resistance among diabetic foot infections in Lebanon. Int Wound J 17: 1764–1773.
19. Mendes JJ, Marques-Costa A, Vilela C, Neves J, Candeias N, Cavaco-Silva P, Melo-Cristino J (2012) Clinical and bacteriological survey of diabetic foot infections in Lisbon. Diabetes Res Clin Pract 95: 153–161.
20. Lipsky BA, Holroyd KJ, Zasloff M (2008) Topical versus systemic antimicrobial therapy for treating mildly infected diabetic foot ulcers: a randomized, controlled, double-blinded, multicenter trial of pexiganan cream. Clin Infect Dis 47: 1537–1545.
21. Uçkay I, Aragón-Sánchez J, Lew D, Lipsky BA (2015) Diabetic foot infections: what have we learned in the last 30 years? Int J Infect Dis 40: 81–91.
22. Citron DM, Goldstein EJC, Merriam CV, Lipsky BA, Abramson MA (2007) Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. J Clin Microbiol 45: 2819-2828.
23. Zemmouri A, Tarchouli M, Benbouha A, Lamkinsi T, Bensghir M, Elouennass M, Haimeur C (2015) Bacteriological profile of diabetic foot and its impact on the choice of antibiotics. Pan Afr Med J 20: 1–7. [Article in French].
24. Lipsky BA (2016) Diabetic foot infections: current treatment and delaying the "post-antibiotic era." Diabetes Metab Res Rev 32: 246-253.
25. Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, Wendel CS, Lipsky BA (2006) Risk factors for foot infections in individuals with diabetes. Diabetes Care 29: 1288-1293.
26. Belefquih B, Frikh M, Benlahlou Y, Maleh A, Jadid L, Bessaibis F, Ghazouni M, Chagar B, Lemnouer A, Elouennass M (2016) Diabetic foot infection in Morocco: microbiological profile. Wounds 28: 89-98.
27. Vatan A, Saltoglu N, Yemisen M, Balkan II, Surme S, Demiray T, Mete B, Tabak F, Study Group, Cerrahpasa Diabetic Foot (2018) Association between biofilm and multi/extensive drug resistance in diabetic foot infection. Int J Clin Pract 72: e13060.
28. Saltoglu N, Ergonul O, Tulek N, Yemisen M, Kadanali A, Karagoz G, Batirel A, Ak O, Sonmezer C, Eraksoy H, Cagatay A, Surme S, Nemli SA, Demirdal T, Coskun O, Ozturk D, Ceran N, Pehlivanoglu F, Sengoz G, Aslan T, Akkoyunlu Y, Oncul O, Ay H, Mulazımoglu L, Erturk B, Yilmaz F, Yoruk G, Uzun N, Simsek F, Yildirmak T, Yaşar KK, Sonmezoglu M, Küçükardali Y, Tuna N, Karabay O, Ozgunes N, Sargın F (2018) Influence of multidrug resistant organisms on the outcome of diabetic foot infection. Int J Infect Dis 70: 10–14.
29. Guillamet CV, Kollef MH (2016) How to stratify patients at risk for resistant bugs in skin and soft tissue infections? Curr Opin Infect Dis 29: 116-123.
30. Saseedharan S, Sahu M, Chaddha R, Pathrose E, Bal A, Bhalekar P, Sekar P, Krishnan P (2018) Epidemiology of diabetic foot infections in a reference tertiary hospital in India. Brazilian J Microbiol 49: 401–406.
31. Wu M, Pan H, Leng W, Lei X, Chen L, Liang Z (2018) Distribution of microbes and drug susceptibility in patients with diabetic foot infections in Southwest China. J Diabetes Res 2018: 9817308.
32. Hartemann-Heurtier A, Robert J, Jacqueminet S, Ha Van G, Golmard JL, Jarlier V, Grimaldi A (2004) Diabetic foot ulcer

and multidrug-resistant organisms: risk factors and impact. Diabet Med 21: 710–715.

33. Kamel NA, Aboshanab KM, Abouelwafa MM, El-Tayeb, Wafaa N (2013) Plasmid mediated extended spectrum beta-lactamase producing strains of Enterobacteriaceae isolated from diabetic foot infections in Egypt. Arch Clin Microbiol 4: 1.

34. Zubair M, Malik A, Ahmad J (2012) Study of plasmid-mediated extended-spectrum β-lactamase-producing strains of Enterobacteriaceae, isolated from diabetic foot infections in a north Indian tertiary-care hospital. Diabetes Technol Ther 14: 315–324.

35. Nedjai S, Barguigua A, Djahmi N, Jamali L, Zerouali K, Dekhil M, Timinouni M (2013) Prevalence and characterization of extended spectrum beta-lactamase-producing *Enterobacter cloacae* strains in Algeria. J Infect Dev Ctries 7: 804–811. doi: 10.3855/jidc.3127.

36. El Hamzaoui N, Berguigua A, Nayme K, Mohamed S, Timinouni M, Louzi L (2020) Prevalence of extended-spectrum beta-lactamases in uropathogenic Enterobacteriaceae isolated from a community setting, Meknes, Morocco. Gene Reports 19: 100652.

37. Dziri O, Dziri R, Maraoub A, Chouchani C (2018) First report of SHV-148-type ESBL and CMY-42-type *AmpC* β-lactamase in *Klebsiella pneumoniae* clinical isolates in Tunisia. Microb Drug Resist 24: 1483–1488.

38. Zorgani A, Almagatef A, Sufya N, Bashein A, Tubbal A (2017) Detection of CTX-M-15 among uropathogenic *Escherichia coli* isolated from five major hospitals in Tripoli, Libya. Oman Med J 32: 322.

39. Cantón R, González-Alba JM, Galán JC (2012) CTX-M enzymes: origin and diffusion. Front Microbiol 3: 110.

40. Messai Y, Benhassine T, Naim M, Paul G, Bakour R (2006) Prevalence of β-lactams resistance among *Escherichia coli* clinical isolates from a hospital in Algiers. Rev Esp Quimioter 19: 144-151.

41. Zenati F, Barguigua A, Nayme K, Benbelaïd F, Khadir A, Bellahsen C, Bendahou M, Hafida H, Timinouni M (2019) Characterization of uropathogenic ESBL-producing *Escherichia coli* isolated from hospitalized patients in western Algeria. J Infect Dev Ctries 13: 291–302. doi: 10.3855/jidc.10702.

42. Iabadene H, Messai Y, Ammari H, Alouache S, Verdet C, Bakour R, Arlet G (2009) Prevalence of plasmid-mediated *AmpC* β-lactamases among Enterobacteriaceae in Algiers hospitals. Int J Antimicrob Agents 34: 340–342.

43. Nedjai S, Barguigua A, Djahmi N, Jamali L, Zerouali K, Dekhil M, Timinouni M (2012) Prevalence and characterization of extended spectrum β-lactamases in *Klebsiella-Enterobacter-Serratia* group bacteria, in Algeria. Med Mal Infect 42: 20–29.

44. Gharout-Sait A, Touati A, Guillard T, Brasme L, de Champs C (2015) Molecular characterization and epidemiology of cefoxitin resistance among Enterobacteriaceae lacking inducible chromosomal *ampC* genes from hospitalized and non-hospitalized patients in Algeria: description of new sequence type in *Klebsiella pneumoniae* isolates. Brazilian J Infect Dis 19: 187–195.

45. Nordmann P, Poirel L (2014) The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin Microbiol Infect 20: 821-830.

46. Touati A, Mairi A (2020) Carbapenemase-producing Enterobacterales in Algeria: a systematic review. Microb Drug Resist 26: 475–482.

47. Abderrahim A, Djahmi N, Pujol C, Nedjai S, Bentakouk MC, Kirane-Gasmi D, Dekhil M, Sotto A, Lavigne JP, Pantel A (2017) First case of NDM-1-producing *Klebsiella pneumoniae* in Annaba University Hospital, Algeria. Microb Drug Resist 23: 895–900.

48. Alsharapy SA, Gharout-Sait A, Muggeo A, Guillard T, Cholley P, Brasme L, Bertrand X, Moghram GS, Touati A, De Champs C (2020) Characterization of carbapenem-resistant Enterobacteriaceae clinical isolates in Al Thawra University Hospital, Sana'a, Yemen. Microb Drug Resist 26: 211–217.

49. Yagoubat M, Ould El-Hadj-Khelil A, Malki A, Bakour S, Touati A, Rolain JM (2017) Genetic characterisation of carbapenem-resistant Gram-negative bacteria isolated from the University Hospital Mohamed Boudiaf in Ouargla, southern Algeria. J Glob Antimicrob Resist 8: 55–59.

50. Dortet L, Poirel L, Nordmann P (2014) Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed Res Int 2014: 249856.

51. Nordmann P, Dortet L, Poirel L (2012) Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med 18: 263-272.

52. Robin F, Aggoune-Khinache N, Delmas J, Naim M, Bonnet R (2010) Novel VIM metallo-β-lactamase variant from clinical isolates of Enterobacteriaceae from Algeria. Antimicrob Agents Chemother 54: 466-470.

53. Rodriguez-Martinez JM, Nordmann P, Fortineau N, Poirel L (2010) VIM-19, a metallo-β-lactamase with increased carbapenemase activity from *Escherichia coli* and *Klebsiella pneumoniae*. Antimicrob Agents Chemother 54: 471-476.

54. Hornsey M, Phee L, Wareham DW (2011) A novel variant, NDM-5, of the New Delhi metallo-β-lactamase in a multidrug-resistant *Escherichia coli* ST648 isolate recovered from a patient in the United Kingdom. Antimicrob Agents Chemother 55: 5952–5954.

55. Samant SA, Marathe N, Vaishampayan A (2015) Detection of *bla*$_{NDM-1}$ gene in *Providencia rettgeri* isolated from diabetic foot ulcers of four patients from India. Int J Pharma Bio Sci 6: 125-130.

56. Nawfal Dagher T, Azar E, Al-Bayssari C, Chamieh AS, Rolain JM (2019) First detection of colistin-resistant *Klebsiella pneumoniae* in association with NDM-5 carbapenemase isolated from clinical Lebanese patients. Microb Drug Resist 25: 925–930.

57. Brinkac LM, White R, D'Souza R, Nguyen K, Obaro SK, Fouts DE (2019) Emergence of New Delhi metallo-β-Lactamase (NDM-5) in *Klebsiella quasipneumoniae* from neonates in a Nigerian hospital . mSphere 4: e00685-18.

58. Khan AU, Nordmann P (2012) NDM-1-producing *Enterobacter cloacae* and *Klebsiella pneumoniae* from diabetic foot ulcers in India. J Med Microbiol 61: 454–456.

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