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Original Article

Carbapenemases and extended-spectrum β-lactamases producing *Enterobacteriaceae* isolated from Tunisian and Libyan hospitals

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Abstract

Introduction: The aim of the study was to investigate the prevalence of extended-spectrum β-lactamase (ESBL) and carbapenemase production among clinical isolates of *Enterobacteriaceae* recovered from Tunisian and Libyan hospitals.

Methodology: Bacterial isolates were recovered from patients in intensive care units and identified by biochemical tests and MALDI-TOF. Antibiotic susceptibility testing was performed by disk diffusion and the E-test method. ESBL and carbapenemase activities were detected using standard microbiological tests. Antibiotic resistance-encoding genes were screened by PCR and sequencing. Clonal relationships between *Klebsiella pneumoniae* strains were carried out using multi-locus sequence typing (MLST).

Results: A total of 87 isolates were characterized, with 51 and 36, respectively, identified as *E. coli* and *K. pneumoniae*. Overall the resistance prevalence was high for aminoglycosides (> 60%), fluoroquinolones (> 80%), and extended-spectrum cephalosporins (> 94%), and was low for imipenem (11.4%). Among this collection, 58 strains (66.6%) were ESBL producers and 10 *K. pneumoniae* strains (11.4%) were carbapenemase producers. The antibiotic resistance-encoding genes detected were $bla_{\text{CTX-M-15}}$ (51.7%), $bla_{\text{TEM-1}}$ (35.6%), several variants of bla_{SHV} (21.8%), and $bla_{\text{OXA-48}}$ (11.4%). The MLST typing of *K. pneumoniae* isolates revealed the presence of multiple clones and three novel sequence types. Also, close relationships between the OXA-48-producing strains from Tunisia and Libya were demonstrated.

Conclusions: This study is the first paper describing the emergence of carbapenemase- and ESBL-producing *Enterobacteriaceae*, sensitive to colistin, isolated in Tunisia and Libya. Active surveillance and testing for susceptibility to colistin should be implementing because resistance to colistin, mainly in *Klebsiella*, has been recently reported worldwide.

Key words: *Escherichia coli*; *Klebsiella pneumoniae*; ESBLs; carbapenemases; Tunisia; Libya.

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Introduction

Enterobacteriaceae are the most common pathogens causing both community-acquired and hospital-acquired infections, including infections of the urinary and gastrointestinal tracts, peritonitis, meningitis, sepsis, and medical device-associated infections [1]. The infections caused by these bacteria are associated with significant morbidity and mortality. In fact, a previous report in Israel demonstrated that the mortality rates investigated in clinical studies ranged from 22% to 72% [2]. Within this family, *E. coli* is a frequent cause of urinary tract infections, and *K. pneumoniae* is an important cause of pneumonia [3]. Emerging resistance in *Enterobacteriaceae* is a significant problem that requires immediate attention. Indeed, resistance phenotype due to the production of extended-spectrum β-lactamases (ESBLs) and carbapenemases is becoming a major public health concern worldwide [1,4]. ESBLs include class A β-lactamases, namely TEM and SHV, which confer resistance to ampicillin, amoxicillin, and other penicillins, as well as cephalosporins [3].

Enterobacteriaceae may also express ESBLs that are not closely related to TEM- or SHV-related species, including CTX-M- and OXA-type ESBLs. These ESBLs are typically plasmid-mediated rather than chromosomally mediated β-lactamases [5]. ESBLs that hydrolyze carbapenems should be distinguished from other β-lactamases [3]. Indeed, they have broader-range activity, covering carbapenems as well as extended-spectrum cephalosporins [6]. The clinically most important groups are the increasingly emerging NDM-1, KPC, and OXA-48 enzymes; their producers are spread around the world, becoming an alarming public health problem [7]. ESBL- and carbapenemase-producing *Enterobacteriaceae* strains are being increasingly reported in Europe, South America, Asia, Oceania, and Africa [8]. The situation is still worse in low-income countries, where there is a lack of antimicrobial-resistance surveys and an absence of adequate policies on antibiotics use [9,10]. The poor state of health has undoubtedly exacerbated the problem of antimicrobial resistance in these countries [10]. In Tunisia and Libya, misuse of antimicrobial agents by the public is widespread. Indeed, in both countries, antimicrobials can be purchased from pharmacies without a prescription, which has led to the rapid emergence of many resistant bacteria. In addition, there is a lack of infection prevention policies and simple control measures, such as hand hygiene, to avoid the spread of pathogens in the hospital setting [9,10].

In view of the emergence of ESBLs and carbapenemases in Tunisia [9,11-13] and because there is no detailed information on the occurrence of these enzymes in Libyan hospitals, this study was undertaken to investigate the prevalence of antibiotic resistance and to characterize the molecular mechanisms of these resistances in *E. coli* and *K. pneumoniae* clinical isolates collected from Libyan and Tunisian hospitals. Herein, we describe the first multiclonal spread of *K. pneumoniae*-harbored $bla_{\text{OXA-48}}$ and ESBL-encoding genes in Libyan hospitals.

Methodology

Bacterial isolates

A total of 87 clinical isolates were collected over 12 months, between March 2014 and March 2015. Among these strains, 47 were recovered from Benghazi Medical Center (BMC), Benghazi, Libya, and 40 strains were isolated from Avicenne Clinic in Tunisia. All strains were isolated from hospitalized patients in intensive care units (ICUs). The isolates were previously identified using biochemical tests, a Phoenix automated microbiology system, and confirmed by matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Antibiotic susceptibility testing

Antibiotic susceptibility was determined on Mueller-Hinton agar using the standard disk diffusion method as described by the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (www.sfm-microbiologie.org). Sixteen antibiotics were tested, including ceftazidime, cefotaxime, amoxicillin-clavulanic acid, ceftriaxon, amoxicillin, aztreonam, ertapenem, imipenem, gentamicin, amikacin, ciprofloxacin, nalidixic acid, nitrofurantoin, cefoxitin, trimethoprim-sulfamid, and colistin (BIORAD, Marnes-la-Coquette, France).

For all isolates, minimum inhibitory concentrations (MICs) of ceftazidime and imipenem were determined using an E-test strip (AB BioMerieux, Grenoble, France). MICs values were interpreted according to the CA-SFM breakpoints.

Phenotypic detection of ESBLs and carbapenemases

ESBL production was detected by a double-disk synergy test (DDST). Enhancement of the inhibition zone between the disks containing clavulanic acid and cefotaxime, ceftazidime, or aztreonam indicated the presence of ESBL production [14]. Imipenem-resistant isolates were screened for carbapenemase production using the modified Hodge test (MHT), the modified Carba NP test (MCNP) and the EDTA test as previously described [15-18].

Molecular detection of antibiotic-resistance-encoding genes

Conventional polymerase chain reaction (PCR) was performed to identify Ambler class A ESBL genes using specific primers for bla_{CTX}, bla_{TEM}, bla_{SHV}, bla_{PER}, bla_{VEB}, and bla_{GES} genes. Real-time PCR and conventional PCR were performed for the bla_{KPC}, $bla_{\text{OXA-48}}$, and bla_{NDM} genes for imipenem-resistant strains [4]. Primers used for PCR and RT-PCR amplification of carbapenemases, ESBLs, and metallo-β-lactamases (MBLs) genes are listed in Table 1. Strains used as positive controls were *K. pneumoniae* KPNASEY (CTX-M, TEM, SHV, and NDM producer), *E. coli* CMUL64 (OXA-48 producer), and *K. pneumoniae* ST512 (KPC producer).

DNA sequencing

PCR products were purified and sequenced using the Big Dye terminator chemistry on an ABI 3730 automated sequencer (Applied Biosystems, Foster City,

Table 1. Primers used for PCR and real-time PCR amplification of carbapenemases, ESBLs, and MBLs genes.

Gene name	Type of PCR	Primer name	Primer sequence (5' ➔ 3')	Amplicon size (bp)
*bla*CTX-M	Standard PCR	CTX-F	TTTGCGATGTGCAGTACCAGTAA	544
		CTX-R	CGATATCGTTGGTGGTGCCATA	
*bla*TEM	Standard PCR	TEM-F	ATGAGTATTCAACATTTCCGTG	861
		TEM-R	TTACCAATGCTTAATCAGTGAG	
*bla*SHV	Standard PCR	SHV-F	TTTATGGCGTTACCTTTGACC	1051
		SHV-R	ATTTGTCGCTTCTTTACTCGC	
*bla*OXA-48	Standard PCR	OXA-48-F	TTGGTGGCATCGATTATCGG	744
		OXA-48-R	GAGCACTTCTTTTGTGATGGC	
	Real-time PCR	OXA-48-F	TCTTAAACGGGCGACCAAG	125
		OXA-48-R	GCGTCTGTCCATCCACTTA	
*bla*NDM-1	Standard PCR	NDM-1-F	CATTTGCGGGGTTTTTATG	1022
		NDM-1-R	CTGGGTCGAGGTCAGGATAG	
	Real-time PCR	NDM-1-F	GCGCAACACAGCCTGACTTT	155
		NDM-1-R	CAGCCACCAAAAGCGATGTC	
*bla*KPC	Standard PCR	KPC-F	ATGTCACTGTATCGCCGTCT	893
		KPC-R	TTTTCAGAGCCTTACTGCCC	
	Real-time PCR	KPC-F	GATACCACGTTCCGTCTGGA	180
		KPC-R	GGTCGTGTTTCCCTTTAGCC	

PCR : polymerase chain reaction ; ESBL : extended-spectrum β-lactamase ; MBL : metallo β-lactamase.

USA). The obtained nucleotide sequences and their deduced amino acids sequences were compared against the NCBI database using, respectively, BlastN and BlastP functionalities. (www.ncbi.nlm.nih.gov).

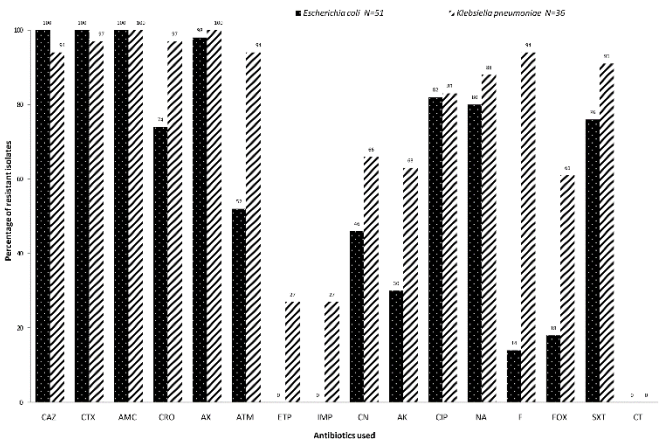
Molecular strain typing

The clonal relationships between imipenem-resistant *K. pneumonie* isolates were studied by multi-locus sequence typing (MLST). Isolates were attributed to a sequence type (ST) number according to the allelic profiles available in the Institute Pasteur's MLST web site (www.pasteur.fr/mlst).

Results

After identification, it was possible to conclude that the collection of 87 isolates included 51 strains of *E. coli* and 36 of *K. pneumoniae*. Antibiotic susceptibility testing for the 87 isolates is summarized in Figure 1. In general, a high prevalence of resistance was observed against the greater part of antibiotics, especially third-generation cephalosporins. Eighty percent of the isolates were very highly resistant to ceftazidime, cefotaxime, amoxicillin-clavulanic acid, amoxicillin, and ciprofloxacin. In addition, 10 strains of *K. pneumoniae* showed a high level of resistance to carbapenems, with MICs for imipenem > 16 mg/L (Table 2). In the present study, only ESBLs and carbapenemase-producing *Enterobacteriaceae* were presented in Table 2 and Table 3. All isolates were susceptible to colistin. The double-disk synergy test showed that 66.6% of isolates were ESBL positive. Detection of *bla*CTX-M, *bla*SHV and *bla*TEM using PCR amplification showed that 58 strains were positive for the genes encoding these enzymes. As shown in Table 2, the nucleotide sequences of the amplicons revealed the presence of sequences that were 100% similar to that of *bla*CTX-M-15, *bla*TEM-1 and many variants of *bla*SHV (Table 3). None of the isolates harbored either the *bla*PER, *bla*VEB or *bla*GES genes. The results of carbapenemase production tests showed that all imipenem-resistant isolates were positive by MHT and MCNP, suggesting carbapenemase production. However, the activity of β-lactamases was not inhibited by EDTA, an indicated that the imipenem-resistant isolates were not MBL producers.

Figure 1. Antibiotic susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* clinical strains.



CAZ: Ceftazidime, **CTX:** Cefotaxime, **AMC:** Amoxicillin-Clavulanic Acid, **CRO:** Cefriaxon, **AX:** Amoxicillin, **ATM:** Aztreonam, **ETP:** Ertapenem, **IMP:** Imipenem, **CN:** Gentamicin, **AK:** Amikacin, **CIP:** Ciprofloxacin, **NA:** Nalidixic Acid, **F:** Nitrofurantoin, **FOX:** Cefoxitin, **SXT:** Trimethoprim-Sulfamid, **CT:** Colistin.

Table 2. Phenotypic and genotypic characteristics features of the Tunisian and Libyan *Klebsiella pneumoniae* clinical isolates producing ESBLs and carbapenemases.

Isolate	Location	Date of isolation	Ward	Type of swab	ESBL synergy test	IMP MIC (µg/mL)	ESBLs and carbapenemases				
							CTX-M-U	TEM	SHV	OXA	ST
2251	Tunis	4-2014	ICU	Tracheal	+	> 16	CTX-M-15	TEM-1	SHV-11	OXA-48	101
8119	Tunis	4-2014	ICU	Tracheal	+	> 16			SHV-28	OXA-48	101
1676	Tunis	4-2014	ICU	Tracheal	+	> 16	CTX-M-15	TEM-1	SHV-11	OXA-48	101
5236	Tunis	4-2014	ICU	Tracheal	+	< 2	CTX-M-15	TEM-1	SHV-38		29
9142	Tunis	4-2014	ICU	Tracheal	+	< 2	CTX-M-15	TEM-1	SHV-1		1065
5933	Tunis	4-2014	ICU	Tracheal	+	< 2	CTX-M-15	TEM-1	SHV-11		969
2295	Tunis	4-2014	ICU	Tracheal	+	< 2	CTX-M-15	TEM-1	SHV-11		17
8332	Tunis	4-2014	ICU	Tracheal	+	< 2	CTX-M-15		SHV-132		111
6086	Tunis	4-2014	ICU	Tracheal	+	< 2	CTX-M-15	TEM-1	SHV-1		1657
6163	Tunis	4-2014	ICU	Tracheal	+	< 2	CTX-M-15		SHV-132		1112
58	Benghazi	4-2014	ICU	Tracheal	-	> 16				OXA-48	100
694	Benghazi	4-2014	ICU	Tracheal	-	> 16				OXA-48	101
330	Benghazi	4-2014	ICU	Tracheal	-	> 16				OXA-48	1949
695	Benghazi	4-2014	ICU	Tracheal	-	> 16				OXA-48	101
76	Benghazi	4-2014	ICU	Tracheal	-	> 16				OXA-48	101
203	Benghazi	4-2014	ICU	Tracheal	-	> 16				OXA-48	1950
82	Benghazi	4-2014	ICU	Tracheal	-	> 16				OXA-48	1951
2	Benghazi	4-2014	ICU	Tracheal	-	< 2	CTX-M-15	TEM-1	SHV-187		20
51	Benghazi	4-2014	ICU	Tracheal	+	< 2	CTX-M-15	TEM-1			414
79	Benghazi	4-2014	ICU	Tracheal	+	< 2	CTX-M-15		SHV-26		101
33	Benghazi	4-2014	ICU	Tracheal	+	< 2	CTX-M-15				1322
88	Benghazi	4-2014	ICU	Tracheal	+	< 2	CTX-M-15				1322

ICU: intensive care unit; IMP: imipenem; MIC: minimum inhibitory concentration; ST: sequence type.

Table 3. Phenotypic and genotypic characteristics features of the Tunisian and Libyan *Escherichia coli* clinical isolates producing ESBLs.

Isolate	Location	Date of isolation (mm-yyyy)	ESBLs		
			CTX- M-U	TEM	SHV
9671	Tunis	4-2014	CTX -M-15		SHV-12
8069	Tunis	4-2014	CTX- M-15	TEM-1	
7592	Tunis	4-2014	CTX- M-15	TEM-1	SHV-11
7898	Tunis	4-2014	CTX- M-15	TEM-1	
6042	Tunis	4-2014	CTX -M-15		
5868	Tunis	4-2014	CTX -M-15	TEM-1	
2631	Tunis	4-2014	CTX -M-15	TEM-1	SHV-11
2360	Tunis	4-2014	CTX -M-15		
4316	Tunis	4-2014	CTX -M-15		
9160	Tunis	4-2014		TEM-1	
8892	Tunis	4-2014	CTX -M-15		
6576	Tunis	4-2014	CTX -M-15		
2584	Tunis	4-2014	CTX -M-15		
5728	Tunis	4-2014	CTX -M-15		
8853	Tunis	4-2014	CTX -M-15		
6194	Tunis	4-2014	CTX -M-15		
1111	Tunis	4-2014	CTX -M-15		
1	Benghazi	4-2014	CTX -M-15	TEM-1	
2	Benghazi	4-2014		TEM-1	
3	Benghazi	4-2014	CTX -M-15	TEM-1	SHV-2
4	Benghazi	4-2014	CTX -M-15		
5	Benghazi	4-2014	CTX -M-15		
6	Benghazi	4-2014	CTX -M-15	TEM-1	
7	Benghazi	4-2014		TEM-1	
8	Benghazi	4-2014	CTX -M-15		
9	Benghazi	4-2014		TEM-1	
10	Benghazi	4-2014		TEM-1	
11	Benghazi	4-2014		TEM-1	
12	Benghazi	4-2014	CTX -M-15	TEM-1	
13	Benghazi	4-2014	CTX -M-15		
14	Benghazi	4-2014	CTX -M-15		
15	Benghazi	4-2013	CTX -M-15	TEM-1	
16	Benghazi	4-2013	CTX -M-15		
17	Benghazi	4-2013	CTX -M-15		
18	Benghazi	4-2013	CTX -M-15		
213	Benghazi	4-2013		TEM-1	
184	Benghazi	4-2013		TEM-1	
113	Benghazi	4-2013		TEM-1	
608	Benghazi	4-2013		TEM-1	
560	Benghazi	4-2013	CTX -M-15	TEM-1	SHV-28
690	Benghazi	4-2013	CTX -M-15	TEM-1	SHV-28
635	Benghazi	4-2013	CTX -M-15		
340	Benghazi	4-2013	CTX -M-15		

ESBL: extended-spectrum β-lactamase

Screening for carbapenemase-encoding genes by PCR showed that all 10 isolates contained a bla_{OXA-48}-like gene (Table 2). All of the isolates were negative against bla_{KPC}-like or bla_{NDM} genes.

All 36 *K. pneumoniae* isolates were analyzed by MLST, and different STs were observed (Table 2). This analysis showed that the 36 isolates of *K. pneumoniae* belong to 15 different STs. The most frequent clone was ST101, corresponding to imipenem-resistant *K. pneumoniae* strains (Table 2).

Using MLST analysis, three isolates of *K. pneumoniae* collected from BMC, Libya, were found to have novel STs. *K. pneumoniae* 330 was assigned as ST1949, *K. pneumoniae* 203 was assigned as ST1950, and *K. pneumoniae* 82 was assigned as ST1951. It is worth mentioning that those isolates were positive for OXA-48.

Discussion

In this study, we characterized carbapenemase- and ESBLs-producing *E. coli* and *K. pneumoniae* clinical isolates isolated from patients admitted to Tunisian and Libyan hospitals. Among the 87 isolates, about two-thirds produced ESBLs. Several studies have also shown the emerging problem of ESBL-producing *E. coli* and *K. pneumoniae* isolates in different geographic regions, including the Mediterranean basin [19] and North Africa [9,20-23]. In our work, $bla_{CTX-M-15}$ was the most frequently detected (54%) gene in the ESBL-positive Tunisian and Libyan isolates. These results suggest that $bla_{CTX-M-15}$ is the most common gene responsible for mediating extended-spectrum cephalosporin resistance in these isolates. These data confirm previous studies showing that this enzyme is widely present in Tunisia [11,21,24-31] (Table 4). Indeed, Mamlouk *et al.* detected the $bla_{CTX-M-15}$ gene in

Table 4. Studies reporting ESBL-producing *Enterobacteriaceae* in Tunisia and Libya.

Country	Location	Year	Species	Enzymes described	Journal	Reference
Tunisia	Tunis	2004	*K. pneumoniae*	SHV-12, SHV-2a	Microb Drug Resist	[25]
Tunisia	Sfax	2006	*K. pneumoniae*	CTX-15, CMY-4	Antimicrob Agents Chemother	[11]
Tunisia	Tunis	2006	*K. pneumoniae*, *E. coli*	CTX-M-15, CTX-M-16	J Clin Microbiol	[30]
Tunisia	Tunis	2007	*E. coli*	TEM-15	Diag Microbiol Infect Dis	[22]
Tunisia	Tunis	2008	*K. pneumoniae*, *E. coli*	OXA-1, TEM-1, SHV-1, SHV-11, SHV-27, SHV-103, CTX-M-15	Int J Antimicrob Agents	[24]
Tunisia	Tunis	2009	*K. pneumoniae*	TEM-164	Microb Drug Resist	[21]
Tunisia	Sousse	2009	*K. pneumoniae*, *C. freundii*, *E. coli*	CTX-M-15, SHV-2a, SHV-12, SHV-28, TEM-1	Clin Microbiol Infect	[26]
Tunisia	Sousse	2010	*K. pneumoniae*, *E. coli*	CTX-M-15, SHV-12, SHV-2a	Microb Drug Resist	[27]
Tunisia	Monastir	2010	*K. pneumoniae*	CTX-M-15, CTX-M-14, CTX-27, SHV-12, SHV-2a	Clin Microbiol Infect	[31]
Tunisia	Mahdia	2012	*E. cloacae*	SHV-12	Microb Pathog	[23]
Tunisia	Tunis	2013	*E. coli*	CTX-M-15, SHV-12	Microb Drug Resist	[29]
Tunisia	Tunis	2014	*E. coli*	CTX-M-15	Folia Microbiol	[28]
Libya	Zawiya	2015	*E. coli*	TEM, CTX	Libyan J Med	[32]

Table 5. Studies reporting OXA-48 producing *Enterobacteriaceae* in North Africa.

Country	Year	Isolate source	Species	Carbapenemase described	References
Tunisia	2012	Urine	*K. pneumoniae*	OXA-48	[23]
Tunisia	2012	NA	*K. pneumoniae*	OXA-48	[36]
Tunisia	2012	Pus, urine, blood	*K. pneumoniae*, *C. freundii*	OXA-48	[13]
Morocco	2011	Urine, rectal swab	*Enterobacteriaceae*	OXA-48	[46]
Morocco	2012	NA	*Enterobacteriaceae*	OXA-48	[45]
Morocco	2012	NA	*E. coli*	OXA-48	[44]
Morocco	2014	Rectal swabs	*Enterobacteriaceae*	OXA-48	[34]
Egypt	2013	Feces, exudate, blood	*E. coli*	OXA-48	[43]
Algeria	2014	NA	*E. coli*	OXA-48	[33]

NA: not available

30% of *Enterobacteriaceae* (35 *E. coli* and 27 *K. pneumoniae*) collected from different wards of Charles Nicolle Hospital in Tunis [30]. More recently, in 2014, Ferjani *et al.* reported that 88% of cefotaxime-resistant *E. coli* strains, isolated from urine of patients in a Tunisian hospital, harbored the $bla_{\text{CTX-M-15}}$ gene [28]. These studies confirm the current spread of the CTX-M-15 encoding-gene, which encodes the most prevalent β-lactamase detected among ESBL-positive *K. pneumoniae* and *E. coli* strains in Tunisian hospitals (Table 4). The increased consumption of cefotaxime and ceftazidime might have contributed to the emergence of ESBLs, and particularly these CTX-M-type enzymes. The occurrence of the $bla_{\text{SHV-11}}$ encoding gene among the ESBL-positive Tunisian strains is consistent with the finding of Abbassi *et al.*, who reported the $bla_{\text{SHV-11}}$ encoding gene in *K. pneumoniae* ESBL-positive isolates recovered in the Centre of Bone Marrow Transplantation of Tunisia [24] (Table 4). However, in Libya, few articles have been published describing the presence of ESBL-producing *Enterobacteriaceae* [32] (Table 4). In this study, similar phenotypes and genotypes related to antibiotic resistance were found in Tunisia and Libya. Indeed, in North Africa, many studies also demonstrated the incidence of ESBL-producing *E. coli* and *K. pneumoniae.* Agabou *et al*. demonstrated the frequency and diversity of ESBLs produced by *E. coli* isolates from patients hospitalized in the Regional Military Hospital of Constantine in Algeria [33]. Additionally, in Morocco, Girlich *et al.* reported the high rate of fecal carriage of ESBL-producing *Enterobacteriaceae* at a university hospital [34]. In Egypt, a neighboring country of Libya, the CTX-M-15 encoding-gene has been found in clinical isolates of *E. coli* from Cairo [35].

Interestingly, we identified the presence of the $bla_{\text{OXA-48}}$ gene in ten imipenem-resistant *K. pneumoniae* isolates in our study. Indeed, three Tunisian and seven Libyan *K. pneumoniae* isolates resistant to imipenem were found to produce OXA-48 (Table 3). Several studies concerning the emergence of OXA-48-producing *K. pneumoniae* have been reported in Tunisia (Table 5), but our study is the first that detected OXA-48-positive *K. pneumoniae* isolated directly from patients hospitalized in Libyan hospitals. Indeed, in Tunisia, two carbapenem-resistant *K. pneumoniae* clinical isolates carrying the plasmid-harbored OXA-48 carbapenemase gene were reported in 2010 [12]. Ktari *et al.* reported the spread of 21 (13.7%) *K. pneumoniae* isolates producing the $bla_{\text{OXA-48}}$ encoding-gene in a Tunisian university hospital [36]. In 2012, Saidani *et al*. screened 21 ESBL-producing *Enterobacteriaceae* with reduced susceptibilities to carbapenems; they found that 5 of the 21 isolates investigated were OXA-48 positive [13]. More recently, among enterobacterial clinical isolates recovered in the Center of Maternity and Neonatology of Monastir, Tunisia, Charfi *et al.* identified one isolate positive for the OXA-48 gene that co-expressed the $bla_{\text{CTX-M-15}}$ gene [37]. These data showed the dissemination of imipenem-resistant *K. pneumoniae* carrying the $bla_{\text{OXA-48}}$ gene in Tunisian hospitals (Table 5).

Concerning Libya, to the best of our knowledge, our study is the first that detected the emergence of OXA-48-producing *K. pneumoniae* in this country. However, three studies reported the presence of OXA-48-producing *K. pneumoniae* isolated from Libyan patients transferred to Europe for treatment. Indeed, Kocsis *et al.* reported that a carbapenem-resistant *K. pneumoniae* carrying the $bla_{\text{OXA-48}}$ gene was recovered from the blood culture of a Libyan patient hospitalized in the ICU of the Sacro Cuore-Don Calabria Hospital in Negrar, Italy [38]. Italy is the country with the highest incidence of OXA-48-producing *K. pneumoniae* isolates, and has been considered to be the epicenter of the spread of this enzyme in Mediterranean basin [39]. Indeed, several isolates of *K. pneumoniae* producing OXA-48 have been reported, proving that the situation in this country is becoming endemic [39,40]. In addition, Pirš *et al*. reported the first case of OXA-48-producing *K. pneumoniae* in Slovenia, isolated from a rectal swab collectedfrom a patient transferred from Libya. The patient was colonized by both ESBL-producing *E. coli* and ESBL- and OXA-48-producing *K. pneumoniae* [41]. Hammerum *et al.* reported that patients transferred from Libya to Denmark carried OXA-48-producing *K. pneumoniae* [42].These studies underscore the importance of an early warning system at the European level and screening upon admission of patients transferred across countries. These findings may also confirm that North Africa, including Tunisia and Libya, are considered to be reservoirs of oxacillinase producers, particularly OXA-48; to date, this enzyme represents the most common carbapenemase type circulating in this region [43-46] (Table 5). Recently, in 2014, Agabou *et al.* reported the first description of OXA-48-producing *E. coli* and the pandemic clone ST131 from patients hospitalized at the Regional Military Hospital of Constantine [33]. In Morocco, Girlich *et al*. showed a high prevalence of multidrug-resistant *Enterobacteriaceae*, and particularly OXA-48 producers, at a university hospital [34] (Table 5). MLST analysis of Tunisian and Libyan

isolates showed the occurrence of multiple clones, with clones belonging to ST101 being the most frequent.

This is the first report using MLST analysis for typing of *K. pneumoniae* isolates in Libya, but MLST analysis studies have reported that ST101 is the most prevalent ST type in Tunisia. Indeed, the results of this work are consistent with the studies of Charfi *et al.*, who assigned the *K. pneumoniae* KP51 strain to ST101, a widespread clone harboring various β-lactamases, mostly OXA-48, which had been already reported in Tunisia and which had also accounted for an outbreak in Spain [37,47]. In addition, Cuzon *et al.* reported a plasmid-mediated OXA-48 in *K. pneumoniae* ST101 from Tunisia [48]. Three novel STs were found among the *K. pneumoniae* isolates from Benghazi, Libya: ST1949, ST1950, and ST1951. These isolates were positive for OXA-48, as were other isolates assigned to previously described STs (20, 100, 101, 414, and 1322). These findings show that BMC, which is a tertiary hospital in Benghazi and covers the eastern part of Libya in terms of providing healthcare services, may suffer from multiclonal spread of nosocomial pathogens; more importantly, the CTX-M-15 and OXA-48-producing *K. pneumoniae* detected in this study demonstrates the long-standing infection control problems needing urgent attention.

Conclusions

This study described the emergence of ESBL- and carbapenemase-producing *Enterobacteriaceae* in Tunisian and Libyan hospitals. These findings are of great concern because of the rapid dissemination of multidrug-resistant bacteria, particularly carbapenem-resistant strains, which represent a major therapeutic and epidemiological threat. The implementation of strict infection prevention and control precautions in addition to regular surveillance studies is urgently needed to contain the increasing spread of nosocomial pathogens in these countries.

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