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Research Article

OCCURRENCE OF CARBAPENEMASES AND EXTENDED-SPECTRUM BETA-LACTAMASES IN UROPATHOGENIC *ENTEROBACTERIACEAE* ISOLATED FROM A COMMUNITY SETTING, SETTAT, MOROCCO

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ABSTRACT

Objective: Urinary tract infections (UTIs) are still commonly diagnosed in outpatients as well as in hospitalized patients. In this study, we investigated the prevalence and performed molecular characterization of extended-spectrum-β-lactamases (ESBL) and carbapenemases produced by *Enterobacteriaceae* isolates that cause community UTIs in Settat city, Morocco.

Methods: From January 2012 to December 2013, all uropathogenic community *Enterobacteriaceae* isolates were collected from the microbiology laboratory of Hassan II Hospital, Settat, Morocco. Antibiotic susceptibility testing was performed as recommended by Clinical and Laboratory Standard Institute. Phenotypic identification of ESBL and carbapenemase producer isolates was confirmed by the double-disk synergy test and the modified Hodge test, respectively. Molecular characterization of β-lactamase genes was performed using polymerase chain reaction (PCR), followed by sequencing of the obtained products.

Results: Among 153 isolates, 31 (20.26%) were multi-drug resistant (MDR). Nine strains (5.88%) were ESBL producers, of which *Klebsiella pneumoniae* (n=5; 20.83%), *Escherichia coli* (n=3; 3.15%), and *Enterobacter cloacae* (n=1; 9.09%) species were identified. The results of ESBL-encoding gene detection by sequencing revealed the presence of CTX-M-15 (n=9) in association with other β-lactamase genes such as temoneira 1 (n=8) and sulfhydryl variable 1 (n=5). According to the modified Hodge test and PCR, three isolates (1.96%) were positive carry the bla_{OXA-48} gene.

Conclusion: The emergence of MDR uropathogenic *Enterobacteriaceae* isolates in our community is highly alarming. Strict measures will be required to control the further spread of these uropathogenic isolates.

Keywords: Carbapenemase, *Enterobacteriaceae*, Extended-spectrum-β-lactamases, Moroccan community.

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INTRODUCTION

Urinary tract infections (UTIs) are common bacterial infections that are still observed in outpatients and hospitalized patients [1]. They are the second most common infection appearing in the community [2]. *Enterobacteriaceae* are known to be the most common cause of UTIs [1]. Indeed, *Escherichia coli* and *Klebsiella pneumoniae* isolates are pathogens responsible for causing serious infections, both in the community and in health-care settings [3]. The past decade has shown an emergence of carbapenemase and extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* isolates in the community setting, the ESBLs being frequently plasmid encoded. These plasmids also carry genes encoding resistance to other antibiotics [4]. The majority of ESBL types are derived from temoneira (TEM) and sulfhydryl variable (SHV) genes by mutations. This extends the spectrum of beta-lactam antibiotics susceptible to hydrolysis by these enzymes are susceptible to hydrolysis by extended-spectrum antibiotics [4]. More than 180 TEM-type and 130 SHV-type β-lactamases had been identified worldwide (www.lahey.org/Studies/). More recently, CTX-M enzymes that preferentially hydrolyze and confer resistance to cefotaxime have been found in several continents around the world. More than 100 CTX-M type β-lactamases have been identified (www.lahey.org/Studies/), divided into five groups (CTX-M-1, 2, 8, 9 and 25).

In Moroccan hospitals, *E. coli* and *K. pneumoniae* isolate producing carbapenemase and ESBL have been the most predominant [5,6]. The β-lactamase genes detected in Moroccan hospital isolates were bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{DHA}, and bla_{OXA} types [5,7,8]. Carbapenemase OXA-48 and NDM-1 producing *Enterobacter cloacae,* specifically *K. pneumoniae*, have been recently reported in Moroccan hospitals [5,7]. In addition, the occurrence of an OXA-48 producer in the environment has also been reported in Morocco [9].

Our study is known to be the first in Settat region, the objective of this study was to report the prevalence and characteristics of ESBL and carbapenemase-producing *Enterobacteriaceae* isolated from community acquired UTIs (CA-UTIs) in the Moroccan community of Settat city. It remains then, highly necessary to apply strict measures and adopt preventive strategies and continuous surveillance, to investigate the epidemiology of these multidrug-resistant organisms in the community.

METHODS

Setting and bacterial isolates

From January 2012 to December 2013, urine specimens were collected from outpatients clinically suspected to have UTI, who had not previously been hospitalized. The urine specimens were analyzed at the microbiology laboratory at Hassan II Hospital, Settat. All urinary specimens with significant bacteremia (≥10^5 colony-forming unit/mL) were further processed. The isolates were identified by conventional methods and biochemical tests using the Api20E collection (Biomerieux, Marcy l'Etoile, France).

The gender and age of the patients were recorded. *Enterobacteriaceae* isolated from currently or previously hospitalized patients were excluded from the study.

Antimicrobial susceptibility testing

Antimicrobial drug susceptibility testing of *Enterobacteriaceae* isolates was performed according to the disk diffusion method on Mueller-Hinton agar plates (Bio-Rad, Marnes-la-Coquette, France), and the results were interpreted according to the recommendations by the Clinical and Laboratory Standards Institute (CLSI) [10]. The following antimicrobial agents (Bio-Rad, Marnes-la-Coquette, France) were tested: Amoxicillin/clavulanic acid (20/10 µg), cephalothin (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), imipenem (10 µg), and ertapenem (10 µg).

Multi-drug resistant *Enterobacteriaceae* (MDRE) were defined as isolates that were non-susceptible to at least one agent in three or more antimicrobial categories [11]. Isolates that did not meet the criteria were described as non-MDRE.

Phenotypic detection of ESBL production

ESBL production was screened using a double-disk synergy test, between cefotaxime, ceftazidime, and aztreonam disks, placed at a distance of 30 mm (center to center) from the amoxicillin/clavulanic disk as previously described [12]. This test is considered as positive if a zone of inhibition is seen between the cephalosporin antibiotics and the amoxicillin/clavulanic disk, after 24 hrs of incubation. The standard strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as a negative and a positive control for ESBL production.

Screening for carbapenemase production

All isolates showing reduced susceptibility to an imipenem disk (diameter of zone of inhibition <22 mm) or an ertapenem disk (diameter of zone of inhibition ≤21 mm) were screened for the production of carbapenemase according to the recommendations in the CLSI guidelines [10].

Phenotypic detection of carbapenemase

Phenotypic carbapenemase production was assessed using the modified Hodge test and interpreted according to the recommendations of the CLSI [10].

The phenotypic detection method for class B metallo-β-lactamase (MBL) production was performed using the combined disk test using two imipenem disks (10 µg), one containing 10 µL of 0.1 M (292 µg) anhydrous ethylene diamine tetra-acetic acid (EDTA) (Sigma Chemicals, St. Louis, MO), placed 25 mm apart (center to center). MBL production was considered positive if the difference in diameter between the inhibition zones with an imipenem disk supplemented with EDTA and an imipenem disk alone was >4 mm [12,13].

Preparation of DNA template for PCR

Total DNA was extracted by the boiling method by suspending a few colonies of an overnight culture of *Enterobacteriaceae* isolates growing on Luria-Bertani agar (Bio-Rad, Marnes-la-Coquette, France) in 500 µL of DNase- and RNase-free water (Invitrogen, Paisley, UK) and boiling at 100°C for 10 minutes in a thermal block (Polystat 5, Bioblock Scientific, France), followed by centrifugation at 19,000 ×*g* for 5 minutes. An aliquot of 1 µL of the supernatant was used as the DNA template for PCR.

Detection of β-lactamase-encoding genes

Enterobacteriaceae isolates were screened by PCR for the following β-lactamase-encoding genes: bla_{CTX-M} of phylogenetic lineage groups 1, 2 and 9, bla_{TEM}, bla_{SHV}, bla_{PER}, and bla_{VEB} genes as described previously [6]. Carbapenem-resistant *Enterobacteriaceae* were tested by using specific primers for carabapenem-hydrolysing enzymes encoding the genes bla_{GES}, bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, and bla_{NDM} as described previously [6].

Amplification reactions were performed in a volume of 50 µL containing 1 µL of DNA template, 2.5 mM $MgCl_2$, 0.4 µM each of the forward and reverse primers, 100 µM of each dNTP, and 2 units of *Taq* DNA polymerase (Promega, Madison, USA) in ×1 PCR buffer as provided by the manufacturer.

The cycling parameters were: 5 minutes of denaturation at 95°C; followed by 30 cycles of denaturation (95°C for 1 minute), annealing (52°C for 1 minute for TEM and IMP; 58°C for 1 minute for NDM; 60°C for 1 minute for CTX-M, KPC, VIM, and SHV), and extension (72°C for 1 minute); ending with a final extension period of 72°C for 7 minutes.

PCR products were detected by electrophoresis on a 1.5% agarose gel (FMC Bioproduct, Rockland, USA) containing 1 µg/mL of ethidium bromide and analyzed under ultraviolet light. The gel picture was captured with an Olympus Digital Camera and analyzed using Digi-Doc-it software (UVP, Upland, USA).

Sequencing of resistance genes

All obtained amplification 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 used for PCR amplification. The nucleotide and deduced protein sequences were analyzed using online software at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

Statistical analysis

The Pearson's χ^2 or the Fisher's exact tests were used (SPSS version 20 software, IBM, Chicago, USA) for comparisons where appropriate. A $p<0.05$ was considered significant for all tests.

RESULTS

Among 1060 urine samples, 190 were positive for UTI, including 153 (80.5%) that contained *Enterobacteriaceae*. Overall, 153 patients living in Settat city with *Enterobacteriaceae*-related CA-UTIs were recruited for this study. The strains were not duplicated. The mean patient age was 45 years (range 1-88 years, median 41 years), and the male/female ratio for these patients was 0.5.

The distribution of the bacteria in outpatients with and without MDRE UTIs is shown in Table 1. The majority of the uropathogens were *E. coli* (62.09%), *K. pneumoniae* (15.69%), *Proteus mirabilis* (9.80%), and *E. cloacae* (7.19%). *E. coli* was significantly more common in patients with non-MDRE UTIs than in those with MDRE UTIs (66.39% versus 45.16%; p=0.02), whereas *K. pneumoniae* and *E. cloacae* were significantly a more common in patients with MDRE UTIs than in those with non-MDRE UTIs (Table 1).

Antibiotic resistance rates for *Enterobacteriaceae* isolates are shown in Table 2.

The overall resistance rates for amoxicillin/clavulanic acid, sulfamethoxazole-trimethoprim, ciprofloxacin, cefoxitin, and gentamicin were 59.48%, 47.7%, 24.18%, 22.88%, and 18.30%, respectively. 12 (7.84%) strains were resistant to the third-generation cephalosporins.

Seven strains (4.58%) were immediately susceptible to ertapenem. Antibiotic resistance rates for MDRE UTIs were significantly greater than for non-MDRE UTIs for the majority of the tested antibiotics (Table 3).

ESBL production was detected in nine (5.88%) *Enterobacteriaceae* isolates with a predominance of *K. pneumoniae* (n=5; 20.83%) followed by *E. coli* (n=3; 3.15%) and *E. cloacae* (n=1; 9.09%). The results of ESBL encoding gene detection by PCR revealed that the strains studied harbored a diversity of β-lactamases, namely CTX-M, SHV, and TEM (Table 4). Further analysis of the bla_{TEM}, bla_{SHV} and bla_{CTX-M} sequences identified the respective subgroups TEM-1 (n=8), SHV-1 (n=5) and CTX-M-15 (n=9). No amplicons were obtained for the other two tested CTX-M subgroups (Table 4), bla_{PER} and bla_{VEB} were not detected in any of the isolates. All combinations of *bla* genes detected are shown in Table 4. Among the nine $bla_{CTX-M-15}$ isolates, eight also harbored bla_{TEM-1} and five harbored bla_{SHV} genes. It is interesting to note that the three β-lactamases genes bla_{SHV}, bla_{TEM} and bla_{CTX-M} were coexpressed in four *K. pneumoniae* isolates.

Table 1: Distribution of *Enterobacteriaceae* isolates with and without multidrug resistance isolated from 153 study patients

	n (%)					
***Enterobacteriaceae* Isolates**	**All isolates**	**MDR-isolates**	**Non-MDR isolates**	**p**	**OR**	**95% CI**
E. coli	95 (62.09)	14 (45.16)	81 (66.39)	0.02	0.41	0.18-0.92
K. pneumoniae	24 (15.69)	9 (29.03)	15 (12.30)	0.02	2.91	1.13-7.50
P. mirabilis	15 (9.80)	1 (3.23)	14 (11.48)	0.19	0.24	0.03-1.96
E. cloacae	11 (7.19)	5 (16.13)	6 (4.92)	0.03	3.71	1.05-13.11
C. freundii	7 (4.58)	2 (6.45)	5 (4.10)	0.57	1.61	0.29-8.74
K. oxytoca	1 (0.65)	0	1 (0.82)	-	-	-
Total	153 (100)	31 (100)	122 (100)	-	-	-

MDR: Multidrug resistant, Non-MDR: Non-multidrug resistant, OR: Odds ratio, CI: Confidence interval, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. mirabilis: Proteus mirabilis, E. cloacae: Enterobacter cloacae, C. freundii: Citrobacter freundii, K. oxytoca: Klebsiella oxytoca*

Table 2: Antibiotic resistance rates among *Enterobacteriaceae* species isolated from study patients

Antibiotics	***E. coli* n=95 (%)**	***K. pneumoniae* n=24 (%)**	***P. mirabilis* n=15 (%)**	***E. cloacae* n=11 (%)**	***C. freundii* n=7 (%)**	***K. oxytoca* n=1 (%)**
AMX	75 (78.95)	-	13 (86.67)	-	-	-
AMC	47 (49.47)	19 (79.17)	6 (40)	-	-	1 (100)
KF	39 (41.05)	19 (79.17)	5 (33.33)	-	-	1 (100)
CAZ	2 (2.11)	7 (29.17)	0	3 (27.27)	0	0
CTX	2 (2.11)	7 (29.17)	0	3 (27.27)	0	0
CRO	2 (2.11)	7 (29.17)	0	3 (27.27)	0	0
AZT	5 (5.26)	6 (25.00)	0	3 (27.27)	0	0
FOX	9 (9.47)	5 (20.83)	4 (26.67)	-	-	0
ERT	1 (1.05)	4 (16.67)	0	2 (18.18)	0	0
IPM	0	0	0	1 (9.09)	0	0
CIP	20 (21.05)	11 (45.83)	1 (6.67)	4 (36.36)	1 (14.29)	0
SXT	43 (45.26)	15 (62.50)	6 (40)	6 (54.55)	3 (42.86)	0
AK	1 (1.05)	2 (8.33)	0	0	0	0
GM	11 (11.58)	9 (37.50)	2 (13.33)	5 (45.45)	1 (14.29)	0
CT	0	0	-	2 (18.18)	1 (14.29)	0

AMX: Amoxicillin, AMC: Amoxicillin/clavulanic acid, KF: Cephalothin, CTX: Cefotaxime, CRO: Ceftriaxone, CAZ: Ceftazidime, AZT: Aztreonam, FOX: Cefoxitin, ERT: Ertapenem, IPM: Imipenem, CIP: Ciprofloxacin, SXT: Sulfamethoxazole/trimethoprim, AK: Amikacin, GM: Gentamicin and CT: Colistin, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae, P. mirabilis*: *Proteus mirabilis*, *E. cloacae*: *Enterobacter cloacae*, *C. freundii*: *Citrobacter freundii, K. oxytoca*: *Klebsiella oxytoca*

Table 3: Antibiotic resistance rates among 153 unique *Enterobacteriaceae* bacterial isolates from study patients, with and without MDR

Antibiotics	**All isolates n (%)**	**MDR n (%)**	**non-MDR n (%)**	**p**	**OR**	**95% CI**
AMX	131 (85.62)	30 (96.77)	101 (82.79)	0.12	3.11	0.69-14.07
AMC	91 (59.48)	26 (83.87)	65 (53.28)	0.001	4.56	1.64-12.65
KF	82 (53.59)	26 (83.87)	56 (45.90)	0.0003	5.10	1.96-13.29
CAZ	12 (7.84)	11 (35.48)	1 (0.82)	>0.0001	66.55	8.14-544.06
CTX	12 (7.84)	11 (35.48)	1 (0.82)	>0.0001	66.55	8.14-544.06
CRO	12 (7.84)	11 (35.48)	1 (0.82)	>0.0001	66.55	8.14-544.06
AZT	14 (9.15)	14 (45.16)	0	>0.0001		
FOX	35 (22.88)	16 (51.61)	19 (15.57)	0.0005	4.21	1.79-9.89
ERT	7 (4.58)	7 (22.58)	0	>0.0001		
IPM	1 (0.65)	1 (3.23)	0	0.04		
CIP	37 (24.18)	27 (87.10)	10 (8.20)	>0.0001	75.60	22.02-259.51
SXT	73 (47.71)	29 (93.55)	44 (36.07)	>0.0001	25.70	5.85-112.89
AK	3 (1.96)	3 (9.68)	0	0.00006		
GM	28 (18.30)	19 (61.29)	9 (7.38)	>0.0001	18.35	6.89-48.84
CT	3 (1.96)	0	3 (2.46)	1		

AMX: Amoxicillin, AMC: Amoxicillin/clavulanic acid, KF: Cephalothin, CTX: Cefotaxime, CRO: Ceftriaxone, CAZ: Ceftazidime, AZT: Aztreonam, FOX: Cefoxitin, ERT: Ertapenem, IPM: Imipenem, CIP: Ciprofloxacin, SXT: Sulfamethoxazole/trimethoprim, AK: Amikacin, GM: Gentamicin, CT: Colistin, MDR: Multidrug resistant, Non-MDR: Non-multidrug resistant, OR: Odds ratio, CI: Confidence interval

Table 4: Characteristics of the ESBL-producing *Enterobacteriaceae* isolates collected from a Moroccan community

Code	Species	Date of isolation (DD/MM/YYY)	Gender/age	β-lactamase	Carbapenemase type	Antibiotic resistance pattern*
Ec1	*E. cloacae*	11/04/2012	M/65	-	OXA-48	FOX, ERT, SXT, CIP, GM
Ec2	*E. cloacae*	07/10/2013	M/60	CTX-M-15, TEM-1		FOX, ERT, SXT, CIP, GM
E1	*E. coli*	04/04/2012	M/60	CTX-M-15, TEM-1	OXA-48	FOX, SXT, GM
E2	*E. coli*	27/06/2012	M/60	CTX-M-15, TEM-1		FOX, SXT, CIP
E3	*E. coli*	17/12/2013	M/65	CTX-M-15, TEM-1		FOX, SXT, CIP, GM
K1	*K. pneumoniae*	16/01/2013	F/70		OXA-48	FOX, SXT, CIP
K2	*K. pneumoniae*	26/01/2012	M/64	CTX-M-15, TEM-1, SHV-1		FOX, SXT, CIP, GM
K3	*K. pneumoniae*	01/06/2013	M/50	CTX-M-15, TEM-1, SHV-1		SXT, CIP, GM
K4	*K. pneumoniae*	02/10/2013	M/65	CTX-M-15, TEM-1, SHV-1		FOX, ERT, SXT, CIP, GM
K5	*K. pneumoniae*	09/11/2012	M/65	CTX-M-15, TEM-1, SHV-1		SXT, CIP, GM
K6	*K. pneumoniae*	11/12/2012	M/66	CTX-M-15, SHV-1		SXT, CIP, GM

*All isolates were resistant to amoxicillin, amoxicillin/clavulanic acid, cephalothin, cefotaxime, ceftriaxone, ceftazidime and aztreonam. M: Male, F: Female, FOX: Cefoxitin, ERT: Ertapenem, SXT: Sulfamethoxazole/trimethoprim, CIP: ciprofloxacin, GM: Gentamicin, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae, P. mirabilis: Proteus mirabilis, E. cloacae: Enterobacter cloacae, C. freundii: Citrobacter freundii, K. oxytoca: Klebsiella oxytoca*, ESBL: Extended-spectrum-β-lactamases

Among seven carbapenem-resistant isolates, three (3/153; 1.96%) were positive in the modified Hodge test and were identified as *K. pneumoniae*, *E. coli,* and *E. cloacae*. Only one carbapenemase-producing isolate (*E. coli* E1) was an ESBL co-producer. The results of carbapenemase gene detection by PCR and sequencing revealed that the strains studied harbored the bla_{OXA-48} gene (Table 4).

DISCUSSION

Carbapenemase and ESBL-producing *Enterobacteriaceae* have become a worldwide problem*. Enterobacteriaceae* have become one of the most important causes of nosocomial and community-acquired infections [14,15]. β-lactams (mainly extended-spectrum cephalosporins, carbapenems) and fluoroquinolones represent the main therapeutic option to treat infections due to these bacteria [2]. However, the extensive use of these antibiotics has caused both the emergence and the progressive spread of this health-threatening problem.

In Morocco, several studies demonstrate a wide dissemination of ESBLs in the environments, in both clinical and community settings [8,16,17], especially in community-acquired UTIs [16,18,19]. Most urinary and intra-abdominal infections with *E. coli* and *K. pneumoniae* are endogenous [20]. The ESBL producing *Enterobacteriaceae* showed a high frequency of resistance to various antimicrobial agents (β-lactams, ciprofloxacin, sulfamethoxazole trimethoprim, aztreonam) which have been reported increasingly worldwide [18,21]. *E. coli* and *Klebsiella spp.* producing β-lactamases play an important role in resistance to β-lactams [22]. The mechanism of resistance to the third-generation cephalosporins in non-ESBL producers could be due to the production of AmpC β-lactamase [23].

Our study reveals a higher prevalence of ESBL-producing *K. pneumoniae* (20.83%) and ESBL-producing *E. coli* (3.26%) in the Moroccan community than recorded in previous Moroccan reports [18].

This indicates that in a 2-year interval, the prevalence of ESBLs among urinary *K. pneumoniae* has been increasing, and shows that ESBL-producing strains continue to disseminate in Moroccan communities.

The inappropriate and extensive use of broad-spectrum antibiotics can lead to the emergence and spread of these ESBL producing strains. Another cause remains a person-to-person transmission via fecal carries between within families as reported by Rodriguez-Bano *et al.* [24]. Furthermore, evidence of ESBL-producing isolates in cattle, poultry, dogs, and cats suggests that food-producing animals and house pets might act as a reservoir for the transmission or acquisition of ESBL genes or ESBL-producing *Enterobacteriaceae* in the community [25,26]. Furthermore, antibiotics are commonly used in animals for prophylaxis or as performance enhancers, and such practices are likely to increase the development of resistance.

In general, the resistance patterns of ESBL-producing bacteria studied here were similar to those frequently described in various studies, i.e., the ESBL producers were resistants to β-lactams, fluoroquinolones, aminoglycosides and trimethoprim/sulfamethoxazole [18,26], which contributes to the selection and persistence of multidrug-resistant ESBL strains and plasmids in both clinical and community settings.

We detected a variety of β-lactamases among *Enterobacteriaceae* isolates, namely SHV-, CTX-M-, and TEM-type enzymes. The CTX-M type was the most common ESBL in our setting [16,19]. The predominance of CTX-M-15 indicates that this variant is now common in Morocco, as in other countries, as a result of worldwide dissemination [27,28]. The higher incidence of CTX-M among ESBL enzymes is probably associated with the mobilization and incorporation of bla_{CTX-M} into mobile genetic elements. Cantón *et al.* report that bla_{CTX-M} genes have been mobilized to plasmids almost ten times more frequently than other class A β-lactamases [29].

The coexistence of different β-lactamase genes within the same isolate, as found in this study, has also been reported in other countries [30,31]. Although the clinical importance of ESBLs in patients carrying ESBL-expressing *K. pneumoniae* is clear, the clinical importance of non-ESBL enzymes is yet to be revealed. Among these non-ESBL enzymes, we identified SHV-1, an intrinsic gene in *K. pneumoniae*. Co-expression of CTX-M enzymes and SHV-1 penicillinases is most commonly found in *K. pneumoniae* isolates [32,33].

The OXA-48-producing *Enterobacteriaceae* isolates described in Moroccan communities and hospitals may have a relationship to those identified in France and the Netherlands [9].

This report illustrates the widespread of carbapenemase-producing *Enterobacteriaceae* in the Mediterranean area and in Europe. Our search showed trends of the emergence of carbapenem-resistant *Enterobacteriaceae* in Moroccan community settings along with the carriage of resistance genes with clinical relevance coexpressed by bla_{OXA-48} [18]. The spread of the same major carrying plasmid between Moroccan cities has been identified. This finding emphasizes the importance of systematic detection to contain the spread of these multidrug-resistant organisms.

CONCLUSION

Our study reported an increase in the prevalence of ESBL and OXA-48 carbapenemase-producing multidrug-resistant *Enterobacteriaceae* in Morocco. These data suggest that specific strategies for regular surveillance and control of antimicrobial resistance agents are required. Antibiotic resistance remains a serious issue, as the rapid spread of these bacteria would eventually limit clinical treatment choices. To avoid UTI treatment failures, it will be necessary to start monitoring ESBL and carbapenemase producers in laboratories using conventional antibiograms.

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