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Original article

Molecular characterization of extended spectrum β-lactamase-producing *Escherichia coli* in a university hospital in Morocco, North Africa

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Urinary;
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Abstract

Introduction: β-Lactams are among the most widely prescribed antibiotics in human medicine. However, because of their massive and usually inappropriate use, resistance to these drugs has increased markedly, especially due to extended-spectrum β-lactamase (ESBL) production.

Objectives: The aims of this study were to determine the prevalence of urinary *Escherichia coli* strains isolated from urine samples taken from patients diagnosed with urinary tract infections (UTIs), to evaluate their current antimicrobial susceptibility pattern and to look for blaSHV, blaTEM and blaCTX-M genes in these multi-drug resistant isolates.

Subject and methods: A retrospective survey was made over 3 years from 2010 to 2012. It included all uropathogenic *E. coli* strains isolated from urine samples taken from consulting and hospitalized patients in the Avicenne Teaching Hospital in Marrakech, Morocco.

Results: E. coli was the etiologic agent in 63% of reported UTIs due to *Enterobacteriacae*. In all, the prevalence of ESBL-producing *E. coli* reached 6% of all urinary *Enterobacteriaceae* isolates in 2012.

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The bacterial resistance rates of ESBL-producing *E. coli* isolates were as follows: amoxicillin/clavulanic acid (100%), trimethoprim/sulfamethoxazole (76%), gentamicin (66%), ciprofloxacin (82%) and amikacin (56%). None of these strains was resistant to carbapenems. The ESBL production patterns observed included single production of CTX-M (70%), SHV (12%) and TEM (0%). Some ESBL-producing *E. coli* isolates produced combinations of 2 ESBLs belonging to different groups: CTX-M+SHV (12%) and CTX-M+TEM (6%).

Conclusion: The results of this work report, for the first time in the Marrakech region, the ESBL production pattern with CTX-M being most common among the ESBL-producing urinary *E. coli*. Moreover, a major finding is the production of multiple ESBL types by some urinary *E. coli* isolates.

Introduction

Escherichia coli, a member of the *Enterobacteriaceae* family, has been reported to be one of the most predominant organisms causing urinary tract infections (UTIs) which are very common reasons for consultation and antibiotic prescription in current practice [1,2]. Massive and usually inappropriate use of antibiotics for treatment of UTIs generates a selective pressure that is followed by the rapid emergence and spread of multi-drug resistant bacterial strains. Nowadays, resistance of uropathogenic *E. coli* to many antibiotic classes is a very common finding in human medicine and is usually associated with increased medical costs, prolonged hospital stays and frequent therapeutic failure [3].

The production of extended-spectrum β-lactamases (ESBL) by urinary *E. coli* strains is a major public health concern in both hospital and community settings [2]. These ESBL-producing strains represent a significant therapeutic challenge as they are resistant to all currently available β-lactam antibiotics but cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g. imipenem and ertapenem) [4].

Resistance to expanded-spectrum cephalosporins by ESBL production is mainly due to members of the TEM and SHV families of enzymes. The distribution of ESBLs has evolved to a predominance of CTX-M enzymes, mainly with *E. coli* as one of the major carriers of ESBL-encoding genes. Nowadays, the class-A ESBLs, TEM, SHV and CTX-M types, are the most widespread and clinically relevant [5].

During the last three decades, ESBLs among urinary *E. coli* have been reported worldwide, and their occurrence has increased in both outpatients and inpatients diagnosed with UTIs. As for most developing countries in Africa, ESBL-producing bacteria have been examined in only a few studies. Limited data from prior studies conducted in Morocco suggest that ESBLs are common in Moroccan hospitals [6,7], with blaCTX-M, blaSHV and blaTEM genes representing the main reported ESBL families [8,9]. ESBL-producing *E. coli* isolates from the Marrakech region have not been characterized previously. Therefore, the aims of this present study were to determine the prevalence of ESBL-producing *E. coli* of both community and nosocomial origin isolated from urine samples taken from patients diagnosed with UTIs, to detect their drug resistance pattern to commonly used antibiotics in medical practice and to detect blaSHV, blaTEM and blaCTX-M genes in these multi-drug resistant isolates.

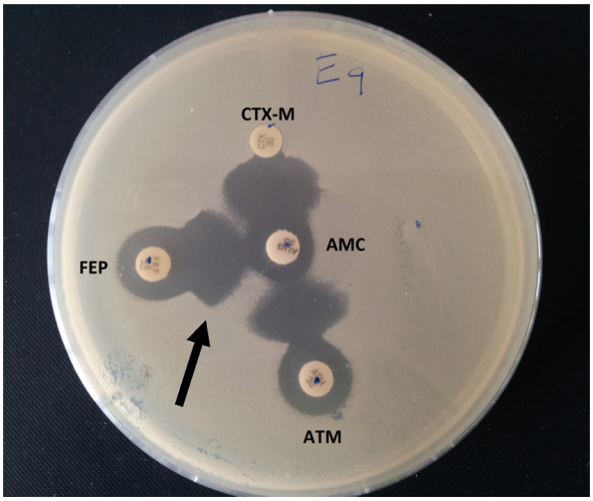

Figure 1 The double-disk synergy test performed on a 100 mm Mueller-Hinton agar plate: a clinical *E. coli* isolate, positive result.

Subject and methods

All clinical non-duplicate *E. coli* isolates isolated from urinary samples at the microbiology laboratory of Avicenne University Hospital (Marrakech, Morocco) over a 3-year period (2010–2012) were included in this study. This university hospital serves wide urban and rural geographic areas in the Marrakech region.

Urinary *E. coli* isolates were identified to the species level, tested for antimicrobial susceptibility and screened for ESBL production using custom MicroScan Walkaway dehydrated broth microdilution panels (MicroScan®, Sacramento, CA, USA) according to the Antibiogram Committee of the French Society of Microbiology (CA-SFM) guidelines [10].

Phenotypic ESBL production was confirmed by the double-disk synergy test "DDST" (Fig. 1). This phenotypic ESBL screening test is based on the demonstration of a synergy image between Amoxicillin/clavulanic acid (20/10 mg) and cefotaxime (30 mg), ceftazidime (30 mg) and aztreonam (30 mg) according to the guidelines of the CA-SFM [10].

Twenty-five percent (17/67) of the ESBL-producing *E. coli* isolates were randomly chosen to undergo ESBL molecular characterization

Table 1 Antimicrobial susceptibility profiles and bla genes detected by PCR in seventeen ESBL-producing *E. coli*.

Isolate no.	DDST	Antimicrobial susceptibility testing															ESBL genes
		Amx	Amc	cf	Tic	Fox	Ctx	Caz	Fep	Imp	Fos	Gm	Tm	Ak	Sxt	Cip	
E. coli 1	+	R	R	R	R	S	R	R	R	S	S	R	R	S	R	R	CTX-M1
E. coli 2	+	R	R	R	R	S	R	R	R	S	S	R	R	R	S	R	CTX-M1
E. coli 3	+	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	CTX-M1
E. coli 4	+	R	R	R	R	S	R	R	R	S	R	R	R	S	R	R	CTX-M1
E. coli 5	+	R	R	R	R	S	R	R	R	S	S	R	R	S	R	R	CTX-M1 no gr 1
E. coli 6	+	R	R	R	R	S	R	R	R	S	S	R	R	R	S	R	CTX-M1
E. coli 8	+	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	CTX-M1 no gr 1
E. coli 9	+	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	CTX-M1
E. coli 10	+	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	CTX-M1
E. coli 11	+	R	R	R	R	S	R	R	R	S	S	R	R	R	S	S	CTX-M1
E. coli 12	+	R	R	R	R	S	R	R	R	S	S	S	S	S	R	R	CTX-M1
E. coli 13	+	R	R	R	R	S	R	R	R	S	S	S	S	S	S	R	SHV
E. coli 14	+	R	R	R	R	S	R	R	R	S	S	R	R	S	S	R	SHV
E. coli 15	+	R	R	R	R	S	R	R	R	S	S	R	R	R	S	S	CTX-M1/SHV
E. coli 16	+	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	CTX-M1/SHV
E. coli 17	+	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	CTX-M1/TEM
E. coli 18	+	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	CTX-M1

Amx: amoxicillin, Amc: amoxicillin/clavulanate; Cf: céfalotine; Tic: ticarcillin; Fox: cefoxitin; Ctx: cefotaxime; Caz: ceftazidime; Fep: cefepime; Fos: fosfomycine; Imp: imipenem; Gm: gentamycine; Tm: tobramycine; Sxt: sulfamethoxazole; Cip: ciprofloxacine, Nal: nalidixic acid; Ak: amikacine; +: positive; −: negative; R: resistant; S: sensible.

Table 2 Nucleotide sequences of polymerase chain reaction (PCR) primers used in this study.

Gene	Primer sequence (5′–3′)
CTX-M group1	CTX-M (F): GGTTAAAAAATCACTGCGTC CTX-M (R): TTGGTGACGATTTTAGCCGC
SHV	SHV (F): CGCCGGGTTATTCTTATTTGTCGC SHV (R): CGCCGGGTTATTCTTATTTGTCGC
TEM	TEM (F): ATAAAATTCTTGAAGACGAAA TEM (R): GACAGTTACCAATGCTTAATCA

(Table 1). The conserved core regions of the blaCTX-M, blaSHV and blaTEM genes, which represent the most commonly reported ESBL families in Morocco, were amplified by PCR to identify the families of ESBL enzymes produced by urinary *E. coli* isolates in the Marrakech region. PCR amplification of bla genes was carried out using the primers listed in Table 2.

Results

During the three-year study period (2010–2012), a total of 1472 *Enterobacteriaceae* isolates was recovered from culture specimens of patients diagnosed with UTIs. Of these, the most frequent *Enterobacteriaceae* strains isolated from urinary samples were *E. coli* (63%, $n = 924$). *E. coli* strains accounted for 86% of the community-acquired UTIs.

The bacterial resistance patterns of non-ESBL producing *E. coli* isolates and ESBL-producing *E. coli* isolates are presented in Table 3. During the study period, the percentage of *E. coli* strains resistant to third-generation cephalosporins by ESBL production increased progressively from 3% in 2010 to 4% in 2011 and 6% in 2012 of all isolated urinary *Enterobacteriaceae* (overall 4.5%; 67/1472). All *E. coli* isolates were susceptible to carbapenems (e.g. imipenem and ertapenem).

Table 3 Antimicrobial resistance rates between non-ESBL and ESBL producing urinary *E. coli strains* recovered in the Marrakech region between January 2010 and December 2012.

Tested antibiotic	ESBL producing *E. coli* strains (%R) ($n = 67$)	Non-ESBL producing *E. coli* strains (%R) ($n = 857$)
Amx	100	65
Aug	100	43
T/S	76	55
Gm	66	14
Cp	82	22
Ak	56	8
Fd	13	11
Fos	9	7
Etp	0	0
Imp	0	0

T/S: trimethoprim/sulfamethoxazole; Aug: amoxicillin/clavulanate; Cp: ciprofloxacin; Gm: gentamicin; Fd: nitrofurantoin; Ak: amikacin; Fos: fosfomycin; Imp: imipenem; Etp: ertapenem.

The distribution of ESBL-producing genes is detailed in Fig. 2. The ESBL production patterns observed included single production of CTX-M (70%), SHV (12%), TEM (0%) and some combinations of 2 ESBLs belonging to different groups: CTX-M1+TEM (6%) and CTX-M+SHV (12%).

Discussion

This study included all *E. coli* isolates responsible for community and nosocomial acquired UTIs recovered at the Avicenne Teaching Hospital of Marrakech, Morocco between 2010 and 2012. Although UTIs caused by ESBL-producing *E. coli* are a cause of concern due to clinical failure of empirical treatment protocols in the Marrakech region [6], there were no available data regarding the prevalence and characteristics of ESBL-producing *E. coli* from the Marrakech

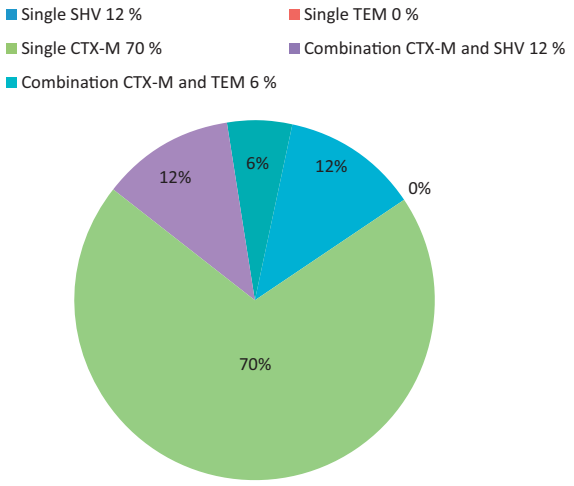

Figure 2 Distribution of genes encoding extended spectrum β-lactamase (ESBL) among 100 clinical isolates of ESBL producing *E. coli*.

region before this study. We found that *E. coli* was the etiologic agent in 63% of UTIs due to *Enterobacteriaceae*. This high isolation frequency of urinary *E. coli* is clearly justified by the preponderance of *E. coli* in the general profile of bacteria responsible for UTIs [11].

Characteristic of antimicrobial resistance are the differences between regions, hospitals and even departments. *E. coli* strains are susceptible to commonly used antimicrobial agents in treatment of UTIs. However, antibiotic resistance of uropathogenic *E. coli* in UTIs is increasing worldwide. Our study showed that non-ESBL producing *E. coli* isolates have alarming bacterial resistance rates to most prescribed antimicrobial agents tested, such as amoxicillin (65%), amoxicillin-clavulanic acid (AMC) (43%), T/S (55%) and ciprofloxacin (22%). The high antimicrobial resistance to these antimicrobial agents widely prescribed in Morocco [12] justifies that they are currently no longer recommended for empirical treatment of UTIs [13]. Many factors have contributed to such high bacterial resistance rates. They include massive use and misuse of antibiotics both in hospitals by health professionals and in the community by self-prescription as well as inadequate antimicrobial surveillance programs.

Over the past decade, the occurrence of ESBLs among *Enterobacteriaceae* isolated from urine samples of both in- and outpatients has been assessed in several studies [6,14–16]. UTIs caused by ESBL-producing *Enterobacteriaceae* have been reported worldwide, and our study disclosed a high prevalence of ESBL-producing *E. coli*.

Antimicrobial resistance testing showed a very important variety when comparing ESBL and non-ESBL producing strains of *E. coli* (Table 3). The mean percentages of resistance to commonly used antibiotics measured in the ESBL-producing *E. coli* were remarkably higher than the antimicrobial resistance percentages displayed by non-ESBL producing *E. coli* isolates. Indeed, all ESBL-producing *E. coli* showed higher resistance rates to aminoglycosides, T/S and fluoroquinolones. This finding suggests that ESBL-encoding genes and genes encoding other classes of antibiotics were on the same plasmids and therefore spread together [17,18]. Fluoroquinolones and T/S are often used to treat UTIs caused by *Enterobacteriaceae* in various clinical settings. Our results showed that 82% and 76% of the ESBL-producing isolates were resistant to ciprofloxacine and T/S, respectively. The association of ESBL production and resistance to fluoroquinolones and T/S has been reported worldwide, and the widespread use of fluoroquinolones has been identified as a risk factor for the emergence of fluoroquinolone-resistant ESBL-producing strains [19–21].

The clinical relevance of ESBLs has been well documented by numerous published reports describing clinical failure with antimicrobial agents supposed to be effective [22]. Thus, the choice of antimicrobial agents effective against ESBL-producing organisms is currently very limited to a few classes of antibiotics, such as carbapenems, that are often the recommended treatment of UTIs caused by ESBL-producing *E. coli.* However, carbapenemase-producing *Enterobacteriaceae* are increasingly being reported and are becoming a major clinical and public health concern in many countries including Morocco [8]. The antimicrobial susceptibility results show that imipenem and ertapenem remain the most effective drugs against urinary *E. coli* isolates. Nevertheless, there remains a need for continuous surveillance and judicious use of this class of antibiotics to prevent the emergence of carbapenem-resistant *E. coli* isolates in our region.

Molecular detection and identification of β-lactamases would be essential for a reliable epidemiological investigation of antimicrobial resistance. Until now, ESBL genes have been studied in different parts of the world but not in the Marrakech region. This is the first systematic study that reports the diversity of ESBL genes among ESBL-producing urinary *E. coli* isolated in this region. Three types of ESBL were detected in our study: CTX-M, SHV and TEM. Clearly, CTX-M ESBLs are the most prevalent among single ESBL types produced by *E. coli* in our study, accounting for 70% of the ESBLs compared with SHV (12%) and TEM (0%). This is a dramatic turnaround from the situation in the 1990s when TEM and SHV ESBLs were dominant and CTX-M types were rarely recognized. The distribution of ESBL-producing genes in our series, with a large predominance of CTX-M, is similar to those reported in several countries including Argentina, the UK, Spain, France and India [5,22–25], among others countries where CTX-M-producing *Enterobacteriaceae* have been described to be endemic.

Multiple ESBL-producing *E. coli* appear to occur worldwide. Among the 17 tested strains of ESBL-producing *E. coli*, 3 strains were found to harbor bla genes for two ESBLs of different families (Table 1). The combination of blaCTX-M with blaSHV predominated in isolates producing two ESBLs (dual ESBLs). This finding confirms the accumulation of multiple types of bla genes in *E. coli* isolated in the Marrakech region.

The class-A ESBLs, TEM, SHV and CTX types, are the most widespread and clinically relevant worldwide [26]. In Morocco, ESBL-producing *Enterobacteriaceae* have been isolated from samples collected in different hospitals [6,7], and the ESBL-positive strains frequently expressed blaCTX-M among other ESBLs such as blaTEM, blaSHV, blaDHA and blaOXA types [27]. CTX-M ESBLs are the most commonly described ESBL types in Tunisia [28] and Algeria [29,30]. This also illustrates the large spread of ESBL-producing *E. coli* in the North African countries.

Originally, ESBLs were most commonly reported to be a hospital-based problem. However, there are now numerous reports that ESBLs are becoming common among community-acquired

pathogens, especially among *E. coli*. In particular, the CTX-M family of ESBLs has been reported to be the most prevalent among the ESBL-produced enzymes in community-onset UTIs worldwide [31].

Conclusion

This is the first systematic study of ESBL types produced by urinary *E. coli* in the Marrakech region. The results of this survey demonstrate a high prevalence of ESBL-producing *E. coli* with important antimicrobial resistance rates to commonly used antibiotics. We also report the diversity of ESBL genes harbored by ESBL-producing *E. coli*, with CTX-M ESBLs being most common. The important dissemination of ESBL-producing *E. coli* has lead to a decrease in therapeutic options and to an increase in hospital costs. Therefore, updates of trends for regional epidemiological data on antimicrobial resistance are crucial in order to promote appropriate antimicrobial therapy as well as an effective infection control and clinical care management.

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Conflict of interest

None declared.

Ethical approval

Not required.

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