Multidrug-resistant community-acquired urinary tract infections in a northern region of Morocco: epidemiology and risk factors

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Abstract

Introduction **Urinary tract infection is very common and is the second most frequent reason for consultation in office-based practice. The incidence varies from one country to another. The diffusion of MDR in the community complicates therapeutic management. The objective of this study was to describe the bacterial epidemiology and to determine the risk factors for the acquisition of MDR in community urinary tract infections in our region.**

Methods **This was a retrospective case-control study conducted in the bacteriology laboratory of the Mohammed V Military Teaching Hospital over an 8-month period from 01 October 2015 to 31 May 2016. Cases were defined as patients with community-acquired urinary tract infection with MDR and controls were defined as patients with a urinary tract infection without MDR.**

Results **Out of 373 isolates, enterobacteria represented 80%. *E. coli* represented 59.2%, followed by *K. pneumoniae* at 15%. The rate of MDR represented 13.4% of which ESBL enterobacteria represented 12.1%. Univariate analysis showed a statistically significant association between male sex (p=0.001), age >65 years (p=0.007), urban origin (p=0.003), previous hospitalization within 3 months (p=0.001) and antibiotic therapy within 6 months (p=0.001) with MDR community-acquired urinary tract infection. On the other hand, multivariate analysis by logistic regression showed that age >65 years (OR=8.4, CI: 2.1-42), previous hospitalization within 3 months (OR=13.4, CI: 3.3-140.2) and antibiotic therapy within 6 months (OR=9.2, CI: 4.1-60.1) were significantly associated to MDR community-acquired urinary tract infection.**

Conclusions **The increase in resistance to enterobacteria in the community prompts a review of the list of antibiotics prescribed for probabilistic management of these infections in our region.**

Keywords **Community-acquired, urinary tract infection, antibiotic resistance, enterobacteria, MDR, ESBL.**

Introduction

Urinary tract infections (UTIs) are very common in both community and hospital settings. They are the second most common reason for consultation in the community.[1] There is little data on community-acquired urinary tract infections. *Escherichia coli* is the most isolated species (70-95%) followed by other enterobacteria (10-25%). *Staphylococcus saprophyticus* is almost exclusively responsible for cystitis (1-7% of cases), particularly in women aged 15-30 years, where it can reach 10%.[1] In recent years, we have seen the

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emergence of strains that are increasingly resistant to antibiotics, raising fears of therapeutic impasses. This problem is essentially hospital-based, and the spread of resistance in the community suggests a major public health problem.[1] The progression of multidrug-resistant bacteria (MDR) in the community complicates the probabilistic management of serious urinary tract infections and those at risk of complication. The emergence of these multidrug-resistant bacteria is favored by a certain number of risk factors, such as antibiotic exposure, hospitalization, residence in a long-term care facility, hemodialysis, and intravascular catheter. Human ingestion of animal and plant products has a high potential for the spread of antibiotic resistance genes via the consumption of antibiotic residues and antibiotic-resistant bacteria and residence in an area endemic for extended-spectrum beta-lactamase-producing bacteria as well are risk factors.[1] The local data are mostly mono-centric and most often without separation between community-acquired urinary tract infections and healthcare-associated infections. These MDR were essentially represented by extended-spectrum beta-lactamase-producing enterobacteria with a rate varying according to the regions ranging from 3.3% to 33.2% after 2010.[1,2] In Morocco, this rate varied from 9% to 11%.[3,4] In this context, we conducted this study to analyze the epidemiology and risk factors for the acquisition of MDR in community-acquired urinary tract infections in our region.

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Methods

This is a retrospective case-control study conducted in the bacteriology laboratory of the Mohammed V Military Teaching Hospital over an 8-month period from 01 October 2015 to 31 May 2016. Cases were defined as patients with a community-acquired urinary tract infection with MDR and controls were defined as patients with a community-acquired urinary tract infection without MDR. Polymorphic urocultures and duplicates were excluded. Enterobacteria producing extended-spectrum beta-lactamase and carbapenemase, cefoxitin (methicillin-resistant *Staphylococcus aureus* – MRSA) and/or glycopeptide-resistant *Staphylococcus aureus*, ampicillin- and/or glycopeptide-resistant *Enterococcus*, ceftazidime- and/or imipenem-resistant *Pseudomonas aeruginosa*, and ceftazidime- and/or imipenem-resistant *Acinetobacter baumannii* were considered as MDR.

For each patient, clinical and microbiological data were collected from the patient's chart and the laboratory's information system. Patient variables included age, sex, geographic origin, history of UTI, co-morbidities, and history of hospitalization or stay in a medical facility in the 3 months prior to the urine cytobacteriological examination, as well as antibiotic treatments received by the patients in the previous 6 months.

The urine of the patients included in our study was subjected to a quantitative uroculture on violet bromocresol agar and a cytological study by the UF-500i machine (Sysmex Europe GmbH, Germany), permitting the enumeration of figurative elements in the urine. The identification of bacterial isolates was based on cultural, morphological and enzymological characteristics such as oxidase test, catalase test, coagulase test, urease test, cocci, bacilli, pigment, and appearance of colonies. Biochemical identification was performed using API20 ready-to-use galleries (bio-Mérieux SA, France).

Community-acquired urinary tract infection was diagnosed when leukocyturia was greater than 10^4 WBCs/mm^3 and bacteriuria greater than 10^3 CFU/mL for *Escherichia coli* and

Staphylococcus saprophyticus or greater than 10^4 CFU/mL for the other species.[5]

Antibiotic susceptibility testing was performed by the agar diffusion method and interpreted according to the recommendations of EUCAST/CA-SFM 2019.[6] The detection of extended-spectrum β-lactamases (ESBL) was performed by a phenotypic method based on synergy detection between the amoxicillin-clavulanic acid disc and three third-generation cephalosporin discs: cefotaxime, ceftazidime and ceftriaxone.

Carbapenemase-producing strains were detected by the Biorad® Carba-test as described by the manufacturer.[7]

The third generation cephalosporins and fluoroquinolones used in our study were ceftriaxone and norfloxacin, respectively. The carbapenems used in our study were ertapenem and imipenem.

Statistical methods

Data were entered into Microsoft Office Excel 2007. Statistical analysis was performed using SPSS version 13 software (SPSS Inc., USA). Comparison of categorical variables was performed by Pearson's and Fisher's Chi-square exact tests and comparison of quantitative variables by Student's and Mann-Whitney U tests according to the normality of the distribution. Multivariate analysis was performed using a logistic regression model. The odds ratio (OR) and their corresponding 95% confidence intervals (CI) for each variable were also calculated. All statistical tests were two-tailed, a p value <0.05 was considered statistically significant.

Results

During the study period, we investigated 1253 patients, of whom 373 fulfilled our inclusion criteria, for a positivity rate of 30%. The patients were of urban origin in 89% of the cases. Females were predominant with a sex ratio M/F of 0.46. The median age was 47±16 years (range 0-89 years). Signs suggestive of simple urinary tract infection were observed in 92% of the cases (343/373). Enterobacteria dominated the epidemiological profile of the isolates (80%; 299/373). *Escherichia coli* was the predominant species (59%; 221/373), followed by *Klebsiella pneumoniae* (15%; 56/373), *Enterobacter cloacae* (4%; 14/373) and *Proteus mirabilis* (2%; 8/373). *Acinetobacter baumannii* and *Pseudomonas aeruginosa* represented 2% (7/373) and 1% (3/373), respectively. Gram-positive cocci represented 17% (64/373), as follows: group B *Streptococcus* (9%; 32/373), *Enterococcus* spp. (3%; 12/373), *Staphylococcus aureus* (2%; 8/373) and *Staphylococcus saprophyticus* (1%; 4/373) – Figure 1. The rate of MDR bacteria was 13% (50/373) of which ESBL producing enterobacteria represented 12% (45/373) with 4 isolates of carbapenem-producing *Enterobacter cloacae*, methicillin-resistant *Staphylococcus aureus* 0.8% (3/373) and ampicillin-resistant *Enterococcus* 0.5% (2/373).

The distribution of ESBL producing enterobacteria (ESBL) according to species showed a clear predominance of *Escherichia coli* (53%; 19/36), followed by *Klebsiella pneumoniae* (11%; 4/36).

Enterobacteria isolates showed a resistance rate to amoxicillin of 33%, amoxicillin-clavulanic acid (43%), ceftriaxone (12%), gentamicin (6%), amikacin (0%), norfloxacin 38%, trimethoprim-sulfamethoxazole 36%, nitrofurantoin (9%) and fosfomycin (1%) – Figure 2.

Isolates of ESBL producing enterobacteria were resistant to fluoroquinolones in 79% of cases, to trimethoprim-sulfamethoxazole in 35% and to gentamicin in 38%. However, these isolates were still sensitive to amikacin (92%), fosfomycin (87%) and nitrofurantoin (84%) – Figure 3.

Univariate analysis showed a statistically significant association between male sex (p=0.001), age >65 years (p=0.007), urban geographical origin (p=0.003), previous hospitalization within 3 months (p=0.001) and antibiotic treatment within 6 months (p=0.001) with MDR community-acquired urinary tract infection (Table 1). However, multivariate analysis by logistic regression showed that age >65 years (OR=8.4, CI: 2.1-42), previous hospitalization within 3 months (OR=13.4, CI: 3.3-140.2) and antibiotic treatment within 6

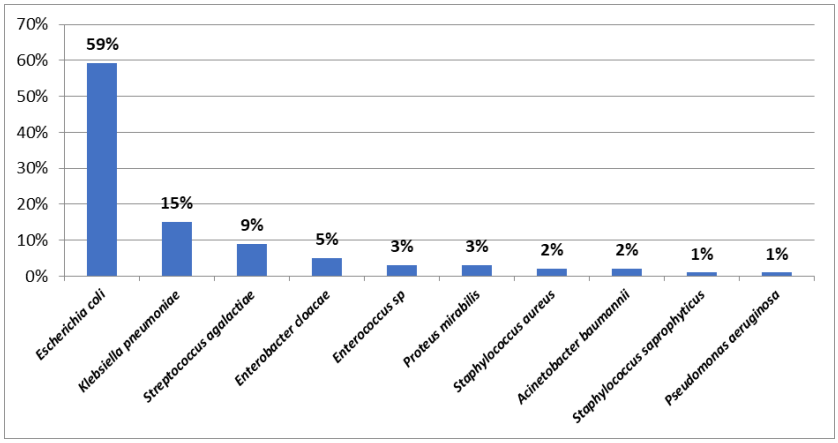
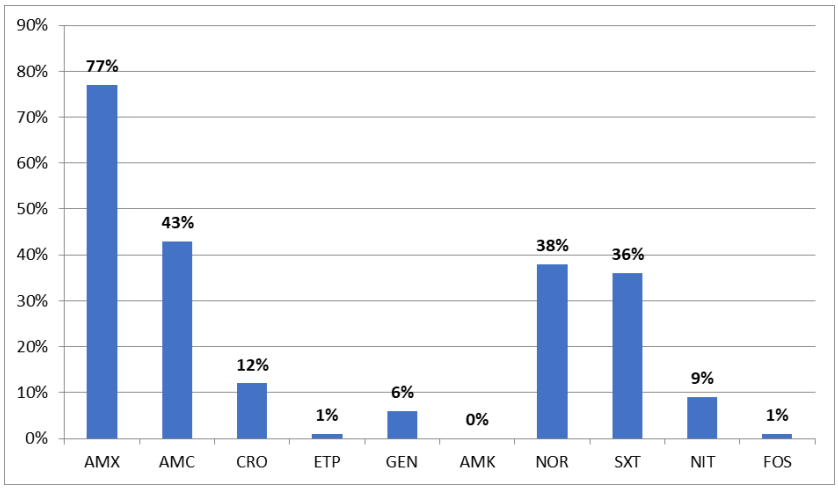


Figure 1. Species distribution of isolates (n=373)



AMX – amoxicillin; **AMC** – amoxicillin-clavulanic acid; **CRO** – ceftriaxone; **ETP** – ertapenem; **GEN** – gentamicin; **AMK** – amikacin; **NOR** – norfloxacin; **SXT** – trimethoprim-sulfamethoxazole; **NIT** – nitrofurantoin; **FOF** – fosfomycin.

Figure 2. Resistance rate of enterobacteria isolates to antibiotics (n=299)

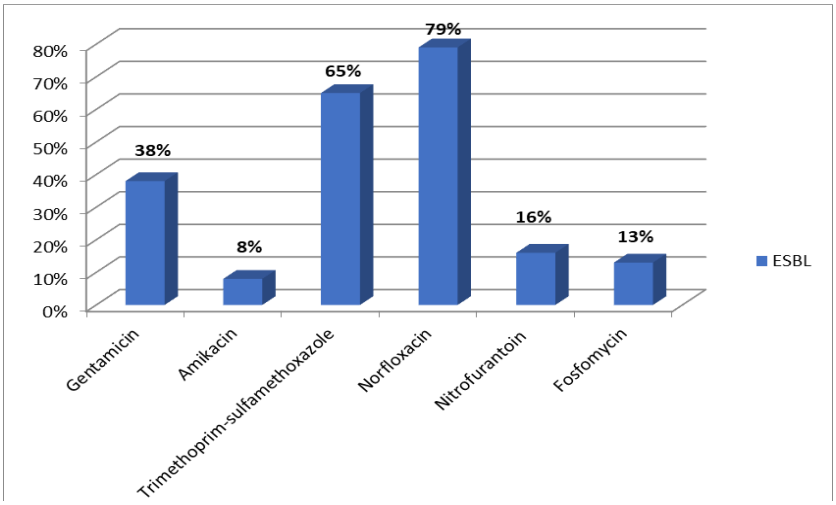


Figure 3. Rate of antibiotic resistance in extended-spectrum beta-lactamase (ESBL) producing isolates (n=50)

Table 1. Univariate analysis of risk factors for community-acquired urinary tract infection with MDR

	Total N=373	Case N=50	Control N=323	p	Odds ratio	95%CI
Male sex	110	34	76	**0.001**	**6.2**	2.2-10.1
Female sex	240	16	224	0.09	1.1	0.9-2.2
Age >65 years	168	32	136	**0.007**	**8.1**	5.4-20.8
Urban origin	331	44	287	**0.003**	**5.42**	1.5-14.1
Recurrent urinary tract infections	43	10	33	0.681	2.1	0.5-8.4
Previous hospitalization within 3 months	57	17	40	**0.001**	**9.51**	6.02-17.3
Antibiotic treatment within 6 months	50	18	32	**0.001**	**7.82**	3.1-24.6
Diabetes	109	9	100	0.841	0.9	0.4-1.9
Neoplasm	20	8	12	0.752	0.66	0.2-1.7
Pregnancy	36	1	35	0.987	0.7	0.2-1.2
Renal insufficiency	21	2	19	0.564	1.1	0.6-2.1

95%CI – 95% confidence interval.

Table 2. Multivariate analysis of risk factors for community-acquired urinary tract infection with MDR

	p	Odds ratio	95%CI
Male sex	0.08	3.88	0.82-6.22
Female sex	0.62	0.84	0.47-1.68
Age >65 years	**0.048**	**8.4**	**2.1-42**
Urban origin	0.29	2.66	0.91-3.08
Recurrent urinary tract infections	0.14	1.01	1.00-1.03
Previous hospitalization within 3 months	**0.003**	**13.4**	**3.3-140.2**
Antibiotic treatment within 6 months	**0.012**	**9.2**	**4.1-60.1**
Diabetes	0.28	2.4	0.5-4.4
Neoplasm	0.32	0.4	0.1-2.8
Pregnancy	0.066	2.2	0.9-5.2
Renal insufficiency	0.41	1.9	0.4-2.2

95%CI – 95% confidence interval.

months (OR=9.2, CI: 4.1-60.1) were significantly associated to MDR community-acquired urinary tract infection (Table 2).

Discussion

Urinary tract infections are a very frequent reason for consultation in the community. This pushes us to understand the bacterial epidemiology and to evaluate the level of antibiotic resistance for a better management of this pathology.

In our study, urinary tract infections were more frequent in women, with a sex ratio M/F of 0.46 and this is due to their physiology (short urethra, pregnancy) which favors the occurrence of this sort of infections. Our data are in agreement with the literature.[2]

Our uropathogenic isolates were dominated by enterobacteria in 80%. Our results are similar to the literature data.[2] This predominance is explained by the anatomical proximity of the gastrointestinal tract with the urogenital system.

E. coli is the most frequently isolated germ in our study (59%), followed by *K. pneumoniae* (15%). Our data are similar to those reported in Tunisia, Saudi Arabia and Iran.[2,8,9]

The resistance profile of enterobacterial isolates showed alarming rates in our region. The MDR rate was 13%, represented almost exclusively by ESBL producing

Enterobacteriaceae (36/373), i.e. 12%. This is related to the production of ESBL with plasmid determinism and therefore high diffusion. This ESBL phenotype has been reported in numerous studies.[10-12] Nationally, this rate varies between 7% and 15% and 1.3% to 4.8% internationally.[13-16] The diffusion of the ESBL phenotype in the community will complicate the management of this infection in the ambulatory setting.

Resistance to third generation cephalosporins was 12% in our series. Our results are higher than the European data.[17] This resistance rate is higher than 10%, which could lead to the exclusion of these molecules from the probabilistic treatment of pyelonephritis and prostatitis in our region. Resistance to norfloxacin in enterobacteria isolates was 38%. Our data remain higher than those reported by European and American studies and lower than those reported by Chinese studies.[18-20] Acquired resistance to these antibiotics results from a combination of several mechanisms: chromosomal, plasmid (*qnr* genes, aac(6')-Ib-cr, etc).[13] This increasing resistance to fluoroquinolones will lead to the exclusion of these molecules from the probabilistic treatment of simple and complicated cystitis, pyelonephritis and prostatitis. Resistance of enterobacterial isolates to trimethoprim-sulfamethoxazole was 36%. Our data are higher than European data (17.8%).[20] This molecule is recommended as first-line treatment according to American and Scottish recommendations and as second-line treatment according to French recommendations. The increase in the rate of resistance to this molecule will lead to its elimination from the probabilistic treatment of urinary tract infections. In our study, the rates of resistance to gentamicin and amikacin were 6% and 0% respectively. These data are similar to those reported by other national and international studies.[18,21] These molecules still display a good sensitivity, which is why it is interesting to use them in combination with other molecules such as third generation cephalosporins, fluoroquinolones, or even carbapenems in the treatment of severe urinary infections. ESBL production will have an impact on resistance to other classes of antibiotics, notably fluoroquinolones, aminoglycosides and cotrimoxazoles. In our study, ESBL isolates showed higher rates of resistance to fluoroquinolones (79%), trimethoprim-sulfamethoxazole (65%), gentamicin (38%), and amikacin (8%) compared to Enterobacteriaceae isolates not producing ESBL for these same antibiotics. Several studies have reported a frequent association between genetic determinants of *qnr* and ESBL.[22] In our series, resistance to imipenem was noted in 4 isolates of *Enterobacter cloacae*. This result is similar to local data and lower than Asian data (4.4%).[4,23]

Besides the above-mentioned antibiotics, which have a good diffusion in the urinary tract and a high impact on the intestinal microbiota (beta-lactams, fluoroquinolones and trimethoprim-sulfamethoxazole), we have fosfomycin and nitrofurantoin which showed low resistance rates even for ESBL isolates and have a low impact on the intestinal microbiota. In our study, the resistance to nitrofurantoin was 9%. This encourages the prescription of this molecule as first-line treatment for simple and complicated acute cystitis in our region. Multicenter studies have shown the value of nitrofurantoin in the management of simple or complicated ESBL cystitis.[5]

Fosfomycin is the most recommended first-line antibiotic for uncomplicated cystitis according to the latest recommendations of the French society of infectious pathology.[5] In our study, the resistance rate was 1%. This rate shows the interest of prescribing fosfomycin in single dose as a probabilistic treatment for simple acute cystitis. Different studies have shown a good efficacy of multidose fosfomycin on ESBL strains.[5] In our study, univariate analysis showed that male sex (p=0.001) and age >65 years were associated with multidrug-resistant community urinary tract infection. Age >50 years and male sex have been reported as risk factors for *E. coli* ESBL urinary tract infection by several studies.[24] Previous hospitalization was associated with multidrug-resistant urinary tract infection in our study. Our data come to consolidate the data in the literature.[24] Antibiotic use within 6 months was strongly associated with multidrug-resistant

urinary tract infection. Larramedy S et al. reported that prior antibiotic use was the most frequently identified risk factor for *E. coli* ESBL urinary tract infection.[24] Urban geographical origin was associated with multidrug-resistant community-acquired urinary tract infection in our series. Multivariate analysis by logistic regression showed that age >65 years, previous hospitalization within 3 months and antibiotic therapy within 6 months were significantly associated to multidrug-resistant community-acquired urinary tract infection. Other risk factors studied in our study such as geographic origin, female gender, diabetes, recurrent urinary tract infections, pregnancy, renal failure and neoplasm were not related to community-acquired urinary tract infection in our study. Larramedy S et al. reported that diabetes was a moderate risk factor for *E. coli* ESBL urinary tract infection.[24]

Our study uses retrospective data from a single hospital with a small sample size and therefore these results should not be generalized.

Similar studies should be conducted in all Moroccan hospitals to accurately determine the prevalence of multidrug-resistant community-acquired urinary tract infections and associated risk factors.

Conclusions

Our results showed a high rate of MDR, which encourages us to be more cautious when prescribing empirical treatment. Fosfomycin and nitrofurantoin have maintained a good sensitivity even in ESBL urinary tract infections and we should therefore encourage the prescription of these two molecules as first line in uncomplicated cystitis. We also found that age over 65 years, hospitalization in the previous 3 months and antibiotic therapy in the previous 6 months are risk factors associated with the acquisition of multidrug-resistant community-acquired urinary tract infections. These results highlight the need for a bacterial resistance surveillance system, first at the local level and then at the national level, to limit the emergence of MDR in the community.

Authors' contributions statement: All authors have made substantial contributions. ME conceived, determined and designed the study. EB, YM, ELB and YB acquired clinical and biological data. EB and ME analysed the data. EB, YM and ME presented and interpreted graphical data. EB and ME drafted, corrected and edited the manuscript. AM, YB and MC performed the linguistic review and critically revised the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

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Availability of data and materials: The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate: Requests for authorization to conduct the study and for collaboration in the research were addressed to the hospital director and the chief of the diagnostic center. As this was a retrospective study, in which the investigator had no contact with the patients, informed consent was not required. However, anonymity and confidentiality were respected for all study data.

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