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Original article

Antimicrobial susceptibility of urinary *Klebsiella pneumoniae* and the emergence of carbapenem-resistant strains: A retrospective study from a university hospital in Morocco, North Africa

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Abstract
Introduction: Urinary tract infections (UTIs) due to multi-drug resistant *Klebsiella pneumoniae* (*K. pneumoniae*) strains are increasing worldwide and have become a major public health problem.
Objectives: The aim of this study was to determine the current and local antimicrobial susceptibility of urinary *K. pneumoniae* isolated from inpatients and outpatients in a university hospital.
Subjects and methods: A retrospective study was carried out, covering a 3-year period from January 2010 to December 2012. It focused on all the *K. pneumoniae* strains isolated from the urine samples analyzed at the microbiology laboratory of the Avicenne Teaching Hospital, Marrakech, Morocco, North Africa.
Results: K. pneumoniae represented 22% of all the urinary *Enterobacteriaceae* isolated during the study period. The bacterial resistance rates of *K. pneumoniae* isolates not producing extended-spectrum β-lactamase (ESBL) were as follows: trimethoprim–sulfamethoxazole "T/S" (61%), amoxicillin/clavulanic acid (51%), ciprofloxacin (32%), gentamicin (21%) and amikacin (11%). ESBL-producing *K. pneumoniae* strains accounted for 25.5% of all the urinary *K. pneumoniae* isolates and showed resistance to T/S (89%), gentamicin (89%), ciprofloxacin (84%) and amikacin (50%). For the first time in our region, we also noted the emergence of carbapenem-resistant strains that accounted for 7% of all the urinary ESBL-producing *K. pneumoniae* isolates.

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Conclusions: Antimicrobial susceptibility testing of urinary *K. pneumoniae* isolates showed a significantly high resistance to commonly used antimicrobial agents. These data highlight the need for regular surveillance of microbial resistance to improve infection control and guide the use of antimicrobial agents.

Introduction

Urinary tract infection (UTI) is a very common reason for consultation and antibiotic prescription in current practice [1,2]. Excessive and/or inappropriate use of antibiotics in treating UTIs is responsible for the emergence and spread of multi-drug resistant urinary bacteria. UTIs caused by multidrug-resistant *Klebsiella pneumoniae* isolates are a major public health problem, since the efficacy of many antimicrobial agents has been restricted, thus reducing the therapeutic options significantly and making the provision of an appropriate antimicrobial therapy more challenging. In addition, multi-drug resistant bacteria are responsible of urinary tract infections that are hard to treat and this increases the medical costs and the mortality and morbidity rates [3].

The development of drug-resistant pathogens in patients with serious infections such as UTIs has generally been ascribed to the widespread use of antimicrobial agents and the limited availability of infection prevention and control programs. As a result, it is increasingly common to encounter individuals infected with bacterial pathogens that are resistant to almost all currently available antibiotics. Of particular concern in the healthcare setting is the emergence of resistant gram-negative pathogens, including ESBL-producing *K. pneumoniae*. While antibiotic resistance was previously noted mainly in nosocomial UTIs, it is nowadays also frequently observed in community-acquired UTIs [1,2]

Globally, nonsusceptibility of urinary *K. pneumoniae* to commonly used oral and parenteral antimicrobial agents is rapidly increasing and shows large variation temporally and regionally [4]. Empirical antibiotic therapy is based on epidemiological data that are updated and adapted geographically [5]. Thus, it is of great importance for institutions to know the local antibiotic resistance patterns of each region in order to implement suitable infection control measures and develop a rational antibiotic policy with local recommendations for antibiotic use. These surveillance data are also used to assess the effectiveness of the measures taken and to identify new points for intervention to control bacterial resistance.

Unlike most developed countries, we unfortunately do not yet have nationwide surveillance programs for monitoring antimicrobial resistance. However, surveillance studies of bacterial resistance are among the most important measures in terms of controlling the spread of resistant bacteria. Until now, antimicrobial surveillance in Morocco has been limited to a few large hospitals. Initial studies indicated that there has been a significant increase in the prevalence of gram-negative bacteria resistant to the commonly used antimicrobial drugs for the treatment of UTIs. Therefore, the objective of this regional study was to add valuable data that can assist the scientific community in the development of a plan for a rational use of antimicrobial agents in the treatment of UTIs due to *K. pneumoniae*.

Subjects and methods

In this retrospective study, all urine samples processed at the microbiology laboratory of the Avicenne University Hospital (Marrakech, Morocco, North Africa) between January 2010 and December 2012 were analyzed. The Avicenne University Hospital serves wide urban and rural geographic areas in the Marrakech region. During the study period, urine samples were collected from outpatients and inpatients hospitalized in different services of our hospital. In cases where multiple urine cultures from the same patient were positive for the same organism only the first episode was reviewed and recorded.

Urinary *K. pneumoniae* isolates were identified to the species level and tested for antimicrobial susceptibility using custom MicroScan Walkaway dehydrated broth microdilution panels (MicroScan®, Sacramento, CA, USA) according to the guidelines of the Antibiogram Committee of the French Society of Microbiology (CA-SFM) [6]. *K. pneumoniae* strains were classified as ESBL producers when there was at least a significant reduction (i.e., $\geq$ three-fold reduction) in the minimum inhibitory concentration (MIC) value of the tested antibiotic (i.e. ceftazidime or cefotaxime) in combination with clavulanic acid as compared to the MIC value of that antibiotic tested alone.

ESBL production was confirmed by the double-disk synergy test "DDST". This phenotypic testing is based on the demonstration of a synergy image between amoxicillin/clavulanic acid (20/10 mg) and cefotaxime (30 mg), ceftazidime (30 mg) aztreonam (30 mg) and cefepime according to the CA-SFM guidelines [6].

The MICs of carbapenems were determined using the agar dilution method in accordance with the CA-SFM guidelines [6]. The isolates showing nonsusceptibility to either ertapenem or imipenem (MICs of $\geq$2 μg/mL) were tested for the production of carbapenemases using the modified Hodge test (MHT) (Fig. 1).

Results

During the study period, a total of 1472 *Enterobacteriaceae* isolates were obtained from culture specimens of patients diagnosed with UTI. The *Enterobacteriaceae* encountered most frequently were *Escherichia coli* (63%, $n = 924$) and *K. pneumoniae* (22%; $n = 321$) (Fig. 2). 263 (82%) strains of *K. pneumoniae* were isolated from community-acquired UTIs.

The antimicrobial resistance patterns of non-ESBL producing *K. pneumoniae* isolates and ESBL-producing *K. pneumoniae* isolates are presented in Table 1. During the study period, the percentage of *K. pneumoniae* strains resistant to third-generation cephalosporins due to ESBL production increased progressively from 18.2% in 2010 to 29.4% in 2011 and 34.1% in 2012 (Overall 25.5%).

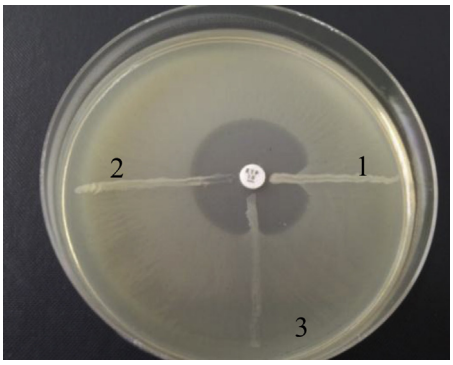

Figure 1 The MHT performed on a 100 mm Mueller-Hinton agar plate. (1) *K. pneumoniae*, positive result; (2) *K. pneumoniae*, negative result and (3) a clinical *K. pneumoniae* isolate, positive result.

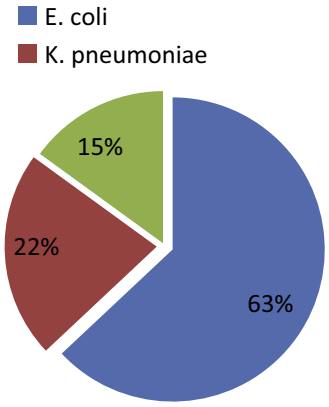

Figure 2 Distribution of *Enterobacteriaceae* species causing UTIs.

Among the urinary ESBL-producing *K. pneumoniae* isolates ($n = 82$), six strains (7%) showed a cross-resistance to carbapenems, which indicates an emergence of carbapenem-resistant strains of *K. pneumoniae*.

Table 1 Antimicrobial resistance rates among Non-ESBL producing *K. pneumoniae* strains and ESBL-producing *K. pneumoniae strains* isolated patients with UTI between January 2010 and December 2012.

Tested antibiotic	ESBL producing *K. pneumoniae* strains (%)	Non-ESBL producing *K. pneumoniae* strains (%)
Aug	100	51
T/S	89	61
Gm	89	21
Cp	84	32
Ak	50	11
Fd	24	13
Fos	6	8
Etp	7	0
Imp	7	0

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

Discussion

UTI is one of the most common infectious diseases [7]. *Escherichia coli* and *K. pneumoniae* have been reported to be the most common organisms causing UTI. We studied the diversity of urinary *Enterobacteriaceae* isolated at the Avicenne Teaching Hospital (Marrakech, Morocco) from 2010 to 2012. As expected, our study showed that *E. coli* and *K. pneumoniae* were implicated in 85% of UTIs due to *Enterobacteriaceae*. This is demonstrated by the prevalence of these two pathogens in the epidemiology of both nosocomial and community-acquired UTIs. Although *E. coli* is a more common cause of UTIs (63%) than *K. pneumoniae* (22%), the latter creates a dilemma for clinicians because of the multi-drug resistance expressed by this pathogen [8]. Therefore, it is often a focus of epidemiologic resistance studies [9].

The prevalence of *Klebsiella* species in this study (22%) is almost the same as the percentages reported in Ethiopia (19–21%) [10] and Cameroon (18.5%) [11]. In Morocco, urinary *K. pneumoniae* was isolated in 10% and 28% of the urine samples in the Meknes [12] and Rabat [13] regions, respectively.

Over the past two decades there has been a wide use of extended broad-spectrum antimicrobial agents to meet the emerging challenge of treating UTIs due to gram-negative bacilli. However, these microbes have developed multiple antimicrobial resistance mechanisms, including enhanced drug efflux, alterations of the drug target and the production of plasmid-mediated β-lactamases [14]. The typical characteristic of antimicrobial resistance is that there are often great differences temporally and regionally [4,15].

Antimicrobial susceptibility testing has shown variable levels of resistance to the tested antibiotics in the treatment of UTIs. Moreover, urinary *K. pneumoniae* isolates have, in general, high rates of resistance to the commonly used antimicrobial agents. All *K. pneumoniae* isolates are naturally resistant to amoxicillin and ampicillin, due to a constitutively expressed chromosomal class-A β-lactamase [16].

The acquisition of resistance to amoxicillin–clavulanic acid (AMC) is a global phenomenon showing widely varying occurrence rates. In the Marrakech region, antimicrobial resistance of urinary *K. pneumoniae* isolates to AMC has been reported to be similar to the resistance rates reported in the Rabat region (Morocco) [13] and in Algeria (50%) [17]. In Tunisia, nonsusceptibility to AMC was almost two times lower (23.7%), as reported in the literature [18].

Trimethoprim–sulfamethoxazole (T/S), used extensively in general practice, was first introduced as a combination drug that inhibits bacterial production of folate, causing a bacteriostatic effect. The frequent use of this antimicrobial agent for the treatment of community-acquired UTIs has led to higher resistance levels. In our study, antimicrobial non-susceptibility to T/S (61%) displayed by urinary *K. pneumoniae* isolates was similar to that reported in Algeria (63.2%) [17]. The high rates of resistance to T/S currently reported in the Marrakech region and in many countries confirm that this antibiotic should no longer be used as a first-line treatment of uncomplicated UTIs.

Urinary *K. pneumoniae* isolates show intrinsic sensitivity to fluoroquinolones. Ciprofloxacin, an orally well absorbed quinolone, is commonly used for empirical UTI treatment. Our study showed

a resistance rate to Ciprofloxacin of 32%. The resistance rate of urinary *K. pneumoniae* to ciprofloxacin was 33% in the Rabat region (Morocco) [13]. Because of treatment failure with routine drugs, fluoroquinolones, such as ciprofloxacin, have been used as an alternative medication, and this might be responsible for the high nonsusceptibility to quinolones in urinary *K. pneumoniae* isolates. Acquired resistance to fluoroquinolones results from a combination of several mechanisms including a decrease in membrane permeability, overexpression of efflux systems and resistance by mutations of the topoisomerase in the quinolone-resistance determining region [19].

With growing resistance of urinary *Enterobacteriaceae* to the commonly used antimicrobial agents, nitrofurantoin and fosfomycin have become increasingly important in the treatment of UTIs. They are known to have less potential for promoting resistance and therefore should be used preferentially. In this study, the susceptibility of *K. pneumoniae* isolates to amikacin, nitrofurantoin and fosfomycin was the lowest with regard to the non-carbapenem antimicrobial agents tested.

One major concern associated with urinary *Enterobacteriaceae* is the emergence of ESBL-producing strains. These strains, reported for the first time in Germany in 1983, confer resistance to all β-lactam antimicrobial agents except cephamycins and carbapenems [20]. Over the last two decades, several studies have reported epidemic outbreaks of ESBL-producing *Enterobacteriaceae* [21].

Although ESBL production has been reported in a variety of gram-negative rods, *K. pneumoniae* is one of the organisms most likely to produce ESBL. The frequency of ESBL production varies substantially from region to region and is increasing significantly. In the Marrakech region, the incidence of ESBL-producing urinary *Enterobacteriaceae* strains increased from 7% in 2008 to 13% in 2012 [22]. This rapidly rising prevalence of ESBL production among *Enterobacteriaceae* is the result of selection pressure due to massive prescription and often misuse of broad-spectrum antibiotics, including cephalosporins, both in hospitals and the community. In our study, the prevalence of ESBL-producing *K. pneumoniae* isolates was 25.5% of all clinical urinary *K. pneumoniae*. Other studies reported a prevalence of 20.2% in Tunisia [18], of 26% in France [23] and of 20.8% in Spain [24].

The high incidence of ESBL-producing *K. pneumoniae* can mainly be explained by the spread of these multi-drug resistant strains. However, only molecular characterization of the ESBL-encoding genes can prove clone association and relatedness of our ESBL-producing *K. pneumoniae* isolates. The class A ESBLs, TEM, SHV and CTX types are the most widespread and clinically relevant worldwide [25]. In Morocco, ESBL-producing *Enterobacteriaceae* have been isolated in different hospitals [12,22], and the ESBL genes detected in Morocco were bla_{TEM}, bla_{SHV}, bla_{DHA} and bla_{OXA} types [26].

The genes encoding ESBL enzymes are generally plasmid-mediated, and additional resistance determinants to aminoglycosides, fluoroquinolones and T/S are often co-transferred on the same plasmid [27]. Furthermore, many of the ESBL isolates express cross-resistance between antimicrobial agents. Therefore, they pose a significant therapeutic challenge to both clinicians and clinical microbiologists.

Antimicrobial resistance testing showed a very important variety when comparing ESBL and non-ESBL producing strains of *K. pneumoniae*. In this study, all ESBL-producing *K. pneumoniae* showed a high level of resistance (84–89%) to ciprofloxacin, gentamicin and T/S. For example, the resistance of ESBL-producing *K. pneumoniae* to ciprofloxacin in this study (84%) was similar to the resistance rate reported in Rabat (capital of Morroco) (85%) [13] and higher than the one reported in Tunisia (67.5%) [18]. The clinical relevance of ESBLs has been well documented by numerous published reports describing clinical failure with the use of commonly used antimicrobial agents [28]. Thus, the choice of antimicrobial agents effective against ESBL-producing organisms is currently very limited.

ESBL production by *K. pneumoniae* isolates is a well-recognized problem and explains the high resistance rates to commonly used antimicrobial agents. Due to this steady decrease in susceptibility to non-carbapenem antibiotics over time, increased carbapenem consumption has been subsequently reported and speculated to be associated with an increasing resistance of *K. pneumoniae* isolates to carbapenems. These antimicrobial agents are considered the treatment of choice for serious infections caused by ESBL-producing and/or AmpC β-lactamase-producing *Enterobacteriaceae* because of their high stability to β-lactamase hydrolysis and the relatively high susceptibility of ESBL producers to carbapenems [29].

For the first time in the region of Marrakech, we have noticed the emergence of carbapenem-resistant *K. pneumoniae* strains. Among the urinary ESBL-producing *K. pneumoniae* isolates ($n = 82$), six strains (7%) showed cross-resistance to both ertapenem and imipenem, which may suggest clonal spread of ESBL-producing strains highly resistant to group I and group II carbapenems. Although a high susceptibility to carbapenem has been demonstrated worldwide, it is noteworthy that since the emergence of carbapenem-resistant organisms the effectiveness of carbapenem therapy is speculated to become more limited. Recently, in Rabat city (Morocco), two isolates of carbapenem-resistant *K. pneumoniae* were obtained from the same patient, with one isolate harboring plasmid-encoded bla-(OXA-48) and the other the bla-(OXA-1) gene [30]. In addition, Barguigua et al. [26] showed the trends of the emergence of carbapenem-resistant *Enterobacteriaceae* in the Moroccan community setting with carriage of resistance genes with clinical relevance co-expressed with bla_{OXA-48} and bla_{IMP-1}.

Avoiding infections reduces the amount of antibiotics to be used and reduces also the likelihood that resistance will develop during therapy. Moreover, promoting hand hygiene and the use of antibiotics as directed and only when necessary are important measures to prevent the spread of drug-resistant infections. Besides, there is an urgent need in Moroccan hospitals to implement prevention and control programs to reduce the spread of drug-resistant pathogens. Updates of trends in the use of antimicrobial agents in hospitals are crucial data for clinical care management and for improving the habits of antibiotic prescribing.

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Competing interests

None declared.

Ethical approval

Not required.

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