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Original Research Article

Antibiotic Resistance Pattern of Uropathogenic *Escherichia coli* Identified in a Healthcare Laboratory (Morocco)

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ABSTRACT

Urinary tract infection is one of the most common infectious diseases worldwide. This study aimed to determine the frequency of Urinary tract infections, identify the microorganisms involved, and determine the antibiotic resistance pattern of *Escherichia coli*. Conventional biochemical techniques identified isolates. The antibiogram was produced using the Muller-Hinton agar disc diffusion method according to the recommendations of the Antibiotic Committee of the French Microbiology Society. During the study period, 21. 4% of the samples were identified as positive. The microorganisms isolated were: *Escherichia coli* (47%), *Klebsiella pneumoniae* (19%) followed by *Trichomonas vaginalis* (9%), *Candida albicans*, and *Pseudomonas* sp (8%). Finally, *Staphylococcus aureus* and *Proteus rettgeri* represent 4% of isolates. The dominant resistance of *E.coli* was to amoxicillin and ticarcillin (55%) followed by cefalotine (39%). Resistance to fluoroquinolones was 27% (norfloxacin). The amikacin and gentamicin resistance rates were 12.5%, and 10.5% respectively. The resistance to the third-generation cephalosporins (ceftazidime, cefotaxime) was 10.5%. *E. coli* were resistant to nitrofurantoin and fosfomycin with percentages of 10.5%, and 6.6% respectively. However, the resistance to imipenem does not exceed 2%. Increasing rates of *E.coli* antibiotic resistance indicate that careful monitoring of their use is necessary.

Keywords: Resistance; Antibiotics; *Escherichia coli*; Urinary Tract Infection

Introduction

Urinary tract infections (UTIs) affect about 150 million people worldwide.[1] In the United States, 10.5 million people consult ambulatory services for symptoms of UTI, and 2-3 million visit emergency departments.[2,3] In France, UTIs represent the second reason for consultation in ambulatory care[4] and emergency services.[5] In Morocco, urinary tract infections are the main reason for hospital consultations.[6] Generally, most infections are acute and uncomplicated cystitis.[7] Urinary tract infection was the leading cause of hospital-acquired infections according to the 2009-2010 prevalence surveys carried out at the Ibn Sina University Hospital Center in Rabat (Morocco).[8] The germ most often isolated from urinary tract infections is *Escherichia coli* (*E. coli*).[9] To treat these infections and avoid the worsening of the disease, appropriate antibiotic therapy is necessary.

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Nevertheless, the use of antibiotics has created resistance of uropathogenic *E. coli* (UPEC) to antibiotics on a global scale, especially in developing countries.[10] This phenomenon is due to certain factors such as i) the excessive consumption of antibiotics,[11] and ii) the permanent change in *E. coli* genetic.[12] The resistance of *E.coli* to antibiotics notably amoxicillin-clavulanic acid is demonstrated by serval studies.[13,14] Controlling infections due to multi-resistant bacteria such as *E.coli* requires regular monitoring of bacterial resistance and control of the use of antibiotics.[15] In this context, the aims of this study were: i) determine the frequency of UTI, ii) identify the microorganisms involved, iii) determine the antibiotic resistance pattern of *E. coli*.

Material and Methods

This study was conducted at the laboratory of Mohammed V Hospital (Meknes Center of Morocco) (33.9038489, -5.541422). A retrospective study was carried out from 01 January 2016 to 31 December 2017 using the archive of the laboratory. Urine was collected in a sterile vial. Each sample underwent a routine cytobacteriological examination of urine (CBEU) comprising a microscopic examination in a Fast Read® cell making it possible to note the elements possibly present (leukocytes and red cells, epithelial cells, crystals…). The interpretation was based on the criteria defined by Kaas (urinary infection parameters: leukocyturia> 10^4 / mL).[9]

The isolation of the microorganisms required the use of different culture media: Bromcresol Purple Lactose Agar (BIOKAR Diagnostics, Beauvais French), MacConkey Agar (Oxoid Ltd. Basingstoke, UK), and Sabouraud + chloramphenicol for fungi or yeasts (BIOKAR Diagnostics, Beauvais French). Conventional biochemical techniques

were used to identify isolates: Galerie API 20NE® and API 20E® (BioMerieux, ® SA, MARCY I'Etolle, France).

Target Strain Susceptibility Testing

The antibiogram profile of the target bacterial strains used in this study was evaluated against 12 antibiotics belonging to various families including amoxicillin, amoxicillin-clavulanic acid, ticarcillin, cefalotine, ceftazidime, cefotaxime, imipenem, norfloxacin, amikacin, trimethoprim/sulfamethoxazole, gentamicin, fosfomycin and nitrofurantoin (Table 1). All antimicrobials were obtained from Oxoid Ltd. Basingstoke, UK.

The standardized disk-diffusion method was performed as described by the Antibiotic Committee of the French Microbiology.[16] The inoculums have been prepared by a direct colony suspension in sterile physiologic and then adjusted to 0.5 McFarland scale. Mueller–Hinton Agar (Oxoid Ltd., UK) plates were inoculated with the bacterial strains. The commercial antibiotic disks were deposited aseptically on the agar surface. After incubation at 37°C for 16 to 18 h, the diameters of the inhibition zones were measured and the strains were categorized according to the standards.[16]

Data quality control

Culture media were produced in accordance with the guidelines provided by the relevant manufacturer. Using reference strains of *E. coli* (ATCC 25922), the sterility and viability of the media were evaluated.

Analytical statistics

Microsoft Office Excel 2010 (beta version) was used to analyze the data, and Chi-square tests were used to determine whether they were statistically significant. Differences with *p-values* of 0.001, 0.01, and 0.05 were regarded as extremely, very, and significantly significant, respectively.

Table 1: Distribution of antibiotics tested according to antibiotic families

Antibiotics Families		Antibiotics	Dose per disk (µg)
Betalactams	Penicillins	Amoxicillin	20
		Amoxicillin/ clavulanic acid	20/10
	Carboxypenicillins	Ticarcillin	75
	Cephalosporins (First generation)	Cefalotine	30
	Cephalosporins (third generation)	Ceftazidime	10
		Cefotaxime	5
	Carbapenems	Imipenem	10
Fluoroquinolones		Norfloxacin	10
Co-trimoxazole		Trimethoprim/Sulfamethoxazole	1,25/23,75
Aminoglycosides		Amikacin	30
		Gentamicin	10
Phosphonic Acids		Fosfomycin	200
Nitrofurantoin		Nitrofurantoin	100

Results and Discussion

Urinary tract infection is one of the most common infectious diseases worldwide.[17] It is caused by Gram-negative bacteria (*E. coli, Klebsiella pneumoniae*) and Gram-positive bacteria (*Staphylococcus saprophyticus*), as well as by fungi (*Candida albicans*).[17] Uropathogenic *Escherichia coli* (UPEC) is a causative agent in the majority of UTIs.[18] UPECs are a major target of antimicrobial therapy because they affect a large proportion of the population.[19] However, the clinical management of urinary tract infections is complicated by the increase in strains of UPEC resistant to commonly used antimicrobial agents.[18] During the study period, 1500 samples (non-redundant) were analyzed and 21.4% were identified as positive. Among positive cultures, 30% were identified in hospitalized patients (Chi-square 2 = 10.4 df = 1, *p-value* <0.5%). This result could be explained by the fact that CBEU is often prescribed for pre-operative or systematic examinations for pregnant women and diabetics without the presence of clinical signs of UTI This result has been demonstrated by several studies.[9] The positivity rate of samples for women is higher than for men with respective rates of 26% and 20%. Statistically, the distribution of UTI according to gender is highly significant (chi-square = 23.13 df = 1, *p-value* < 0.001). These results could be explained by the anatomical aspect of the female reproductive system (proximity of the anal and vaginal orifices) and physiological (pregnancy).[17] The majority of studies have shown that UTI affects women more than men.[6]

The most isolated microorganisms were *Escherichia coli* (47%), *Klebsiella pneumoniae* (19%), followed by *Trichomonas vaginalis* (9%), *Candida albicans* (8%), and *Pseudomonas* sp (8%). Finally, *Staphylococcus aureus* and *Proteus rettgeri* represent 4% of isolates (Figure 1). Using the chi-square statistical test, *E. coli* is the most frequent germ (chi 2 = 130.3, df = 1, *P-value* <0.001). The most isolated microorganisms are enterobacteria (*E. coli* and *Klebsiella pneumoniae*).[18]

Of the 151 UPEC isolates studied, 83 (55%) were resistant to Amoxicillin, and 77 (51%) were resistant to amoxicillin + clavulanic acid. Bacteria isolated were resistant to ticarcillin and cefalotine with respective rates of 55%, and 39%. While resistance to Imipenem did not exceed 2%. Of the 151 UPEC isolates, 27% were resistant to norfloxacin. The isolates studied were resistant to ceftazidime, cefotaxime, gentamicin, and nitrofurantoin with a percentage of 10.5% (Table 2). These enterobacteria are generally treated with antibiotics of the beta-lactam family (penicillins, cephalosporins, carbapenems).[19] However, *E. coli*, which has been naturally sensitive to antibiotics, over the years, has developed important resistance, limiting the indications of many first-line antibiotics.[10] This study showed that penicillins were the least active antibiotics against *E.coli* (amoxicillin (55%), amoxicillin-clavulanic acid (51%)). The resistance of UPEC to amoxicillin-clavulanic acid is higher in developing countries (48–83%) compared to developed countries (3.1–40%).[13] Several UPEC isolates are resistant to amoxicillin-clavulanic acid.[14] Studies in Morocco have shown that the resistance rate of uropathogenic *E. coli* to amoxicillin or amoxicillin-clavulanic acid is between 50 and 70%.[20] A high resistance rate was observed for ticarcillin (55%). Resistance to this antibiotic has been noted by several studies.[21] The spread of resistance to ticarcillin would be explained by the excessive consumption of these antibiotics.[22]

Table 2: Antibiotic resistance among 151 UPEC isolates (n=151)

Antibiotics	n (%)
Amoxicillin (AM)	83 (55)
Amoxicillin clavulanic acid (AMC)	77 (51)
Ticarcillin (TIC)	83 (55)
Cefalotine (CF)	59 (39)
Ceftazidime (CAZ)	16 (10.5)
Cefotaxime (CTX)	16 (10.5)
Imipenem (IPM)	3 (2)
Trimethoprim/Sulfamethoxazole (SXT)	83(55)
Norfloxacin (NOR)	41 (27)
Amikacin	19 (12.5)
Gentamicin (GEN)	16 (10.5)
Fosfomycin (FOS)	10 (6.6)
Nitrofurantoin	16 (10.5)

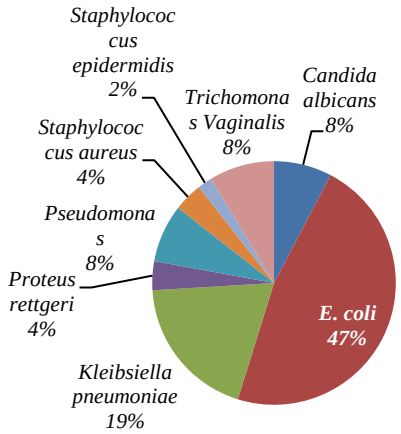

Figure 2: Percentage of microorganisms isolated from CBEU

High rates of resistance of UPEC against these antibiotics limit the recommendation of these molecules to treat UTI due to *E. coli*.

Of the 151 UPEC isolates studied, 39% were resistant to cefalotine (First-generation Cephalosporin), 10.5% to ceftazidime and cefotaxime (Third-generation Cephalosporin). UPEC strains are also susceptible to cefotaxime.[23] In Europe, UPEC's resistance against third-generation cephalosporins reached 11.8%.[24] UPEC isolated from patients in England were found to be resistant to third-generation cephalosporins (13.8% for cefotaxime, 21.3% for ceftazidime).[25] Other research demonstrated that the resistance rate to ceftazidime was >50% in Iran[26] and 21% in Morocco.[20] Carbapenems remain the most powerful family of antibiotics against *E. coli* and for treating urinary tract infections.[27] In this study, resistance against imipenem does not exceed 2%. Several studies have found *E. coli* sensitive against imipenem.[28]

The epidemiological situation of resistance of *E. coli* strains to fluoroquinolones remains variable, with 70% in China,[29] and 18.6% in France.[30] In this study, 27% of *E. coli* isolates were resistant to norfloxacin. High rates of resistance against norfloxacin were noted in Casablanca between 2004 and 2008 (31.2% to 36.6%). [7]

According to this study, the resistance against gentamicin and amikacin of *E.coli* isolated was 10.5%, and 12.5%, respectively. In Pakistan, the resistance of *E. coli* against gentamicin and amikacin remained at 29% and 4%.[31] In Mexico, the resistance rates to gentamicin and amikacin were 28.2%, and 10%, respectively.[32] The rate of gentamicin resistance was 63% in China,[29] 24.6% in Tunisia,[14] and 17% in Morocco.[20]

The isolates studied were resistant to nitrofurantoin and fosfomycin with respective percentages of 10.5%, and 6.6 %. In European countries, the percentage of nitrofurantoin-resistant UPEC isolated from patients ranged from 3% to 3.8%.[33,34] Benhiba et *al.* (2015) have shown that *E. coli* has been resistant to the main families of antibiotics except for fosfomycin and nitrofurantoin.[35] Therefore, it recommended using these antibiotics as first-line therapy of UPEC.[13]

Conclusion

The findings of this study reveal that the most common causative agents of UTIs were *E. coli*. These bacteria showed high levels of antimicrobial resistance to commonly prescribed drugs such as amoxicillin, AMC, norfloxacin, and SXT followed by first-generation cephalosporins (cefalotine). However, *E.coli* remains relatively sensitive to ceftazidime, cefotaxime (third-generation cephalosporins), nitrofurantoin, amikacin, and gentamicin. In this study, *E. coli* are very sensitive to fosfomycin, and imipenem.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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