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Antibiotic resistance profile of *Escherichia Coli* isolated from patients with urinary tract infections

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Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections whether they are community or hospital acquired. Several risk factors are associated with UTIs, such as, amongothers, sex, previous UTIs, vaginal infections, and genetic susceptibility. In the present study, weanalyzed the resistance profile of *Escherichia coli* which is the bacterium frequently associated with urinary tract infections. *E. coli* strains were isolated by culture from urine from 150 episodes of urinary tract infections. Our results showed that among the 150 episodes tested, 102 were from females (69%) and 45 were from males (31%). The antibiogram results showed that *E. coli* strains exhibited high resistance against ampicillin and amoxicillin because these two antibiotics are the most widely used in the medical field. Imipenem and aminoglycosides, on the other hand, remain the most active molecules. Nevertheless, it is prudent to use these antibiotics, in order to reduce the risk of developing resistance against them.

Key words: urinary tract infections, UTIs, antibiotic resistance, *E. coli*,

1. Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections in both community and hospital facilities. Urinary tract infections vary in severity and can affect all patients regardless of age (Bergogne-Bérézin, 2006). They come in the second rang of reasons for consultation and prescription of antibiotics after respiratory infections (Akpabie & Prieur, 2001). More 150 million cases of urinary tract infections are diagnosed each year in the world (Lavigne et *al.*, 2005). Overall, we estimated that almost half of women and 12% of men will suffer from at least one urinary tract infection during their lifetime, and that a quarter of these people will have the recurrent form of the disease (Brumbaugh et *al.*, 2013). These infections constitute a real public health problem due to the additional cost linked to the care they cause (Elfane et *al.*, 2016). Several risk factors are associated to UTI, including gender, previous UI, sexual activity, vaginal infection, diabetes obesity, and genetic susceptibility (Foxman, 2014; Hannan, 2012). Uncomplicated urinary tract infection is commonly seen in patients with a healthy urinary system and without using medical devices, which is often the case for outpatient (community acquired infections) (Hooton, 2012; Lichtenberger & Hooton, 2008; Mann et *al.*, 2017). The diagnosis of urinary tract infection is based on the presence of suggestive clinical signs and the existence of significant bacteriuria and leukocyturia. Cytobacteriological examination of urine (CBEU) is the gold standard that allowsconfirmation of infection by identifying and isolating the causative agent mostly followed bydetermination of the sensitivity of isolated bacteria to antibiotics. Most UTIs are particularlycaused by bacteria, the most common of which belonged to the *Enterobacteriaceae* family(*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Citrobacter* and *Enterobacter*) with a predominance of *E. coli*. Other bacteria such as *Streptococcus* and *Enterococcusfaecalis* may also be detected (Asadi-Karam et *al.*, 2019). Antibiotics were considered to bepowerful weapons that could eradicate infectious diseases of bacterial origin (Monnet D L, 2000). Routine treatment of UTIs relies on the use of antibiotics such as β-lactams, trimethoprim, nitrofurantoin and quinolones in many countries (Asadi-Karam et *al.*, 2019). Unfortunately, the widespread and misuse of these antibiotics has led to an increase in the rate of resistance to these antibiotics and such resistance remains to date a major public health problem. The increasing rate of antibiotic resistance among uropathogens, particularly among *Escherichia coli* and *Klebsiella pneumoniae* as the most common etiological agents of urinary tract infections, causes difficulty in choosing an adequate empirical therapy and treatment success (Arana et *al.*, 2017). Moreover, the widespread use of antibiotics imposes strong selection pressure for the development of antibiotic resistance (Yoneyama & Katsumata, 2006). Herein, we analyzed the resistance profile of *E. coli* which is the bacterium frequently associated with urinary tract infections. *E. coli* strains were isolated by CBEU test from urine retrieved from episodes of urinary tract infections.

2. Materials and methods

2.1. Patients and specimens

In total, our study included episodes of community urinary tract infections collected from 150 patients for whom *E. coli* strains were isolated from theirs urines by CBEU. All urines specimens were received and different analyses were carried out at Mirouh medical laboratory which is localized in Ferdjioua, Mila, in the northeastern of Algeria.

2.2. Cytobacteriological testing

The microbiological diagnosis was based on the cytobacriological urinary examination of the urine. The urine is collected from the first urination in the morning in order to obtain urine that has stayed for a long time (at least 3 to 4 hours) in the bladder. After evacuation of the first 20ml of the urine, the following 20ml are collected in a sterile tube (Gonthier, 2000). The urine is seeded on an agar medium. For these patients, CHROMagar^{tm} Orientation culture medium was used. The cultures were then incubated at 37°C for 18 to 24 hours. Phonotypical identification was carried out by using VITEK 2 system.

2.3. Antibiotic resistance testing

The antibiotic susceptibility testing is a test that allows determination of the sensitivity of a microbial strain to a given antibiotic or to a panel of antibiotics. Depending on the results of the testing, the doctor can direct his choice of treatment in order to better adapt it to the pathology (Dupeyron, 2014). Herein, antibiotic susceptibility was performed using the fully VITEK 2 compact 15 automated susceptibility testing system as recommended by the supplier.

2.4. Biochemical, Serological and hematological testing

Biochemical parameters were performed using Abbott Architect C 4000 automaton. Serological and hematological parameters were performed using VIDAS biomérieux (ELFA) automaton, Abbott Architect i1000 sr automaton or Architect C4000 automaton as needed. Cell blood count was performed by flow cytometry on a sysmex XN-350 automaton. All parameters were performed and analyzed according to the recommendations of the manufacturers of the apparatus.

2.5. Statistical analysis

Statistical analysis was performed using Graph-Pad program. Student t-test, chi2 test or Fischer exact test were used as needed. *P* values less than or equal to 0.05 were considered significant.

3. Results and discussion

3.1. Patients sex and clinical data outcomes

Our study included 150 episodes of community-acquired urinary tract infections from which *E. coli* was isolated by culture. Of these episodes, 102 were from women (69%) and 45 were from men (31%) (Figure 1). For all of our patients the average age was 43.07 ± 25.76 years, with domination of young women whose age is below 38 years old (38%).

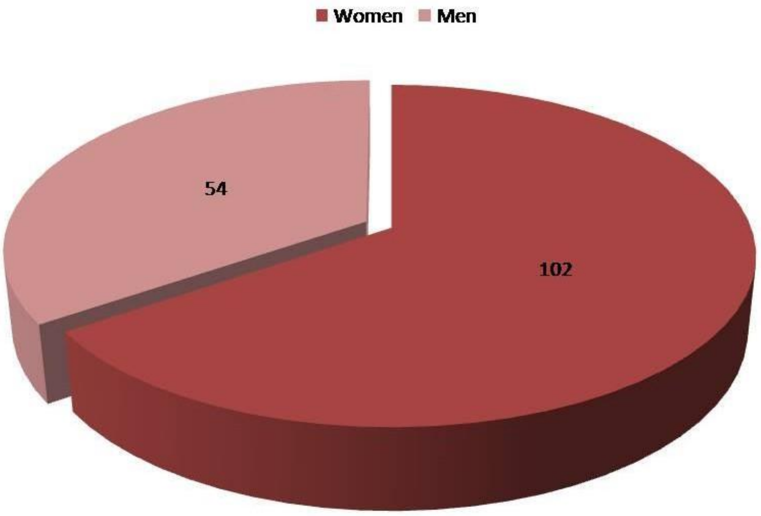

Figure 1. frequency of urinary tract infections depending on sex.

These results suggested that women are more susceptible to UTIs than men, as previously reported (Jeandel & Blain, 2004), which may be due to several risk factors such as the difference of the anatomy of the urinary tract apparatus between men and women. In fact, the urethra is shorter in women and it is located near the vagina and anus (Jeandel & Blain, 2004). Men are relatively protected due to their anatomical structure of the urinary system where the distance from the anus to the urethra can further reduce fecal contamination. Concerning clinical data, our results showed that pregnancy was observed among 6 women while 3 patients were diabetics (Table 1).

Table 1. clinical data of patients

Parameters	Frequency
Age (Years)	43,07 ± 25 ,76
Women	102 (69%)
Men	48 (31%)
Pregnancy	6 (5.88%)

Diabetes	3 (2%)
Rubella IgG	5
Rubella IgM	Negative
Toxoplasmosis IgG	3
Toxoplasmosis IgM	Negative
Hepatitis B virus	Negative
Hepatitis B virus	Negative
HIV	Negative

3.2. Comparative analysis of clinical parameters depending on sex

To identify probable variation in clinical parameters related to UIs between men and women, we performed statistical comparison of available clinical parameters between the two groups (Table 2). The obtained results showed a statistically significant difference particularly in age, as well as in titers of creatininemia that were detected in high averages among men compared to women. Also, while the difference was not statistically significant, urinary leucocytes where detected at high rates among males than females. In contrast, titers of calcemia were in highly detected among women compared to men. Our results showed that for the majority of affected patients whose age ranged between 18 and 38 years were females, which inspires a female predisposition to UTIs in this age group. In women, it has been reported that the frequency increases with age, with 2 peaks, one at the start of sexual activity and the other in the postmenopausal period. In contrast, our results showed that the predisposition to UTIs after 65 years is almost masculine (Table 2). This result agrees with the results of Dia et al., published in 2015, which showed that a high frequency of UTIs was found among people over60 years who were particularly men (Dia et *al.*, 2015). Among men, frequency of UTIs increases after 50 years and it is particularly in relation to prostate pathology.

Table 2: clinical data of patients depending on sex

Parameters	**Women**	**Men**	**P Value**
Patients number	102	48	/
Age (years)	34.13 ±21.40	61.02±25.09	**0.0001**
Blood urea (g/L)	1.24±2.11	0.23±0.08	0.7305
Creatininemia (mg/L)	0.23±0.08	8.84±2.11	**0 .0270**
Calcemia (mg/L)	88±4.24	0	**0.0306**
White blood cells (013/µL)	8.86±2.7	8.83±2.62	0.9490
Platelets (103/µL)	249.64±106.03	259.2±106.03	0.6072

C reactive protein (mg/L)	98.62±91.93	81.96±81.43	0.9032
TSH 3 (µUI/ml)	1.57±1.05	0	/
Urinary leukocytes (/mm3)	1327,20±2483,60	4076.83±15996,50	0.0914
Rubella IgG	5	Negative	/
Rubella IgM	Negative	Negative	/
Toxoplasmosis IgG	3	Negative	/
Toxoplasmosis IgM	Negative	Negative	/
Hepatitis B virus	Negative	Negative	/
Hepatitis B virus	Negative	Negative	/
HIV	Negative	Negative	/

3.3. Resistance profile of *E. coli* to different antibiotics

The study of susceptibility of the 150 stains of *E. coli* against different types of antibiotics showed high levels of resistance to ampicillin (84%) because it is the antibiotic mostly used in the medical institutions. Resistance against Trimethoprim coupled to sulfamethoxazole and amoxicillin coupled to clavulanic acid, but at moderate levels, was also showed (42.67% against Trimethoprim coupled to sulfamethoxazole, and 34.67% amoxicillinc oupled to clavulanic acid) (Figure 2). The high resistance of *E.coli* to ampicillin is concordant with what has been previously reported in other regions in Algeria. Indeed, a study conducted by Benyagoub et *al.* in2013 at Bechar, Algeria, reported that the rate of resistance of *E. coli* against amoxicillin and clavulinic acid, cotrimoxazole and ampicillin was significant (Benyagoub et *al.* in 2013). Moreover, according to a study by Bouzenoune et *al.* in 2007, the resistance to antibiotics of bacteria isolated from UTI at the hospital of Ain M'lila, Algeria, showed that ampicillin has become the least active antibiotic on *E. coli* (Bouzenoune et *al.* 2009). This result has also been confirmed by other studies conducted round the world (Messai et *al.*, 2006; Larabi et *al.*, 2003). Nevertheless, except these antibiotics previously mentioned, our study showed that strains of *E. coli* studied are marked by a high sensitivity to many different antibiotics (Fig. 2). In fact, all of the *E.coli* trains that where tested showed total sensitivity to amikane (100%) and Imipenem (100%). High levels of sensitivity were also observed against Fosfomycin (98.67%), Gentamycin (92%), Ciprofloxacin (86.67%), Cefotaxime (82%), Ceftazidime (82%) and Cefazolin (71.33%) which may be due to the rare use of these antibiotics as previously demonstrated by researchers Kalantar E et *al.* in 2008 (Kalantar et *al.* in 2008). In fact, Imipenem and aminoglycosides remain highly active on *enterobacteriaceae* as well as in others countries such as Tunisia and Turkey, and their use must therefore be favored (Larabi et al., 2003. Yüksel et al., 2006). In contrast, according to the study by Thabet et al. in 2010 carried outat the Aziza Othmana Hospital in Tunis showed high resistance of *E. coli* to antibiotics (Thabetet *al.*, 2010). This high resistance could be explained by the fact that the majority of these patients were hospitalized, and that the large use of antibiotics in the hospital environment, which means that a reasoned antibiotic therapy in hospitals remains highly recommended in order to ovoid development of multi-resistant strains.

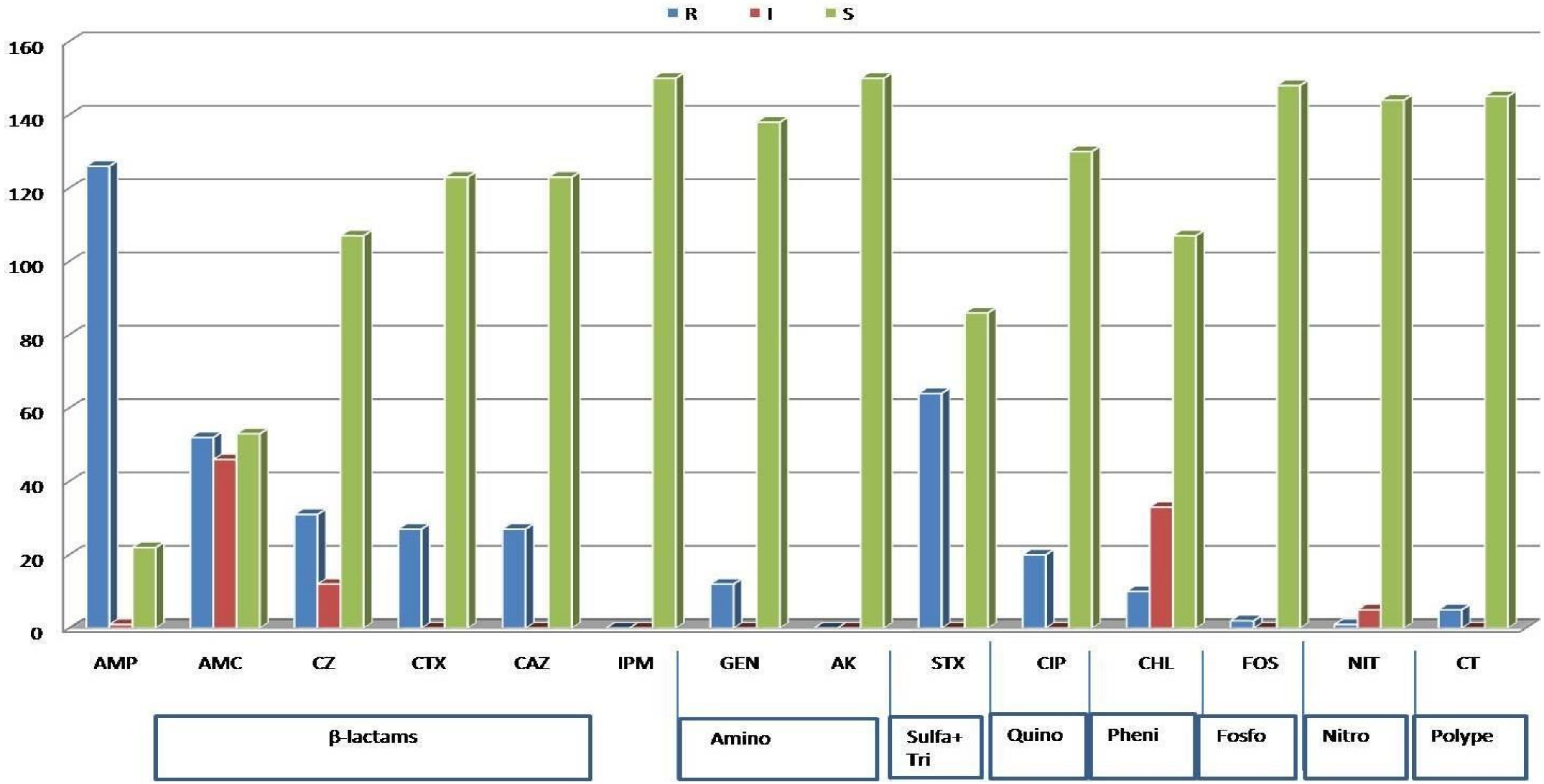

Figure 2: resistance profile of 150 strains of *E. coli* to different antibiotics. AMP= Ampicillin. AMC= Amoxiclav. CZ= Cefazolin. CTX= Cefotaxime. CAZ= Ceftazidime. IPM= Imipenem. GEN= Gentamycin. AK= Amikacin. SXT=Trimethoprim+Sulfamethoxazole. CIP= Ciprofloxacin. CHL= Chloramphenicol. FOS= Fosfomycin. NIT= Nitrofurantoide. CT= Colistin. R, Resistant; I, Intermediate, S, sensitive.

4. Conclusion

E. coli is the most widely recognized extra-intestinal Gram-negative pathogen isolated from urine culture in patients with complicated or uncomplicated UTIs. The bacterium is implicated in 70 to 80% of community-acquired infections as well as in 40-60% of healthcare-associated infections. Furthermore, such a situation has not changed much in recent years and *E. coli* is continuing to rank first among the isolated uropathogens. Moreover, knowledges about the antibiotic resistance profile of this bacterium is a valuable tool for the choice of first-line antibiotic therapy, which needs to be adapted to eradicate the pathogen. Bacteriological diagnosis of urinary tract infections that must be supplemented by aantibiotic sensibility testing for resistance analysis is the most effective mean allowing better therapeutic care and management of patients, and the inadequate use of antibiotics is responsible for a large part of developing resistance against these antibiotics. Additionally, resistance to antibiotics is becoming higher in recent years, reaching worrying levels for some of them. Our study shows that *E. coli* associated to UTIs developed resistance particularly oriented against ampicillin and amoxicillin due to their wide use. However, imipenem and aminoglycosides, especially amikacin, remain, on the other hand, the most active molecules. Nevertheless, it is recommended to avoid excessive use of these molecules, in order to reduce the risk of development resistance against them.

Conflict of interest: none

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