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Antibiotic sensitivity profile of uropathogenic *Escherichia coli* strains at the China-Guinea Friendship Hospital of Kipé in Conakry (Guinea)

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Abstract

Introduction: *Escherichia coli* (*E. coli*) is one of the main bacterial species associated with urinary tract infections. Nowadays, this bacterium is becoming more and more resistant to antibiotics. Objective: The aim of this study was to determine the antibiotic sensitivity profiles of all strains of *E. coli* isolated from urine during the period from September 1st, 2018 to March 13th, 2019 at the Biomedical Laboratory of the China-Guinea Friendship Hospital of Kipé in Conakry.

Materiel and Methods: Cultures were done on different agar media. Bacterial identification, antibiograms and determination of minimum inhibitory concentrations (MIC) were performed on the Vitek 2 Compact 15 automated system.

Results: A total of 66 strains of *E. coli* have been isolated from patients of both sexes. The sex ratio (M/F) was 0.43. The mean age of the patients was 50.83 years. The majority of strains were sensitive to imipenem (96.96%), amikacin (96.96%), ertapenem (94.73%), gentamicin (69.23%), tobramycin (60, 60%), cefoxitin (64.28%), cefotaxime (62.50%), piperacillin/tazobactam (77.4%), amoxicillin/clavulanic acid (50.00%) and nitrofurantoin (87%). In contrast, the majority of strains were resistant to ampicillin (81.81%), cefalotin (62.02%), ticarcillin (88.00%), nalidixic acid (82.75%), ciprofloxacin (56.06%), ofloxacin (56.00%) and combination of trimethoprim/sulfamethoxazole (83.60%), sometimes with high MICs.

Conclusion: Our results show that urinary tract infections due to *E. coli* are more frequently observed in females than in males. Some of these strains studied exhibited multidrug resistance profiles to antibiotics. Among the classes of antibiotics tested, carbapenemes, nitrofurans, aminoglycosides, appear to be more active on *E. coli* uropathogenes in Guinea.

Keywords: Escherichia coli; Antibiotics; Sensitivity; UTI; Kipé/Conakry

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1. Introduction

Escherichia coli is a Gram-negative bacterium belonging to the family of *Enterobacteriaceae*. It is a bacterial species often associated with various infections in humans including the urinary tract infections (UTI's) [1-4]. Likewise, urinary tract infections are among the most common infections found in humans around the world [5]. However, their frequency depends on sex and age [6-8]. The female sex is generally more affected than the male [4, 6, 9] due to the proximity of the anus and the urinary meatus. This facilitates the urethra's access to intestinal bacteria originating from the rectum [10, 11]. The anatomical relationship of the female urethral meatus to the vagina tends to expose it to increased chances of trauma during sexual intercourse [10, 12].

In France, studies have shown that urinary tract infections are the second reason for consulting and prescribing antibiotics in the doctor's office and in emergency services, but they are probably the first cause of bacterial infections in the country [13, 14].

There are an estimated 150 million urinary tract infections per year worldwide [15, 16].

In the United States, urinary tract infections result in approximately 8 million physician visits per year [15, 17].

A study conducted in 2006 by Roos et al., (2006), showed that UTIs affected more than ten million people in Western Europe [18]. Katongole et al. (2020) reported in Uganda that *E. coli* causes 80 to 90% of community urinary tract infections and 30 to 50% of those contracted in hospitals [19, 20].

Escherichia coli (*E. coli*) is considered to be one of the bacterial species most frequently associated with uropathogenic infections in humans [19, 21, 22]. It is the species most involved in asymptomatic urinary tract infections [18].

According to the World Health Organization (WHO), urinary tract infections with *Escherichia coli* (*E. coli*) are by far the most common in the hospital and in the community [5].

This bacterial species is also among those which more frequently include multiresistant bacterial strains (MDRs) to most families of antibiotics [19, 20, 23]. Resistance by production of extended spectrum beta-lactamases (ESBL) is one of the resistance mechanisms most frequently described in uropathogenic *E. coli* as in other species of *Enterobacteriaceae* [2].

In 2018, Makanéra *et al.*, described in Guinea a multidrug resistance in a single strain of *Leclercia adecarboxylata* from urinary tract infections [24]. This species belonging to *Enterobacteriaceae* family showed multi-resistance to most families of antibiotics [24]. The increase of antibiotic resistance and appearance of multi-drug resistant (MDR) pathogens in the course of urinary tract infections is related to high rates of inadequate antibiotic empirical therapies prescribed without the antibiotic susceptibility testing and finally result in an ineffective urinary tract infections treatment [1,25].

The aim of this prospective study was to determine the sensitivity profile of *E. coli* strains isolated from urinary tract infections between September 1^{rst}, 2018 and March 13th, 2019 in the Biomedical Laboratory the China and Guinea Friendship Hospital of Kipé, Conakry (HASIGUI).

2. Material and methods

Study setting: Our study was carried out at the Biomedical Laboratory of the Sino-Guinean Friendship Hospital (HASIGUI) in Kipé.

2.1. Inclusion and non-inclusion criteria

Inclusion criteria were included in this study, all urine samples positive for *Escherichia coli* between September 1st, 2018 to March 13th, 2019 at the Biomedical Laboratory of the China-Guinea Friendship Hospital of Kipé in Conakry.

Non-inclusion criteria: Not included in our study, all urine samples not meeting the inclusion criteria.

Variables: the variables studied group together biological variables (ECBU, antibiogram) epidemiological variables (age, sex) socio-demographic variables (residence, profession, marital status).

Variables: the variables studied include biological variables (identification, antibiogram) epidemiological variables (age, sex) socio-demographic variables (residence, occupation, marital status).

Collection, processing and analysis of data: the data was collected using a survey form developed according to the objectives and variables through the collection of information and samples taken from patients followed by the analysis. Finally, the data collected was entered into the Word 2013 software, processed and analyzed by the Excel xl Slata software.

The results were presented in the form of texts and tables.

2.2. Bacterial identification and antibiograms

The urine sample with a volume of 50ml collected in a sterile vial was subjected to the cytobacteriological examination in the fresh state by observation on photonic binocular microscope (Microscope XS-213, Nanjing BW Optics Co., Ltd., Jiangsu, China). Gram stained smears were also observed on the same photonic binocular microscopy.

Gram-Hücker kits (RAL Diagnostics, Martillac, France) were used for Gram stains. The sample was then cultured on different agar media: nutrient agar (Liofilchem, Roseto DA, Italy), Mac Conkey agar (Biomérieux, Marcy l'Etoile, France) and CLED (Biomérieux, Marcy l'Etoile, France). Incubation was carried out for 18 to 24 hours in the GRP 9080 (Sumsung Laboratory Instrument CO., Ltd, Shanghai, China). Uniform bacterial colonies isolated from the cultures were stained by the Gram method in order to verify their purity, a key step preceding analyzes with the Vitek 2 Compact 15 automated system (Biomérieux, Marcy'Etoile, France). Bacterial identification, antibiograms and determination of minimum inhibitory concentrations (MIC) were carried out using the Vitek 2 compact 15 automated system (Biomérieux, Marcy'Etoile, France). Vitek 2 GN cards were used for identification and Vitek 2 AST-N 233 cards (Biomérieux, Marcy l'Etoile, France) were determined according to Clinical and Laboratory Standards Institute (CLSI + Natural Resistance) Interpretation Criteria and Standards. The Vitek 2 Compact's Advanced Expert System (AES) software compares the MICs of the instrument and the identity of the identified germ to the standard phenotypes of that germ (CLSI + Natural Resistance).

Limitations: The small size of the sample collected during a short study period due to the absence of Urology department (a specialized department).

3. Results

3.1. Epidemiological profile of patients

Table 1 Distribution of 66 patients according to gender.

Gender	Effectif	%
Male	20	30
Female	46	70
Total	66	100

During the study, we collected 66 strains of *E. coli* isolated from patients presented with a urinary tract infection of which 46 (70%) were female and 20 (30%) were male, for a sex ratio of 0.43.

Distribution of patients according to departments shows that the majority of patients included in this study came from the Cardiology department, followed by outpatients (from other health structures in the city of Conakry). The Neurology and Emergency departments occupied the third and fourth position respectively (Figure 1).



Figure 1 Distribution of patients according to the requesting service.

 Table 2 Sociodemographic characteristics of patients.

Age groups (years)	Number	%
≤ 20	2	3
21 - 40	21	32
41 - 60	18	27
61 - 80	23	35
≥ 81	2	3
Total :	66	100
Professions		
Administration officers	12	18
Trade Agents	12	18
Health workers	1	1
Security agents	1	1
Pupils/Students	5	8
Teachers	3	5
Housewives	22	33
Workers	7	11
Peasants	3	5
Total	66	100
Marital Status Married	53	80
Singles	8	12
Divorced	3	5
Widowers	02	3
Total	66	100
Residence		
Kaloum	1	1,5
Dixinn	4	6
Matam	1	1,5
Ratoma	28	43
Matoto	17	26
Others*	15	23
Total	66	100

Others *: patients from other cities in the country (Coyah, Boké, Dubréka, and Kankan)

The 61 to 80 year olds and 21 to 40 year olds were the most affected by urinary tract infection with *E. coli*, respectively 35% and 32%, followed by that of 41 to 60 years (27%) (Table 2). The socio-professional category most affected was that of Housewives 22 (33%), followed by those of sales and administration officers, 12% each (Table 2). As for marital status, the results showed that married couples were the most affected, with 80% of the sample (Table 2). Patients from the municipalities of Ratoma and Matoto were the most affected, respectively 43% and 26%. They are followed by those coming from other cities of Guinea, or 23% (Table 2).

3.2. Antibiotic sensitivity of Escherichia coli strains

Table 3 Breakdown of antibiotics used according to their therapeutic class.

Class	Effective	%
β- lactams	10	56
Aminoglycosides	3	17
Quinolones	3	17
Sulfonamides	1	5
Nitrofurans	1	5
Total	18	100

This table indicates that 5 therapeutic classes are used for antibiotic sensitivity tests and the most used class of antibiotics in our study is that of β -lactams (56%), followed by that of aminoglycosides and quinolones with 17% each one (Table 3).

Table 4 Antibiotic susce	ptibility profile o	of strains of uro	pathogenic <i>E. coli</i>
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Antibiotics	Sensitivity (%)	Intermediate (%)	Resistance (%)
Ampicillin	9,09 (4/44)	4/44 (9,09)	36/44 (81,81)
Cefalotin	7/29 (24,13)	4/29 (13,79)	18/29 (62,06)
Ticarcillin	6/50 (12,00)	0/50 (0,00)	44/50 (88,00)
Piperacillin/tazobactam	47/61 (77,04)	5/61 (8,19)	9/61 (14,75)
Amoxicillin/Clavulanic acid	14/28 (50,00)	9/28 (32,14)	5/28 (17,85)
Cefoxitin	18/28 (64,28)	6/28 (21,42)	4/28 (14,28)
Cefotaxime	15/24 (62,5)	1/24 (4,16)	8/24 (33,33)
Ceftazidime	24/64 (37,5)	4/64 (6,25)	36/64 (56,25)
Ertapenem	36/38 (94,73)	1/38 (2,63)	1/38 (2,63)
Imipeneme	64/66 (96,96)	0/66 (00,00)	2/66 (3,03)
Amikacin	64/66 (96,96)	0/66 (00,00)	2/66 (3,03)
Gentamicin	45/65 (69,23)	0/65 (00,00)	20/65 (30,76)
Tobramycin	40/66 (60,60)	8/66 (12,12)	18/66 (27,27)
Nalidixic Acid	5/29 (17,24)	0/29 (00,00)	24/29 (82,75)
Ciprofloxacin	29/66 (43,93)	0/66 (00,00)	37/66 (56,06)
Ofloxacin	11/25 (44,00)	0/25 (00,00)	14/25 (56,00)
Trimethoprim/sulfamethoxazole	10/61 (16,39)	0/61 (00,00)	51/61 (83,60)
Nitrofurantoin,	34/39 (87,17)	2/39 (5,12)	3/39 (7,69)

Table 4 shows that the majority of strains of *E. coli* were susceptible to imipenem amikacin, ertapenem, gentamicin, cefoxitin and cefotaxime. In contrast, the majority of these strains of *E. coli* were resistant to ticarcillin, ampicillin, trimethoprim / sulfamethoxazole, nalidixic acid, and cefalotin.

Antibiotic	Sensitivity (µg/l)	Antibiotic families	Detected phenotypes
Ampicillin	R (>16)	Bêta-lactams	ESBL + Carbapenemases
Amoxicillin/Clavulanic acid	R (>16)		(metallo or KPC), resistance carbapenems
Ticarcillin	R (>64)		
Piperacillin/tazobactam	R (>64)		
Cefalotin	R (>32)		
Cefoxitin	R (>32)		
Cefotaxime	R (>32)		
Ceftazidime	R (>4)		
Ertapenem	R (>4)		
Imipenem	R (=0,5)		
Amikacine	R (=8)	Aminosides	Resistance GEN TOB NET AMI (AAC(6') + ?)
Gentamicin	R (>8)		
Tobramycine	R (>8)		
Nalidixic acid	R (>16)	Quinolones	Resistance
Ciprofloxacin	R (>2)		
Ofloxacin	R (>4)		
Nitrofurantoine	R (=256)	Furanes	Resistance
Trimethoprime/sulfa-methoxazole	R (>160)	Sulfamides	Resistance

Table 5 Resistance phenotypes detected in one of the strains of uropathogenic E. coli

Table 5 shows the resistance phenotype of one of the strains of *E. coli* to carbapenems determined by the Vitek 2 Compact 15 analyzer was resistance to by production of extended spectrum beta-lactams (ESBLs) and carbapenemase, while resistance to aminoglycosides was AAC type (6) + ?).

4. Discussion

4.1. Socio-demographic characteristics

The distribution of patients by sex showed that the female sex was predominant with 69.69% (46/66) against 30.30% (20/66) for the male sex. The sex ratio (Male / Female) was 0.43 (Table 1). These frequencies are comparable to those reported in Morocco by Ismael in his Doctoral thesis in Medicine, with 56.51% female and 43.49% male [26]. Many other studies relating to uropathogenic *E. coli* maintain that the female sex generally remains predominant because of its anatomical structure, due to the proximity of the anus and the urinary meatus [1,6,9]. Another reasons for lower incidence of uropathogenic *E. coli* in male than female is the antibacterial property of prostatic fluid [6].

The distribution of patients according to age showed that the age group 61-80 years was the most affected (35%) followed by that of 21-40 years (32%) (Table 2). This could be explained by the fact that the frequency of urogenital infections with *E. coli* increases with age. In the literature, this observation had been made by other authors [1,6]. In addition, the 21 to 40 years old age group is among the most sexually active.

Women housewives were the most represented (33%). This category belonging to the female sex is very represented in the country (Table 2).

As for marital status, we notice that married couples were the most affected by UTI, with 80,30% of the sample (Table 2). This can be due to intense sexual intercourse, pregnancy or other situations such as anatomical and physiological factors in women thus promoting the installation of germs [27]. Otherwise, sexual activity is rather normal and frequent among married couples. This is justified by the social and religious condition and traditional context in Guinea.

According to the requesting services (figure 1), it can be said that apart from urological problems, the patients still suffered from other health problems. The cardiology department was the most represented with 38%. Physiotherapy and trauma services are the least represented with 2% each. The high prevalence in the Cardiology department could be explained on the one hand by the fact that there are more patients who were consulted in the Cardiology department compared to other departments and on the other hand, the service of Cardiology most often Cytobacteriological examinations of urine.

4.2. Antibiotic susceptibility of uropathogenic E. coli

4.2.1. Sensitivity of Escherichia coli strains to β -lactams

These results show a high sensitivity of *E. coli* to imipenem (96.96%), ertapenem (94.73%), piperacillin/tazobactam (77.4%), cefotaxime (62.5%) and amoxicillin / clavulanic acid (50.0%). In contrast, strains of *E. coli* generally showed resistance to ticarcillin (88.0%) and ampicillin (81.81%), ceftazidime (56.25%) (Table 4).

Many authors have reported high sensitivity of ESBL producing uropathogenic *E. coli* to carbapenemes (imipeneme and ertapeneme) of uropathogenic ESBL beta-lactamase-producing strains of uropathogenic ESBL [1,9,28]. The susceptibility to carbapenemes of strains of *E. coli* uropathogenes found during this present work is comparable to that reported in Mexico by Ramirez-castillo et al., (2018) who found total susceptibility of all strains of *E. uropathogenic coli* analyzed during their study [9]. Reazi et al. (2015) reported that most uropathogenic ESBL-producing strains of *E. coli* were susceptible to carbapenemes [28]. The beta-lactam sensitivity of the majority of *E. coli* studied in this present work, is globally comparable to that reported by some authors [1,28].

The strains studied in this present work were generally resistant to ampicillin, ticarcillin, ceftazidime is due, on the one hand, to the fact that these molecules (in particular ampicillin) are used in Guinea in an abusive manner, and 'on the other hand by the fact that most of these strains are ESBL producers. Shahbazi *et al.* (2018) has found that higher number of ESBL-producing uropathogenic *E. coli* isolates were resistant to amino glycosides and quinolones when compared to the uropathogenic *E. coli* strains that not produce ESBL [29]. Carbapenems (imipenem and meropenem) represent the best option for the treatment of UTIs caused by ESBL-producing strains [30]. Cephalosporins, penicillins, and monobactams should be used with β -lactamase inhibitors [31].

The frequency of ESBL-producing *E. coli* isolates is different in various parts of the world and sometimes even in various hospitals within the country. In addition to resistance to β -lactam antibiotics, ESBLproducing *E. coli* isolates are also resistant to other antimicrobial agents, such as aminoglycosides, tetracycline, and trimethoprim/sulfamethoxazole [28]. Shahbazi *et al.* (2018) has found that higher number of ESBL-producing UPEC isolates were resistant to amino glycosides and quinolones when compared to the UPEC strains that not produce ESBL [29]. Carbapenems (imipenem and meropenem) represent the best option for the treatment of UTIs caused by ESBL-producing strains [30]. Cephalosporins, penicillins, and monobactams should be used with β -lactamase inhibitors [31].

One of the most prominent and concerning findings in our study is the high resistance to broad spectrum antibiotics such as carbapenems which is in contrast with other studies that reported lower resistance (about 34%) to imipenem and meropenem in India [32], Malaysia [33], Columbia, Saudi Arabia [34], and Iran [28,35-37]. Although we found carbapenems as the most effective agent against the ESBL but the high rate of resistance, in comparison with other studies, is still very concerning. Recently, Alikhani et al. study in Iran showed 75% susceptibility among ESBL pathogens to carbapenems [9]. The main reason for large difference in the rate of resistance among different countries and different regions within the same country is due to the extensive use of broad spectrum antibiotics especially third generation cephalosporins and persistence of the resistant strains in health care facilities. Extensive usage of broad spectrum antibiotics specially third generation cephalosporins was reported by Salehifar *et al.*, [23]. The rate of antibiotics consumption in our setting was significantly higher than other centers [23].

4.2.2. Sensitivity of Escherichia coli Strains to Aminoglycosides

In this table, we have found that the aminoglycosides (Amikacin, Gentamycin and Tobramycin) have been shown to be very active on strains of *E. coli*. Indeed, these strains of *E. coli* were generally sensitive to amikacin (96.96%), gentamicin (69.23%) and tobramycin (60.60%).

These results above are different from those found in India by Shahid et al. (2008) in terms of aminoglycosides antibiotic sensitivity frequencies [38]. Indeed, these authors reported that strains of *E. coli* analyzed in their study showed a sensitivity of 57.1% to amikacin, followed by tobramycin with 38.5% and gentamicin with 31.9%. This shows that these molecules were more active on strains of *E. coli* in this present study than those of these authors. However, in our study the susceptibility frequencies of strains of *E. coli* with aminoglycosides showed amikacin to be more active than gentamicin, and the latter to be more active than tobramycin. Thus, conversely the resistance phenotype detected in strains of *E. coli* were generally lower and the phenotypic resistance detected is the mainly as following: Resistance GEN TOB NET AMI (AAC (6') + ?).

4.2.3. Sensitivity of Escherichia coli strains to Quinolones

The majority of strains of *E. coli* were quite resistant to quinolones. Indeed, this resistance from *E. coli* to quinolones was 82.75% for nalidixic acid, 56.0% for both ciprofloxacin and ofloxacin.

These results reflect those of other work done elsewhere around the world. Indeed, Akaya et al. (2015) reported metadata from 53 studies on strains of uropathogenic *E. coli*, carried out between 2001 and 2011 on quinolones [39]. Compilation of these data showed high resistance of uropathogenic *E. coli* to these molecules.

Indeed, overall resistance of these strains to nalidixic acid, ciprofloxacin, norfloxacin and ofloxacin were 42.3%, 28.2%, 48.5% and 24.1%, respectively [39].

Thus, the resistance observed in strains of uropathogenic *E. coli* isolated at the Sino-Guinean Friendship hospital in Kipé is well above the average resistance observed by these authors. This situation would be favored by the excessive use of quinolones in Guinea, considered to be broad-spectrum antibiotics. They are potent inhibitors of bacterial DNA gyrase and topoiso-merase IV [40]. Resistance to quinolones is on the rise in all bacterial species around the world. The mechanisms of acquired resistance are mainly chromosomal (modification of targets, impermeability/active efflux) while plasmid resistance is frequently detected in *Enterobacteria* such as *E. coli* [1,40].

4.2.4. Sensitivity of Escherichia coli to Nitrofurans

Nitrofurantoin was the only antibiotic molecule representing the Furan class. This molecule was active on the majority of strains of *E. coli* analyzed. In fact, 87.17% of these strains were sensitive to this molecule.

These results are similar to many others reported around the world. Indeed, in 2019, Kot reported thatthe resistance of uropathogenic *E. coli* to nitrofurantoin is very low, favoring its use as a first-line antibacterial agent. Studies conducted by Sanchez et al. (2016) showed that in the United States nitrofurantoin retains a high level of antibiotic activity against *E. coli* isolated from urinary tract infections [1,41]. A comparison of the reports from the period of 2003 to 2012 revealed that resistance of *E. coli* isolates from adults to nitrofurantoin only slightly increased (from 0.7% to 0.9%). Kresken et al., (2016) reported that studies carried out in Germany, Belgium and Spain showed that *E. coli* is usually susceptible to nitrofurantoin. Indeed, the rates of uropathogenic *E. coli* resistance in these countries in the period 2013–2014 were below 1.5% [1,42].

According to the European Association of Urology guidelines [43], nitrofurantoin is recommended for the treatment of uncomplicated cystitis as first-line empiric therapy.

In Argentina and Brasilia uropathogenic *E. coli* isolates are usually susceptible to nitrofuantoin. Indeed, a slightly higher percentage of uropathogenic *E. coli* resistant to nitrofurantoin was observed among isolates from elderly hospitalized patients in Argentina (2.3%) [44]. While in Brazil the rate of uropathogenic *E. coli* isolates resistant to nitrofurantoin was 6.6% [45].

4.2.5. Sensitivity of Escherichia coli to Sulfonamides (trimethoprim/ sulfamethoxazole)

The sulfonamide class was represented by the combination trimethoprim / sulfamethoxazole. This combination of sulfonamides was not very active on the majority of strains of *E. coli* studied. In fact, 83.60% of these strains were resistant to the sulfonamides tested.

This observation has been made beforehand by many other authors. Indeed, in Mexico City, Ramifez-Castillo et al., (2018) reported in their study a high frequency of resistance of strains of *E. coli* to the trimethoprim / sulfamethoxazole combination which was 72.7% [9]. In Iran, Rezai et al. (2015) reported a resistance frequency of 65% of strains of uropathogenic *E. coli* in the combination trimethoprim/sulfamethoxazole [28]. A similar rate of resistance of uropathogenic *E. coli* isolates against Trimethoprim/Sulfamethoxazole (50.6%) was observed in Brazil [45]. These resistance frequencies of *E. coli* in the combination of antibiotics is lower than that found in this present work. The high frequency of uropathogenic *E. coli* to combined antibiotics (trimethoprim and sulfamethoxazole) is believed to be due to the uncontrolled use of this molecule, often used in Guinea during respiratory infections and diarrhea.

5. Conclusion

Our results showed that urogenital infections caused by *E. coli* seem to be more frequently encountered in females than in females. Carbapenems (Imipenem and ertapeneme), nitrofurantoin and aminoglycosides (amikacin, gentamicin and tobramycin) as well as some cephalosporins (piperacillin / tazobactam combination,) seem to be the antibiotics of choice of first line in the treatment of urogenital infections with *E. coli*.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

Statement of informed consent

The informed consent of all patients to participate in the study was acquired and confidentiality was observed throughout the data collection process. The results were used for strictly scientific purposes.

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