

Antibiotic Susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* Strains, Urinary Tract Infections Cases in Bobo-Dioulasso, Burkina Faso

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Abstract

Background: Urinary tract infections are a health issue in community and hospital areas. In order to successfully improve the urinary tract infection treatment, we aimed to study the susceptibility of drugs against bacterial strains from urine. The specimens were from bacteriology laboratory of Centre Muraz, Bobo-Dioulasso, Burkina Faso.

Methods: We received 810 specimens for drug susceptibility test. The identification and susceptibility are performed using the standard method of susceptibility testing (agar disk diffusion), as recommended by the Antibiogram Committee of the French Society for Microbiology.

Results: About 24.07% of tested specimens were positive for at least one pathogenic germ with 58.46% of *E. coli* and 28.2% of *K. pneumoniae*. Most of *K. pneumoniae* strains (94.4%) or *E. coli* strains (86%) were resistant to amoxicillin. The sensibility was only observed with the use of some molecules or combination such as imipenem (100% for *K. pneumoniae* and 95.6% for *E. coli*) or piperacillin + tazobactam (95.7% for *K. pneumoniae* and 98.2% for *E. coli*).

Conclusions: Based on these results, which show the high level of antibiotics resistance by *Enterobacteriaceae* mostly involved in urinary tract infection cases, frequent assessment of antibiotics susceptibility to targeted strains during antibiotic therapy is necessary. There is a need to educate our countries in the use of antibiotics only after antibiotics susceptibility test and to provide them with the need for these tests, in order to not return to a post-antibiotic era, in which common infections can once again kill.

Keywords: *Escherichia coli*; *Klebsiella pneumoniae*; Antibiotics resistance; urinary tract infection; Burkina Faso

Abbreviations

UTI: Urinary Tract Infections; CBEU: Cytobacteriological Examination of the Urine

Introduction

Urinary Tract Infections (UTI) are the most common in community and hospital level in the world [1,2]. Even if they are often seen as ordinary and benign, the UTI complications can disturb kidney function [3]. In pediatrics, they are the most severe bacterial infections with serious long-term consequences [4]. In Bangladesh, the antibiotics prescription for UTI treatment is in the second place of most common use [5]. The main causative agents of UTI are from the Enterobacteriaceae family. In Burkina Faso Ouedraogo/Yugbare and collaborators have identified enterobacteria in 74% of UTI cases [6] in pediatric hospital. In Algeria and Tunisia, enterobacteria were identified respectively in 60% and 80% of UTI [3,7]. The management of UTI requiring antimicrobial therapy must be made conventionally from an antibiotic susceptibility test to avoid aggravations, partial healing or recurrence. This is needed because of the spread of resistance to common antibiotics [8], not only in developing countries but also in developed countries [9-11]. Yet few studies have indicated that the lack of medical bacteriology laboratories or their non-functionality often leads doctors in developing countries to prescribe broad spectrum antibiotics to overcome this deficit [2,12,13]. Burkina Faso, like other developing countries, is experiencing the same difficulties.

Work conducted in Burkina Faso have shown resistance to amoxicillin and cotrimoxazole rate of about 80% for *K. pneumoniae* and 74% for *E. coli* [14,15]. Many studies on UTI concerned *E. coli* and *K. pneumoniae* worldwide [1,5,8,9,16]. Zhang and collaborators study [17] found some resistant cases of *K. pneumoniae* isolates to Carbapenems. The survey conducted by Tansarli and collaborators [8] indicated that *E. coli* and *K. pneumoniae* were most commonly uropathogenic germs encountered during the various studies in Africa and these strains exhibited good sensitivity to ATB as cefotaxime, imipenem, fosfomycin, and ciprofloxacin.

Considering the scale of the emergence of resistance to antibiotics, frequent assessment of antibiotics susceptibility to targeted strains during antibiotic therapy is necessary. This assessment will reveal among other new trends in terms of resistance to ATB to contribute to improving the therapeutic care of UTI caused by bacteria. In this context, this study was conducted to assess the susceptibility to antibiotics of *E. coli* and *K. pneumoniae* strains isolated from urine in Burkina Faso hospital.

Material and Methods

This is a cross-sectional study and it was conducted in bacteriology laboratory of Centre Muraz on a six-month period.

The study population was patients of all ages coming for the Cytobacteriological Examination of the Urine (CBEU) at the bacteriology laboratory of Centre Muraz (Bobo-Dioulasso, Burkina Faso) during the study period.

The morning urine or urine that have remained for at least four hours in the patient's bladder was collected in a clean, sterile jar and this before any antibiotic treatment or after antibiotic break of at least 72 hours. In the laboratory analyzes are performed after assigning a code to each specimen.

For macroscopic examination in a fresh state, it was to appreciate the appearance of urine. The count was done using a Lemaure cell with total homogenized urine. The results are in leukocytes/mm³ and/or red blood cells/mm³ relating to the counting cell used.

Direct examination of urine was performed after the Gram stain X100 [18] with a microscope, objective X100.

For culture, 10 µL of the urine was homogenized and stretched on agar with bromocresol purple using a sterile and calibrated loop close to the flame. Petri dishes were incubated at 37°C for 18 to 24 hours. The identification was based on the bacteria characteristics: crop, morphology and biochemistry through macroscopic examination, the oxidase test and by using API 20E.

After identifying the bacteria, specimens positive in the screening of *E. coli* and/or *K. pneumoniae* were selected for the antimicrobial susceptibility testing.

Antimicrobial susceptibility was used to estimate their sensitivity/resistance to antibiotics on Mueller-Hinton culture medium (MH). Antibiotics used were mostly from the family of cephalosporins, macrolides, quinolones and aminoglycosides. Disks were placed in Petri round dishes of 90 mm, then the plates were incubated at 37°C, 24 to 48 hours, and the lid in the bottom. The diameter of inhibition around each antibiotic was measured in millimeters with a Vernier caliper under a good light and interpreted according to the recommendations of the Antibiogram Committee of the French Society for Microbiology [19]. Phenotypic detection of the β-lactamases was performed according to the screening of β-lactam susceptibility.

Statistical analysis

Data were entered and analyzed using Excel 2010 and EpiData Software Version 3.1. Statistical significance level was set at α = 5%.

Ethical consideration

Patient consent was obtained and anonymity was preserved by assigning identification number.

Results

Patients sociodemographic characteristics and frequency of pathogens isolated

Between August 2013 and January 2014, 810 cytotobacteriological urinalysis (urine culture) were performed, revealing that 24.07% (195/810) were positive for at least one enterobacterium (CI95% [18 - 30]). Among these 195 specimens, *E. coli* was identified in 114 specimens, so a frequency of 58.46% (CI95% [49 - 67]), against 55 specimen's positives for *K. pneumoniae*, i.e. a frequency of 28.2% (CI95% [16.2 - 40.2]). Other identified germs were: *Staphylococcus epidermidis* 5.64% (11/195), *Staphylococcus aureus* 4.10% (8/195), *Candida albicans* 1.03% (2/195), and other enterobacteria 2.56% (5/195). The majority of specimen diagnosed positive were of male: 64.0% among those diagnosed positive to *K. pneumoniae* and 55.3% of those positive for *E. coli* (Table 1).

The majority of women with positive CBEU were aged between 15 to 65 years: 92.31% among women with urine infected by *E. coli* and 69.23% among those with urine infected by *K. pneumoniae* (Table 1).

Age (year)	Gender	Pathogens frequency (%)	
		<i>E. coli</i> (n=114)	<i>K. pneumoniae</i> (n=55)
< 15	M	2.17	0
	F	3.84	4.35
15 - 65	M	58.70	69.23
	F	92.31	65.22
> 65	M	39.13	30.77
	F	3.85	30.43
Total	M	63 (55.3%)	35 (64.0%)
	F	51 (44.7%)	20 (36.0%)

Table 1: Patients characteristics according to age, gender and identified germs.
F: Female; M: Male.

Antibiotic susceptibility of predominantly bacteria isolated from urine

Antimicrobial susceptibility was carried out on each of the 114 *E. coli* strains and 55 *K. pneumoniae* strains.

From the antimicrobial susceptibility testing, only piperacillin associated with tazobactam presented a very good activity against *E. coli* and *K. pneumoniae* strains with a sensitivity of 98.2% and 96.4% respectively. High resistances were observed with molecules such as amoxicillin (86% for *E. coli* and 94.4% for *K. pneumoniae*), ticarcillin (84.2%; 89.1%) and ticarcillin + clavulanic acid combination (84.2%; 83.6%) (Table 2).

Antibiotics	Critical values (mm)	Susceptibility and frequencies (%)					
		<i>E. coli</i>			<i>K. pneumoniae</i>		
		S	I	R	S	I	R
Amoxicillin	16 - 23	2.6	11.4	86	00	5.5	94.5
Augmentin	13 - 18	27.2	28.9	43.9	32.7	29.1	38.2
Piperacillin	18 - 22	19.3	28.9	51.8	29.1	9.1	61.8
Piperacillin +Tazobactam	17 - 21	98.2	0.0	1.8	96.4	3.6	00
Ticarcillin	22 - 24	14.9	0.9	84.2	7.3	3.6	89.1
Ticarcillin +Clavulanic acid	22 - 24	14.9	0.9	84.2	9.1	7.3	83.6

Table 2: Frequency of the susceptibility of isolated bacterial strains to penicillins.
S: Sensitive; I: Intermediate; R: Resistant.

The susceptibility of *E. coli* and *K. pneumoniae* strains to first and second generations of cephalosporins molecules used was less than 50% for most of them. Also, sensitivity to 3rd generation of cephalosporins was greater than 50% for *E. coli* strains and less than 50% for *K. pneumoniae* (Table 3).

	Antibiotics	Critical Values (mm)	Susceptibility and frequencies (%)					
			<i>E. coli</i>			<i>K. pneumoniae</i>		
			S	I	R	S	I	R
1 st , 2 nd generations	Cefalotin	12 - 18	49.1	14	36.9	34.5	3.6	61.8
	Cefoxitin	15 - 22	58.8	32.4	8.8	63.6	20.0	16.4
	Cefuroxime	22 - 25	34.2	25.4	40.4	32.7	7.3	60.0
	Cefamandole	15 - 22	41.2	22.8	36	32.7	9.1	58.2
3 rd generation	Ceftriaxone	23 - 26	64	0.0	36	43.6	56.3	1.8
	Cefotaxime	23 - 26	64	0.9	35.1	43.6	0.0	56.4
	Cefixime	22 - 25	50.9	11.4	37.7	43.6	0.0	56.4
	Ceftazidime	19 - 21	62.3	5.3	32.4	49.1	1.8	49.1
4 th generation	Cefepime	19 - 21	63.2	1.7	35.1	47.3	3.6	49.1

Table 3: Frequency of the susceptibility of *E. coli* and *K. pneumoniae* strains to cephalosporins.
S: Sensitive; I: Intermediate; R: Resistant.

Frequency of susceptibility to carbapenems and monobactams of strains isolated

All isolates were sensible to imipenem. But for aztreonam, only the *E. coli* strains were sensible (Table 4).

Drug class	Antibiotic	Critical Values (mm)	Susceptibility and frequencies (%)					
			<i>E. coli</i>			<i>K. pneumoniae</i>		
			S	I	R	S	I	R
Carbapenems	Imipenem	17 - 24	95.6	3.5	0.9	100	0.0	0.0
Monobactams	Aztreonam	22 - 23	64	3.5	32.5	49.1	0.0	50.9

Table 4: Frequency of the susceptibility of isolated strains to carbapenems and monobactams.

S: Sensitive; I: Intermediate; R: Resistant.

Resistance phenotypes in *E. coli* strains isolated

Resistance phenotypes to beta-lactams in *E. coli* (bacteria mostly isolated) are ranked in the following order: Penicillinase expressed at high level 31%; Cephalosporinase expressed at high level 18%; Penicillinase expressed at low level 16%; Penicillinase resistant to inhibitors and Extended Spectrum Beta-Lactamase (ESBL) 12%; Wild type 11%.

Discussion

In this study of 810 CBEU, 195/810 were positive for at least one enterobacterium namely a frequency of 24.07%. These results are reliable with Moukrad and collaborators, who revealed in Morocco 25% of positive specimens in at least one enterobacterium [20]. But in 2013, Prakash and Saxena [21] in India had found a higher frequency (53.82%) of enterobacteria in Urinary Tract Infection (UTI) cases.

The prevalence of infections caused by *E. coli* and *K. pneumoniae* was 58.46% and 28.2% respectively among the positive specimens to CBEU. These values confirm the major place of these bacteria in UTI cases [22]. Similar proportions of *K. pneumoniae* (28% and 29%) were reported respectively in Abidjan and Ouagadougou by Kouassi-M'bengue and collaborators in 2008 [16] and Ouedraogo/Yugbare and collaborators in 2012 [6].

The gender male represented the largest population while in general it is women who are more prone to UTI [3,21,23]. The high number of men in this study could be explained by their age, and also because many of them come from Urology Service of Souro Sanou University Hospital (Bobo-Dioulasso, Burkina Faso), who are mostly patients with prostate problems.

In our study, concerning the results of the susceptibility to penicillins (not associated with penicillinases inhibitors) of *E. coli* and *K. pneumoniae* strain isolated, it proved that there was high prevalence of resistance against most molecules (> 50%). The antimicrobial susceptibility testing showed that amoxicillin is the molecule with the highest resistance rates: 86% for *E. coli* and 94.4% for *K. pneumoniae*. This means that these strains were producing beta lactamase and confirms their natural resistance to aminopenicillins as demonstrated by some studies [3,9,24]. The study conducted by Dibua and collaborators in 2014 revealed that amoxicillin was not active on any Gram-negative strains of their urine specimens [23]. Indeed, amoxicillin is no longer used as first line in developed countries [10]. However some studies reveal a massive use of this antibiotic in developing countries, which poses a risk of therapeutic impasse becoming increasingly frequent, especially with the emergence of bacteria becoming less susceptible to the third generation of antibiotics [25,26]. Also, many bacteria are already adapted to the most commonly used drugs, such as those of the class of penicillin. The high rates of enterobacteria resistance to amoxicillin (86 - 94%) obtained in this study confirm those of previous studies in Sudan or Ethiopia [27,28] and by Mathlouthi and collaborators in Tunisian and Libyan hospitals [26]. Lower prevalence's were previously obtained in Burkina Faso (74% and 71%) respectively with Karou and collaborators [15] and Ouedraogo and collaborators [14] and in Senegal (73,1%) with Sire et collaborators [29].

Antibiotics combination for therapy helped to overcome the action of penicillinase. For example the case of combinations amoxicillin + clavulanic acid or piperacillin + tazobactam with good sensitivity observed in our study, which match with results reported in 2013 by El Fassi and collaborators in Tetouan [30] or those of Abdoullah and collaborators in Karachi [12]. Unfortunately, with the use, some of the combinations are losing their effectiveness as this study reveals with Augmentin. Because, through being exposed to antibiotics, bacteria eventually develop enzymes able to degrade antibiotics [31]. For molecules which have maintained their effectiveness, it could be explained by the non-misuse.

Regarding cephalosporin, during this study, cases of resistance to cephalosporins were detected both for *E. coli* and for *K. pneumoniae*. This could be explained by the most misuse of this antibiotic class and also by the fact that, with time, some strains have developed enzyme enabling them to resist to cephalosporins. The results obtained in this study matched with those of Tansarli and collaborators who reported that in Africa the sensitivity of *E. coli* strains to first generation of cephalosporins (Cefalotin) ranged from 44 to 75% [8].

In our study, Imipenem had a very good activity against *E. coli* and *K. pneumoniae* strains. This result is similar to that found in India by Prakash and Saxena [21], in Karachi by Abdullah and collaborators [12] who reported a sensitivity to Imipenem between 97.7% and 100%. The excellent activity of imipenem on these strains can be explained by the fact that this molecule is not usually used for urinary tract infections treatment.

Six beta-lactam resistant phenotypes in *E. coli* strains (mainly isolated bacterium) isolated, have been identified. The main phenotype was the penicillinases expressed at high level by 31% of the isolates, followed by cephalosporinases at 18%. However, we observed that other *E. coli* strains (12%) were secreting Extended Spectrum Beta-Lactamase (ESBL). This corroborates the fact that *E. coli* is resistant to antibiotics by producing several types of beta-lactamases [9,24]. However, the prevalence and phenotypic characteristics may vary from one to another health area depending on the conditions of use of these molecules [3]. Lower rates of ESBL-producing enterobacteria (1.3%) were reported by Khalifa and Khedher in 2010 [3]. Also, Bourouis and collaborators in 2015 drew attention to the fact that some of *Enterobacteriaceae* species were not only beta-lactamases producing but could adapt remarkably to antibiotics [32].

The non-restriction of the use of antibiotics, the incorrect dosage of antibiotics, as well as the too long or too short treatment duration is also implicated in case of resistance [10,33-35]. It would be advantageous to encourage the controlled and appropriate use of antibiotics.

Infections caused by bacteria with already acquired resistance to some antibiotics are becoming more frequent [30] due to their massive and repeated use. Amoxicillin alone should no longer be used to treat UTI in our health services due to the fact that it is an inactive molecule on the responsible strains. But it would take also regulation of the use of antibiotics so that there is no increase in strains that are not sensitive to any antibiotic. Because as revealed by INSERM, it will be to return to the days before the discovery of antibiotics.

Conclusion

This study reveals high resistance rates of *E. coli* and *K. pneumoniae* strains to beta-lactams. The rapid emergence of resistance is particularly worrying as it concerns the beta-lactams which are the most accessible and widely used in developing countries including Burkina Faso. These results warn on the magnitude of this public health issue, especially in a context where the marketing authorization of new antibiotics is rare while bacterial infections are growing. Strict regulation of the prescription and the use of antibiotics in general and especially beta-lactam would assist in controlling of the increasing resistances of bacteria responsible for the urinary tract infections.

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Conflict of Interests

No conflict of interests is declared.

Author's Contribution

Somda Diane and Koné Alicia have contributed equally to the work of this article

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