Resistance to antibiotics of *Escherichia Coli, Enterobacter Gergoviae* and *Klebsiella Pneumoniae* during urinary infections at CHUJRA Antananarivo, Madagascar

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Abstract

Introduction: Infections of the urinary tract remain among the most common problems facing the clinician. This work aims to determine the antibiotic resistance of *E. coli*, *E. gergoviae* and *K. pneumoniae* during urinary tract infections in the Microbiology laboratory of the Joseph Ravoahangy Andrianavalona Antananarivo University Hospital Center to allow a better therapeutic approach. **Material & Methods:** We carried out a retrospective and descriptive study on all of the *E. coli*, *E. gergoviae* and *K. pneumoniae* strains isolated from the urine of patients who attended the laboratory of Microbiology on CHUJRA Antananarivo with a suspected UTI between January 2004 and december 2014. Susceptibility to antibiotics was assessed by the disc diffusion technique on Mueller–Hinton agar (MH) or MH + 5% sheep blood, as recommended by the Antibiogram Committee of the French Microbiology Society as the antibiotics tested. **Results:** *E. coli*, *E. gergoviae* and *K. pneumoniae* were isolated from 577 of the 5836 patients who submitted a urine sample (9.89%). Almost three quarters of isolates were *E. coli* (72.96%), *E. gergoviae* was found in 7.80% of cases and *K. pneumoniae* in 19.23% of cases.Ampicillin, amoxicillin + clavulanic acid, ticarcillin, first generation cephalosporins, second generation cephalosporins, cyclins, and cotrimoxazole represent a very high resistance level for *E. coli*, *E. gergoviae* and *K. pneumoniae* in the vast majority of cases. Since 2008, the presence of ESBL has been lower than in previous years ($p=0.02$). Antibiotics that remain effective in the majority of UTI cases are nitrofurans, colistin, imipenem, amikacin and Piperacillin / tazobactam. **Conclusions:** These results confirm the presence of multi- resistant strains as in hospitals and community settings and the associated resistance to the most accessible antibiotics inevitably limits the choice of antibiotic in the establishment of adequate treatment.

Keywords: Resistance, Antibiotics, ITU, CHUJRA Antananarivo

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Introduction

Infections of the urinary tract remain among the most common problems facing the clinician. It is remarkable because of the different clinical varieties related to different topographic sites of infection and broad concept, ranging from asymptomatic infection to pyelonephritis with sepsis. Cause identification and severity of infection are generally established through the evaluation of clinical presentation, biochemical and urinary cultures. They are common in both community and hospital settings. They represent more than 40% (always increasing proportion) of nosocomial infections [1]. This frequency is related to favoring factors and uropathogenicity factors of the germs in question [2].

However, excessive use of antibiotics including broad spectrum in other conditions contributes to the development of resistance in bacteria responsible for urinary tract infection and causes difficulty in the treatment of these. Levels of bacterial resistance vary from country to country and from year to year. Also, knowledge of the local situation and its evolution are necessary for the choice of antibiotic therapy for first-line. Very little study concerning the antimicrobial resistance of bacteria isolated from urinary tract infections in Antananarivo have been published to date. However, with the emergence of resistant urinary pathogens observed in certain community and hospital settings, a change of practice in the management of patients is to be considered thus requiring a revision of the choices.

All bacterial strains are not capable of inducing a urinary tract infection, some strains have specific virulence factors allowing rapid diffusion from the faecal flora to the renal parenchyma.

These are enterobacteria represented mainly by *Escherichia coli,* as well as *Enterobacter gergoviae* and *Klebsiella pneumoniae*. This work aims to determine the antibiotic resistance of *E. coli*, *E. gergoviae* and *K. pneumoniae* during urinary tract infections in the Microbiology laboratory of the Joseph Ravoahangy Andrianavalona Antananarivo University Hospital Center (CHUJRA) to allow a better therapeutic approach.

Methods

Study design: The present study was a retrospective and descriptive study on all of the *E. coli*, *E. gergoviae* and *K. pneumoniae* strains isolated from the urine.

Study setting: Patients who attended the laboratory of Microbiology on CHUJRA Antananarivo with a suspected urinary tract infection.

Duration of study: between January 2004 and December 2014.

Inclusion criteria: only patients who had pyuria (>10 white blood cells/mL) and significant bacteriuria (>105cfu/mL) were included in the microbiological analysis. Only one specimen per patient was included in the study.

Exclusion criteria: Species other than *E. coli*, *E. gergoviae* and *K. pneumoniae* were exclude from the study.

Methods: the bacteria were identified by standard microbiological techniques. Susceptibility to antibiotics was assessed by the disc diffusion technique on Mueller–Hinton agar (MH) or MH + 5% sheep blood, as recommended by the Antibiogram Committee of the French Microbiology Society (ACFMS) as the antibiotics tested [3]. After 24 h at 37°C, the zone of inhibition was measured. To detect extended-spectrum b-lactamases (ESBLs), discs of ceftazidime and ceftriaxone were placed 30 mm from an amoxicillin/clavulanate (20/10 mg) disc. Enhancement of the zone of inhibition between the clavulanate disc and any one of the third-generation cephalosporin discs indicated the presence of an ESBL. The distance between the discs could be reduced when the synergy was not easily observed as in cephalosporinase-producing strains. Data analysis: the data were processed and analyzed by the software Epi-info 7.1.3.

Results

E. coli, *E. gergoviae* and *K. pneumoniae* were isolated from 577 of the 5836 patients who submitted a urine sample (9.89%). Among them, 413 were women and 164 were men (mean age 28.4 years, range 0-77 years, sex ratio 0.40).

There was no significant difference concerning the species according to age classes or gender. Almost three quarters of isolates were *E. coli* (72.96%)*, E. gergoviae* was found in 7.80% of cases and *K. pneumoniae* in 19.23% of cases (Table 1).

Ampicillin, ticarcillin, cyclins, and cotrimoxazole represent a very high resistance rate for *E. coli* with respectively 89.07%, 87.38%, 76.43%, and 75.77% of cases. Regarding the antibiotic resistance of *K. pneumoniae*, amoxicillin + clavulanic acid, first-generation cephalosporins (C1G), cyclins, and cotrimoxazole represent a very high resistance rate in almost all cases with respectively 88.29 %, 88.29%, 91.89%, and 91.89%.

Ticarcillin, cyclins, cotrimoxazole and second-generation cephalosporins (C2G) represent a very high resistance rate for *E. gergoviae* with 85.71%, 82.22%, 86.36% and 82.22% respectively (Table 2). There was no significant difference concerning the species according to the provenance and antibiotics sensibility.

R: resistant, S: sensible, Amoxi-clav: amoxicillin + clavulanic acid, C1G: first-generation cephalosporins, C2G: second-generation cephalosporins, C3G: third generation cephalosporins, Pipe/tazo: piperacillin/tazobactam

E. coli, *K. pneumoniae* and *E. gergoviae* secretory ESBL were found in 54 cases, 38 cases and 22 cases, respectively whether 19.76% of cases of diagnosed urinary tract infection.ESBL producing to *E. coli* is more common in the community (38 cases, or 59.37%) while in hospital, *K. pneumoniae* and *E. gergoviae* produce more ESBL with 28 case (65.11%) and 18 case (69.23%) respectively.

The presence of very high rate of *E. coli*, *K. pneumoniae* and *E. gergoviae* secreted by ESBL was noted during the years 2004 until 2007. Then this rate decreases since 2008 and then stabilizes for the rest of the years. This result is significant (p=0.02).

Table-1: Distribution by the provenance of the sample.

Germs	Hospitalised (n)	Communautary (n)	Total (n/%)	
Escherichia coli	181	240	421	72.96
Enterobacter gergoviae	26	19	45	7.80
Klebsiella pneumoniae	65	46	111	19.23

Table-2: Antibiotic susceptibility of strains of *E. coli*, *K. pneumoniae*, and *E. gergoviae*.

Antibiotics	E. coli		K. pneumoniae		E. gergoviae	
	R (n/%)	S (n/%)	R (n/%)	S (n/%)	R (n/%)	S (n/%)
Ampicillin	375 (89.07)	46 (10.93)	111 (100)	0	45 (100)	0
Ticarcilin	368 (87.38)	53 (12.62)	111 (100)	0	45 (100)	0
Amoxi-clav	286 (67.93)	135 (32.07)	98 (88.29)	13 (11.71)	45 (100)	0
C1G	299 (71.02)	122 (28.98)	98 (88.29)	13 (11.71)	45 (100)	0
C2G	173 (41.09)	247 (58.71)	74 (66.67)	37 (33.33)	37 (82.22)	8 (17.78)
C3G	64 (15.09)	357 (84.91)	43 (38.74)	68 (61.26)	26 (57.77)	19 (42.23)
Nitrofuran	18 (4.29)	402 (95.71)	12 (10.81)	99 (89.19)	4 (8.89)	41 (91.11)
Gentamicin	71 (16.86)	350 (83.41)	36 (32.43)	75 (67.57)	23 (51.11)	22 (48.89)
Tobramycin	71 (16.86)	350 (83.41)	36 (32.43)	75 (67.57)	24 (53.33)	21 (46.67)
Netilmycin	71(16.86)	350 (83.41)	36 (32.43)	75 (67.57)	24 (53.33)	21 (46.67)
Kanamycin	75 (17.81)	346 (88.19)	46 (41.44)	65 (58.56)	26 (57.78)	19 (42.22)
Fluoroquinolone	167 (39.67)	254 (60.33)	82 (73.87)	29 (26.13)	35 (77.78)	10 (22.22)
Cyclins	321(76.43)	99 (23.57)	102 (91.89)	9 (8.11)	37 (82.22)	8 (17.78)
Cotrimoxazole	319 (75.77)	102 (24.23)	102 (91.89)	9 (8.11)	38 (86.36)	7 (13.64)
Chloramphenicol	44 (10.53)	374 (89.47)	23 (20.91)	87 (79.09)	0	45 (100)
Amikacin	3 (0.71)	417 (99.29)	0	111 (100)	0	45 (100)
Pipe/tazo	10 (2.51)	388 (97.49)	4 (3.67)	107 (95.41)	0	45 (100)
Colistin	0	420 (100)	3 (2.70)	108 (97.30)	32 (85.71)	5 (14.29)
Imipenem	0	420 (100)	0	111 (100)	12 (25)	33 (75)

Antibiotics that remain effective in most cases of urinary *E. coli*, *K. pneumoniae*, and *E. gergoviae* infections have been nitrofuran, colistin, imipenem, amikacine and piperacillin/tazobactam (Table 2).

Discussion

The study focuses on the antibiotic resistance of *E. coli*, *K. pneumoniae* and *E. gergoviae* isolated from urinary tract infection because urinary tract infection is still a very common infection with an incessant increase in resistance to certain antibiotics [4].

Among the bacteria studied, *E. coli* is isolated in almost three quarters of cases in hospitalized patients or not (Table 1). Ampicillin, amoxicillin + clavulanic acid, ticarcillin, first generation cephalosporins (C1G), second generation cephalosporins (C2G), cyclins, and cotrimoxazole represent a very high resistance level for *E. coli*, *E. gergoviae* and *K. pneumoniae* in the vast majority of cases (Table 2). Some data from the literature confirm that nosocomial or community urinary tract infections represent an increase in antibiotic resistance. This would be due to the misuse of antibiotics because it is possible that exposure to

another family of antibiotics may select bacteria resistant to other families of antibiotics by cross mechanisms [5]. These results confirm the presence of multiresistant strains as in hospitals and community settings and the associated resistanceto the most accessible antibiotics inevitably limits the choice of antibiotic in the establishment of adequate treatment. Moreover, the worrying resistance to amoxicillin-clavulanic acid association are described in the treatment of first resort hinders the empiric antibiotic therapy of urinary tract infection [6].

However, studies of a larger population need to be undertaken to statistically support these findings and to evaluate other risk factors and the determinants of the carriage of these multidrug-resistant bacteria in community and hospital settings. The emergence of strains of third-generation cephalosporin-resistant bacteria that are the

ESBLs that constrain the treatment of infection is today a scourge known by several countries including Madagascar [7]. This resistance, more commonly known by ESBL, constitutes a very large heterogeneous family of bacterial enzymes which inactivates most beta-lactams except cephamycins and carbapenems. They are most often of plasmid origin, which explains their rapidity of diffusion and evolution [8]. This constantly increasing resistance must attract the attention of all treating physicians because if it is poorly controlled, this could lead to several complications such as irreversible kidney damage, severe sepsis and could even lead to death of the patient [5].

The studies carried out in Antananarivo by Ramilitiana B. and al which found a rate of ESBL at 8.3% or that of Rasamiravaka T. and al, which show an ESBL level at 11.7%, confirm the results of the present study [7, 9]. A study by Shekshok Y. et al showed a high level of C3G-resistant *Klebsiella pneumoniae* (62%), which would be a consequence of the selection pressure due to the wide use of ß-lactam antibiotics and furthermore, these resistances acquired from made of their plasmid determinism, have a great power of dissemination [10]. ESBL-producing *E. coli* is more common in the community (59.37% of cases) while in hospital, *K. pneumoniae* and *E. gergoviae* produce more ESBL. Some data from previous studies or those in the literature show that *E. coli* is the main pathogen of nosocomial or community urinary tract infection, an increase in antibiotic resistance [5].

These ESBL infections are a public health problem as many are of community origin. The approach must therefore be multidimensional and integrate two components that are the improvement of standard precautions and the rationalization of antibiotic consumption and their prescription following the protocols established in Madagascar because the first-line treatment may vary depending on realities of each country [9,11].

Nitrofurans, colistin, imipenem, amikacin and Piperacillin / tazobactam are the antibiotics that remain effective in cases of urinary tract infection found in the present study. A study by Y. Sekhsokh and al states that ESBL-producing bacteria are resistant to the majority of antibiotics but retain a high sensitivity to imipenem and amikacin [10]. This is the recognized and undisputed treatment of with ESBL-producing enterobacteria is based on carbapenems, but the place of the other molecules remains to be debated. Indeed, in the current threatening epidemiological situation, no molecule can be ruled out, if only to diversify the prescribed antibiotherapies.

The variability of the problems posed by the ESBL-producing bacteria implies the establishment of a national surveillance network, the microbiology laboratory playing a key role in the detection and monitoring of resistance. Indeed, it is important to understand the extent and development of antibiotic resistance over time to manage this problem. To control antibiotic resistance, it is clear that reliability can no longer be on the development of new molecules whose prospects are very limited, but it is possible to slow the development of resistance by prevention strategies. Prevention of the emergence of resistance and diffusion of ESBL secretory bacteria represents a major public health issue for health care institutions. This rise in resistance is generally correlated with, among other things, an increase in antibiotic consumption as suggested by Grude et al. [12]. In Morocco, the consumption of antibiotics increased by 42%, according to El Mdaghri et al. [13], hence the importance of raising awareness of the proper use of antibiotics and monitoring to control the spread of antibiotic resistance.

The likely relationship between resistance and high antibiotic consumption on the one hand, the insufficient application of standard hygiene precautions to limit cross-transmission of ESBL on the other, favor this phenomenon [14].

It is therefore necessary to implement strategies to control these pathogens. The fight can be articulated around three main objectives such as the control of the spread of ESBL by cross-transmission, the prevention of the emergence of ESBL linked to the too wide, too abusive use of antibiotics.

Also, it is necessary to spread to the entire medical world (doctors, dentists, midwives and paramedics, employees and liberals) training and information on the epidemic spread of ESBL secretory enterobacteria, which exposes, ultimately, the risk of therapeutic impasse.

All microbiologists must also be made aware of the problem of the epidemic spread of ESBL-secreting enterobacteria and their resistance genes, and the means that must be used to identify this type of resistance. The information will focus on the risk of cross-transmission of these bacteria and their resistance genes, whose natural reservoir is mainly digestive and urinary. A strong presence of *E. coli*, *K. pneumoniae* and *E. gergoviae* secretory by ESBL was found from 2004 to 2007, then this rate decreased significantly since 2008 and stabilized thereafter. At the national level in Madagascar, real access to medicines began in 2007 as part of the national health policy which was associated with strengthening the training of health personnel in quality and quantity with adequate staff distribution qualified in all health centers. This measure is to ensure at least 85% availability and accessibility of essential drugs, medical consumables, reagents in all health facilities by extending the range of Salama (essential drug supplier) for drugs and consumables hospitals.

This has been accompanied by a rationalization of the prescription and also the rational use of medicines with the objective of improving the health system in Madagascar until 2011. Better access to medicines and their rational prescription in a hospital environment can explain the decrease of the ESBL found in our center during the year 2008. However, the private practices, which escape the trainings and supervisions of the Ministry of Health, continued in the irrational prescription of the drugs, in particular the antibiotics [15].

The decrease of the pharmacological pressure by the changes made at the level of protocols probably led to the decrease of the resistances. Several studies have reported decreases in resistance following the suppression or restriction in the prescription of certain antibiotics [16].

A survey conducted by Raisin France has determined an increase in EBLSE from 38% in 2006 to 60% in 2012 and for the same reasons that have been mentioned previously [17]. At the national level, epidemiological surveillance of ESBL-secreting enterobacteria must be ensured. Specific studies should be considered to complete this scheme.

However, this study has limitations because the samples are not representative of all the cases of urinary infection that could be encountered throughout Madagascar.

Conclusion

In almost all cases, ampicillin, amoxicillin + clavulanic acid, ticarcillin, first-generation cephalosporins, second-generation cephalosporins, cyclins, and cotrimoxazole represent a resistance very high rate for *E. coli*, *E. gergoviae* and *K. pneumoniae.* Since 2008, the presence of ESBL has been lower than in previous years. Antibiotics that remain effective in the majority of UTI cases are nitrofurans, colistin, imipenem, amikacin and Piperacillin / tazobactam. The increasing strength of community and hospital-acquired strains of *E. coli*, *E. gergoviae* and *K. pneumoniae* antibiotics has become an alarming phenomenon in Antananarivo.

This encourages reflection on the management of patients consulting for urinary tract infection. To avoid the occurrence of resistance to these molecules, it would be wise to have the results of the bacteriological examination before prescribing them.

The rules of antibiotic prescriptions and the introduction of antibiotic prophylaxis should also be applied to limit the scourge of ESBL secretory bacteria. Indeed, the treatment of urinary tract infections should be reconsidered and be the subject of a therapeutic consensus taking into account the national situation of antibiotic resistance.

What the study adds to the existing knowledge?

The data obtained from the present study can be used to determine trends in antimicrobial resistance of *E. coli*, *E. gergoviae* and *K. pneumonia* during community or nosocomial urinary tract infections and to reformulate the antibiotics to be prescribed. This will assist clinicians in the rational selection of antibiotic therapy for effective management of patients.

Author's contribution

- **Andry Maharo Andrianarivelo:** Concept, design, preparation and editing of manuscript.
- **Arthur Bien Aimé Ratsimbazafy:** Preparation and editing of manuscript.
- **Zakasoa Mbololona Ravaoarisaina:** Preparation and editing of manuscript, literature search.
- **Solofo Sarah Rafaramalala:** Data Collection, data compiling.
- **Ianja Iorenantsoa Razanadrakoto:** Data collection, data compiling.
- **Lalaina Mamenosoa Rakotondraoelina:** Data collection and data analysis.
- **Annie Armandine Raharisoa:** Assisted in data analysis.
- **Christian Rafalimanana:** Final approval
- **Andry Rasamindrakotroka:** Final approval

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