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RESEARCH ARTICLE

UROPATHOGENS FREQUENCY AND PROFILE OF ANTIMICROBIAL SUSCEPTIBILITY OF *ESCHERICHIA COLI* FROM PATIENTS ATTENDED BY BRAZILIAN UNIFIED HEALTH SYSTEM

Flávia de Mello Santos Franco de Paula, Armindo Antônio Alves and Ana Laura Remédio Zeni Beretta*

Postgraduate Program Programa de Pós Graduação-Mestrado em Ciências Biológicas/Centro Universitário Hermínio Ometto - Uniararas, Araras, São Paulo, Brazil

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ABSTRACT

Aims
Due to the increase of multiresistant microorganisms causing urinary tract infection (UTI), this study aimed to identify the frequency of uropathogens in 2000 patients with clinical suspicion and profile of antimicrobial susceptibility of *Escherichia coli* facing the positive urine cultures.

Methods
The samples were processed in a clinical laboratory convened exclusively by the Unified Health System, and the Microbiological Research Lab - Uniararas in the period between April and July 2012.

Results
The UTI was present in 247 (12.35 %) patients. The frequency of major uropathogens were *Escherichiacoli* 75.71%; *Klebsiella pneumoniae* 8.50%; *Burkholderiacepacia*3.24%; *Enterobacter* sp 2.83%; *Proteus mirabilis* 2.43%; *Pseudomonas aeruginosa* 2.43%; *Staphylococcus aureus*2.03%; *Streptococcusagalactiae* 1.22%; 1.21% *Staphylococcus saprophyticus* and *Streptococcus* sp 0.40%. It was observedthat the *E. coli* was the most frequent uropathogen, with significant difference ($p < 0.05$).Among outpatient samples, 2.14% of *E. coli* producing strains showed beta-lactamase and 0.53% resistantto imipenem, which proved the most effective antibiotic against *E. coli* followed by the amikacin with resistance 2.14%. Moreover, *E. coli* was resistant to cephalothin in 88.70% of the tests, while beingresistant to nalidixic acid of 48.67%.

Conclusion
The results reported here demonstrate that the etiology of urinary tract infections is similar to that found in other parts of the world, and the highest frequency was *E. coli*. The resistance pattern of this uropathogen may have different characteristics according to the historical consumption of antimicrobials in each community. Thus, it is important that epidemiological data on the frequency of infectious agents along with the profile of antimicrobial susceptibility are announced periodically with the intention of helping the medical and clinical information for new therapeutic options.

INTRODUCTION

Urinary tract infection (UTI) is commonly diagnosed in medical practice, and is a frequent site of infection in the community patients and patients admitted to hospitals, representing a major cause of care-associated infections in health. Are present mainly in children, young adults, sexually active women and elderly (Horner *et al*., 2006; Masson *et al*., 2009; Kahlmeter & Poulsen, 2012).

The occurrence of the causative pathogen of UTI varies and the susceptibility profile requires monitoring with the aim of acquiring new information for guidance of therapeutic options. A major concern regarding the use of drugs is related to the use of antimicrobials. Increased resistance to several antimicrobial agents entails difficulties in controlling infections and contributes to the rising costs of health care and own hospitals (Castro *et al*., 2002).

The majority of studies on the isolation and identification of microbial strains multiresistant has been performed in hospitalized patients, however, we believe that resistant organisms may be also agents of UTI in the community (Pavanello *et al*., 2009).

Corresponding author:* **Ana Laura Remédio Zeni Beretta
Programa de Pós Graduação-Mestrado em Ciências Biomédicas/Centro Universitário Hermínio Ometto - UNIARARAS, Araras, SãoPaulo, Brazil

Most etiologic al agents involved in community-acquired UTI are in order of frequency: *Escherichia coli, Staphylococcus saprophyticus, Proteus* sp., *Klebsiella* sp. and *Enterococcus faecalis*. The *E. coli* is responsible for 70% to 85% of community-acquired UTIs and 50% to 60% in elderly patients (Rahn, 2008).

When is acquired UTI in hospitalized patients, the etiological agents are very diverse, predominantly enterobacteriaceae, with reduction in the frequency of *E. coli*, although usually remains the first cause, and a increase of *Proteus* spp., *Pseudomonas aeruginosa*, *Klebsiella* sp., *Enterobacter* sp., *E. faecalis* and yeasts, especially *Candida* sp. (Ksycki & Namias, 2009; Martins *et al*., 2010).

Epidemiological studies of UTIs and the pattern of susceptibility / resistance of uropathogens agents increases in importance in the face of treatment failure, which in most cases is empirical, and the antimicrobial susceptibility test drives the new therapeutic approach (Cheong *et al*., 2007; Pires *et al*., 2007). Improper use of antibiotics to treat this kind of illness induces a selective pressure on the agent, favoring the multiplication of resistant bacterial strains. Thus, the use of short or single doses is not recommended in the treatment of UTIs, as it may induce microbial resistance (Sanz-Garcia *et al*., 2009).

Patients with UTI caused by resistant pathogens are at risk because, receiving inadequate empirical antibiotic therapy, may exhibit increased morbidity. The high rates of antibiotic resistance in E. coli isolated from patients with urinary tract infection has been reported worldwide (Beraldo-Massoli *et al*., 2012; Jeon *et al*., 2012).

The present study aimed to identify the frequency of uropathogens in patients with clinical suspicion and profile of susceptibility of *Escherichia coli* isolated in the face of positive urine cultures in a clinical laboratory with an exclusive agreement by the Unified Health System in a laboratory clinical analysis of the state of São Paulo (Brazil).

METHODS

This study was conducted after approval by the ethics and research committee by Hermínio Ometto-UNIARARAS University Center through the e National Research Ethics Committee - Platform Brazil, in the opinion 278 237 dated from 21/05/2013.

This study was conducted by the Microbiology section of the Municipal Hospital Dr. Tabajara Ramos, located in the city of Mogi Guaçu, São Paulo State, Brazil, which sees patients exclusively through the SUS with low complexity. With the collaboration of the Laboratory of Microbiological Research from University Center Hermínio Ometto- UNIARARAS/ Araras, SP, Brazil.

Through a model of retrospective cohort study 2,000 urine samples collected in the period between April and July 2012, previously analyzed for physic-chemical form and sediment to exclude contamination with fecal material, ointments and vaginal secretion. Samples free of contamination were from patients at community level (Basic Health Units, Center for Medical Specialties, Unit of Emergency, Specialty Outpatient Clinics and Home for the Elderly Father Longinus and Home Care, the latter two being high in patients who catheter and urinary catheter long term) and patients admitted to the Intensive Care Unit of clinical Oncology and clinically suspected UTI.

The material analysis was performed according to routine microbiology sector. Urine samples were seeded using calibrated loop (0.001 mL) in MacConkey media CLED and incubated for 24 hours at 37 ° C. Samples with number ≥ 105 Colony Forming Units / milliliter (CFU/mL) and ≥ 104 CFU/mL which grew a single organism were considered positive. Thus, we could observe that there is not a fixed number of significant bacteriuria, which can be applied to all types and all ITU circumstances. The technique applied in the culture was the quantification of colonies (Grabe *et al*., 2010). Biochemical evidence of identification of microorganisms were performed according Koneman (Koneman, 2010).

After identifying the uropathogen, testing sensitivity to antibiotics (TSA) was performed. The profile of antimicrobial susceptibility was determined using the Kirby-Bauer method, based on the diffusion of antimicrobial discs in Müller-Hinton agar according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2000). The standardization of the TSA and its interpretation are updated annually by the Clinical and Laboratory Standards Institute (CLSI, 2012). The following antimicrobials were tested: nalidixic acid, amikacin, amoxicillin-clavulanic acid, aztreonam, cephalothin, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, nitrofurantoin, norfloxacin and sulphazotrim.

Extended spectrum beta lactamase (ESBL) strains were checked by double disc diffusion method, where a disk of ceftazidime was placed 20 mm from the beta-lactamase inhibitor clavulanic acid + amoxicillin and the same distance from a disk cefotaxime having a hard perpendicular aztreonam. The appearance of a "ghost area" or enlargement of the zone of inhibition of the production of cephalosporin confirms ESBL.
In interpreting the results were measured sizes of the halos formed and analyzed for indications of sensitivity and resistance to CLSI (2012) table and informative NCCLS (2000) was used.

The statistical analysis was performed in Microsoft Excel® software, in which were made the systematization of matrices, charts and graphs that correlate the data and the Fisher significance test to detect p value.

RESULTS AND DISCUSSION

The *E. coli* was the main etiological agent of UTI in 64.78% women and 10.94% men, with prevalence in individuals aged between 61 and 70 (Figure 1).

Among outpatient samples, 2.14% of *E. coli* producing strains showed beta-lactamase and 0.53% resistant to imipenem (Figure 2), which proved the most effective antibiotic against *E. coli* followed by the amikacin with resistance 2.14%. Moreover, *E. coli* was resistant to cephalothin in 88.70% of the tests, while being resistant to nalidixic acid of 48.67% (Figure 3).

The UTIs are among the infections found more frequently in clinical practice. In this context, the rise of antibiotic resistant organisms is a serious problem which is associated with the use empirical and sometimes inappropriate and unnecessary use of antibiotics that enhance the appearance of such resistance (Kahlmeter & Poulsen, 2012). In this context, it is important to choose the appropriate antimicrobial and study the susceptibility of uropathogens in each institution profile.

In this study 247 (12.35%) positive samples analyzed 2000 urine cultures were found, the affected population predominantly female (64.78%), aged between 61 and 70 years. These data are in accordance with other studies where approximately 60% of clients reported at least one episode of UTI throughout life, especially in postmenopausal women (Tenover & Hughes, 2006; Sanz-Garcia *et al*., 2009).

The frequency of positive urine cultures in this study was lower than that found by Astal *et al*. (2002) who found percentages ranging between 28.7 and 75%. This is due to the different characteristics of the populations studied. The prevalence of Gram negative bacteria of the family Enterobacteriaceae, *E. coli* is most frequently in the outpatient setting and in hospitalized patients with reports of corroborate Martins *et al*.(2010); Ksycki; Namias (2009).

While this study turns to Gram-positive uropathogens, another realizes that the most frequent, *S. aureus* is at variance with the reports described by Rahn (2008), when quoting *S. saprophyticus* as the most prevalent in young women.

The property profile of antimicrobial susceptibility is an aspect of relevance, it may be significantly different to be associated with local (Astal *et al*. 2002) selective pressures. Therefore, the use of appropriate antibiotics is extremely important to decrease the mortality rate of individuals suffering from UTI, and should be used when needed, with dosage and time appropriate to achieving success in therapeutic treatment.

Studies by Koch (2008) indicate the highest prevalence of bacterial resistance of the strains tested trimethoprim-sulfamethoxazole (46.90%), followed by cephalothin (46.70%), nalidixic acid (27.60%) and nitrofurantoin (22.30%). These data show disagreement with the results of this study since the *E. coli* strains were resistant to cephalothin in 88.70% of cases, nalidixic acid 48.67%, sulfamethoxazole-trimethoprim in 44.39% and nitrofurantoin in 26.20%.

Such data are closely related to self-medication and flaws in the empirical treatment of infections. This question is not municipal or local, but national order, because antibiotics were used in this study period, indiscriminately until now have their dispensation in Brazil regulated by the National System of Management of Controlled Products (NSMCP), ie, red stripe with retention of prescription has to be submitted in two copies, one for the patient and one for retention in the dispensing room, with electronic sales information.

Studies by Warren (1999) trimethoprim-sulfamethoxazole recommended that it should not be shown empirically in cases of resistance between 10 and 20%. This study identified and profiled antimicrobial susceptibility in hospital and community levels of *E. coli* uropathogen and pointed out that such strains have indices greater than 20% resistance to cephalothin, nalidixic acid, and sulphazotrim was not found, norfloxacin, ciprofloxacin and nitrofurantoin. So and, this study indicated discourages these antibiotics empirically for the treatment of UTI caused by this microorganism.

In outpatient, study samples, the *E. coli* strains isolated were producing beta-lactamases, as consistent with reports that described by Esmerino *et al*. (2003) it is important to note the relationship between positive strains producing beta-lactamase (ESBL) and the occurrence of multidrug resistance to antibiotics.

Studies performed in 40s already indicated the presence of producing enzyme beta-lactamase, both in Gram-positive bacteria, Gram-negative as in (Abraam & Chain, 1940; Kirby, 1944) with genes capable of encoding ESBL may be located in plasmids, which enables the transfer between species or the bacterial chromosome universal in some bacterial species (Valdevenito & Pablo, 2008). The ESBL producing *E. coli* are a cause of concern to the microbiologist as well as to the clinicians, particularly the multi drug resistant strains (Dalela, 2012).

Carbapenems such as imipenem antibiotics have broad-spectrum bactericidal activity with improved stability against ESBL-producing organisms, must be indicated in severe hospital infections with other drugs for the treatment of bacterial resistance has failed (Maina *et al*., 2013). In this study, a strain resistant to imipenem was found in a community environment, probably due to incorrect use of this potent antimicrobial. The contribution portrays the goal of this study can be compared to studies of Horner *et al*. (2006) Menezes *et al*. (2005) observed that when it is observed that the tables are increasing resistance among clinical isolates of UTI.

Figures and Tables

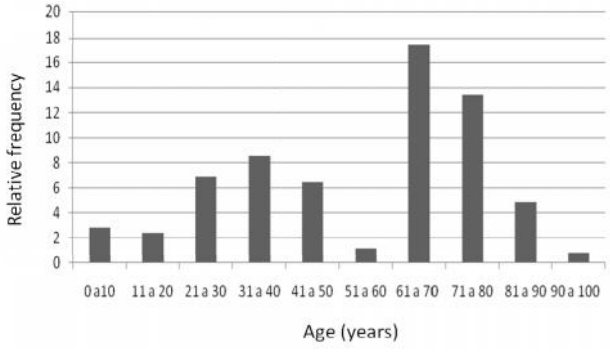

Figure 1 Age of the patients. Relative frequency of age of patients with UTI caused by *E. coli*.

Table 1Absolute and relative frequency of microorganisms The absolute and relative frequency of microorganisms isolated from patients with urinary tract infection aided by the Unified Health System (SUS) in a Clinical Laboratory located in the state of São Paulo-Brazil, considering its origin.

Microorganism isolated	Community origin	Hospital Admission				Total Cases
	Health Posts *	First Aid	Oncology	Intensive Care Unit	Medical Clinic	
Burkholderia cepacea	2,44 (6)			0,40 (1)	0,40 (1)	3,24 (8)
Enterobacter sp.	2,83 (7)					2,83 (7)
Escherichia coli	69,71(172)	1,60(4)	2,8 (7)	0,80 (2)	0,80 (2)	75,71 (187)
Klebsiella pneumoniae	6,90 (17)			0,40 (1)	1,20 (3)	8,50 (21)
Proteus mirabilis	2,43 (6)					2,43 (6)
Pseudomonas aeruginosa	2,43 (6)					2,43 (6)
Staphylococcus aureus	2,03 (5)					2,03 (5)
Staphylococcus saprophyticus	0,81 (2)		0,40 (1)			1,21 (3)
Streptococcus agalactiae	1,22 (3)					1,22 (3)
Streptococcus sp.	0,40 (1)					0,40 (1)

* Basic Health Units, Center for Medical Specialties, Specialty Outpatient Clinics and Home for the Elderly Father Longinus, Home Care.

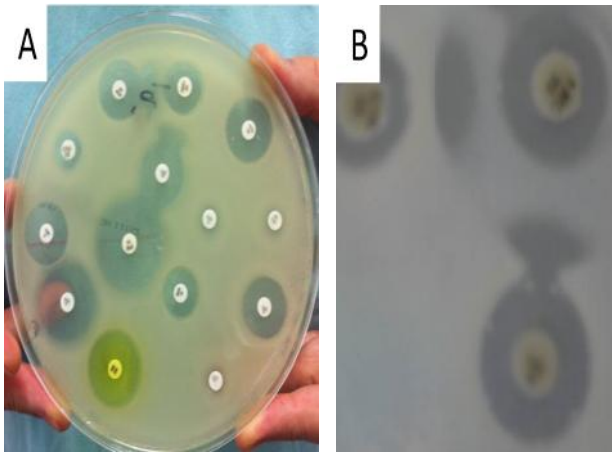

Figure 2 Extended spectrum beta lactamase (ESBL) in *E. coli.* This figure shows the characteristics of the presence of beta-lactamase of extended spectrum in *E. coli*. A: General view; B: Details of the formation of inhibition zone.

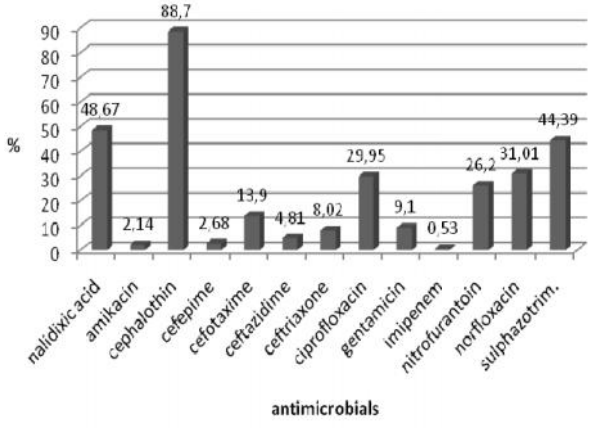

Figure 3Profile antibiotic resistance from *E. coli*. This graph illustrates the profile of antimicrobial resistance of E. coli, in which high resistance is observed with cephalothin (88.70% of cases).

CONCLUSIONS

The ITU was frequent in men and women of all ages and virulence factors are important because they are the same that determine the severity of the infection. The most frequent uropathogen among patients attended by the Health System in a clinical analysis laboratory located in the state of São Paulo, Brazil, was *E. coli*, which corroborate with other published reports.

The sensitivity profile of this uropathogen showed the epidemiological importance of *E. coli*, mainly for their antibiotic resistance profile. ESBL producing *E. coli* has tremendously increased worldwide and it is one of the most common causes of morbidity and mortality associated with hospital-acquired infections. The results suggest the need for greater attention to empirical indication, demonstrating that the most prevalent uropathogens has not satisfactorily respond to antimicrobial agents indicated part of the clinic.

Competing interests

There are no conflicts of interest for any of the authors in relation to this work. The lead authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Authors' contributions

FMSFP designed and conducted all phases of the research study. She also analyzed and interpreted the data and drafted the manuscript.
AAA advised and assisted with all phases of the project and revised the manuscript.
ALZB had the original idea for the study and advised on all aspects of the study and revised the manuscript.

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