Original Article

Do we really know the prevalence of multi-drug resistant *Escherichia coli* in the territorial and nosocomial population?

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Abstract

Introduction: The purpose of this work was to evaluate the prevalence of the Quinolones resistant *Escherichia coli* and/or ESBL producers in the population of our catchment area and hospital component.

Materials and Methods: From January 2008 to December 2010, all data concerning urine cultures in patients with suspected urinary tract infection and/or asymptomatic bacteriuria referring at our center located in the south of Milan were prospectively evaluated.

Results: In 2008, 2136 outpatient and 1232 hospital urine cultures were analyzed. The presence of quinolone-resistant strains was 21% at a local level and 53% in hospitals. ESBL-producing strains were isolated in 3.5% of cases at a local level and 20.5% in hospitals. In 2009, 2396 outpatient and 1320 hospital urine cultures were analyzed. The presence of quinolone-resistant strains was 21% at a local level and 46% in hospitals. ESBL-producing strains were isolated in 5.4% of cases at a local level and 20% in hospitals. In 2010, 2601 outpatient and 1717 hospital urine cultures were analyzed. The presence of quinolone-resistant strains was 34% at a local level and 26% in hospitals. ESBL-producing strains were isolated in 6.7% of cases at a local level and 20.6% in hospitals. The multidrug resistance was significantly ($P < 0.01$) higher in ESBL-positive strains.

Conclusion: Due to rising antibiotic resistance among uropathogens, it is important to have knowledge of the organisms causing urinary tract infections and their antibiotic sensitivity patterns. In areas with high prevalence of *E. Coli* resistance, performing urine culture before every surgical procedure became mandatory, in order to prevent fatal sepsis.

Key Words: *Escherichia coli*, hospital, resistance, territory, urinary tract infection

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INTRODUCTION

Urinary tract infections (UTIs) have been reported to affect up to 150 million individuals annually worldwide.[1] Unfortunately, in Europe, there are no reliable data concerning the prevalence of various types of UTIs and their impact on the quality of life of the affected population, on economics in general and of the health care system in particular.

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Antimicrobial resistance of human pathogens emerges as the use of antimicrobial agents for human infections increases. This limits the usefulness of antimicrobial therapy and leads to more clinical failures in treating patients with bacterial infections.

Quinolones, with their enhanced systemic activity against many gram-negative aerobes, have been widely used since the first

introduction of ciprofloxacin in 1987.[2] Fluoroquinolones are included among the antibiotics recommended by the Infectious Disease Society of America (IDSA) and European Association of Urology (EAU) for urinary tract infection. Despite this, resistance to fluoroquinolones has been reported in urinary tract infection worldwide.

During the past two decades, antimicrobial-resistant strains producing extended-spectrum-lactamases (ESBLs) have emerged amongst the Enterobacteriaceae. ESBLs are plasmid mediated bacterial enzymes that are able to hydrolyze oxyimino-β-lactams (broad-spectrum cephalosporins and aztreonam). The marked increase in the incidence of infections due to ESBL-producing *Escherichia coli* in recent years is of great concern, since the therapeutic options for these organisms are limited.

There is tremendous variability of antimicrobial resistance in not only different geographic regions, but also over time in specific areas. These phenomena make continuous surveillance of the extent and trends of antimicrobial resistance essential for guiding effective empiric therapy in every continent, country, city, hospital or even health care unit.

Recently, we have observed an increase of sepsis due to multidrug-resistant bacteremia in patients undergoing prostate biopsy and we report the detection of ESBL-producing *E. coli* from these patients.[3] The increase in multidrug-resistant strains of *E. coli* has limited therapeutic options and the early identification of infections due to these multidrug resistance organisms is necessary for appropriate treatment in order to reduce the mortality and mobility rate in affected patients.

The purpose of this work was to evaluate the prevalence of the Quinolones resistant *E. coli* and/or ESBL producers in the population attending the our catchment area and from the hospital component, to know if there is a variability in years and to understand the clinical implication of this phenomena.

MATERIALS AND METHODS

Patients diagnosed as having urinary tract infection in the outpatient clinic or emergency room or patients diagnosed within 48 hours after hospitalization were classified as having outpatient urinary tract infection, and patients diagnosed during the hospitalization period were classified as having inpatient urinary tract infection.

From January 2008 to December 2010, all data concerning urine cultures in patients with suspected urinary tract infection and/or asymptomatic bacteriuria referring at our centre were prospectively evacuate [Table 1].

The bacteria isolated were identified by colony morphology and biochemical reactivity (ATB Expression Sys bioMérieux). The resistance to quinolones and third generation cephalosporins has been tested by the method of microdilution (ATB Expression Sist. bioMérieux). While the presence of ESBL was assessed by the agar diffusion test recommended by the CLSI (Clinical and Laboratory Standards Institute). At that time, amoxicillin/clavulanic acid, cefazolin, cefotaxime, ceftazidime, ceftriaxone, cefotetan, cefuroxime, ciprofloxacin, imipenem, and trimethoprim/sulfamethoxazole were used as antibiotics.

Statistical methods

Prevalence data were compared by means of logistic regression, the odds ratio estimate and its 95% confidence limits were reported on Tables 2, 3 and 4. To correct for overdispersion or underdispersion, the covariance matrix was multiplied by the estimate of the dispersion parameter defined by the deviance divided by its degrees of freedom. All statistical evaluations were performed by SAS software package vers.9.1.3 and α value of 0.05 was considered as statistically significant.

Table 1: Details of urine cultures per year

	2008	2009	2010
Territorial urine culture	2136	2396	2601
Hospital urine culture	1232	1320	1717
Territorial positive culture for *E. coli*	237	260	282
Hospital positive culture for *E. coli*	112	116	155
Territorial *E. coli* ESBL+culture	7	15	19
Hospital *E. coli* ESBL+culture	24	25	31
Territorial *E. coli* resistant to quinolone	47	56	96
Hospital *E. coli* resistant to quinolone	51	42	39

Table 2: Statistical analysis of isolation of *E. coli* over time (years 2008, 2009, 2010)

Comparison	Years	OR estimate [95% C.L.]	*P* value
E. coli territorial	2008 Vs 2010	1.03 [0.9; 1.2]	0.7568
	2009 Vs 2010	1.00 [0.8; 1.2]	0.8810
E. coli hospital	2008 Vs 2010	1.01 [0.8; 1.3]	0.8493
	2009 Vs 2010	0.97 [0.8; 1.3]	0.7764
ESBL territorial	2008 Vs 2010	0.26 [0.1; 0.6]	0.0160
(Overall)	2009 Vs 2010	0.53 [0.3; 1.1]	0.9375
ESBL hospital (Overall)	2008 Vs 2010	1.09 [0.6; 2.0]	0.8849
	2009 Vs 2010	1.10 [0.6; 2.0]	0.8522
Quinolone resistant	2008 Vs 2010	0.22 [0.1; 0.3]	0.0001
Territorial (females)	2009 Vs 2010	0.24 [0.2; 0.4]	0.0013
Quinolone resistant	2008 Vs 2010	0.59 [0.3; 1.3]	0.4068
Territorial (Males)	2009 Vs 2010	0.63 [0.3; 1.3]	0.5649
Quinolone resistant	2008 Vs 2010	0.22 [0.1; 0.3]	<0.0001
Territorial (Overall)	2009 Vs 2010	0.25 [0.2; 0.4]	0.0005
Quinolone resistant	2008 Vs 2010	0.39 [0.2; 0.6]	0.0056
Hospital (Females)	2009 Vs 2010	0.28 [0.2; 0.4]	0.0001
Quinolone resistant	2008 Vs 2010	0.22 [0.1; 0.5]	0.0062
Hospital (Males)	2009 Vs 2010	0.17 [0.1; 0.3]	0.0048
Quinolone resistant	2008 Vs 2010	0.23 [0.2; 0.4]	0.0015
Hospital (Overall)	2009 Vs 2010	0.16 [0.1; 0.2]	<0.0001

Table 3: Analysis of the incidence rates at local and hospital level

Infection	Territorial rate (%)	Hospital rate (%)	OR estimate [95% C.L.]	*P* value
ESBL+(Females)	2.80	12.0	4.74 [2.7; 8.2]	<0.0001
ESBL+(Males)	3.24	8.88	2.91 [1.7; 5.1]	0.0001
ESBL+(Overall)	6.03	20.9	4.11 [2.8; 6.1]	<0.0001
Quinolone resistance (Females)	23.3	51.9	3.57 [2.7; 4.7]	<0.0001
Quinolone resistance (Males)	6.04	12.8	2.28 [1.5; 3.5]	0.0002
Quinolone resistance (Overall)	29.3	34.5	1.27 [0.97; 1.7]	0.0807

Table 4: Analysis of the isolation of the bacterium with respect to sex[8]

Infection	Males Rate (%)	Females Rate (%)	OR estimate [95% C.L.]	*P* value
ESBL territorial	3.24	2.95	0.91 [0.5; 1.7]	0.7540
ESBL hospital	12.01	8.88	1.40 [0.9; 2.2]	0.1577
Quinolone resistance territorial	6.03	23.3	4.72 [3.3; 6.8]	<0.0001
Quinolone resistance hospital	12.8	21.7	1.89 [1.3; 2.8]	0.0013

RESULTS

Epidemiology of ESBL-producing bacteria in UTI and antimicrobial susceptibility

In Table 1, the details of urine cultures per year were reported.

In 2008, 2136 outpatient and 1232 hospital urine culture were analyzed. The isolation of *Escherichia coli*, respectively, occurred in 237 samples (11.0% of the total and 72.7% of positive urine cultures) and in 112 samples (9.1% of the total and 48.1% of positive urine cultures). The presence of quinolone-resistant strains was 21% at a local level and 53% in hospitals. ESBL-producing strains were isolated in 3.5% of cases at a local level and 20.5% in hospitals. In total, the cross presence of quinolones-resistance and ESBL enzymes is present in 100% of the strains isolated in the outpatient setting and 66% of the strains isolated in hospitals.

In 2009, 2396 outpatient and 1320 hospital urine culture were analyzed. The isolation of *Escherichia coli*, respectively, occurred in 260 samples (10.8% of the total and 68% of positive urine cultures) and in 116 samples (8.8% of the total and 46.2% of positive urine cultures). The presence of quinolone-resistant strains was 21% at a local level and 46% in hospitals. ESBL-producing strains were isolated in 5.4% of cases at a local level and 20% in hospitals. In total, the cross presence of quinolones-resistance and ESBL enzymes is present is in 94.4% of the strains isolated in the outpatient setting and 70% of the strains isolated in hospitals.

In 2010, 2601 outpatient and 1717 hospital urine culture were analyzed. The isolation of *Escherichia coli*, respectively, occurred in 282 samples (10.8% of the total and 70% of positive urine cultures) and in 155 samples (9.0% of the total and 43.3% of positive urine cultures). The presence of quinolone-resistant strains was 34% at a local level and 26% in hospitals. ESBL-producing strains were isolated in 6.7% of cases at a local level and 20.6 % in hospitals. In total, the cross presence of quinolones-resistance and ESBL enzymes is present in 100% of the strains isolated in the outpatient setting and 73.8% of the strains isolated in hospitals.

ESBL-producing strains are also significantly resistant ($P < 0.01$) to ampicillin, piperacillin, piperacillin/tazobactam, trimethoprim/sulfamethoxazole, tetracycline and gentamicin. The multidrug resistance was significantly ($P < 0.01$) higher in ESBL-positive strains. All strains were susceptible to amikacin and carbapenems.

Statistical analysis of the data

Analysis of the isolation of *E. coli* over time (years 2008, 2009, 2010) [Table 2]: This initial analysis suggests that the isolation of this bacterium at a local level and in hospital results constant during these years. Statistically there is an increased rate of isolation of ESBL+ form at a local level, while in the hospital setting remains unchanged. Resistance to quinolones is progressive increase in the territorial area, due to an increase in these strains in women. Instead, the sensitivity to quinolones is increasing in the territorial male. In hospitals there is a decreasing of fluoroquinolones resistance in both genders.

Analysis of the incidence rate of the bacterium at local and hospital level [Table 3]: ESBL-positive strains isolated in hospitals are more than 4 times that at a local level and especially in women. Resistance to quinolones is high among women in the hospital, and is about 4 times greater than at a local level.

Analysis of the isolation of the bacterium with respect to sex [Table 4]: ESBL + strains do not prefer one sex over the other, while the resistance to quinolones is significant increase in women, especially in the territorial area.

DISCUSSION

We observed that the increase of multidrug-resistant bacteremia in men undergoing prostate biopsy suggested that surveillance for ESBL-producing bacteria is necessary in all departments at our hospital and in outpatients. Therefore, the aim of this study was to obtain epidemiologic data on the bacterial antibiotic resistance patterns in our catchment area in order to understand the dimension of these phenomena and guide our daily clinical practice. So, the title of this article reflects the facts that the real prevalence of some type of bacteria and in particular the study of their resistance patter are not well known and are generally under-estimated by the physicians.

After performing these evaluations we were amazed for the prevalence of these strains in the population. When data were expressed as the prevalence of ESBLs+, our data results the higher reported in Italy respect to previous data extrapolated from previous studies.[4,5]

The development of antibiotics has contributed greatly to reducing mortality caused by infection; nonetheless, as the use of antibiotics becomes generalized, the vicious circle of the development of the emergence of resistant bacteria and the use of new more efficacious antibacterial molecules cannot be severed.

E. coli is the main etiologic agent of acute urinary tract infections, which are usually treated with an empiric therapy with quinolones and fluoroquinolones. It was suggested that the rapid bactericidal activity of quinolones would be advantageous for minimizing resistance.[6] However, an association between the increase of fluoroquinolones prescriptions and an increase in bacterial resistance to the class has been reported from several different countries and resistance rates have been shown to vary markedly by center, with some hospital laboratories reporting >25% of their *E. coli* isolates as fluoroquinolones resistant.[7,8] With regard to susceptibility or resistance, the fluoroquinolones appear to exemplify a class effect, such that any decrease in susceptibility (i.e. increased Minimal Inhibitory Concentration-MIC) to one drug means a simultaneous decrease for all.[9] Our prevalence of *E. coli* resistance to Ciprofloxacin in the hospital setting results elevated in previous years. In 2010, the reduction of this value could be attributed to the understanding of the resistance pattern and the shifting to other class of antimicrobial agents such Amoxicillin/Clavulanic Acid, Trimethoprim/Sulfamethoxazole and Cephalosporins in the treatment of symptomatic urinary tract infections or in the prophylaxis of endo-urological surgical procedures.

In a worldwide survey conducted in 2004, 10% of the *E. coli* strains were found to be ESBL producers.[10] For *E. coli,* an increase in the prevalence of b-lactam resistance, especially concerning the third generation cephalosporins (in the period from 2002 to 2009, from 1.7% to 8%), has been observed recently, according to the annual report of the European Antimicrobial Resistance Surveillance System.[11] In the last 3 years, we observed an impressive rising of ESBL + *E. coli* sepsis after prostatic biopsy. Also, we noted that a growing in hospitalizations for symptomatic infections to the urinary tract due to these multi-resistant strains.

Antibiotics that can be used for the treatment of multidrug-resistant bacteria including ESBL-producing bacteria in urinary tract infection are limited.[11] In our study, similarly, excluding imipenem and amikacin, antibiotics with sensitivity higher than 50% to ESBL-producing *E. coli* were absent. Early identification of ESBL production is becoming increasingly important in terms of appropriate treatment and effective infection control in hospitals. In fact, the emergence of ESBL-producing *E. coli* in the community has been increasingly recognized as a global problem in recent years. Patients with infections caused by ESBL producers may experience delay in the initiation of appropriate therapy compared with patients with non-ESBL infections. In addition, infection with ESBL-producing bacteria raises mortality, and it not only prolongs hospital stay but also increases relative treatment costs.

This finding is interesting because the therapeutic options for ESBL producing bacteria might be limited. Current endemic areas now exist throughout the world, with ESBL types demonstrating geographic variation. In the United States, occurrence of ESBL production in Enterobacteriaceae show a national average around 3%, in Europe varies greatly from country to country (for example in the Netherlands is less than 1%), in Japan is less than 0.1%, while is 12% in Hong Kong.[12] Our values of prevalence of ESBL + *E. coli* results very elevated.

Therefore, it is very important to assess the risk factors for the emergence of ESBL-producing bacteria in order to prevent such resistance. ESBL-producing bacteria is more frequent in patients with a past history of hospitalization, a past history of exposure to antibiotics, a past history of catheterization, and a past history of urogenital surgery.[13-15] Overall, on 111 patients, 63 were hospitalized in the last year, 78 were exposed to quinolones and four to third-generation cephalosporins and five have bladder catheter for chronic urinary retention. This suggests two main points of action: Blocking infection within hospitals could reduce the prevalence of ESBL-producing bacteria and could lower the spread to communities while the respect of the strictly indications for the administration of antibiotics could reduce the rising of resistance.

CONCLUSIONS

The resistance to antibacterial agents is a major public health problem. The presence of *E. coli* ESBL producers at outpatient setting, although modest, should not be under-estimated, probably because at the base of the wider spread phenomenon in hospital. Due to rising antibiotic resistance among uropathogens, it is important to have local hospital based knowledge of the organisms causing UTI and their antibiotic sensitivity patterns. This information would be relevant not only to the local hospital but would also be a vital regional database. Monitoring of the antimicrobial resistance of *E. coli* is important because resistance has been reported to be associated with increased patient morbidity and mortality, and contributes to escalating health care cost. These results must

be considered in relation to the empiric antibiotics therapy in symptomatic urinary tract infections, in case of various urological procedures that we face on patients in particular that recently hospitalized. So for urologist, in areas with high prevalence of *E. coli* resistance, performing urine culture before every surgical procedure became mandatory, in order to prevent fatal sepsis.

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