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In vitro antimicrobial activity of several antimicrobial agents against *Escherichia coli* isolated from community-acquired uncomplicated urinary tract infections

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Abstract. – AIM: For frequency *Escherichia (E.) coli* is the agent of urinary tract infections (UTIs). The objective of the present study was to evaluate in vitro activity of several antimicrobial agents to guide empirical treatment of uncomplicated UTIs. In vitro antimicrobial susceptibility of 429 *E. coli* strains, isolated in urine specimens from community-acquired uncomplicated UTIs in outpatients, was studied during the 1st semester 2011.

MATERIALS AND METHODS: Urine samples were processed with Robobact/Uriset system. *E. coli* strains were identified with GN card for VITEK-2 system and API 20E gallery, as confirmatory assay. *E. coli* strains were tested, for antimicrobial susceptibility, using VITEK-2 system and confirmed with disk diffusion method.

RESULTS: Penicillins have exhibited sensitivity percentage of 50.9%, inhibitor-protected penicillins 88.9%, cephalosporins 91.6%, carbapenem 100%, aminoglycosides 91.5%, fluoroquinolones 71.8%, nitrofuran 99.3% and sulfonamide 74.1%.

CONCLUSIONS: *In vitro* imipenem, nitrofurantoin, cephalosporins and aminoglycosides (>90% of isolates) have the best sensitivity in community-acquired UTIs. In particular nitrofurantoin has showed a low MIC distribution and high sensitivity percentage. Therefore, it could be suggested in empirical treatment of these infections.

Key Words:
Antimicrobial agent, *E. coli*, MIC, UTI(s).

Introduction

Uncomplicated community acquired urinary tract infections (UTIs) is the second most common infectious disease in community medical practice[1]. *Escherichia (E.) coli* is the most clinically relevant organism of uncomplicated UTIs[2]. It is the aetiologic agent in 75-90% of these infections[3]. It has been reported that in Western countries, antimicrobial susceptibility test may be unnecessary in these infections, for high cost/benefit[4]. Unfortunately, significant increase in prevalence of resistance to antibiotics has been observed in common human and animal pathogens in the worldwide[5-7]. The appearance and spread of resistance to antimicrobial agents resulted an increase of morbidity, mortality, and cost of health care[8]. Bacteria, resistant to commonly used antimicrobial molecules, have long been detected and studied[9,10]. Misuse and overuse of antimicrobial agents are important and relevant risk factors for emergency and amplification of bacterial resistances[11]. Since 1980, e.g., *Enterobacteriaceae* have acquired resistance mechanisms, producing extended-spectrum beta-lactamases (ESBL) and also, in recent years, carbapenemase[12,13]. Furthermore, broad use of fluoroquinolones has been followed by emergency of resistance[14], which has been due mainly to chromosomal mutations in genes encoding the subunits of the drugs' target enzymes (e.g. DNA gyrase and topoisomerase IV, etc.). Minimum inhibitory concentration (MIC) distribution of ampicillin (AP), piperacillin (PIL), piperacillin/tazobactam (PTZ), amoxicillin/clavulanate (AUG), cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPM), imipenem (IMI), ciprofloxacin (CIP), norfloxacin (NOR), amikacin (AK), gentamicin (GM), nitrofurantoin (NI) and trimethoprim/sulfamethoxazole (SXT) has been evaluated against *E. coli* strains isolated from community-acquired uncomplicated UTIs.

The goal in the present study was to identify *in vitro* antimicrobial molecules, characterized by low MIC and high percentage of sensitivity to guide empirical treatment of uncomplicated UTIs.

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Materials and Methods

In vitro antimicrobial susceptibility of 429 *E. coli* strains, isolated in urine specimens from community-acquired uncomplicated UTIs, were studied in outpatients during the 1st semester 2011. All urine samples were processed with Robobact/ Uriset system (DIESSE, Siena, Italy), according to the manufacturer's instructions. It is a system for culture, isolation and count of pathogenic organisms and contains a chromogenic growth medium and Columbia Blood Agar medium (Uribact-Chrom Columbia Blood). After incubation within this system in ambient air at 35° ± 1°C for 24 hours, the solid media were read by investigators, considering significant a count ≥5 × 10^4 CFU/mL (Colony Forming Unit/mL). Manufacturer's instructions were followed to identify the isolates according to the color, shape, and size of colonies. All pink colonies, grown in chromogenic medium, were inoculated into Sheep Blood agar plates (Bekton Dickinson, Erembodegem-Aalst, Belgium) and incubated in ambient air at 35° ± 1°C for 24 hours, to obtain well isolated bacterial colonies. *E. coli* strains were identified by standard laboratory techniques. Identification tests were GN card for VITEK-2 system (BioMérieux, Marcy l'Etoile, Craponne, France) and, as confirmatory methods, API 20E gallery (BioMérieux, Marcy l'Etoile, Craponne, France), according to the manufacturer's instructions. All *E. coli* strains were tested for antimicrobial susceptibility using VITEK-2 system. Susceptibility tests with the VITEK-2 system were performed with the AST-N27 card, according to the manufacturer's instructions. However, VITEK-2 system has some antibiotic-microrganism limitations, e.g. PTZ-*E. coli*, erithromycin-*S. agalactiae*, AK-*A. baumannii,* etc., as described in manufacturer's package insert of antibiotic susceptibility testing (AST) cards. Therefore, Kirby Bauer disk diffusion method was conducted for each *E. coli* isolates, as reference antimicrobial test. Standardized 0.5 McFarland saline bacterial suspensions were used to inoculate the Mueller Hinton agar (Becton Dickinson **Er**embodegem-Aalst, Belgium**)** with cotton swab in order to obtain confluent growth. The plates were incubated at 35° ± 1°C for 18 ± 2 hours in ambient air within 15 minutes from application of the disks (Bio-Rad, Milan, Italy). The resultant zones of inhibition were measured with Osiris System (Bio-Rad, Milan, Italy), according to the manufacturer's instructions. The Osiris system is able to deduce the MIC on the size of the inhibition zones. The concentration of antimicrobial agents for disk diffusion methods were as follows: AP 10 mcg, PRL 30 mcg, PTZ 30/6 mcg, AUG 20/10 mcg, CTX 5 mcg, CAZ 10 mcg, CPM 30 mcg, IMI 10 mcg, CIP 5 mcg, NOR 10 mcg, AK 30 mcg, GM 10 mcg, SXT 1.25/23.75 mcg and NI 100 mcg. Quality controls (QC) for identifications and antimicrobial assays were used; QC strains employed for the identification of Gram negative bacteria was *Klebsiella oxytoca* ATCC 700324 (Oxoid, Milan, Italy), whereas for the antimicrobial susceptibility test was the *E. coli* ATCC 25922 (TCS Biosciences Ltd, Buckingham, UK). The tested dilutions for the antimicrobial susceptibility were: AP from ≤2 to ≥32, PRL from ≤4 to ≥128, PTZ from ≤4 to ≥128, AUG from ≤2 to ≥32, CPM from ≤1 to ≥64, CTX from ≤1 to ≥64, CAZ from ≤1 to ≥64, IMI from ≤1 to ≥8, GM from ≤1 to ≥16, AK from ≤2 to ≥64, CIP from ≤0.25 to ≥4, NOR from ≤0.5 to ≥16, NI from ≤16 to ≥128, SXT from ≤1 to ≥16. The interpretation of results was performed following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria of 2011. Evaluation of certain mechanisms of resistance (ESBL or carbapenemase) was carried out through the use of phenotypic tests described in literature (double disk test and modified Hodge test)[15,16]. The epidemiological data of antimicrobial susceptibility are obtained by Mercurio software (NoemaLife, Bologna, Italy). The patients enrolled in this study are selected on the base of following anamnestic data: increased urinary frequency and/or urgency, dysuria, macrohematuria, mild to moderate fever, and no significant back pain; whereas patients with urinary or faecal incontinence, genital disorder, hospitalization in the last three weeks, and subsequent isolations within 15 days from 1st *E. coli* detection were excluded. They were 250 female and 179 male, with mean age 56.4 (age range 15-96 y.o.) and distributed as follows: ≥15 y.o. in 45% of cases and ≥55 y.o. in 55% of cases.

Results

Table I shows MICs distribution of *E. coli* strains isolates related to antimicrobial agents tested. Penicillins exhibit sensitivity percentage of 50.9% (AP 50.1% and PRL 51.7%), inhibitor-protected penicillins 88.9% (PTZ 96% and AUG 81.8%), cephalosporins 91.6% (CPM 92.5%, CTX 90.2% and CAZ 92.1%), carbapenem (IPM) 100%, aminoglycosides 91.5% (GM 91.4% and AK

Table I. MICs distribution of *E. coli* strains.

Antimicrobial agents	No of strains with following MIC value (mcg/mL)[a]									
	0.25	0.5	1	2	4	8	16	32	64	128
AP	–[b]	–[b]	–[b]	**166**	**34**	**15**	4	210	–[b]	–[b]
PRL	–[b]	–[b]	–[b]	–[b]	**214**	**8**	4	1	3	199
PTZ	–[b]	–[b]	–[b]	–[b]	**329**	**83**	7	3	3	4
AUG	–[b]	–[b]	–[b]	**180**	**87**	**84**	58	20	–[b]	–[b]
CPM	–[b]	–[b]	**397**	7	5	2	1	3	14	–[b]
CTX	–[b]	–[b]	**387**	0	5	2	0	0	35	–[b]
CAZ	–[b]	–[b]	**395**	1	6	4	15	0	8	–[b]
IMI	–[b]	–[b]	**429**	0	0	0	–[b]	–[b]	–[b]	–[b]
GM	–[b]	–[b]	**375**	**17**	2	0	35	–[b]	–[b]	–[b]
AK	–[b]	–[b]	–[b]	**294**	**82**	**21**	32	0	0	–[b]
CIP	**310**	**11**	8	5	95	–[b]	–[b]	–[b]	–[b]	–[b]
NOR	–[b]	**295**	6	27	1	2	98	–[b]	–[b]	–[b]
NI	–[b]	–[b]	–[b]	–[b]	–[b]	–[b]	**380**	**34**	**12**	3
SXT[c]	–[b]	–[b]	**313**	**5**	0	0	111	–[b]	–[b]	–[b]

[a]The numbers, in bold style, indicate susceptible category by current EUCAST recommendations; [b]Dilutions not displayed by VITEK-2 analysis; [c]Trimethoprim: sulfamethoxazole in the ratio 1:19; the breakpoint are expressed as the trimethoprim concentration.

92.5%), fluoroquinolones 71.8% (CIP 74.8% and NOR 68.8%), nitrofuran (NI) 99.3% and sulfonamide (SXT) 74.1%. The 10.3% (44/429) of *E. coli* isolates produced ESBL, with significant prevalence in age range ≥55 y.o. (41/44); conversely was not revealed strains carbapenemase-producing. According to EUCAST guidelines, it is interesting to point that, in cases of *E. coli* isolates produced ESBL, the MIC value ≤1 for cephalosporins was revealed and categorized as sensitive in 27.3% of isolates for CPM (12/44), in 4.5% for CTX (2/44) and in 22.7% of for CAZ (10/44). Bacterial load was $>10^5$ CFU/mL in 86.7% of cases (372/429). Moreover, the statistical analysis, through the use of Cohen's kappa coefficient (Kc)[17,18], has revealed that, between VITEK-2 automated system and disk diffusion manual method, subsist an agreement from substantial to almost perfect. In fact substantial agreement has been highlighted for TZP assay (Kc 0.67), while an excellent agreement has been found for AK assay (Kc 0.89) and for the assays of the other antimicrobial agents (Kc ranged from 0.93 to 0.99). In addition, a moderate agreement was revealed for antimicrobial agent PTZ against producing-ESBL strains (Kc 0.60), as observed by Tout et al[19], instead almost perfect agreement in other strains (Kc 0.86).

Discussion

Today, the increase in bacterial resistance and the concomitant global financial crisis require the rationalization of antimicrobial prescriptions. At the same time, surveillance studies of *in vitro* antimicrobial activity are necessary in order to avoid therapeutic failures, and then to reduce morbidity, mortality, and consequently, health care costs. Among the antimicrobial agents tested, the obtained results display that *in vitro* imipenem, nitrofurantoin, cephalosporins and aminoglycosides have the best sensitivity in community-acquired UTIs (>90% of isolates). However, the use in medical practice of cephalosporines and carbapenems, in these localized infections, may cause the emergence of some resistance mechanisms (broad-spectrum beta-lactamases and carbapenemase)[20]. Aminoglycosides are systemic antimicrobial agents, associated with some limitations in patients with renal diseases or may be related to renal injury[21,22]. Conversely, nitrofurantoin has localized activity in urinary tract and consequently a minor selective pressure on bacteria. Despite high use of fluoroquinolones in medical practice[5,23,24], they show a lower activity *in vitro* than nitrofurantoin and trimethoprim/sulfamethoxazole against *E. coli* strains studied. Therefore, nitrofurantoin could be suggested in empirical treatment of community-acquired UTIs[25], for excellent activity against almost all *E. coli* isolated in the present study, for the low MIC distribution, oral administration, low-cost therapy, localized activity and easy availability in community. In addition, the use of this antimicrobial agent in clinical practice could reduce also the *E. coli* producing ESBL prevalence.

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