PREVALENCE AND SUSCEPTIBILITY TO CHEMOTHERAPEUTIC AGENTS OF BACTERIAL SPECIES ISOLATED FROM URINARY TRACT INFECTIONS

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Abstract

Objectives: The susceptibility to chemotherapeutic agents was tested in 3,810 strains of bacteria isolated from urinary tract infections in the period 1st January 2002–30 May 2009, in outpatients aged 18 to 94 years (78.1% females and 21.9% males).

Material and methods: The susceptibility to chemotherapeutic agents was determined by the standard disc-diffusion method.

Results: Of the 3,810 strains of bacteria, 76.8% were Escherichia coli, 7.1% were Proteus spp., 6.3% were Klebsiella spp. and 9.8% strains were other enterobacteria, Pseudomonas spp., Staphylococcus aureus and Enterococcus spp. strains. All the tested strains of Escherichia coli were susceptible to colistin, over 90% strains were susceptible to some third generation cephalosporins and amikacin. Proteus spp., Klebsiella spp. and the other Enterobacteriaceae strains showed a high susceptibility to fluoroquinolones and third generation cephalosporins, Pseudomonas spp. strains presented a high rate of susceptibility to colistin, ceftazidime, imipenem and amikacin. Staphylococcus aureus and Enterococcus spp. showed a high susceptibility to nitrofurantoin, amoxicillin-clavulanic acid. Of the tested strains, 15 strains of Escherichia coli, 3 strains of Klebsiella spp., 1 strain of Citrobacter farmeri and 1 strain of Enterobacter spp. produced an extended spectrum beta-lactamase (ESBL). The associations between bacteria or between bacteria and Candida spp. were noted.

Conclusions: Escherichia coli strains presented a high susceptibility to some third generation cephalosporins, to amikacin and to ceftazidime. Some fluoroquinolones and cephalosporins were active on Proteus spp. and Klebsiella spp. strains; 20 strains of enterobacteria produced ESBL.

Keywords: urine, bacteria, susceptibility, ESBL.

PREVALENŢĂ ŞI SENSIBILITATEA FAŢĂ DE CHIMIOTERAPICE A SPECIILOR BACTERIENE IZOLATE DIN INFECŢIILE TRACTULUI URINAR

Rezumat

Objective: A fost testată sensibilitatea faţă de chimioterapice pentru 3810 tulpini bacteriene, izolate din infecţii ale tractului urinar în perioada 1 Ianuarie 2002–30 Mai 2009, la pacienţii din ambulator în vârstă de 18-94 ani (78,1% femei şi 21,9% bărbaţi).

Material şi metodă: Sensibilitatea faţă de chimioterapice s-a determinat prin metoda difuzimetrică standard.

Rezultate: Din cele 3810 tulpini bacteriene, 76,8% au fost tulpini de Escherichia coli, 7,1% au fost Proteus spp., 6,3% Klebsiella spp. şi 9,8% au fost alte enterobacterii, Pseudomonas spp., Staphylococcus aureus şi Enterococcus spp. Toate
Introduction
Urinary tract infections (UTI) are among the most frequent bacterial infections encountered both in the outpatient units and in nosocomial infections. They can involve any age group and are often followed by recurrences which increase the risk of long term kidney lesions. Urinary infections are frequently caused by enterobacteria, Escherichia coli occupying the first place in their etiology. The choice of the antibacterial treatment is based on the knowledge of the predominant pathogenic agents and of their susceptibility to chemotherapeutic agents. The selection and spreading of the strains resistant to the antibiotics is a clinical aspect of great importance in the present period, requiring the continual monitoring of this phenomenon [1,2,3,4,5,6,7].

This paper analyses the results relating to the susceptibility to chemotherapeutic agents of certain bacteria isolated in the outpatient unit from patients with UTI in the period 1 January 2002–30 May 2009.

Material and methods
The urine samples were obtained using the clean-catch midstream urine and were analysed in the outpatient unit. The sensitivity to chemotherapeutic agents was tested in 3,810 bacterial strains, isolated from 2,974 (78.1%) females and 836 (21.9%) males, aged 18 to 94 years, in the period 1 January 2002–30 May 2009. This patients presented the signs of urinary tract infections (dysuria, polyuria, hematuria). Clinical diagnosis of the subjects from which the 3,810 strains were isolated was acute or recurrent cystitis in the most part of the cases (3552 cases) and an important number of severe renal diseases (258 cases) was detected in the period 1 January 2005–30 May 2009 (e.g. nephritis, nephropathies, kidney stones, renal failure, renal myeloblastosis, renal or urinary bladder disfunctions, surgically unique kidney) (Fig.1). Of the 258 severe renal cases, 201 (77.9 %) were produced by E. coli strains. These severe infections were detected especially in the patients presenting repeated UTI usually determined by enterobacteria multiple resistant to chemotherapeutic agents or/and with bacterial associations. Thus, of the 258 strains isolated from the severe renal cases, 95 (36.8%) strains presented multiple drug resistance, and also of the 201 Escherichia coli strains, 75 (37.3%) strains were multiple drug resistant.

Fig. 1. Clinical diagnosis of the 258 severe renal diseases detected in the period 2005-2009.

The bacteria were isolated by using semi-quantitative urocultures, seeding the culture media: Levine or EMB-Agar (Bio-Rad) and CLED-Agar (cystine-lactose-electrolyte deficient) (Biolab) with the taken urine using calibrated
bacteriologic loop with an inner diameter of 5 mm (the urine volume in a loop being 0.01 ml). The following formula was used: Number of bacterial cells/ml = number of colonies developed x 100, and the presence of a number of 100,000 bacteria cells/ml was considered significant. The bacterial strains were identified according to the aspect of the colonies on the media and by the biochemical tests: T.S.I. – triple sugar iron (Biolab); M.I.U. - mobility, indol, urea (Oxoid), Simmons citrate medium (Mast Diagnostics); Api10S or Api 20E or Api Staph (bioMérieux); the discs for oxidase and the tests of catalase and coagulase; the bile-esculine agar medium (Oxoid). Müller-Hinton medium (Bio-Rad) with NaCl 4% were used for testing Staphylococcus spp. to oxacillin. The colonies suspected for a mycosis were isolated on Sabouraud medium (Bio-Rad).

The susceptibility to chemotherapeutic agents was tested by the standard disc-diffusion method according to the CLSI/NCCLS standards using Müller-Hinton medium and discs provided by Oxoid, Bioanalyse Ltd., ABTEK Biologicals Ltd.: nalidixic acid (NA), colistin (CT), nitrofurantoin (F), norfloxacin (NOR), ofloxacin (OFX), ciprofloxacin (CIP), cefaclor (CEC), cefamandole (MA), cephalixin (CL), cefuroxime (CXM), ceftazidime (CAZ), cefoperazone (CEP), cefotaxime (CTX), ampicillin (AM), amoxicillin-clavulanic acid (AMC), sulbactam-ampicillin (SAM), trimethoprim-sulphamethoxazole (SXT), amikacin (AK), gentamicin (CN), imipenem (IPM). Only Staphylococcus aureus and Enterococcus spp. were tested to penicillin (P), oxacillin (OX), and the strains of Enterococcus were tested to amoxicillin (Amx), too.

Extended spectrum beta-lactamase (ESBL)-producing strains were identified by the double-disk synergy test between a third generation cephalosporin producing strains were identified by the double-disk synergism test between a third generation cephalosporin (CAZ) and AMC (CLSI/NCCLS standard).

Results

Of the 3,810 bacterial strains, 2,926 (76.8%) strains were Escherichia coli, and these strains with the other isolated strains are represented in Fig. 2. The 2,926 strains of Escherichia coli (Fig.3) showed that over 90% strains were susceptible to CAZ, CEP, CTX, AK, all the strains were susceptible to CT, a low rate of susceptibility was noted to SXT and to AM; 15 strains produced ESBL. A number of 5 strains multiple resistant to chemotherapeutic agents were tested to IPM and were susceptible.

Over 70% strains of Proteus spp. were susceptible to CAZ, CRO and in a very low rate the strains were susceptible to F (Fig.4); 53 strains were tested to IPM (51 strains susceptible, 1 strain intermediate, 1 strain resistant). Clinical intolerance (urticaria) to CIP and OFX was reported in a woman of 80 years old with UTI produced by Proteus mirabilis strain susceptible to NOR.

Other bacteria strains (88 strains) were isolated in a small number (Fig.2). Serratia spp., Enterobacter spp. and Citrobacter spp. strains were 100% susceptible to IPM, AK and in a high percentage were susceptible to CAZ and NOR. The most part of the strains of Morganella morgani and Providencia rettgeri were susceptible to IPM and cephalosporins of third generation (CAZ, CTX). One
strain of Citrobacter farmeri and one strain of Enterobacter spp. produced ESBL. Of the 3 strains of Acinetobacter baumanii, one strain was susceptible to CAZ, AK, and the other 2 strains were susceptible only to CT. The strain of Pantoea spp.2 was susceptible to CAZ, F and CT.

Two strains susceptible to F (10 strains resistant), 50% of the strains tested to OFX, CIP and to CN were susceptible. Two strains tested to IPM were susceptible and 24 strains were found resistant to SXT.

The most frequent associations between isolated bacteria were: Proteus spp. with E. coli (10 cases) or with Pseudomonas spp. (6 cases), E. coli with Pseudomonas spp. (6 cases). Also, were found: Candida spp. associated with Citrobacter spp (1 case) or with E. coli (2 cases) or with Staphylococcus aureus (3 cases).

**Discussions**

More than 70% of the tested strains were isolated from urinary tract infections in women. The same situation was reported by many other authors [1,2,3,8,9,10]. The increased incidence of the urinary infections in women is conditioned by favouring anatomic factors, by hormonal changes and by the urodynamic disturbances occurring with age. Nearly 80% of the 3,810 identified strains were E. coli (Fig.2). Numerous authors attested that E. coli occupies the first place in the etiology of urinary infections [1,2,4,5,9,11], similarly with the data of our investigation. Arslan and co-workers [6] isolated E. coli in 90% of the uncomplicated UTI and in 78% complicated UTI. It was noted that in complicated infections the frequency of the isolation of E. coli is decreased and the ratio of non-Escherichia coli Gram-negative bacteria and of Gram positive bacteria increases [4,12]. Of the 3,810 isolated strains, 7.1% of the strains were Proteus spp. and 6.3% were strains of Klebsiella spp. (Fig.2), the situation being similar to that found by Andreu et al. [5] in Spain. In USA and Canada, Zhanel et al. [9] isolated Klebsiella pneumoniae from 12.4% cases of urinary infections, and in India Akram et al. [1] found Klebsiella pneumoniae in 22% of the cases, as compared with a decreased percentage determined by us (6.3%). In Romania, Ungureanu et al. [10] isolated 16% strains of Klebsiella from UTI in 1999 and, Țenea and Dorobăț [11] isolated 8.4% strains of Klebsiella spp. from UTI in 2008. In our paper, other species of Gram negative or Gram positive bacteria (Serratia, Enterobacter, Citrobacter, Morganella, Providencia, Pseudomonas, Staphylococcus) represented a small proportion of the 3,810 isolated ones, similarly with the data reported by other authors [5,9,13].

Of the 2,926 strains of E. coli isolated from us, a significant proportion of these strains were found resistant to AM (60.4%) and to SXT (51%), similarly with the data found by other authors [1,2,3,9,11,14,15]. Some researches reported over 90% E. coli strains susceptible to F and to fluoroquinolones [2,4,9], this rate of susceptibility being more increased than that reported in this paper (74.2-79.8% susceptible strains). During the last years, however, a decreased susceptibility of E. coli to quinolones was recognised especially in the strains isolated from complicated urinary infections in elderly, and previously treated with fluoroquinolones [5,14]. Studies performed in Spain by Gobernado et al. [14] showed that 18% of E. coli strains isolated in the outpatient units were
resistant to quinolones and for the empiric treatment of UTI recommended fosfomycin, CXM or AMC, to which they found less than 3% resistant strains, as compared with the strains isolated by us (Fig.3). Based on a study carried out on 14,319 E. coli strains, Junquera et al. [15] concluded that penicillins, quinolones and SXT cannot be considered any more an election treatment of UTI caused by E. coli. Similar researches performed in Latin America [3] established that AK, piperacillin-tazobactam, aztreonam, carbapenems represent the reasonable therapeutic options because 91%-100% strains in that area were susceptible to these chemotherapeutic agents. Of the 2,926 E. coli strains analysed in this paper, 94.3% strains were susceptible to AK. Țenea and Dorobăț [11] found high percentages of ESBL-positive E. coli strains resistant to quinolones, aminoglycosides, SXT, while ESBL-negative strains were more susceptible to the tested chemotherapeutic agents.

In the case of Proteus spp. and Klebsiella spp. a high proportion of these strains were resistant to F and SXT (Fig.4 and Fig.5). Gales et al. [3] assert that the only therapeutic option in UTI caused by Klebsiella spp. are carbapenems, considering that they found more than 30% isolates producing extended spectrum beta-lactamases (ESBL), a percentage more elevated that the value found in this paper. In our study, of the strains tested to IPM, 25 strains of Klebsiella spp. were susceptible and 51 strains of Proteus spp. were found susceptible, too. For the treatment of other types of enterobacteria isolated in a small number, F, AK, some third generation cephalosporins or some fluoroquinolones could be recommended [16]. Also, other authors isolated a small number of these strains and some of them observed that the rate of susceptibility to these chemotherapeutic agents decreased [9,17,18,19].

The 112 tested strains of Pseudomonas spp. showed a high susceptibility to IPM, CT and AK (Fig.6). Carbapenems, forth generation cephalosporins and AK proved to be efficient in the case of Pseudomonas spp. strains isolated from UTI in Asia [20], such as the results of our investigation. In infections with Pseudomonas aeruginosa some authors recommended the return to the treatment with CT [21].

Tessema et al. [22] reported high percentages of S. aureus resistant to tetracyclines (80%), SXT (53.3%) and AM (43.3%), similarly with the situation showed in Fig.7. Jha and Bapat [23] found very high percentages of susceptibility to cephalosporins (88.8%) and to AK (80.6%); the most part of the strains were susceptible to F (77.7%) and NOR (65.5%), similarly with the data of our study (Fig.7).

Of the 3,810 bacterial strains isolated, 20 strains produced ESBL, as compared with high percentages of enterobacteria strains-producing ESBL detected by other authors [7,24,25,26]. The confirmed ESBL-producing strains are considered resistant to all penicillins, cephalosporins and monobactams, however, part of the strains studied by some authors proved to be susceptible to cefepime [7]. Lee et al. [27] replaced the treatment with cephalosporins by the association of piperacillin-tazobactam, obtaining the reduction of the spreading of the ESBL strains, especially in the case of the strains of Klebsiella pneumoniae. Other authors [8,28] found that ESBL-producing E. coli strain is susceptible to carbapenems, cephapycin, aminoglycosides, fluoroquinolones. In this study, 0.52% tested strains produced ESBL, but ESBL-producing E. coli strains represented a rate of 0.39%, and all these percentages were smaller than those noted in other papers [1,25,26]. Akram et al. [1] isolated ESBL-producing E. coli strains in 34.4% of the UTI in the outpatient unit, showing that these bacteria can escape detection by using the disc-diffusion method, leading to an inadequate use of the chemotherapeutic agents and to ineffectivity of the treatment, recommending the synergic effect test of some third generation cephalosporins and of AMC, similarly with the method used in this paper.

Of the 3,810 tested strains, only 1.9% strains were Enterococcus spp., a lower percentage, as compared with that reported by other authors which found 4-6% strains of Enterococcus spp. involved in UTI in the last years [11,26,29,30,31,32]. The 72 strains of Enterococcus spp. tested by us proved to have a high susceptibility to aminopenicillins (88.3% susceptibility to Amx and 100% susceptibility to AMC) and 81.8% strains were susceptible to F, similarly with the results reported by other authors [4,26,33]. In Romania, Țenea and Dorobăț [11] found that Enterococcus faecium strains isolated from ITU presented 100% resistance to beta-lactamines and 78-92% strains were resistant to quinolones and CN, as compared with the present study, where half of the strains tested to OFX, CIP and to CN were susceptible. The most part of the authors did not found resistance to glycopeptides in enterococci isolated recently from UTI [26,31,32,33,34], while high percentages of the strains were resistant to CN [32,33,35]. The Japanese authors consider that at present the important issues of UTI would be the increased incidence of ESBL-producing Gram-negative bacilli, their resistance to fluoroquinolones and the increased involvement of enterococci multiple resistant to chemotherapeutic agents [36]. The phenomenon of antibiotic resistance requires a continual supervision for a better orientation of the treatment in UTI.

Conclusions
1. Of the 3,810 bacterial strains isolated from UTI, a percentage of 78.1% of the strains were isolated from females and 21.9% from males.
2. A number of 2,926 (76.8%) isolates were E. coli, 7.1% Proteus spp., 6.3% Klebsiella spp., 2.9% of the strains were Pseudomonas spp., 2.67% were strains of Staphylococcus aureus, and in 4.23% of the cases other species of enterobacteria and Enterococcus spp. were identified.
3. Over 90% of E. coli strains were susceptible to some third generation cephalosporins and AK, and in a low ratio the strains were susceptible to AM and SXT; over 70% of Proteus spp. strains were susceptible to some third generation cephalosporins; CT, OFX and CXM, CAZ, were more active on Klebsiella spp. strains, as compared with other chemotherapeutic agents.

4. A number of 15 strains of E. coli, 3 strains of Klebsiella spp., 1 strain of Citrobacter farmeri and 1 strain of Enterobacter spp. were ESBL-producing strains.

References
27. Huang Y, Zhuang S, Du M. Risk factors of nosocomial