

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 cefuroxime. Some fluoroquinolones and cephalosporins were active on *Proteus* spp. and *Klebsiella* spp. strains; 20 strains of enterobacteria produced ESBL.

Keywords: urine, bacteria, susceptibility, ESBL.

PREVALENȚA ȘI SENSIBILITATEA FAȚĂ DE CHIMIOTERAPICE A SPECIILOR BACTERIENE IZOLATE DIN INFECȚIILE TRACTULUI URINAR

Rezumat

Obiective: A fost testată sensibilitatea față de chimioterapice pentru 3810 tulpini bacteriene, izolate din infecții ale tractului urinar în perioada 1 Ianuarie 2002–30 Mai 2009, la pacienții din ambulator în vârstă de 18-94 ani (78,1% femei și 21,9% bărbați).

Material și metodă: Sensibilitatea față 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. și 9,8% au fost alte enterobacterii, *Pseudomonas* spp., *Staphylococcus aureus* și *Enterococcus* spp. Toate

tulpinile de *Escherichia coli* testate au fost sensibile față de Colistin, peste 90% tulpini au fost sensibile față de unele cefalosporine de generația a 3-a și Amikacin. Tulpinile de *Proteus spp.*, *Klebsiella spp.* și alte *Enterobacteriaceae* au prezentat o sensibilitate crescută față de fluoroquinolone și Cefalosporine de generația a 3-a, *Pseudomonas spp.* au prezentat o rată crescută de sensibilitate față de Colistin, Cefazidime, Imipenem și Amikacin. *Staphylococcus aureus* și *Enterococcus spp.* au avut o sensibilitate crescută față de Nitrofurantoin, Amoxicilina-Acid clavulanic. Dintre tulpinile testate, 15 tulpini de *Escherichia coli*, 3 tulpini de *Klebsiella spp.*, 1 tulpină de *Citrobacter farmeri* și 1 tulpină de *Enterobacter spp.* au produs beta-lactamază cu spectru extins (BLSE). S-au constatat asocieri bacteriene și asocierea bacteriilor cu *Candida spp.*.

Concluzii: Tulpinile de *Escherichia coli* au prezentat o sensibilitate crescută față de unele Cefalosporine de generația a 3-a, Amikacin și Cefuroxime. Unele Fluoroquinolone și Cefalosporine au fost active față de tulpinile de *Proteus spp.* și *Klebsiella spp.*; 20 tulpini de enterobacterii au produs BLSE.

Cuvinte cheie: urină, bacterii, sensibilitate, BLSE.

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 1st 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 1st 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.

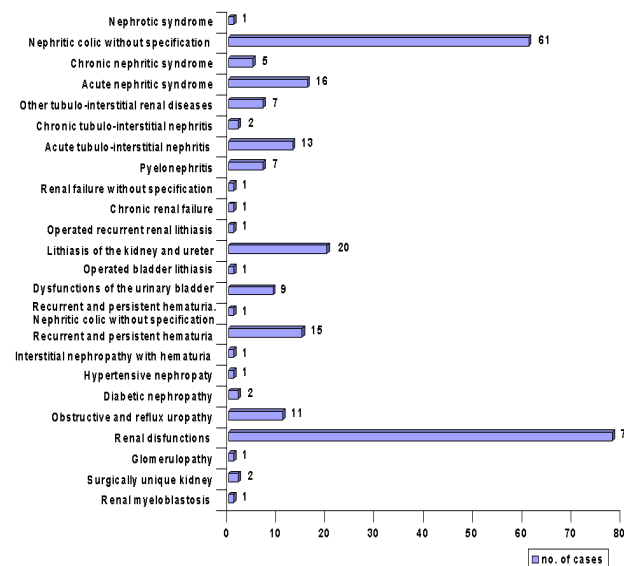


Fig. 1. Clinical diagnosis of the 258 severe renal diseases detected in the period 2005-2009.

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), ceftriaxone (CRO), 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 (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.

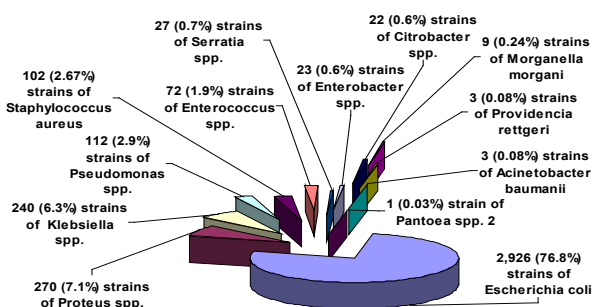


Fig. 2. Bacterial species isolated from UTI.

The standard disc-diffusion method performed for

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.

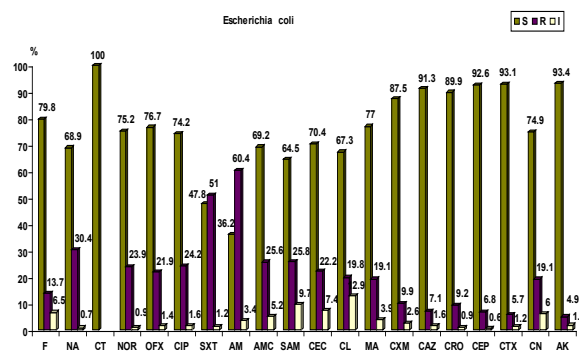


Fig. 3. The susceptibility to chemotherapeutic agents of *Escherichia coli* strains.

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.

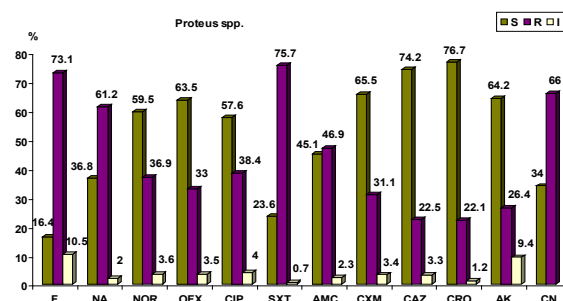


Fig. 4. The susceptibility to chemotherapeutic agents of *Proteus* spp. strains.

The strains of *Klebsiella* spp. presented a high susceptibility to CT and OFX and a low susceptibility to F (Fig.5); 27 strains were tested to IPM (25 strains susceptible, 2 were resistant); 3 strains produced ESBL.

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 morganii* 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 baumannii*, 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.

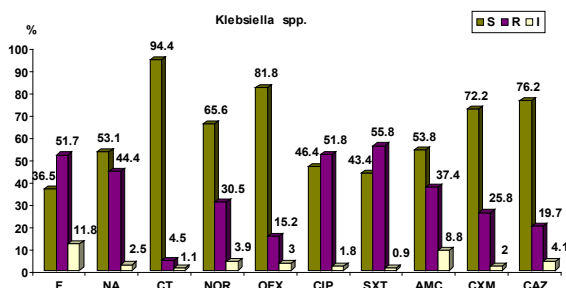


Fig. 5. The susceptibility to chemotherapeutic agents of *Klebsiella* spp. strains.

The strains of *Pseudomonas* spp. presented over 80% susceptibility to CT, IPM, AK and a very low percentage of susceptibility to OFX (Fig.6).

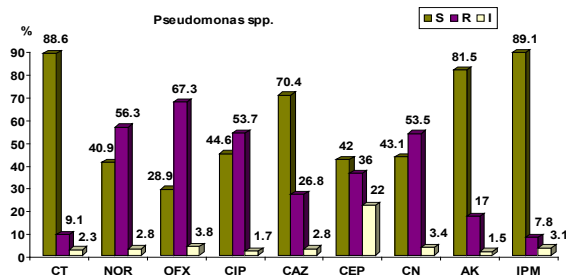


Fig. 6. The susceptibility to chemotherapeutic agents of *Pseudomonas* spp. strains.

The strains of *Staphylococcus aureus* showed over 80% susceptibility to F and OFX and over 40% susceptibility to AM and SXT (Fig.7).

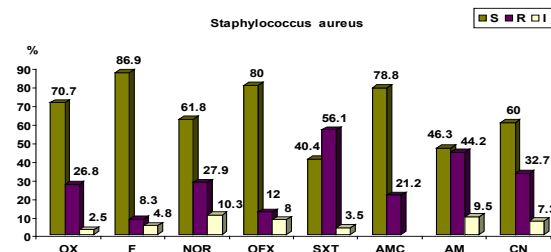


Fig. 7. The susceptibility to chemotherapeutic agents of *Staphylococcus aureus* strains.

Enterococcus spp. strains were 100% susceptible to AMC, 88.3% strains were susceptible to Amx (6 strains resistant, 1 strain intermediate), 81.8% strains were 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 **can not 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 than 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, cephamycin, 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 inefficacy 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.

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