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ANTIBIOTIC SUSCEPTIBILITY OF ESCHERICHIA COLI ISOLATED FROM URINE CULTURES AT THE GALAŢI INFECTIOUS DISEASES HOSPITAL

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SUMMARY

Frequent antibiotic prescriptions for urinary infections (UI) promote the development and spread of antibiotic resistance. In order to identify effective antibiotics for the first line treatment of UI at the Galaţi Infectious Diseases Hospital, we performed a retrospective study based on disk diffusion antibiotic sensitivity testing on bacterial strains from urine cultures. Urine samples were collected from hospitalized patients admitted with symptomatic UI during 2010. In total, 659 records were found of certified bacteriological UI, of which 82% were caused by Enterobacteriacea. Escherichia coli isolates were the most commonly encountered species of Enterobacteriaceae (83%). The median age of the patients with urinary E. coli was 48 [3; 87], sex ratio M/F= 56/399 and 9.8% of patients were HIV positive. Using the Carmeli scoring system for antibiotic resistance risk, we found that 86% of the E. coli isolates were community acquired infections. High level of resistance was evident for Ampiciline at 85%, Neoxazole at 54,6%, TMP-SMX at 45,6% and Amoxi-Clavulanate at 40,2%. The best sensitivity was obtained with 3rd generation cephalosporins and carbapenemes. The incidence of ESBL-positive strains was 7.4% (34/421). No ESBL-positive strain was identified in community acquired UI (Carmeli score 1). In conclusion, it is strongly recommended that the first line of antibiotic therapy at the Galaţi Infectious Diseases Hospital should involve 3rd generation cephalosporins, while carbapenems should be reserved to patients with a Carmeli score of 2 or 3.

Introduction

Enterobacteriaceae are the most common bacterial cause of both community acquired and nosocomial infections [1;2]. The most commonly prescribed antibiotics for Enterobacteriaceae infection treatment are broad-spectrum beta-lactams and quinolones. According to the study of the *European Antimicrobial Resistance Surveillance Network* (EARSS-Net) carried out by 198 laboratories from 22 participating countries, resistance to these antibiotics increased from 2002 to 2010 in Europe [3,4]. The emergence of antibiotic resistance is commonly characterized by the expression of plasmids encoding for various beta-lactamases, and the consequent inactivation of 3rd generation cephalosporins, penicillins and aztreonam. The plasmid location of beta-lactamases allows the mobility of these genes and promotes easy transfer of resistance to other bacteria. Extended-spectrum beta-lactamases (ESBL-positive) have been found in a large number of Enterobacteriaceae, mainly in *K. pneumoniae, K. oxytoca and Escherichia coli*, and more

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rarely in *Enterobacter* sp, *Salmonella enterica, M. morganii, Proteus mirabilis, S. marcescens and Ps. Aeruginosa [5].*
Resistance to fluoroquinolones, aminoglycosides or trimethoprim-sulfamethoxazole (TMP-SMX), as well as advanced nosocomial and community multidrug resistance, are frequent in ESBL-positive Enterobacteriaceae infections [6,7]. Since 1983, when the first ESBL-positive strain was reported in Western Europe, more than 100 ESBL-positive enzymatic subtypes with variable geographical distribution have been described around the world [8,9,10,11,12,13]. Surveillance of ESBL-positive strains is being carried out with several studies in Europe. The present frequency of ESBL-positive bacteria in Europe varies from 0% in Iceland, 1% in Estonia and 2% in France to 6.5% in Spain. Resistance rates of over 10% are expected in Hungary, Romania, Bulgaria, Russia and Turkey [4].

Requirements and objectives of the study

A total of 1340 bacterial isolates from various biological products were identified in the Microbiology Laboratory of the Galaţi Infectious Diseases Hospital during 2010. Almost half of bacteria-positive results were obtained from urine cultures (49.17%; 659/1340). Escherichia coli was isolated in approximately one third of the bacterial cultures (33.95%). These data account for the interest in assessing the antibiotic susceptibility in order to develop a local protocol for urinary infection therapy. The objectives of the study were to assess the antibiotic sensitivity of Escherichia coli to antibiotics, to evaluate the frequency of ESBL-positive strains and to compare the clinical epidemiology of urinary infections with ESBL-positive and -negative Escherichia coli.

Methods and materials

A retrospective study was carried out based on the antibiotic sensitivity and resistance of Escherichia coli isolated from urine cultures at the Galaţi Infectious Diseases Hospital from 01.01.2010 to 31.12.2011. The urine samples were collected from symptomatic patients with fever and/or urinary manifestations. Antibiotic susceptibility of Escherichia coli isolates was assessed by the classic disk diffusion method. Diagnosing the ESBL-positive strains involves the initial step of identifying the cephalosporin-resistant strains, followed by highlighting the synergy between the cephalosporin line and amoxicillin-clavulanate by the double disk method. Duplicate results were excluded from our analysis. Demographic and clinical data were collected from medical records. The risk of antibiotic resistance was estimated using the Carmeli algorithm, considering the highest score from three criteria (Table 1) [14]. Statistical analysis

A. Health care system contact: (1) No contact (2) Health care system contact, noninvasive procedures (3) Over 5 days hospitalization and/ or invasive procedures
B. Antibiotic treatment: (1) No antibiotic treatment (2) Antibiotic treatment
C. Patient Characterisctics (1) Young, no co-morbidities (2) Age over 65 or multiple co-morbidities (3) Immunosuppression: diabetes, cancer, AIDS, neutropenia, cystic fibrosis, Imunosuppressive therapies, other
Calculation: Maximum score (1, 2 or 3) between A, B or C criteria **Interpretation:** (1)Community infection, low risk of antibiotic resistance (2)Health care associated infection, medium risk of antibiotic resistance (3)Nosocomial infection, high risk of antibiotic resistance

Table 1: Carmeli score for stratification of antibiotic resistance risk [14] (*Source:* Carmeli Yehuda. Antimicrobial resistance and patient outcomes: the hazards of adjustment. Crit Care.2006;10(5)::64)

was carried out with the XLStat-Tool Pack Analysis software.

Results

Out of the 659 bacterial isolates obtained from the urine cultures, 82% were Enterobacteriacea sp, 15% Enterococus sp and 3% Non-Fermentative Gram-negative bacteria. The most common aetiology was Escherichia coli, accounting for 83% (455/544) of the Enterobacteriaceae and 69% (455/659) of the bacterial urine isolates. Disk diffusion antibiogram highlighted the following rates of beta-lactamine susceptibility: 17% Ampicillin (AMP), 60% Amoxi-clavulanate (AMC), 78% Cefuroxime (CXM), 83% Cefixime (CFM)/ Ceftibuten (CBT), 93% Cefoperazone (CEP)/ Cefotaxime (CTX)/Ceftriaxone (CRO). The incidence of ESBL-positive Escherichia coli strains was 7.4% (34/421) (Figure 1). The highest antibiotic susceptibility registered was for Carbapenem (95%). Decreased antibiotic susceptibility was also recorded in other antibiotic classes: 77% for aminoglycosides, 79% for quinolones, 53.4% for TMP-SMX, 45.4% for Neoxazole (Nx), and 75% for Nitrofurantoin (Nif).

The median age of patients with Escherichia coli-positive urinary infections was 48 [3; 89], with a predominance of female patients (399/455, 87.2%) and those living in urban areas (292/455, 64.2%). Immunosuppression caused by HIV infection was determined in 9.8% (46/455) of the Escherichia coli isolates. A recurrent infection pattern was recorded in 7.4% (34/455) of the cases.

Comparative analysis of ESBL-positive and -negative Escherichia coli isolates in urinary infections was more prevalent in male (21.4% vs. 12.8%) and older patients (median age 57 vs. 46 years). A Carmeli score of 3 was over two times higher in the ESBL-positive group (26.4% vs. 12.8%), while a score of 1 was found exclusively in the ESBL-negative group (Table 2). HIV/ AIDS was the most frequent co-morbidity factor associated with a Carmeli score of 3 in our hospital, and should be considered a predictor of antibiotic resistance. The incidence of recurrent Escherichia coli urinary

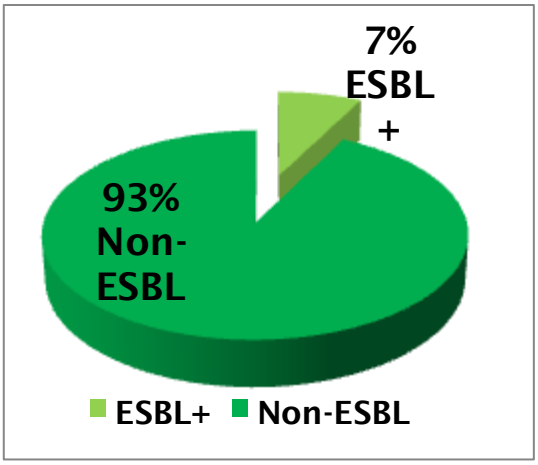

Figure 1: Rate of ESBL + strains of E. coli in the Galați Infectious Diseases Hospital

	E. Col ESBL+	E. coli ESBL-
Number	34	421
Sex M/F	6/28	50/371
Living area R/U	9/24	154 / 268
Average age (range)	57 (18-79) years	46 (3-89) years
Carmeli Score 1/2/3	0/25/9	269/98/54
HIV+	5/34	41/421

Table 2: Comparative characteristics of the patients with ESBL+ versus ESBL- E. coli

infections was significantly higher in HIV positive patients: p = 0,004; OR = 3.14; CI 95% [1.43; 6.87].

Discussion

According to the assessment carried out using the Carmeli algorithm, the majority of urinary E. coli isolates at the Galaţi Infectious Diseases Hospital were community acquired infections. Consequently, the rate of ESBL-positive E. coli strains is consistent with the community resistance level in our geographical area. Except for HIV/AIDS patients, other immunosuppressive co-morbidities associated with urinary infections were rare in the patient pool of our hospital. The low level of TMP-SMX susceptibility has probably been induced by the frequent use of this antibiotic for pneumocystis prophylaxis in AIDS patients.

Some data on the antibiotic resistance surveillance in other Romanian hospitals are available from the reports of the prospective multi-centric *Study for Monitoring Antibiotic Resistance Trends* (SMART), carried out in tertiary centres at Bucharest, Iaşi, Constanţa and Timişoara. Analogously with EARSS reports, higher resistance rates of invasive Enterobacteriaceae infections in Romania were detected with disk diffusion resistance tests carried out by the SMART [15,16]. With the exception of a lower susceptibility to Ampicillin (15.8% vs. 36.91%) and aminoglycosides (76.6% vs. 89%), the antibiotic susceptibility pattern in Galaţi is similar to that found by the SMART in other parts of Romania [Figure 2]. The geographical proximity of Galaţi to the Romanian SMART participant centres, as well as the analogous antibiotic prescribing practices, could explain the similar antibiotic susceptibility observed.

In view of the antibiotic susceptibility rates observed in our study, the first line therapy for urinary infections at the Galaţi Infectious Disease Hospital should ideally involve 3rd generation cephalosporins for patients with a Carmeli score of 1 and carbapenems for patients with a Carmeli score of 2 or 3. Other antibiotics should be prescribed following a de-escalation pattern based on the antibiogram results.

The results of this study may be affected by the difficulty in interpreting the retrospectively collected data and by poor antibiogram methodology with inconsistent use of the antibiotics tested. The significance of the 5% resistance to carbapenems recorded by us may not be relevant since only 20 strains were tested, and thus this finding highlights a single resistant sample.

Development of a new quantitative E-test for antibiotic susceptibility and the consequent use of standardised methods in antibiotic resistance testing are necessary to improve the diagnostics and surveillance of antibiotic resistance in the Galaţi Infectious Diseases Hospital.

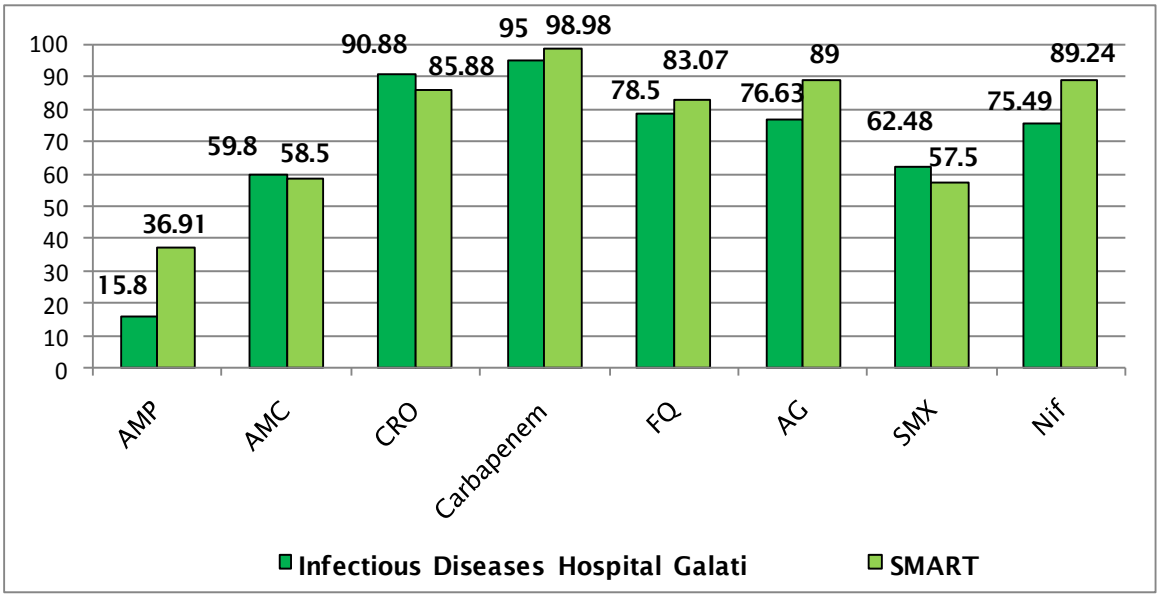

Figure 2: Comparative susceptibility of E. coli in the Galaţi Infectious Diseases Hospital and Romanian SMART (N=2,150) [15]

In conclusion, Escherichia coli was found to be the main aetiology of urinary infections (69%). The incidence of ESBL-positive urinary Escherichia coli was 7.4%. It is strongly recommended that the first line of antibiotic therapy in the Galaţi Infectious Diseases Hospital should involve 3rd generation cephalosporins for patients with a Carmeli score of 1, while carbapenems should be reserved for those with a Carmeli score of 2 or 3.

References

1.Valverde A, Grill F, Coque TM, Pintado V, Baquero F, Cantón R, et al. High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients. J Clin Microbiol..2008 Jan;46:2796-9.

2.Nicolas-Chanoine MH, Jarlier V; 'La Collégialé de Bactériologie-Virologie-Hygiène Hospitalière de l'Assistance Publique, Hôpitaux de Paris, France. Extended-spectrum beta-lactamases in long-term-care facilities. Clin Microbiol Infect. 2008 Jun;14 Suppl: 111-6.

3.World Health Organization (WHO). World Health Day – 7 April 2011. Antimicrobial resistance and its global spread. Geneva: WHO; [Accessed 17 May 2010]. Available from: http://www.who.int/world-health-day/en/

4.Coque TM, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Eurosurveillance,2008;47:1-11.

5.Cantón R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, et al. Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect. 2008;14 Suppl 1: 144-53.

6.Grundmann H, Livermore DM, Giske CG, Canton R, Rossolini GM, Campos J, et al. Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euro Surveill. 2010;15. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19711

7.Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother. 2007; 59:165-17.

8.Bochicchio GV, Baquero F, Hsueh PR, Paterson DL, Rossi F, Snyder TA, et al. In vitro susceptibilities of Escherichia coli isolated from patients with intra-abdominal infections worldwide in 2002-2004: results from SMART (Study for Monitoring Antimicrobial Resistance Trends). Surg Infect (Larchmt). 2006;7:537-45.

9.Andreu A, Planells I; Grupo Cooperativo Español para el Estudio de la Sensibilidad Antimicrobiana de los Patógenos Urinario. Etiology of community-acquired lower urinary infections and antimicrobial resistance of Escherichia coli: a national surveillance study. Med Clin (Barc). 2008;130:481-6.

10.Kjerulf A, Hansen DS, Sandvang D, Hansen F, Frimodt-Møller N. The prevalence of ESBL-producing E. coli and Klebsiella strains in the Copenhagen area of Denmark. APMIS 2008;116:118-24.

11.Galas M, Decousser JW, Breton N, Godard T, Allouch PY, Pina P; et al. Nationwide study of the prevalence, characteristics, and molecular epidemiology of extended-spectrum-beta-lactamase-producing Enterobacteriaceae in France. Antimicrob Agents Chemother. 2008;52:786-9.

12.Empel J, Baraniak A, Literacka E, Mrówka A, Fiett J, Sadowy E, et al. Molecular survey of beta-lactamases conferring resistance to newer beta-lactams in Enterobacteriaceae isolates from Polish hospitals. Antimicrob Agents Chemother 2008;52:2449-54.

13.Korten V, Ulusoy S, Zarakolu P, Mete B; Turkish MYSTIC Study Group. Antibiotic resistance surveillance over a 4-year period (2000-2003) in Turkey: results of the MYSTIC Program.Diagn Microbiol Infect Dis. 2007;59:453-7.

14.Carmeli Yehuda. Antimicrobial resistance and patient outcomes: the hazards of adjustment. Crit Care. 2006;10:164.

15.Popescu G, Popescu C, Nicoara E, et al. Supravegherea rezistenţei germenilor la antibiotice – un studiu multicentric în spitalele de boli infectioase. Infectio..ro, 2009;19: 10-17.

16.Rugina S., Iancu AM., Dumitru I., Ruginǎ C. Managementul infecţiilor urinare determinate de enterobacterii secretoare de betalactamaze cu spectru extins. Infectio.ro, 2009; 19: 18-23.