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EPIDEMIOLOGY AND ANTIBIOTIC RESISTANCE OF GRAM-NEGATIVE URINARY PATHOGENS IN PEDIATRIC PATIENTS*

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SUMMARY: Gür D, Kanra G, Ceyhan M, Seçmeer G, Kanra B, Kaymakoğlu İ. (Infectious Diseases Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Epidemiology and antibiotic resistance of Gram-negative urinary pathogens in pediatric patients. Turk J Pediatr 1999; 41: 37-42.

In order to determine the etiological agents and the rate of resistance to various antibiotics, 209 consecutive Gram-negative bacteria isolated from children admitted to Hacettepe University Children's Hospital with urinary tract infections were investigated over a three-month period. Of these, 46 (22%) were nosocomial isolates. The most frequently isolated organism was E.coli (n: 141) followed by Klebsiella spp. (39), Proteus spp. (19), Pseudomonas spp. (8) and Enterobacter spp. (2). In vitro susceptibilities were evaluated by microbroth dilution method, following NCCLS guidelines. Overall, 75 percent of the isolates were resistant to ampicillin, 52 percent were resistant to TMP/SMX and 25 percent to cefuroxime. Amikacin was the most active aminoglycoside; 93 percent of the isolates were susceptible to this agent, while resistance to gentamicin was 21 percent. Resistance to ceftazidime and ceftriaxone was 12 percent and 19 percent, respectively. Overall, resistance to imipenem was one percent and to ciprofloxacin three percent. These in vitro results should be taken into account before initiating empirical therapy; broad spectrum antibiotics should not be used if the isolate is susceptible to the older drugs in order to prevent the increase in resistance. *Key words: antibiotic resistance, urinary tract pathogens.*

Empirical therapy of urinary tract infections (UTI) depends on knowledge of the causative agents and their susceptibility patterns to antimicrobial agents in each center. These factors may change over time and according to the location. Hence, periodic surveillance studies are required in each center to follow the frequency of the etiological agents and their susceptibility profile as well as to provide a guide to the clinician for empirical treatment. In this study, the prevalence of Gram-negative bacteria isolated from urine cultures in Hacettepe University Children's Hospital was investigated and their susceptibilities to commonly prescribed antimicrobial agents were evaluated in hope of producing a guide to the clinician.

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Material and Methods

Gram-negative organisms which were isolated from consecutive urine cultures with significant growth (10^5 cfu/ml) were collected over a three-month period in 1996. One isolate from each patient was included in the study. The isolates were identified by Sceptor ID (Becton Dickinson, USA). Microbroth dilution tests were employed to determine the in vitro efficacy of the antimicrobial agents. These were supplied from manufacturers in their powder form. The antibiotics and their suppliers were as follows: amikacin and gentamicin (FAKO İlaçları A.Ş.); netilmicin and cefixime (Eczacıbaşı A.Ş.); tobramycin (Nobel A.Ş.); ampicillin sulbactam/ampicllin, cefoperazone and sulbactam/cefoperazone (Pfizer İlaçları A.Ş.); cefuroxime and ceftazidime (Glaxo Wellcome İlaçları A.Ş.); ceftriaxone and TMP/SMX (ROCHE A.Ş.); imipenem (Merck Sharp ve Dohme İlaçları A.Ş.) and ciprofloxacin (Bayer A.Ş.).

Two-fold dilutions of the antibiotics were prepared so that the concentration in the first microwell was 256 mg/L. For TMP/SMX and ciprofloxacin only, the concentration in the first microwell was 16 mg/L. Mueller-Hinton broth (Oxoid) was used in the tests and the results were evaluated according to the NCCLS break points[1].

Results

A total of 209 Gram-negative bacteria were isolated during the study period. The frequencies of these bacteria are shown in Table I. E. coli was the most frequently isolated organism followed by Klebsiella spp. While most of the E. coli were isolated from community-acquired infections with only 17 nosocomial isolates (12%), 18 (46%) of the Klebsiella spp. were isolated from hospitalized patients. Similarly, half of the Pseudomonas spp., six of the Proteus spp. and one of the Enterobacter spp. were nosocomial isolates.

Table I: Gram-Negative Urinary Tract Pathogens (n: 209)

Organism	Number	%
E. coli	141	67
Klebsiella spp.	39	18
Proteus spp.	19	9
Pseudomonas spp.	8	3
Enterobacter spp.	2	< 1

In vitro efficacy of the 15 antibiotics for all the organisms is shown in Table II. Imipenem, ciprofloxacin and amikacin were the most effective antibiotics in vitro against urinary tract isolates. However, the level of resistance varies according to the pathogen.

Comparative in vitro activity of the antibiotics against E. coli and Klebsiella spp. is shown in Tables III and IV, respectively.

Table II: In Vitro Susceptibility of UTI Isolates to Antimicrobial Agents (mg/L)

Antibiotic	Range	MIC_{50}	MIC_{90}	Resistance (%)
Amikacin	≤ 0.125-64	1	16	7
Netilmicin	≤ 0.125-> 256	0.50	64	16
Gentamicin	≤ 0.125-> 256	0.50	64	21
Tobramycin	≤ 0.125-> 256	0.50	32	20
Ampicillin	0.50-> 256	> 256	> 256	75
Sulbactam/Ampicillin	0.25-> 256	32	> 256	64
Cefuroxime (oral)	≤ 0.125-> 256	4	> 256	25
Ceftriaxone	≤ 0.125-> 256	≤ 0.125	64	19
Ceftazidime	≤ 0.125-> 256	≤ 0.125	32	12
Cefoperazone	≤ 0.125-> 256	4	256	43
Sulbactam/Cefoperazone	≤ 0.125-> 256	1	32	12
Cefixime	≤ 0.125-256	0.25	32	19
Imipenem	≤ 0.125-8	≤ 0.125	2	1
TMP/SMX	≤ 0.008-> 16	> 16	> 16	52
Ciprofloxacin	≤ 0.008-> 16	0.01	0.06	3

Table III: In Vitro Susceptibility of E. coli to Antimicrobial Agents (mg/L)

Antibiotic	Range	MIC_{50}	MIC_{90}	Resistance (%)
Amikacin	≤ 0.125-32	1	4	2
Netilmicin	≤ 0.125-128	0.25	1	4
Gentamicin	< 0.125-256	0.50	2	4
Tobramycin	≤ 0.125-64	0.50	2	6
Ampicillin	0.50-> 256	> 256	> 256	65
Sulbactam/Ampicillin	0.25-> 256	16	> 256	58
Cefuroxime (oral)	0.25-> 256	4	16	14
Ceftriaxone	≤ 0.125-> 256	≤ 0.125	2	8
Ceftazidime	≤ 0.125-> 256	≤ 0.125	0.50	2
Cefoperazone	≤ 0.125-> 256	1	256	32
Sulbactam/Cefoperazone	≤ 0.125-> 256	1	16	8
Cefixime	≤ 0.125-256	0.25	1	7
Imipenem	≤ 0.125-8	≤ 0.125	0.25	< 1
TMP/SMX	≤ 0.008-> 16	4	> 16	49
Ciprofloxacin	≤ 0.008-16	≤ 0.008	0.03	3

Table IV: In Vitro Susceptibility of Klebsiella spp. to Antimicrobial Agents (mg/L)

Antibiotic	Range	MIC_{50}	MIC_{90}	Resistance (%)
Amikacin	050-64	2	32	17
Netilmicin	≤ 0.125-128	2	128	43
Gentamicin	≤ 0.125-> 256	8	128	51
Tobramycin	≤ 0.125-> 64	32	256	53
Ampicillin	4-> 256	> 256	> 256	97
Sulbactam/Ampicillin	2-> 256	64	> 256	74
Cefuroxime (oral)	≤ 0.125-> 256	4	256	46
Ceftriaxone	≤ 0.125-> 256	1	> 256	41
Ceftazidime	≤ 0.125-> 256	2	256	41
Cefoperazone	≤ 0.125-> 256	64	> 256	66
Sulbactam/Cefoperazone	≤ 0.125-> 256	8	128	25
Cefixime	≤ 0.125-256	1	> 256	43
Imipenem	≤ 0.125-8	0.25	0.50	2
TMP/SMX	≤ 0.01-> 16	0.25	> 16	38
Ciprofloxacin	≤ 0.008-16	0.03	0.25	3

Discussion

Several studies point to E. coli as the most frequent causative agent in both community and hospital-acquired infections[2]. According to the results of our study, E. coli was the predominant Gram-negative organism in our patients with urinary tract infections, with 67 percent of the cultures yielding this organism. Klebsiella spp. was the second most frequent pathogen and was isolated in 18 percent of the cultures. The most striking difference between these organisms is that Klebsiella strains are mostly nosocomial isolates, whereas most of the E. coli strains were isolated from outpatients. This is in accordance with other studies[2,3]. However, its rate of isolation is reported to be higher in patients with community-acquired infections[3,4]. In some reports, Proteus spp. are the second most frequent organism, but recent studies indicate that there have been changes in the urinary pathogens and that Proteus spp. have been overtaken by Klebsiella spp. and Enterobacter spp. in the hospital. This has been attributed to the use of antibiotics, as well changes in hospital hygiene[2].

Overall resistance to aminoglycosides in these strains is quite high. Amikacin is the most effective agent among this group of antibiotics. The level of resistance is higher in Klebsiella spp., with 17 percent resistance to amikacin and approximately 50 percent to gentamicin and tobramycin. The resistance rate for netilmicin is also high in these isolates (Table IV). Resistance to aminoglycoside antibiotics is related to development of the strains producing aminoglycoside-modifying enzymes active against these agents[5]. It is evident that AAC-6, the

enzyme which is active against amikacin, is rarer than the enzymes modifying netilmicin, gentamicin and tobramycin. This has been reported from most of the centers in Turkey[5, 6]. However, extensive use of this antibiotic may cause an increase in resistance in the near future and therefore must be used with caution.

In general, there is a very high rate of resistance against ampicillin. Nearly all Klebsiella spp. and 65 percent of E. coli are resistant to this agent. Addition of sulbactam had no significant effect on resistance in this in vitro study, suggesting that resistance may be due to inhibitor-resistant β-lactamases[7]. These isolates are reported frequently in community-acquired infections especially among organisms responsible for urinary tract infections[8]. However, a definite conclusion can only be drawn by further studies investigating the mechanisms of resistance in these isolates. Resistance to new β-lactams is not an unexpected result in Klebsiella spp., as these agents are widely used in our hospital. Although broad-spectrum β-lactamases were not investigated in this study, high resistance rates for the cephalosporins suggest that these enzymes were present in our isolates. However, the definite proportion of these enzymes can only be determined by studies using specific methods[9].

TPM-SMX resistance is reported to be higher in developing countries. In a study by Murray et al.[10], 44 percent resistance was observed among E. coli isolated at a pediatric hospital in Chile and 40 percent in Thailand. Most isolates in that study were from urinary tract infections. Overall resistance to TMP/SMX was 52 percent in urinary tract isolates in our study and the rate of resistance was higher in E. coli than in Klebsiella spp., reflecting the extensive use of this antimicrobial agent in urinary tract infections in Turkey. TMP/SMX resistance was nine percent in a U.S.A. study in 1991 and 19 percent in E. coli in a study from the U.K. in 1992[2, 11].

Our results indicate that imipenem is the most effective β-lactam agent against urinary tract isolates, followed by ciprofloxacin. However, these agents should only be used in severe cases as their overuse may lead to a resistance problem sooner than expected. This has been reported by Thomson et al.[11], who point out that noncritical use of the fluoroquinolones over a period of four years in the United States has resulted in the emergence of fluoroquinolone resistance at a greater rate than was originally anticipated. Empirical therapy, when needed, must be initiated according to the resistance pattern of the etiological agents until the susceptibility test results are reported to the clinicians. For safe and effective therapy, broad-spectrum agents are not usually necessary.

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