Resistance patterns to cephalosporins of members of the family enterobacteriaceae isolated from urinary tract infection.

*Abdul Wahab A.R. Hadi & *Mithaq S. Al-Nassiry

*Assistant Professor
Microbiology Department
College of Medicine
Al-Mustansiriyah University
Baghdad, Iraq.

*Laboratorial
Al-Kindy Teaching Hospital
Baghdad, Iraq.

Abstract:

This study was conducted at the Al-Yarmouk Teaching Hospital to determine the resistance patterns to cephalosporins of members of the family Enterobacteriaceae isolated from urinary tract infections (UTIs). A total of 270 urine specimens were collected from February, 2004 to May, 2004.

Escherichia coli was found to be the most organism, followed by Klebsiella spp. The results of susceptibility of isolates under study to different cephalosporins were moderately or highly resistant to many of the test agents. The observations on the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) for the cephalosporins explained the high level of resistance to cephalothin and cefaclor, and a moderate level of resistance to cefotaxime, ceftazidime and cefixime.

The increasing MIC of cephalosporins, especially third generation, indicates decreasing susceptibility of these organisms to these types of β-lactam agents due to the production of extended spectrum β-lactamases (ESBLs).

Kew Words: Cephalosporins, Enterobacteriaceae, UTI.

JBMS 2006; Volume 6:37-44

Introduction:

Urinary tract infections remain the most common among acquired infections. Their importance lies in the fact that a considerable proportion of population may acquired asymptomatic infections[1].

This state of bacteruria, particularly, in female has related to more serious subsequent infection of the upper urinary tract[2]. Urinary tract infections, including cystitis and pyelonephritis are the most frequent nosocomial and community-acquired infections[3].

When antibacterials are selected for the empiric treatment of UTIs, knowledge of most likely causative organisms and the prevalence of resistance of pathogens to antibacterial agents are essential[4]. Such infections are caused by a variety of Gram-negative bacteria that ascend into the urinary tract and establish bacteruria often at levels more than or equal to 10^5 CFU/ml. of urine[4]. Escherichia coli dominates as the causative agent in all patient populations according to international data.

Others would include Staphylococcus saprophyticus, Enterobacteriaceae group (Klebsiella spp., Proteus spp., Enterobacter spp., etc.) and Pseudomonas aeruginosa [4]. The goals in the treatment of UTI is to prevent or treat systematic symptoms, to releave symptoms, to eradicate sequestered infection, to eliminate uropathogenic bacterial strains from fecal or vaginal reservoirs, and to prevent long-term sequelae all at minimal cost, with the lowest rate of side effects, and with the least selection of an antibiotic resistant flora. A steady increase of resistance patterns to antimicrobials, particularly to cephalosporins has been documented[5].

It was, therefore, decided to carry out this study to provide some knowledge on the determination of resistance patterns to cephalosporins of some members of the family Enterobacteriaceae.

Special emphasis has been paid to evaluate the MICs of third generation cephalosporins and decreasing susceptibility of β-lactam agents due to ESBLs production.

Materials & Methods:

Patients seen at Al-Yarmouk Teaching Hospital with UTI, i.e. with urinalysis result showing more than 10 pus cells per high power field, and with no previous history of antibiotic intake, are included in the study.

Urine specimens were then processed for culture, and susceptibility testing using the Kirby-Bauer method. The following antibiotic discs were used: cephalothin, cephalexin, cefazolin, cefuroxime, cefoxitin, cefaclor, cefprozil, cefotaxime, ceftazidime, ceftriaxone, cefixime, ceftibutin and ceftizoxime. The minimum inhibitory concentration (MIC) was determined by twofold dilution method, and a standard strain, E.coli ATCC 25922, was included in the study.

The identification of members of the family Enterobacteriaceae was performed according to the biochemical tests indicated in the scheme of Farmer and his co-workers[6].

Results:

A total of 270 urine specimens were collected from patients admitted to Al-Yarmouk Teaching Hospital for the period from February,2004 to May,2004. From a total of 150 specimens which yielded positive growth, there were 105 isolates belonging to members of the family Enterobacteriaceae (70%). Twenty-five isolates were identified as Ps.aeruginosa (16.66%). The other isolates were as follows: 15(10%) Gram-positive cocci and 5 (3.33%) yeasts. Escherichia coli was the most common microorganism, representing 32 isolates (30.4%). Klebsiella spp. were found to be the second most common, making up 29% of the total isolates.

Furthermore, it was found that Proteus spp. represented 21 isolates (19.9%). The Citrobacter spp. made (7.6%) of the isolates. However, the Enterobacter spp. represented (3.75%). The Providencia spp., Serratia spp. and Morganella morgani accounted for (3.75%), (2.85%) and (1.9%) of the isolates respectively. These results are shown in Table (1).

The sensitivity tests to cephalosporins were performed on 105 isolates of members of the family Enterobacteriaceae by the Kirby-Bauer method[7].

The results were interpreted according to the diameters of inhibition zones as resistant or sensitive (Table 2). Table (3) gives the cephalosporins employed in this study; whereas, the resistance patterns of the isolates are illustrated in Table (4).

In the present study, 39 (37%) of isolates were subjected to MIC and MBC determinations. All have given complete resistance (100%) to the various cephalosporins employed, as shown in Table (5). Table (6) gives the MICs for cephalosporins, carried out by the twofold broth dilution method, as described by Ericsson and Sheries[8] (1971). The MBC was determined by comparing the number of colonies present on subculture from the antibiotic-free broth before it was incubated, with the numbers on the plate subculture from the incubated antibiotic-containing tubes[9]. It should be indicated, that in this work, all isolates had previously been examined for β-lactamase production by the acidometric method of Sykes and Matthew[10].

Discussion:

Urinary tract infection results from the ascension of faecally derived organisms and periurethral tissue into the bladder and kidneys. These adhere to the bladder receptor sites, and if they possess the virulence factors for pyelonephritis, organisms ascend the ureter to reach the kidneys.

These bacteria elicit an inflammatory response stimulating release of cytokines and other pro-inflammatory substances[11]. The most common organism isolated in our study of 270 patients is a Gram-negative bacillus. The clinically relevant members of the Enterobacteriaceae can be considered as two groups: Opportunistic pathogens and overt pathogens. The first group most commonly includes: Citrobacter spp., Klebsiella spp., Proteus spp., Providencia spp., and Morganella spp. Although considered opportunistic pathogens, these may produce significant virulence factors. Although E.coli is a normal bowel inhabitant, its pathogenic classification is somewhere between the overt and opportunistic pathogens. Some strains express potent toxins and cause serious gastrointestinal infections[12].

The results of susceptibility of isolates under study to different cephalosporins (see Table 3) have shown that many isolates were moderately or highly resistant to many of test agents (Table 4).

This reflects the heavy exposure to antibiotic pressure and long duration of therapy. The resistance to the first generation cephalosporins was (80%) to cephalexin, (78%) to cephalothin and (40.9%) to cefazolin. Most isolates have given moderate resistance to cefuroxime and cefprozil, and high degree resistance to cefaclor.

Furthermore, the majority of the isolates were shown to possess moderate resistance to the major types of the third generation cephalosporins. Resistance to cefotaxime, ceftazidime and ceftriaxone was (69.5%), (68.5%) and (70.4%) respectively. On the other hand, low degree of resistance was shown to ceftizoxime, which is similar to cefazolin (44.7%). This was more effective than other types of third generation cephalosporins.

As shown in Table (6), the present results of MICs and MBCs for cephalosporins explain the high level of resistance to cephalothin, cefaclor, and the moderate level of resistance to cefotaxime, ceftazidime and cefixime. The increasing MIC of cephalosporins indicates decreasing the susceptibility of the organisms under study to these types of β-lactam agents due to the production of ESBLs (such as TEM or SHV types ESBLs)[13].

The result of MIC and MBC for the ESBL-producing isolates reveals that it is affected by the level of activity of antimicrobial agent and the inoculum size of the organism[14]. The inoculum size of β-lactamase-producing isolates should be 10^4-10^5 CFU/ml. as recommended by the National Committee for Clinical Laboratory Standards guidelines[15,16,17]. The data presented in Table (7) explain the increasing level of resistance to cephalosporins agents.

The percentage of β-lactamase production by these organisms varies according to different geographical areas. The result of β-lactamase production of the present study was very high, in contrast to what was reported in other countries, because they employed molecular techniques, and isoelectric focusing for detection of β-lactamase production. These techniques are of higher sensitivity and specificity than the conventional ones (e.g. iodometric, acidometric and nitrocefin method) [18].

Extended-spectrum β-lactamases can be difficult to detected because they have different levels of activity against cephalosporins. Thus, the choice of antimicrobial agent to test is critical. For example, one enzyme may actively hydrolyze ceftazidime, resulting in ceftazidime MICs of 256 µg/ml., but may have poor activity on cefotaxime, producing MICs of only 4 µg/ml[19].

Table (1): Isolation of Enterobacteriaceae isolated from urine specimens of patients admitted at Al-Yarmouk Teaching Hospital.

%	Number of cases	Bacterial species
30.4 18 9.5 8.5 8.5 3.8 3.8 2.8 2.8 2.8 1.9 1.9 1.9 0.95 0.95 0.95	32 19 10 9 9 4 4 3 3 3 2 2 2 1 1 1	*E.coli P.mirabilis K.pneumoniae K.oxytoca K.ozaenae C.freundii C.koseri(diversus) K.aerogenes E.cloacae P.rettgeri M.morganii P.vulgaris S.marcescens S.liquifaciens E.aerogenes P.stuartii*
100%	105	Total No. of isolates

Table (2): NCCLS-Performance Standards for Antimicrobial Disc Sensitivity Test. 7th Ed. M2-A7, 2000.

Antibiotic	Disc potency/µg	Inhibition zone (mm).			MIC µg/ml	
		Sen.	Int.	Res.	S	R
Cephalothin Cephalexin Cefazolin Cefuroxime Cefoxitin Cefaclor Cefprozil Cefotaxime Ceftazidime Ceftriaxone Cefixim Ceftizoxime Ceftibutin Ampicillin Amoxicillin Piperacillin Augmentin Combactam*	30 30 60 60 30 30 30 30 30 30 30 30 30 10 20 100 30 60	≥23 ≥20 ≥23 ≥18 ≥23 ≥20 ≥20 ≥23 ≥20 ≥20 ≥26 ≥23 ≥23 ≥20 ≥20 ≥26 ≥20 ≥20	22-20 19-17 22-20 17-15 22-20 19-17 19-17 22-15 19-17 19-17 25-23 22-20 22-20 19-17 19-17 25-20 19-17 19-17	≤19 ≤16 ≤19 ≤14 ≤19 ≤16 ≤16 ≤14 ≤16 ≤16 ≤22 ≤19 ≤19 ≤16 ≤16 ≤19 ≤16 ≤16	≤8 ≤8 ≤8 ≤8 ≤8 ≤8 ≤8 ≤8 ≤8 ≤8 ≤8 ≤8 ≤8 ≤8 ≤8 ≤16 ≤8/4 ≤8/4	≥32 ≥32 ≥32 ≥32 ≥32 ≥32 ≥32 ≥32 ≥32 ≥32 ≥32 ≥32 ≥32 ≥32 ≥32 ≥128 ≥32/16 ≥32/16

Penicillins	Third generation Cephalosporins	Second generation Cephalosporins	First generation Cephalosporins
Ampicillin Amoxicillin Piperacillin Augmentin(Amox.+Clavulenat) Combactam(Amp.+Sulbactam)	Cefotaxime Ceftazidime Ceftriaxone Cefixim Ceftizoxime Ceftibutine	Cefaclor Cefprozil Cefoxitin Cefuroxime	Cephalothin Cephalexin Cefazoline

Table (4): Resistance of Enterobacteriaceae isolates to β-lactam antibiotics.

Total		Isolates E.coli K. K. K. K. P. P. M. C. C. E. E. P. P. S. S. ozaenae aerogenes pneumoniae oxytoca mirabilis vulgaris morgani freundii koseri aerogenes claecae rettgeri stuartii liquifaciens marcescens N N N N N N N N N N N N N N N N	Antibiotics
%	No.		
78 80 40.9 70.4 50.4 80.9 77.1 69.5 68.5 70.4 76.2 44.7 72.4 93.3 86.6 71.4 71.4 64.7	82 84 43 74 53 85 81 73 72 74 80 47 76 98 91 75 75 68	25 7 2 9 7 15 1 2 2 2 1 3 3 0 1 2 25 7 2 9 8 16 1 2 2 3 1 3 2 0 1 2 18 3 1 3 3 5 1 1 1 1 1 3 1 0 0 1 22 7 2 8 7 14 1 1 2 2 1 3 1 1 1 1 18 3 1 5 3 9 1 2 1 3 1 3 1 1 1 1 24 7 2 9 9 15 2 2 2 3 1 3 2 1 1 2 24 7 2 9 7 15 2 2 2 2 1 3 2 0 1 2 24 7 2 9 6 13 1 1 1 1 1 3 2 0 0 2 25 7 2 9 7 10 1 1 1 1 1 3 2 0 0 2 24 7 2 7 7 13 1 1 1 2 1 3 2 1 1 1 23 7 2 9 9 16 1 1 1 3 1 3 2 0 1 1 13 4 2 4 9 5 1 1 1 1 1 3 1 0 0 1 22 7 2 9 9 14 1 2 1 3 1 3 2 1 1 2 29 9 3 9 8 18 2 2 3 4 1 3 3 1 1 2 28 7 3 8 8 19 2 2 2 2 1 3 3 1 1 2 25 7 1 9 6 14 1 1 1 1 1 3 2 1 0 2 29 5 2 9 6 12 1 2 1 1 1 3 2 0 1 1 17 7 2 7 7 15 1 1 1 1 1 3 2 0 1 1	Cephalothin Cephalexin Cefazoline Cefuroxime Cefoxitin Cefaclor Cefprozil Cefotaxime Ceftazidime Ceftriaxone Cefixime Ceftizoxime Ceftibutine Ampicillin Amoxicillin Piperacillin Augmentin Combactam
100	105	32 9 3 10 9 19 2 2 4 4 1 3 3 1 1 2	Total No of isolates

Table (5): Resistance of isolates to all β-lactam agents

%	No. of resistance isolates	Number of isolates	Bacterial species
40.6 40 33.3 33.3 33.3 26.3 50 50 25 25 100 100 33.3 0 50 0	13 4 3 3 1 5 1 1 1 1 3 1 1 0 1 0	32 10 9 9 3 19 2 2 4 4 3 1 3 1 2 1	*E.coli K.pneumoniae K.ozaenae K.oxytoca K.aerogenes P.mirabilis P.vulgaris M.morgani C.freundii C.koseri E.cloacae E.aerogenes P.retgerii P.stuartii S.marcescens S.liquifaciens*
37	39	105	Total No. of isolates

Table (6): MIC/MBC (μg/ml) for members of Enterobacteriaceae isolated from UTI patients.

Bacterial species	MIC/MBC(μg/ml) Cephalothin Cefazolin Cefaclor Cefotaxime Ceftazidime Cefixim Cetizoxime Amoxiciilin Augmentin
E.coli K.pneumoniae K.ozaenae K.oxytoca K.aerogenes P.mirabilis P.vulgaris M.morgani C.freundii C.koseri E.cloacae E.aerogenes P.rettgeri S.marcescens	>1024 / >1024 32-64 / 64-128 1024 / >1024 64-128 / 128-256 64-128 / 64-256 64-128 / 128-256 32-64 / 128-256 ≥1024 / ≥1024 64-128 / 128-256 1024 / >1024 32-64 / 64-128 512-1024 / ≥1024 64-128 / 128-256 64-128 / 128-256 64-128 / 128-256 32-64 / 64-128 ≥1024 / ≥1024 64-128 / 128-256 1024 / >1024 64 / 128 512-1024 / ≥1024 64-128 / 128-256 64 /128 64-128 / 128-256 32 / 64 1024 / >1024 64 / 128 1024 / >1024 32-64 / 64-128 1024 / >1024 64-128 / 128-256 64-128 / 128-256 128 / 256 64 / 128 1024 / >1024 64-128 / 128-256 1024 / >1024 64 / 128 512 / 1024 128 / 256 64 / 128 128 / 256 64 / 128 1024 / >1024 64 / 128 512-1024 / ≥1024 32-64 / 64-128 512-1024 / ≥1024 64-128 / 128-256 32-64 / 54-128 64-128 / 128-256 32-64 / 64-128 1024 / >1024 64-128 / 128-256 512 / 1024 32 / 64 256 / 512 64 / 128 32 / 64 64 / 128 32 / 64 1024 / >1024 64 / 128 1024 / >1024 32 / 64 512 / 1024 64 / 128 64 / 128 64 / 128 32 / 64 1024 / >1024 64 / 128 512 / 1024 32 / 64 256 / 512 64 / 128 32 / 64 64 / 128 32 / 64 512 / 1024 64 / 128 512 / 1024 32 / 64 512 / 1024 64 / 128 64 / 128 64 / 128 32 / 64 1024 / >1024 64 / 128 1024 / >1024 32-64 / 64-128 512-1024 / ≥1024 64-128 / 128-256 64-128 / 128-256 128-256 /256-512 32-64 / 64-128 1024 / >1024 64-128 / 128-256 1024 / >1024 64 / 128 1024 / >1024 64 / 128 64 / 128 128 / 256 64 / 128 1024 / >1024 64 / 128 512 32 / 64 512 / 1024 64 / 128 32 / 64 64 / 128 32 / 128 1024 / > 1024 64 / 128 1024 / >128 64 / 128 1024 / >1024 64 / 128 64 / 128 128 / 256 32 / 64 1024 / >1024 64 / 128
Standard strain *E.coli* ATCC 25922	2 / 4 1 / 2 2 / 4 0.5 / 1 0.5 / 1 1 / 2 0.5 / 1 4 / 8 2 / 4

References:

1. Santoro J, Kaye D. Recurrent urinary tract infections, pathogensis and management. Med. Clin. North. Am. 1978; 62: 1005-1009.
2. Rapkin RH. Urinary tract infection in childhood. Peadiatrics. 1977; 60: 508-512.
3. Hooton TM, Stamm WE. Diagnosis and treatment of uncomplicated urinary tract infection. Infect. Dis. Clin. North. Am. 1997; 11: 551-554.
4. Annablelle TD, Jennifer AC. Surveillance of pathogens and resistance patterns in urinary tract infection. Phil. J. Microbiol. Infect. Dis. 1999; 28(1): 11-14.
5. Manhardt MG. Host defense within the Urinary tract. Paediatr. Nephrol. 1996; 10(5): 568-572.
6. Farmer JJ, Davis RB, Hickman-Brenner FW. Biochemical identification of new species and biotypes of Enterobacteriaceae isolated from clinical specimens. J. Clin. Microbiol. 1985; 21: 46-76.
7. Bauer AW, Kirby WMM, Sherries JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Path. 19966; 45: 493-496.
8. Ericsson HM, Sherries JC. Antibiotic sensitivity testing. Report of an International Collaborative Study. Acta Pathologica Microbiologica Scandinavica. 1971; Section B, Suppl. 217.
9. Working Party of British Society for Antimicrobial Chemotherapy. A guide to antibiotic sensitivity testing. J. Antimicrob. Chem. 1991; 27: 1-50.
10. Sykes RB, Matthew M. The β-lactamase of gram-negative bacteria and their role in resistance to β-lactam antibiotics. J. Antimicrob. Chemother. 1976; 2: 115-157.
11. Ronald MB. Complicated urinary tract infections. Infect. Dis. Clin. North. Am. 1992; 11(3): 583-592.
12. Bailey & Scott`s Diagnostic Microbiology. M. Mosby (1998). 10th ed. Ch; 37:509-510.
13. Alain P, Guillaume A, George AJ. Plasmid-determined AmpC-type β-lactamases. Antimicrob. Agents and Chemother. 2002; Jan: pp. 1-11.
14. Bradely DJ, David JR, Richard R, Thomas CB, Daniel FC. In vitro activities of various β-lactam antimicrobial agents against clinical isolates of E.coli and Klebsiella spp. resistant to oxyimino-cephalosporins. Antimicrob. Agents Chemother. 1995; May: pp. 187-190.
15. National Committee for Clinical Laboratory Standards. Approved standard M7-A3. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards. 1993; Villanova, Pa.
16. National Committee for Clinical Laboratory Standards. Fifth informational supplement M100-S5. National Committee for Clinical Laboratory Standards. 1994; Villanova, Pa.
17. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5 and informational supplement M100-S10. National Committee for Clinical Laboratory Standards. 2000. Wayne, Pa.
18. Bradford PA. Extended-spectrum β-lactamases in the 21st century: Characterization, Epidemiology and Detection of this important resistance Threat. Clin. Microb. Reviews. 2001; Oct.: pp.933-951.
19. National Committee for Clinical Laboratory Standards. Performance standards for microbial susceptibility testing. Approved standard M100-S9. National Committee for Clinical Laboratory Standards. 1999. Wayne, Pa.