Cephalosporins Susceptibility Test in Urinary Tract Infection

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ABSTRACT:
BACKGROUND:
This study was conducted at the Al-Kindy 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, 2008 to May, 2008.
OBJECTIVE:
Determination the Resistance patterns to cephalosporins in Enterobacteriaceae isolated from urinary tract infection.
METHODS:
Urine specimens were processed for culture, and susceptibility testing using Kirby-Bauer method. The minimum inhibitory concentration (MIC) was determined by twofold dilution.
RESULT:
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.
CONCLUSION:
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).
KEY WORDS: cephalosporins, enterobacteriaceae, urinary tract infection.

INTRODUCTION:

Urinary tract infections remain the most common among acquired infections. Their importance lies in the fact that a considerable proportion of population may acquire 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 antibacterial 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 dominate as the causative agent in all patient populations according to international data. Others would include *Staphylococcus saprophyticus, Enterobacteriace group (Klebsiella spp., Proteus spp., Enterobacter spp., etc.)* and *Pseudomonas aeruginosa* [4]. 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[5].

The goals in the treatment of UTI is to prevent or treat systematic symptoms, to relieve symptoms, to eradicate sequestered infection, to eliminate uropathogenic bacterial strains from fecal or vaginal reservoirs, and to prevent long-term squeal at minimal cost, with the lowest rate of side effects, and with the least selection of an antibiotic

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resistant flora. A steady increase of resistance patterns to antimicrobials, particularly to cephalosporins has been documented[6].

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 AND METHODS:

Patients seen at Al-Kindy 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 processed for culture, and susceptibility testing using the Kirby-Bauer method [7]. The following antibiotic discs were used: cephalothin, cephalexin, cefazolin, cefuroxime, cefoxitin, cefaclor, cefprozil, cefotaxime, ceftazidime, ceftriaxone, cefixime, ceftibutin and ceftizoxime, the concentrations of the discs are shown in table (1).

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[8].

RESULTS:

A total of 270 urine specimens were collected from patients admitted to Al-Kindy Teaching Hospital for the period from February, 2008 to May, 2008.

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 (Table2).

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 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%) respectively. These results are shown in Table (1). The resistance patterns of the isolates to cephalosporines are illustrated in Table (3), resistance to cephaclor was the highest.

In the present study, 39 (37%) of isolates were subjected to MIC and MBC determinations. All have given resistance to the various cephalosporins employed; *Enterobactor* spp. gives 100 % resistance as shown in Table (4)

Table (5) gives the MICs for cephalosporins, carried out by the twofold broth dilution method, as described by Ericsson and Sheries[9]. The MBC was determined by comparing the number of colonies present on subculture from the antibiotic-free broth after incubation, with the numbers of colonies subcultured from the antibiotic-containing tubes[10].

Table 1: NCCLS-performance for antimicrobial disc sensitivity test. 7th Ed. M2-A7,2000

Antibiotic	Disc potency/µg	Inhibition zone(mm)			MIC µg/ml	
		Sensitive	intermediate	Resistance	S	R
Cephalothin	30	≥23	22-20	≤19	≤8	≥32
Cephalexin	30	≥20	19-17	≤16	≤8	≥32
Cefazolin	60	≥23	22-20	≤19	≤8	≥32
Cefuroxime	60	≥18	17-15	≤14	≤8	≥32
Cefoxitin	30	≥23	22-20	≤19	≤8	≥32
Cefaclor	30	≥20	19-17	≤16	≤8	≥32
Cefprozil	30	≥20	19-17	≤16	≤8	≥32
Cefotaxime	30	≥23	22-15	≤14	≤8	≥32
Ceftazidime	30	≥20	19-17	≤16	≤8	≥32
Ceftriaxone	30	≥20	19-17	≤16	≤8	≥32
Cefixim	30	≥26	25-23	≤22	≤8	≥32
Ceftizoxime	30	≥23	22-20	≤19	≤8	≥32
Ceftibutin	30	≥23	22-20	≤19	≤8	≥32

Table 2: Bacterial species isolated from urine

Bacterial species		Number of cases	%
Enterobacteriacae	E.coli	32	30.4
	Klebsiella spp.	31	29
	Proteus spp	21	20
	Citrobacter pp.	8	7.6
	Enterobacter spp.	4	3.75
	Providencia spp.	4	3.75
	Morganella spp.	2	1.9
	Serratia spp.	3	2.85
Pseudomonas pp.		25	16.66
Gram positive cocci		15	10
yeast		5	3.33
Total number of isolates		150	100%

Table 3: Resistance of Enterobacteriacae isolates to cephalosporin antibiotics.

Bacterial species	Number of isolates	Number of resistance isolates	%
E.coli	32	13	40.6
Klebsiella spp.	31	11	35.4
Proteus spp.	21	6	28.5
Morganella spp.	2	1	50
Citerobacter spp.	8	2	25
Enterobacter spp..	4	4	100
Providencia spp.	4	1	25
Serratia spp.	3	1	33
Total number of isolates	105	39	37.14 %

Table 4: Isolated bacteria subjected to MIC and MBC to β-lactam agent.

Antibiotics	ISOLATES									
	E.coli. No.	Klebsiella spp. No.	Proteus Spp No.	Morganella spp. No.	Citerobacter spp. No.	Enterobacter spp. No.	Provedencia Spp. No.	Serratia spp. No.	Total No.	%
Cefaclor	24	27	17	2	4	4	3	3	85	80.9
cephalexin	25	26	17	2	5	4	2	3	84	80
cephalothin	25	25	16	2	4	4	3	3	82	78
Cefprozil	24	25	17	2	4	4	2	3	81	77.1
cefixime	23	27	17	1	4	4	2	2	80	76.2
ceftibutine	22	27	15	2	4	4	3	3	80	76.19
cefuroxime	22	24	15	1	4	4	2	2	74	70.4
ceftriaxone	24	23	14	1	3	4	3	2	74	70.4
cefotaxime	24	24	14	1	2	4	2	2	73	69.5
ceftazidime	25	25	11	1	2	4	2	2	72	68.5
cefoxitin	18	12	10	2	4	4	2	2	53	50.4
ceftizoxime	13	14	6	1	2	4	1	1	47	44.7
cefazolin	18	10	6	1	2	4	1	1	43	40.9
Total No.	32	31	21	2	8	4	4	3	105	100

Table 5: MIC/MBC (µg/ml) for members of Enterobacteriaceae isolated from U

Bacterial species	MIC/MBC(µg/ml)							
	Cephalothin	Cefazolin	Cefaclor	Cefotaxime	Ceftazidime	Cefixim	Cetizoxime	
E.coli	>1024 / >1024	32-64 / 64-128	1024 / >1024	64-128 / 128-256	64-128 / 64-256	64-128 / 128-256	32-	
Klebseilla spp	64 / 128-256							
Proteus spp.	1024 / >1024	32-64 / 64-128	512-1024 /≥1024	64-128 / 128-256	64-128 / 128-256	64-128 / 128-256	32-	
Morganella spp.	64 / 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-	
Citrobacter spp.	64 / 64-128							
Enterobacter spp.	1024 / >1024	32 / 64	512 / 1024	64 / 128	64 / 128	64 / 128	32 /	
	64							
Providencia spp.	512 / 1024	32 / 64	256 / 512	64 / 128	32 / 64	64 / 128	32 /	
	64							
Serratia spp.	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							
	512 / 128	32 / 64	512 / 1024	64 / 128	32 / 64	64 / 128	32	
	1024 / >128 / 64	64 / 128	1024 / >1024	64 / 128	64 / 128	128 / 256	32	
Standard strain*E.coli* ATCC 25922	2 / 4	1 / 2	2 / 4	0.5 / 1	0.5 / 1	1 / 2	0.5 / 1	

DISCUSSION:

Urinary tract infection results from the ascension of faecally derived organisms and periurethral area into the bladder. They adhere to the bladder receptor sites, and if they possess the virulence factors (e.g. hemolysin, K Ag that protect bacteria from complement mediated lyses, and endotoxin) for pyelonephritis, they will ascend to the ureter reaching kidneys eliciting an inflammatory response [5].

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[11].

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) [12].

As shown in Table3, The isolates shows moderate to high resistance to the first and second generation cephalosporin antibiotics .This reflected the heavy exposure to antibiotic pressure and long duration of therapy [13,14].

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. This results is in agreement with a study done by Mauqein et al [15], they found that all enterobacteriacae were intermediately susceptible or resistant to cefotaxime. On the other hand, 25 from 32 isolates of E.coli (78.12%) and 25 from 31 isolates of *K.spp.* (77.41%) are resistant to ceftazidime and 24 from 32 (75%) of E.coli isolates are resistant to cefotaxime and 24 from 31 of *K. spp.* isolates (80.64%) are resistant to cefotaxime. This is in keeping with the finding of Okesola et al and Kumar et al where they found that the resistance of *K. spp.* to cefotaxime was 69.3%and 85% respectively and Kumar et al found that E.coli is more resistant to ceftazidime than cefotaxime [16,17] , but both of the above studies found that the resistance of *K. spp.* to ceftazidine is much less than the result in this study (45.2%and 37% as compared to 80.64% in this study), this is explained by the fact that the regional variants of resistance to antibiotics may be explained in part by different local antibiotic practices due to excessive &/or inappropriate antibiotic use especially for broad spectrum antibiotic that is prescribed empirically [18,19].

The resistance pattern of *E.coli* and *K. spp.* to third generation cephalosporin especially cefotaxime and ceftazidime and the increasing MIC of cephalosporins (as shown in table 4) indicates decreasing the susceptibility of the organisms under study to these types of β-lactam agents due to the production of extended spectrum β lactamases (ESBLs) (such as TEM or SHV types ESBLs) that is most commonly produced by these two bacteria [20, 21].

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[22]. 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[23, 24, 25].

The percentage of β-lactamase (ESBL) production by enterobacteriacae varies according to different geographical areas [26]. A study in Richmond, Virginia, reported 1.5% of isolates produced ESBLs. TEM-12 and TEM- 26 seem most common in the USA. In Europe, ESBLs occur in 20-25% of *Klebsiella* spp. from patient in intensive care Unit., although they have been found in up to 30-40% in France. TEM-3 seems to be most common in France. This fact can explain why the resistance pattern of *K. spp.* to ceftazidime in this study is higher than in other studies.

Extended-spectrum β-lactamases can be difficult to detect 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[27].

CONCLUSION:

1. The most common members of Enterobacteriaceae isolated from patients with UTI were *E.coli,* following by *Klebsiella spp., Proteus spp., Citobacter spp., Enterobacter spp., Providencia spp., Serratia spp.,* and *Morganella morgani.*

2. The results of susceptibility testing of isolates, under study, to different β-lactam agents have shown that many isolates of the members of the family Enterobacteriaceae were moderately to highly resistance to many of the tested agent.

3. The MICs and MBCs of vast majority isolates of Enterobacteriaceae recovered from UTI were higher for cephalothin and cefaclor than for cefotaxime, ceftazidime, and cefixim; and than cefazolin and ceftizoxime.

4. Poor permeability of bacteria by the drug, lack of penicillin binding protein for a specific drug, and degradation of drug by beta lactamases are the most important causes that lead to increase antibiotic resistance.

REFERENCES:

1. Santoro J, Kaye D. Recurrent urinary tract infections, pathogensis and management. Med. Clin. North. Am. 1978; 62: 1005-9.

2. Rapkin RH. Urinary tract infection in childhood. Pediatrics. 1977; 60: 508-12.

3. Hooton TM, Stamm WE. Diagnosis and treatment of uncomplicated urinary tract infection. Infect. Dis. Clin. North. Am. 1997; 11:551-54.

4. Annablelle TD, Jennifer AC. Surveillance of pathogens and resistance patterns in urinary tract infection. Phil. J. Microbiol. Infect. Dis. 1999; 28:11-14.

5. Bailey & Scott's Diagnostic Microbiology. M. Mosby (2007). 12th ed.

6. Manhardt MG. Host defense within the Urinary tract. Paediatr. Nephrol. 1996; 10:568-72.

7. Bauer AW, Kirby WMM, Sherries JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Path. 19966; 45:493-96.

8. 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.

9. Ericsson HM, Sherries JC. Antibiotic sensitivity testing. Report of an International Collaborative Study. Acta Pathologica Microbiologica Scandinavica. 1971; Section B, Suppl. 217.

10. Working Party of British Society for Antimicrobial Chemotherapy. A guide to antibiotic sensitivity testing. J. Antimicrob. Chem. 2004;27: 1-50.

11. 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: 115-57.

12. Bradford PA. Extended-spectrum β-lactamases in the 21st century: Characterization, Epidemiology and Detection of this important resistance Threat. Clin. Microb. Reviews. 2001:933-51.

13. Massova I, Mobashery S. Kinship and diversification of bacterial penicillin-binding proteins and beta-lactamases. Antimicrob Agents Chemother 1998;42:1-17.

14. Davis J. Inactivation of antibiotics and the dissemination of resistant genes. Science 1994; 264; 375-82.

15. Mauqein J, Perrier F, Cony makhoul p, Fourche J, Armaillac V. In vitro activity of six beta-lactams against 295 strains of Enterobacteriacae and *P. aeruginosa* isolated from neutropenic patients. Pathol. Biol (Paris). 1995; 43:253-7.

16. Okesola A, Makonjuola O.Resistance to third generation cephalosporins and other antibiotics by enterobacteriacae in western Nigeria. American Journal of infectious disease 2009;5:17-29.

17. Kumar, M.S., Lakshmi V and Rajagopalan R. Occurrence of extended-spectrum beta-lactamases among *Enterobacteriacea* spp. isolated at a tertiary care institute. Ind. J. Med. Micro. 2006;24:208-11.

18. Sannes R, Kuskowski A, Johnson R. Geographical distribution of antimicrobial resistance among Escherichia coli causing acute uncomplicated pyelonephritis in the United States. FEMS Immu-nol Med Microbiol 2004;42:213-8.

19. Beunders J. Development of antibacterial resistance: the Dutch experience. J Antimicrob Chemother 1994;33:17-22.

20. Jacoby, G.A. and Medeiros A,. More extended-spectrum beta-lactamases. Antimicrob Agents Chemother, 1991;35:1697-1704.

21. Alain P, Guillaume A, George AJ. Plasmid-determined AmpC-type β-lactamases. Antimicrob. Agents and Chemother. 2002:1-11.

22. Bradley J, David R, Richard R, Thomas B, Daniel C. 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. 1995:187-190.

23. 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.

24. National Committee for Clinical Laboratory Standards.1994; Villanova, Pa. Fifth informational supplement M100-S5.

25. 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.
26. Livermore DM. Beta-lactamases in laboratory and clinical resistance. Clin Micro Review 1995; 8:557-84.
27. National Committee for Clinical Laboratory Standards. Performance standards for microbial susceptibility testing. Approved standard M100-S9. National Committee for Clinical Laboratory Standards 2004.