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Antibiotic Susceptibility of ESBL-Producing Urinary Isolates at a Tertiary Care Hospital in Tiruchirappalli, South India

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The aim of this study was to obtain data on susceptibility patterns of bacterial pathogens from patients who attended the CSI Mission General Hospital, in Tiruchirappalli, South India from June 2004-June 2005. During the one-year period of study, the most prevalent etiological agent was *Escherichia coli* (44.02%), followed by *Klebsiella pneumoniae* (14.53%) and other species of *Enterobacteriaceae* (41.45%). These isolates were susceptible to many antimicrobial agents, but those that produced extended spectrum *β-lactaniases* (ESBLs) were resistant to most of the antimicrobials. Of all the *Enterobacteriaceae* tested over a period of 6 months, 236 (25.2%) among 936 were ESBL producers. ESBL production was determined by double disc synergy test. The antibiograms of the ESBL and non ESBL-producers were also compared. Many of the ESBL producers were found to be multidrug-resistant.

Key words: ESBL, double disc synergy test, urinary tract infection

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INTRODUCTION

There are an estimated 150 million urinary tract infections per annum worldwide (Stamm and Norrby, 2001). Urinary tract infections are the most common bacterial infections and account for significant morbidity and health care costs (Gupta *et al*., 2001). Despite the widespread availability of antibiotics, UTI remains the most common bacterial infection in the human population (Sharma, 1999). Antibiotics are usually given empirically before the laboratory results of urine culture are available. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory (Gruneberg, 1984). In this context, the present study was carried out for hospitalized patients with UTI and those attending the outpatient department with UTI. Clinical laboratory records of cases of UTI were studied for the spectrum of bacterial isolates and their antibiotic susceptibility results were analyzed for recommending suitable therapy. Also the differences between the antimicrobial susceptibility patterns of ESBL and non ESBL-producers were analyzed.

MATERIALS AND METHODS

Bacterial isolates: Both the out patients and inpatients with UTI were included in the study. The study was carried out between June 2004 and June 2005. Only urine samples from patients who had pyuria and significant bacteriuria (Clarridge *et al*., 1998) were obtained. No mixed infections were encountered. Only one specimen per patient was accepted. Organisms were identified to species level by conventional methods (Koneman *et al*., 1997).

Antimicrobial agents: The antibiotics tested were ampicillin (10 mcg), amikacin (30 mcg), Co-Trimoxazole (1.25/23.75 mcg), nitrofurantoin (300 bcg), gentamicin (10 mcg), norfloxacin (10 mcg), cephotaxime (30 mcg), ceftazidime (30 mcg), netromycin (30 mcg), ofloxacin (5 mcg), imipenem (10 mcg) and levofloxacin (5 mcg).

Antibiotic susceptibility testing: Antibiogram of the isolates was done by Kirby Bauer method (Bauer *et al*., 1966) using antibiotic discs from Hi-media, Mumbai. The above mentioned antibiotics were used. The results were interpreted as per the National committee for clinical Laboratory Standard recommendations (NCCLS, 1993). The multidrug- resistant organisms that were suspected as ESBL-producers were tested for ESBL production by Double Disk Synergy Test (DDST) (Miles and Amyes, 1997). Methicillin resistance in *Staphylococci* was detected using an oxacillin disc. For the quality control of susceptibility tests, *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC were used as negative and positive controls, respectively.

RESULTS AND DISCUSSION

More than 90% of the isolates belonged to *Enterobacteriaceae*. The most frequently isolated species from UTI was *E. coli* (44-02%) followed by *Klebsiella* sp. (14.53%) (Table 1).

Results of the *in vitro* susceptibility testing to antimicrobial agents of UTI isolates are shown in Table 2 and that of ESBL-producing urinary isolates in Table 3.

Of the 936 Enterobacteeriaceae tested, 236 strains were capable of producing ESBLs as shown by double-disc synergy test. The distribution of ESBL-positive species are shown in Table 2. Among the 15 antimicrobials tested, most of the ESBL-positive strains were susceptible to cotrimoxazole and nitrofurantoin. All of them were susceptible to imipenem and few to amikacin, linezolid and ofloxacin.

This research explains the study undertaken to evaluate the susceptibility patterns of several gram negative bacterial strains isolated from UTI in a tertiary care hospital in Tiruchirappalli. Table 1 shows that 58.55% (458/936) of the organisms were *E. coli* and *K. pneumoniae*. These have been reported already as predominant organisms (Tice, 1999; Stamm and Hooton, 1993; Ferry *et al*., 1988). The antibiogram of the ESBL and non-ESBL isolates show an increased resistance of the ESBL isolates to several antimicrobials. This is in accordance with the previous reports of increased resistance of ESBL producers (Gruneberg, 1994). Multi-drug resistance was usually related to production of ESBL. According to various reports, the overall percentage of ESBL-producers ranged between 20 and 40% our data of 25.2% of ESBL-producers also corresponded with the earlier reports (Gupta *et al*., 2002; Cunney *et al*., 1992). Table 2 shows that most of the isolates, including *E. coli* are still susceptible to many antimicrobial agents (Tankhiwale, 2004). Among the several the isolates under study, *Pseudomonas* sp. was found to be highly resistant. Table 3 shows that the most resistant, multi-drug resistant ESBL-producers were susceptible to imipenem. On the other hand, majority of the ESBL isolates exhibited decreased susceptibility towards aminoglycosides as amikacin and netilmicin. This emerging resistance may be attributed to the indiscriminate use of these antibiotics. In addition, the ESBL strains were resistant to the third generation cephalosporins like cefotonime and ceftazidime.

Table 1: Species distribution of UTI isolates

Urinary isolates	No. of isolates	No. of ESBL producers	% of ESBL producers
Escherichia coli	412	100	42.37
Klebsiella pneumoniae	136	36	15.25
Acinetobacter baumannii	52	18	7.67
Pseudomonas aeruginosa	58	10	4.23
Aeromonas hydrophila	78	20	8.47
Citrobacter freundii	36	8	3.38
Enterobacter aerogenes	28	14	5.93
Proteus mirabilis	6	4	1.69
Providencia stuarti	4	-	-
Morganella morgani	54	10	4.23
Other nonfermenter gram negative bacilli	66	16	6.78
Total	936	236	100.00

Table 2: Antibiotic resistance pattern of ESBL-producers (S = Susceptible)

S. No.	ESBL producing organisms (n = 236)	A	At	A-C	Cu	Co	Nf	G	Nx	Cf	Ce	Ca	Nt	Of	I	Le
1.	*Escherichia coli*	(96)	(21)	(72)	(83)	(71)	(62)	(73)	(89)	(92)	(74)	(78)	(62)	(62)	S	(56)
	(n = 100)	96%	21%	72%	83%	71%	62%	73%	89%	92%	74%	78%	62%	62%	-	56%
2	*Klebsiella pneumoniae*	(34)	(23)	(28)	(26)	(22)	(10)	(22)	(28)	(12)	(29)	(30)	(30)	(25)	-	(25)
	(n = 36)	93%	65%	78%	73%	62%	28%	62%	78%	33%	80%	84%	83%	70%	S	70%
3.	*Pseudomonas aeruginosa*	(10)	(6)	(8)	(10)	(9)	(8)	(6)	(8)	(7)	(9)	(6)	(9)	(8)	-	(7)
	(n = 10)	100%	62%	80%	100%	90%	80%	60%	80%	70%	90%	60%	90%	80%	S	70%
4.	*Citrobacter freundii*	(8)	(4)	(5)	(8)	(7)	(5)	(4)	(6)	(7)	(5)	(6)	(5)	(7)	-	(7)
	(n = 8)	100%	50%	60%	100%	90%	60%	50%	80%	90%	60%	70%	60%	90%	S	90%
5.	*Acinetobacter banumanii*	(18)	(3)	(13)	(9)	(8)	(3)	(2)	(3)	(5)	(3)	(5)	(2)	(2)	-	-
	(n = 18)	100%	20%	65%	50%	45%	20%	10%	20%	30%	20%	30%	10%	10%	S	S
6.	*Aeromonas hydrophila*	(20)	-	(20)	(12)	(4)	(4)	(20)	(20)	(20)	(20)	(20)	-	(20)	-	-
	(n = 20)	100%	S	100%	60%	10%	10%	100%	100%	100%	100%	100%	S	100%	S	S
7.	*Enterobacter aerogenes*	(13)	(4)	(6)	(7)	(3)	(3)	(4)	(6)	(3)	(3)	(5)	(3)	(1)	-	(1)
	(n = 14)	90%	30%	40%	50%	20%	20%	30%	45%	20%	20%	35%	20%	10%	S	10%
8.	*Proteus mirabilis*	(4)	(3)	(3)	(3)	(2)	(3)	(3)	(3)	(2)	(2)	(2)	(2)	(1)	-	(1)
	(n = 4)	100%	80%	90%	80%	40%	70%	90%	80%	50%	50%	50%	50%	50%	S	30%
9.	*Morganella morganii*	(9)	(1)	(2)	(6)	(3)	(1)	(1)	(2)	(1)	(6)	(7)	(2)	(3)	-	(4)
	(n = 10)	90%	10%	20%	60%	30%	10%	10%	20%	10%	60%	70%	20%	30%	S	40%

Table 3: Susceptibility of non-ESBL-producing bacteria from UTI patents to antimicrobial agents

S. No.	Organism	A	Ak	A-C	Cu	Co	NF	G	Nx	Cf	Ce	Ca	Nt	Of	I	Le
1.	*Escherichia coli*	R	S	R	MS	S	MS	S	S	S	S	S	S	S	S	S
2.	*Klebsiella pneumoniae*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3.	*Salmonella typhi*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
4.	*S. paratyphi A*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
5.	*Pseudomonas aeruginosa*	R	S	R	R	R	S	S	S	S	R	MS	S	S	S	S
6.	*Citrobacter freundii*	S	S	S	S	S	S	S	R	S	S	R	R	S	S	S
7.	*Acinetobacter baumannii*	R	S	R	MS	S	S	S	S	S	S	S	S	S	S	S
8.	*Aeromonas hydrophila*	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
9.	*Enterobacter aerogenes*	S	S	S	S	S	-	S	S	S	S	S	S	S	S	S
10.	*Proteus mirabilis*	R	S	S	S	S	S	S	R	S	S	S	S	S	S	S

S = Susceptible, R = Resistant, MS = Most Susceptible, - = Absent

The ESBL strains were highly resistant to the quinolones like nalidixic acid, norfloxacin and ciprofloxacin, which are the most commonly used drugs against UTI. These observations were in accordance with the precious reports (Elliot *et al.*, 1987). It has been reported that since the mechanism of action of these quinolones is almost same, emergence of resistance against one will increase the activity of other quinolones, because the resistance trait is plasmid borne and can be easily transmitted to the non-ESBL organisms. This may be the cause for the greater increase in ESBL strains in nosocomial infections. Also since the antibiotic susceptibility patterns of the ESBL and non-ESBL producers vary, it is important to carefully monitor organisms for ESBL production (Tankhiwale, 2004). In the present study, all the ESBL-producers were found resistant to two or more drugs, whereas multi-drug resistance in non-ESBL producers was less comparatively. Our study showed that ESBL production was high among uropathogens and that the ESBL producers were mostly multidrug resistant. Hence routine ESBL testing for uropathogens is essential. Antibiogram patterns would be useful for required therapy.

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