ID: JCDR/2012/4201:0025

Original Article

Microbiology Section

Antibiotic Resistance Pattern in Uropathogens at a Tertiary Care Hospital at Jhalawar with Special Reference To ESBL, AmpC β-Lactamase and MRSA Production

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ABSTRACT

Background: Urinary tract infections (UTIs) are one of the most common bacterial infections in humans, both in the community as well as in the hospital settings. Worldwide, the data show that there is an increasing resistance among the organisms which cause UTI, to the conventional drugs. A study on the changing antibiotic resistance pattern is pertinent for an appropriate treatment and for the prevention and control of the different mechanisms of resistance.

Aim: To find out the drug option for the treatment of UTI due to the presence of various clinical isolates in our geographical area, in the current scenario of increasing antimicrobial resistance, with special reference to ESBL, AmpC β-lactamase and MRSA production.

Materials and Methods: A total of 184 clinical isolates from the urine of various patients who presented to the outpatient and inpatient departments of Jhalawar Medical College and Hospital, Jhalawar, Rajasthan, India, were studied from January 2011 to September 2011. The antimicrobial susceptibility to various drugs was studied by the disc diffusion method, by following the CLSI guidelines. Confirmation of the extended spectrum β-lactamase (ESBL), ampC β-lactamase and methicillin resistant *Staphylococcus aureus* (MRSA) production was done by the phenotypic confirmatory disc diffusion test (PCDDT), the ampC disk test (ADT) and the oxacillin E test respectively.

Results: The antibiotic sensitivity pattern of the gram negative bacilli (GNB) revealed that the maximum sensitivity was seen for imipenem (95.1%), followed by cefoxitin (79.6%), piperacillin/tazobactum (71.8%), cefepime (71.8%), and amikacin (66.9%), and that of the gram positive cocci (GPC) showed that the maximum sensitivity was seen for vancomycin and linezolid (100%), followed by amikacin (95.2%), gentamicin (69.1%) and nitrofurantoin (61.9%). High resistance was seen against amoxycillin/clavulanate, co-trimoxazole, cefotaxime, doxycycline and norfloxacin. Overall, the prevalence of ESBL and ampC β-lactamase and the coexistence of the phenotype (ESBL + ampC β-lactamase) and MRSA in the urinary isolates was found to be 66.9%, 21.1%, 3.5% and 42.4% respectively.

Conclusion: Among the oral drugs, amoxycillin/clavulanic acid, norfloxacin, doxycycline and co-trimoxazole should no longer be considered as the first line drugs for the empirical treatment of clinically evident UTI, because of the very high resistance rates. Nitrofurantoin can be used as an alternative drug only after the sensitivity testing. Parentral drugs such as aminoglycosides, carbapenams and piperacillin/tazobactum can be the alternative choice for complicated UTI. Also, control measures which include the judicious use of antibiotics, antibiotic cycling, the implementation of appropriate infection control measures and the formulation of an antibiotic policy must be done, to prevent the spread of these strains.

Key Words: UTI, Antibiotic resistance, ESBL, AmpC β-lactamase, MRSA

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans, both in the community as well as in the hospital settings [1-3]. In almost all the cases, there is a need to start the treatment before the final microbiological results are available. Area specific monitoring studies which are aimed to gain knowledge about the type of pathogens which are responsible for UTIs and their resistance patterns may help the clinicians to choose the right empirical treatment. Knowledge on the antibiotic resistance patterns of the pathogens is important not only to provide an appropriate therapy, but also for the prevention of resistance amongst the microbes, as the treatment is given without considering the prevalent microbe and its antibiotic resistance pattern results in the selection of more resistant strain [4] and also increase in the prevalence of resistance mechanisms. The aim of the study was to obtain data on the resistance patterns of the major pathogens from patients with UTIs, to the antimicrobial agents which are currently used in the treatment of UTIs, along with the production of resistance mechanisms such as ESBL, AmpC β-lactamase and MRSA.

MATERIALS AND METHODS

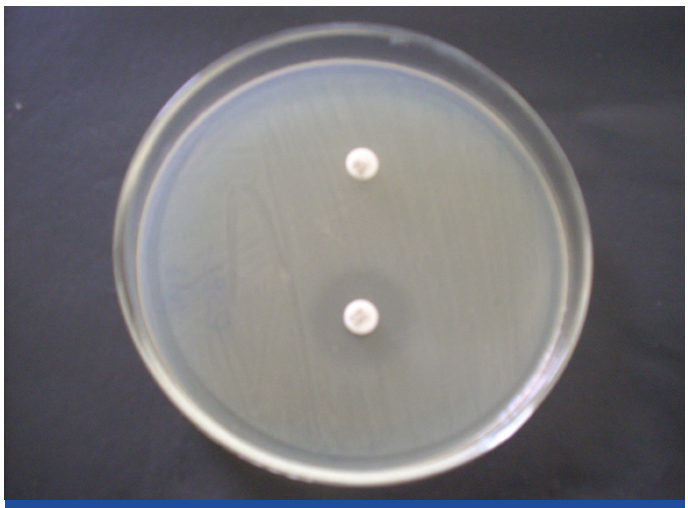
Bacterial isolates: A total of 184 consecutive, non-repetitive, clinical isolates which were obtained from the patients of UTI in the clinical bacteriology laboratory, Jhalawar Medical College, Jhalawar, Rajasthan, India from January 2011 to September 2011, were included in the study.

Antimicrobial susceptibility testing: The isolates were tested by disc diffusion method (modified Kirby-Bauer method) on Muller Hinton agar (Hi-Media), by following the zone size criteria which was recommended by the CLSI [5]. The antibiotics (μg) which were included for the gram negative isolates were amikacin (30), piperacillin (100), piperacillin/tazobactum (100/10), cefepime (30),

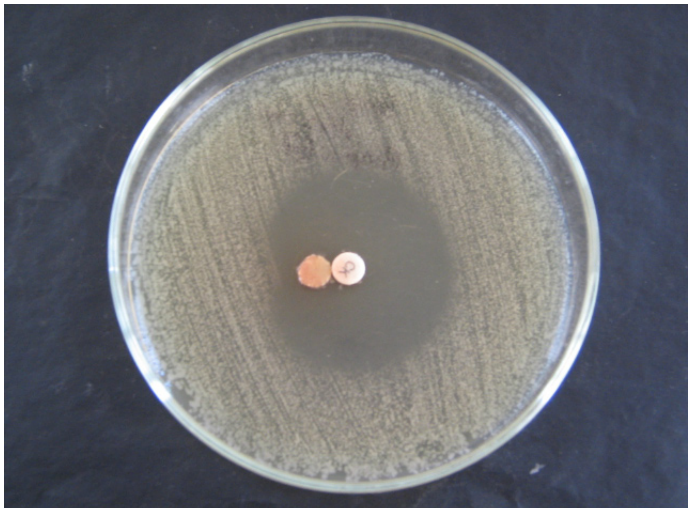
cefotaxime (30), ceftriaxone (30), ceftazidime (30), amoxyclav (20/10), cotrimoxazole (25), norfloxacin (10), imipenam (10), doxycycline (30), azithromycin (15), nitrofurantoin (300) and cefoxitin (30). The gram positive clinical isolates were tested with amoxyclav (20/10), cephalexin (30), linezolid (30), azithromycin (15), doxycycline (30), cefotaxime (30), norfloxacin (10), amikacin (30), gentamicin (10), vancomycin (30), cotrimoxazole (25) and nitrofurantoin (300).

Criteria for the selection of the ESBL producing strains: The isolates were tested for their susceptibility to the third generation cephalosporins (3GCs) e.g. ceftazidime (30 μg), cefotaxime (30 μg) and ceftriaxone (30 μg) by using the standard disc diffusion method, as was recommended by the CLSI [5]. If a zone diameter of ≤ 22 mm for ceftazidime, ≤ 27 mm for cefotaxime and ≤ 25 mm for ceftriaxone were recorded, the strain was considered to be "suspicious for ESBL production" [5]. Only those isolates which were resistant to one of the 3 GCs were selected for the study and they were processed for the ESBL production.

The phenotypic confirmatory disc diffusion test (PCDDT): All the strains which were screened out for the ESBL production were subjected to confirmation by using the PCDDT, as was recommended by the CLSI [5]. In this test, ceftazidime (30 μg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic Acid, 30/10 μg) discs, were applied onto a plate of Mueller Hinton Agar (MHA) which was inoculated with the test strain. An increase of ≥ 5mm in the zone of inhibition of the combination discs in comparison to that of the ceftazidime disc alone was considered to be a marker for ESBL production [5] [Table/Fig-1].



[Table/Fig-1]: A > 5 mm increase in zone of inhibition for ceftazidime/clavulanic acid (CAC) versus its zone diameter when tested alone by ceftazidime confirmed an ESBL producing organism



[Table/Fig-2]: AmpC disc test (ADT) shows indentation on the plane disc side (inoculated with several colonies of test organism) of cefoxitin inhibition zone showing ampC b-lactamase producing organism

The AmpC Disc Test (ADT): All the screened isolates were tested for the confirmation of AmpC β-lactamase production by using ADT. A lawn culture of *Escherichia coli* ATCC 25922 was prepared on an MHA plate. A sterile disc (6 mm) was moistened with sterile saline, it was inoculated with several colonies of the test organism and it was placed besides a cefoxitin disc (almost touching) on the inoculated plate. The plate was incubated overnight at 35^{0}C aerobically for 16-18 hours. A positive test was indicated as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc. An undistorted zone showed a negative test [6] [Table/Fig-2].

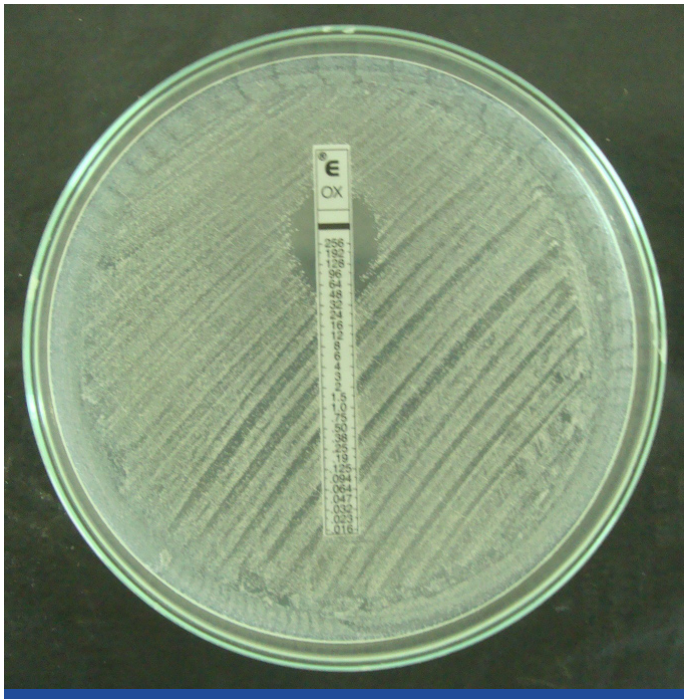
The Oxacillin E test: The MICs of oxacillin were determined by the E-test (AB Biodisk, Solna, Sweden), according to the instructions of the manufacturer [7]. The plates were inoculated by swabbing the surfaces with a 0.5 McFarland's standard bacterial suspension on the MHA medium which was supplemented with 2% NaCl. The E-test strips were placed on the medium, and the plates were then incubated at 35°C for 24 hrs. The results were analyzed on the basis of the CLSI guidelines [5] [Table/Fig-3].

Quality control: *Escherichia coli* ATCC 25922 (β-lactamase negative), *Klebsiella pneumoniae* ATCC 700603 (ESBL-producing), *Staphylococcus aureus* ATCC 25923 (Oxacillin susceptible) and *Staphylococcus aureus* ATCC 43300 (Oxacillin resistant) were used as the control strains [5].

Statistical analysis: The statistical analysis was performed by using the Chi-square test and a *p* value of less than 0.05 was considered as statistically significant.

RESULT

The present study was conducted in the Clinical Bacteriology Laboratory, Department of Microbiology, Jhalawar Medical College,



[Table/Fig-3]: E-test strip showing MRSA producing organism having a MIC of 48μg/ml

Jhalawar, Rajasthan, from January 2011 to September 2011, to know the antibiotic resistance patterns of the uropathogens in our geographical area, in the current scenario of increasing antimicrobial resistance, with special reference to the various mechanisms of drug resistance which were observed at our tertiary health care centre.

The antibiotic sensitivity pattern of the gram negative bacilli revealed that the maximum sensitivity was seen for imipenem (95.1%), followed by cefoxitin (79.6%), piperacillin/tazobactum (71.8%), cefepime (71.8%), amikacin (66.9%) and nitrofurantoin (54.2%). The maximum resistance was seen against ceftazidime and amoxycillin/clavulanate (82.4%), piperacillin (80.3%), cotrimoxazole (78.9%), ceftriaxone (78.2%), cefotaxime and doxycycline (77.5%) and norfloxacin (67.6%) [Table/Fig-4].

The antibiotic sensitivity pattern for gram positive cocci revealed that 100% sensitivity was seen for vancomycin and linezolid, followed by amikacin (95.2%), gentamicin (69.1%), nitrofurantoin (61.9%) and norfloxacin (52.4%). The maximum resistance was seen against cotrimoxazole (83.3%), cefotaxime (59.5%), azithromycin and cephalexin (57.1%), amoxycillin/clavulanate and doxycycline (50%) [Table/Fig-5].

Overall, the prevalence of ESBL and ampC β-lactamase and the coexistence of the phenotype (ESBL + ampC β-lactamase) and MRSA in the urinary isolates was found to be 66.9%, 21.1%, 3.5% and 42.4% respectively [Table/Fig-6 and 7].

Only 2 ESBL producer strains, one of which was *Citrobacter freundii,* was sensitive to ceftazidime and the second, *Proteus mirabilis,* was intermediately sensitive to ceftazidime. All the other isolates showed resistance to ceftazidime, thus indicating that ceftazidime was a good drug for the detection of the ESBL activity. This showed a significant correlation (*p* value <0.01). One isolate of *Klebsiella pneumoniae* and 4 isolates of *Escherichia coli* showed coexistence of their phenotypes. Only one cefoxitin sensitive isolate of *K. pneumoniae* showed the production of ampC β-lactamase. It showed a significant correlation (*p* value <0.01) along with the production of ESBL. All the cefoxitin resistant GNB and GPC were ampC β-lactamase and MRSA producers respectively, thus indicating that cefoxitin was a good drug for the detection of ampC β-lactamase as well as MRSA production. This showed a highly significant correlation (*p* value <0.001)

The maximum ESBL activity was seen in *Escherichia coli* (73.5%), followed by *Acinetobacter* spp. (66.1%), *Proteus vulgaris* (66.7%) and *Klebsiella pneumoniae* (59.1%). The maximum ampC β-lactamase production was found in *Providencia* spp. (100%), *Citrobacter freundii* (50%), *Proteus vulgaris* (33.3%), *Klebsiella pneumoniae* (31.8%) and *Escherichia coli* (18.4%). The MRSA production was maximally seen in *Staphlococcus saprophyticus* (50%) and *Staphylococcus aureus* (41.4%). Out of 23 oxacillin resistant strains, only 14 isolates were resistant to cefoxitin, which were further confirmed as MRSA strains by the oxacillin e-test, which showed a highly significant correlation (p value <0.001) [Table/Fig-6 and 7].

GNB (n=142)	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	94 (66.2)	2 (1.4)	46 (32.4)
Norfloxacin	44 (31.0)	2 (1.4)	96 (67.6)
Cefepime	102 (71.8)	0 (0)	40 (28.2)
Doxycycline	30 (21.1)	2 (1.4)	110 (77.5)
Piperacillin	28 (19.7)	0 (0)	114 (80.3)
Piperacillin/ tazobactam	102 (71.8)	1 (0.7)	39 (27.5)
Imipenam	135 (95.1)	0 (0)	7 (4.9)
Azithromycin	71 (50)	5 (3.5)	66 (46.5)
Co-trimoxazole	29 (20.4)	1 (0.7)	112 (78.9)
Amoxycillin/ clavulanate	23 (16.2)	2 (1.4)	117 (82.4)
Nitrofurantoin	78 (54.9)	4 (2.8)	60 (42.3)
Cefoxitin	113 (79.6)	0 (0)	29 (20.4)
Cefotaxime	30 (21.1)	2 (1.4)	110 (77.5)
Ceftriaxone	30 (21.1)	1 (0.7)	111 (78.2)
Ceftazidime	24 (16.9)	1 (0.7)	117 (82.4)

[Table/Fig-4]: Antibiotic susceptibility pattern of gram negative bacilli in various clinical isolates

GPC (n=42)	Sensitive (%)	Intermediate (%)	Resistant (%)
Amoxycillin/ clavulanate	21 (50)	0 (0)	21 (50)
Cephalexin	18 (42.9)	0 (0)	24 (57.1)
Linezolid	42 (100)	0 (0)	0 (0)
Azithromycin	18 (42.9)	0 (0)	24 (57.1)
Doxycycline	21 (50)	0 (0)	21 (50)
Cefotaxime	16 (38.1)	1 (2.4)	25 (59.5)
Norfloxacin	22 (52.4)	0 (0)	20 (47.6)
Amikacin	40 (95.2)	0 (0)	2 (4.8)
Gentamicin	29 (69.1)	0 (0)	13 (30.9)
Vancomycin	42 (100)	0 (0)	0 (0)
Co-trimoxazole	7 (16.7)	0 (0)	35 (83.3)
Nitrofurantoin	26 (61.9)	1 (2.4)	15 (35.7)

[Table/Fig-5]: Antibiotic susceptibility pattern of gram positive cocci in various clinical isolates

	ESBL (%)	AmpC (%)	ESBL + AmpC (%)
Klebsiella	13/22 (59.1)	7/22 (31.8)	1/22 (4.6)
Escherichia coli	72/98 (73.5)	18/98 (18.4)	4/98 (4.1)
Pseudomonas	2/7 (28.6)	1/7 (14.3)	0/7 (0)
Acinetobacter	2/3 (66.7)	0/3 (0)	0/3 (0)
Proteus mirabilis	1/2 (50)	0/2 (0)	0/2 (0)
Proteus vulgaris	2/3 (66.7)	1/3 (33.3)	0/3 (0)
Citrobacter koserii	2/4 (50)	1/4 (25)	0/4 (0)
Citrobacter freundii	1/2 (50)	1/2 (50)	0/2 (0)
Providencia	0/1 (0)	1/1 (100)	0/1 (0)
Total	95/142 (66.9)	30/142 (21.1)	5/142 (3.5)

[Table/Fig-6]: Prevalence of ESBL, ampC β-lactamase and co-existance of resistance (ESBL + ampC β-lactamase) among gram negative bacilli in various clinical isolate.

	Oxacillin		Cefoxitin		Confirm-atory E-test (%)
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	
Staphylococcus aureus (n = 29)	10 (34.5)	19 (65.5)	17 (58.6)	12 (41.4)	12 (41.4)
CONS (n = 4)	0 (0)	4 (100)	2 (50)	2 (50)	2 (50)
Total (n = 33)	10 (30.3)	23 (69.7)	19 (57.6)	14(42.4)	14 (42.4)

[Table/Fig-7]: Comparison of oxacillin and cefoxitin disc diffusion test for confirmation of MRSA production

Resistance	OPD (%)	IPD (%)
ESBL	75/112 (66.9)	20/30 (66.7)
AmpC	20/112 (17.9)	10/30 (33.3)
ESBL + AmpC	3/112 (2.7)	2/30 (6.7)
MRSA	7/22 (31.8)	7/11 (63.6)

[Table/Fig-8]: Prevalence of ESBL, AmpC, ESBL + AmpC and MRSA in OPD and IPD

	ESBL producer (n = 95) (%)	Non-ESBL producer (n = 47) (%)
Amikacin	25 (26.3)	21 (44.7)
Norfloxacin	66 (69.5)	30 (63.8)
Cefepime	16 (16.8)	24 (51.1)
Doxycycline	76 (80)	34 (72.3)
Piperacillin	82 (86.3)	32 (68.1)
Piperacillin/tazobactam	23 (24.2)	16 (34.0)
Imipenam	5 (5.3)	2 (4.2)
Azithromycin	52 (54.7)	14 (29.8)
Co-trimoxazole	79 (83.2)	33 (70.2)
Amoxycillin/clavulanate	79 (83.2)	38 (80.9)
Nitrofurantoin	40 (42.1)	20 (42.5)
Cefoxitin	4 (4.2)	25 (53.2)
Cefotaxime	79 (83.2)	31 (65.9)
Ceftriaxone	74 (77.9)	37 (78.7)
Ceftazidime	93 (97.9)	24 (51.1)

[Table/Fig-9]: Comparison of antibiotic resistance pattern in both ESBL producers and non ESBL producers

The prevalence of ESBL and ampC β-lactamase and the co-existence of the phenotype and the MRSA production in the outdoor patients was 66.9%, 17.9%, 2.7% and 31.8% respectively, while in the indoor patients, it was 66.7%, 33.3%, 6.7% and 63.6% respectively, thus indicating that the prevalence of the resistance mechanisms were more common in the indoor patients as compared to that in the outdoor patients, thus indicating a highly significant correlation (*p* value <0.001) [Table/Fig-8].

ESBL producers have less resistant isolates for piperacillin/tazobactum (24.2%), amikacin (26.3%), cefepime (16.8%) and cefoxitin (4.2%) as compared to their counterpart non ESBL producers (*p* value >0.05), because the non ESBL producers can have different mechanisms for their resistance patterns, such as the production of ampC β-lactamase, metallo-betalactamase, etc. in having more drug resistant isolates. For other antibiotics, the resistance pattern was quiet more in the ESBL, ampC β-lactamase and the MRSA isolates as compared to their counterparts, thus showing a highly significant correlation (p value <0.001). In the ampC β-lactamase producers, amoxycillin/clavulanate and ceftriaxone showed 100% resistance [Table/Fig-9, 10 and 11].

DISCUSSION

Most of the nosocomial UTIs are caused by gram-negative bacteria, particularly *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and organisms from the *Enterobacteriaceae* group. Collectively, they account for more than 80% of the culture positive cases of UTIs and the rest are caused by gram positive cocci such as Staphylococcus aureus and fungi, e.g. candida species. Fungal infections have gained increased prevalence with the advent of HIV/AIDS and with the widespread use of broad spectrum antibiotics.

	AmpC producer (n = 30) (%)	Non-AmpC producer (n = 112) (%)
Amikacin	22 (73.3)	24 (21.4)
Norfloxacin	28 (93.3)	68 (60.7)
Cefepime	28 (93.3)	12 (10.7)
Doxycycline	23 (76.7)	87 (77.7)
Piperacillin	29 (96.7)	85 (75.9)
Piperacillin/tazobactam	21 (70)	18 (16.1)
Imipenam	2 (6.7)	5 (4.5)
Azithromycin	16 (53.3)	50 (44.6)
Co-trimoxazole	21 (70)	91 (81.3)
Amoxycillin/clavulanate	30 (100)	87 (77.7)
Nitrofurantoin	18 (60)	42 (37.5)
Cefoxitin	29 (96.7)	0 (0)
Cefotaxime	28 (93.3)	82 (73.2)
Ceftriaxone	30 (100)	81 (72.3)
Ceftazidime	29 (96.7)	88 (78.6)

[Table/Fig-10]: Comparison of antibiotic resistance pattern in both AmpC producers and non AmpC producers

	MRSA (n = 14) (%)	Non-MRSA (n = 19) (%)
Amoxycillin/clavulanate	14 (100)	7 (36.8)
Cephalexin	11 (78.6)	7 (36.8)
Linezolid	0 (0)	0 (0)
Azithromycin	12 (85.7)	7 (36.8)
Doxycycline	9 (64.3)	7 (36.8)
Cefotaxime	13 (92.9)	6 (31.6)
Norfloxacin	7 (50)	9 (47.4)
Amikacin	2 (14.3)	0 (0)
Gentamicin	7 (50)	2 (10.5)
Vancomycin	0 (0)	0 (0)
Co-trimoxazole	14 (100)	15 (78.9)
Nitrofurantoin	9 (64.3)	4 (21.1)

[Table/Fig-11]: Comparison of antibiotic resistance pattern in both MRSA producers and non MRSA producers

This also causes an increase in the prevalence of multidrug resistant organisms.

Continuous monitoring systems and effective infection control measures are absolutely required to prevent the rapid and world-wide spread of ESBL, ampC β-lactamase and MRSA producing organisms. The therapeutic options for the infections which are caused by these organisms have also become increasingly limited. Although most of the outbreaks were limited to the high risk patient care areas such as ICUs, oncology units, etc., the first report of an outbreak in nursing homes appeared in the literature in the year 1999 [8]. Therefore, now- a- days the threat of the ESBL, ampC β-lactamase and the MRSA producing isolates is not limited to the ICUs or the tertiary care hospitals only, but they are also found in OPD patients.

The Clinical and Laboratory Standards Institute (CLSI) have issued recommendations for ESBL and MRSA screening and for their confirmation. No CLSI recommendations exist for ESBL detection, for the reporting for organisms other than *Escherichia coli* and *Klebsiella* spp. [9] and also for the detection of ampC β-lactamase, which were included in our study.

The prevalence of the ESBL producers as in previous studies from India was reported to be 6.6 to 68%. Subha *et al* [10] reported 6.6% ESBL producers among *Klebsiella pneumoniae* from children, whereas Babypadmini *et al* [11] showed 40.3% ESBL producers in their study cohort. The occurrence of ESBL producers among the gram negative bacilli in the current study was 95/142 (66.9%), while 73.5% *Escherichia coli*, 66.7% *Acinetobacter lowffi* and *Proteus vulgaris*, 59.1% *Klebsiella pneumoniae*, 50% *Proteus mirabilis*, *Citrobacter koserii* and *Citrobacter freundii* and 28.6% *Pseudomonas aeruginosa* were found to elaborate ESBLs. The ESBL production which was reported among gram negative bacteria by Mathur *et al* [12], Singhal *et al* [13] and C. Rodrigues *et al* [14] correlated well with that which was found in our study.

We observed that 73.5% *Escherichia coli* and 59.1% *Klebsiella pneumoniae* isolates were ESBL producers. Although *K. pneumoniae* was more often reported as an ESBL producer in other studies, in our study, the ESBL production was more common in the *Escherichia coli* isolates as compared to that in the *K. pneumoniae* isolates. [11, 15, 16]

The ESBL production in *Pseudomonas aeruginosa* is less (28.6%) as compared to that in other gram negative bacilli, because their resistance mechanism is mediated by the production of metallobetalactamase, lack of drug penetration due to mutations in the porins or loss of certain outer membrane proteins and an efflux pump. [13, 17, 18]

Recently, the co-existance of both ampC β-lactamase and ESBL in some gram negative bacilli has also been reported. This could be because plasmid mediated ampC β-lactamase has been disseminated among the *Enterobacteriaceae*, sometimes in combination with ESBL [13]. Such strains may give false negative tests in the detection of ESBL. 3.5% (5/142) of the isolates in the present study probably represented co-existance of the phenotypes of both the ampC β-lactamase and the ESBL producers, as was mentioned by Singhal et al [13]. 1.25% (1/80) of such a co-existence among the *Escherichia coli* isolates was demonstrated. With *Klebsiella* spp. and *Escherichia coli*, clavulanic acid may induce the expression of high-level AmpC production, and it may then antagonize rather than protect the antibacterial activity of the partner β-lactam, thus masking any synergy which might have arisen from the inhibition of an ESBL. A much better inhibition was achieved with the sulfones such as tazobactam and sulbactam [19]. Another reasonable approach is to rely on cefepime as an 'indicator drug'. High-level AmpC production has a minimal effect on the activity of cefepime, thus making this drug a more reliable detection agent for ESBLs in the presence of an AmpC β-lactamase [19].

There were 21.1% AmpC β-lactamase producers in our study. 37.5% and 47.8% have been reported from Chennai and Kolkata, respectively [20, 21]. Cefoxitin resistance can be used to screen the isolates for detecting any possible ampC β-lactamase production. But the lack of permeation in the porins has also been reported as one of the resistance mechanisms of cefoxitin in the ampC non-producers [22]. AmpC β-lactamase production in the cefoxitin susceptible isolates may have a mechanism which is similar to that of the ESBL producing organisms. Only one cefoxitin sensitive isolate of *K. pneumoniae* showed the production of ampC β-lactamase along with the production of ESBL. Thus, although studies have indicated that the screening methods which use cefoxitin in the standardized methods to detect the ampC harbouring isolates are useful, they are not still perfect [23] as compared to genotyping.

The identification of the *mec*A gene is the most reliable method for detecting the MRSA isolates. However, not all laboratories can include molecular biology techniques in their routine clinical practice. So, it is essential that phenotypic techniques which are able to detect the MRSA isolates in a rapid and accurate manner are made available, in order to ensure the correct antibiotic treatment and to avoid the spread of the MRSA isolates in the hospital environment. This study confirms that those antibiotics which are able to induce the expression of methicillin resistance, e.g. cefoxitin, are the most appropriate drugs for detecting the MRSA isolates, as was found in our study also. In our study, the confirmation of the MRSA isolates was done by the oxacillin E-test which gave a correlative study with cefoxitin, but the oxacillin disc showed false positivity. The *mec*A-positive isolates were detected with the cefoxitin disc (30 μg) in predicting oxacillin resistance has been reported [24-26]. It has been suggested that no special medium or incubation temperature is required for cefoxitin [25], so that it can be used as better indicator for the detection of MRSA.

The prevalence of MRSA was 42.4% in our study, while Sanjana RK et al [27] in Nepal, detected the prevalence of MRSA as 39.6%, Rajaduraipandi K et al [28] in Coimbatore found 31.1% strains of MRSA and Anupurba S et al [29] in eastern Uttar Pradesh found a 54.85% prevalence of MRSA, which correlated well with the findings of our study. Onanuga A et al [30] in Nigeria have reported a high prevalence of 69%, while Coombs GW et al [31] in Australia found it to be very low as 16%.

A majority of the urinary isolates were susceptible to imipenem (95.1%) and piperacillin/tazobactum and cefepime (71.8%). Similarly, in a study from Coimbatore, all the members of *Enterobacteriaceae* were found to be susceptible to imipenem and piperacillin/tazobactum [32]. In both the studies, amikacin also showed good activity against the gram negative bacteria. Therefore, imipenem is the most active drug for the treatment of infections which are caused by the ESBL producers, followed by piperacillin/tazobactum and amikacin. Carbapenem must be kept in reserve for non-life-threatening infections, where other susceptible antibiotics can be used [14]. The heavy use of carbapenem, in fact, may favour the selection of *Stenotrophomonas maltophilia* (a species which is naturally resistant to these drugs) [33].

Only 2 ESBL producer strains, one of which was *Citrobacter freundii* was sensitive to ceftazidime and the second, *Proteus mirabilis* was intermediately sensitive to ceftazidime. All the other isolates showed resistance to ceftazidime, thus indicating that ceftazidime was a good drug for the detection of ESBL production in our study. Therefore, cefpodoxime and ceftazidime have been proposed as the indicators of ESBL production as compared to cefotaxime and ceftriaxone [34].

The use of a three day course of co-trimoxazole, as recommended by the Infectious Disease Society of America (IDSA), is a first line treatment, except in communities with a high rate of resistance (>10-20%) among uropathogens [35]. A very high resistance rate (78.9% in case of GNB and 83.3% in case of GPC) was found against this drug and so this drug couldn't be used in our geographical area.

Norfloxacin, as it is an oral drug which is cost effective and which has an easy dosing schedule, is commonly prescribed for the treatment of UTI, not only in India, but also in other countries [36]. It showed a high resistance rate (67.6% in GNB and 47.6% in GPC) in our study, which reflects that an increased quinolone resistance was seen in our area, which was showed by other studies also [37-39].

Among other oral antibiotics, nitrofurantoin was found to be more effective in the treatment of UTI, only after the culture and sensitivity testing studies were done. This finding has been corroborated by other studies also [4, 40, 41]. Akram M et al, in Aligarh, found a very high resistance rate (80%) to nitrofurantoin in patients with community acquired UTI [38]. The inappropriate and empirical usage of wide spectrum antibiotics, insufficient hygiene, immunosuppression and a prolonged stay in the hospital are some of the major aetiological factors that elevate the chances of infection [39]. Continuous analysis of the antibiotic resistance pattern acts as a guide in initiating the empirical treatment of UTI and the therapy must be started only after the urine culture and the sensitivity testing have been done. This acts as a gold standard test and it helps in avoiding the treatment failure. So, the rapid dissemination of the antibiotic resistance and its mechanism can be prevented.

CONCLUSION

It is essential to report ESBL, AmpC β-lactamase and MRSA production along with the routine susceptibility testing, which will help the clinicians in prescribing proper antibiotics. The addition of ceftazidime + clavulanic acid for the detection of ESBL and the addition of cefoxitin for the detection of AmpC β-lactamase and MRSA must be done for the reporting of resistant organisms, because the restricted use of antibiotics can lead to the withdrawal of selective pressure and the resistant bacteria will no longer have a survival advantage against these antibiotics. In the end, it is felt that there is a need to formulate strategies to detect and prevent the emergence of resistance for an effective treatment of the infections which are caused by them.

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FINANCIAL OR OTHER COMPETING INTERESTS:
None.

Date of Submission: **Feb 26, 2012**
Date of Peer review: **Mar 17, 2012**
Date of Acceptance: **Mar 29, 2012**
Date of Publishing: **May 31, 2012**