Research article

Study of drug resistance pattern of principal ESBL producing urinary isolates in an urban hospital setting in Eastern India

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Abstract

A total number of 3690 urine samples were processed in the Microbiology Department during a one year period, of which 589 were culture positive. Among them, *Escherichia coli* (45%), Klebsiella spp. (15%), *Enterobactor cloacae* (12%), *Staphylococcus aureus* (6%), *Enterococcus faecalis* (9.8%), *Pseudomonas spp*. (9%), coagulase negative Staphylococci(1.1%) and Acinetobactor spp. (2.1%) were the predominant organisms. Out of the two principal isolates, 31.6% of *Escherichia coli* and 20.4% of *Klebsiella pneumoniae* were Extended Spectrum β Lactamase (ESBL) producers. They also showed multidrug resistance to quinolones and aminoglycosides. The proportion of carbapenemase producers among these isolates was also high.

Introduction

The incidence of extended spectrum β lactamase (ESBL) producing strains among clinical isolates has been steadily increasing over the past few years, resulting in the limitation of therapeutic options.[1] Microorganisms responsible for Urinary Tract Infection (UTI), especially *Escherichia coli* and Klebsiella spp. have the ability to produce ESBLs in large quantities. These enzymes are encoded by transferable conjugative plasmids, which often code resistance to cephalosporins as well as to other antibiotics. The most frequent co-resistances found in ESBL producing organisms are to aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol

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and sulfamethoxazole-trimethroprim.[2] The multidrug resistant strains that produce ESBL are commonly isolated from surgical care patients and patients with indwelling Foley's catheter (79%).[2]

Adequate data on the prevalence of ESBL producers in urinary isolates is not available in this part of India. Hence the present study was undertaken to find the prevalence of ESBL producing organisms in common urinary pathogens like *E. coli* and Klebsiella spp. in a tertiary care hospital in Kolkata and to assess the susceptibility pattern to other non-beta lactam drugs in ESBL producers and non-producers.

Materials and Methods

A total number of 3690 urine samples from the Out Patients Department (OPD) (n=390) and in-ward patients (n=3300) were processed and organisms isolated in significant numbers were identified by standard methods. Antibiotic sensitivity testing was performed using the modified CLSI method.[3] All *E. coli* and Klebsiella spp. isolated in significant numbers were included in this study. Drug sensitivity tests were performed according to CLSI guidelines by using the commercially available discs (Hi-media, India) on Mueller- Hinton agar (Hi-media, India) plates. The discs used were Gentamicin(10µg), Amikacin(30µg), Netilmicin(30µg), Nitrofurantoin(300µg), Ciprofloxacin(25µg), Norfloxacin(10µg), Gatifloxacin(10µg), Cotrimoxazole (1.25/23.75µg), Cefotaxime (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), and Imipenem(10µg). The diameter of the zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate or susceptible according to CLSI criteria. ESBL production was tested by the Double Disc Approximation test[4] and CLSI Confirmatory test.[3] Combination discs were available commercially (Hi media, India). All four discs were applied on one Mueller Hinton Agar (MHA) plate for each individual strain and incubated for 16-24 hrs. An increase in zone diameter for each antimicrobial agent tested in combination with Clavulanic acid versus its zone when tested alone was observed. For Ceftazidime, an increase in zone diameter of >5mm and for Cefotaxime, >3mm was considered to indicate an ESBL producer. The resistance pattern to other antimicrobials was also noted. Standard ATCC strains of *E. coli* ATCC 25922 and *K pneumoniae* ATCC 700603 were included in the study as control strains.[5]The MIC's (Minimum Inhibitory Concentration) of ESBL producing strains for Ceftazidime, Cefotaxime and Ceftriaxone were determined by agar dilution test.[6] Carbapenemase production was tested by the Hodge test.[7]

Results:

Of 3690 urine samples processed, 589 (15.9%) were culture positive. Out of 390 OPD samples 49(12.5%) and out of 3300 samples from inpatients 540 (16.3%) were culture positive. Out of them 316 were *E.coli*(53.6%), 88 were Klebsiella spp. (15%), 59 were *Staphylococcus aureus*(10%), 58 were Enterococci spp.(9.8%), 53 were Pseudomonas spp.(9%) and 15 were coagulase negative Staphylococci (2.5%). *E.coli* and Klebsiellaspp. were selected for study. Of these isolates, 100 (31.6%) *E.coli* strains and 18 (20.4%) Klebsiella strains were ESBL producers. Among the isolates from OPD samples, only 5 *E. coli* strains and 2 Klebsiella spp.were ESBL producers. Standard ATCC strains (*E.coli* ATCC 25922 and *K. pneumoniae*

ATCC 700603) were tested for MIC and found to be within CLSI control limits. Ten (10%) *E. coli* and 3 (14%) Klebsiella spp. were found to be carbapenemase producers.[7]

Table I: Susceptibility pattern of ESBL producing *E.coli* and Klebsiella spp.

Drugs	*E.coli* (n=100)		Klebsiella spp. (n=18)	
	Susceptible	Resistant	Susceptible	Resistant
Imipenem(10µg)	90(90%)	10(10%)	15(86%)	03(14%)
Nitrofurantoin(300µg)	90(90%)	10(10%)	02(11.1%)	16(88.9%)
Amikacin(30µg)	84(84%)	16(16%)	12(66.6%)	06(33.4%)
Netilmicin(30µg)	70(70%)	30(30%)	11(61.1%)	07(38.9%)
Gentamicin(10µg)	24(24%)	76(76%)	05(27.7%)	13(72.3%)
Cefotaxime(30µg)	31(31%)	69(69%)	01(5.5%)	17(94.5%)
Ceftazidime(30µg)	36(36%)	64(64%)	01(5.5%)	17(94.5%)
Cotrimoxazole (1.25/23.75µg)	17(17%)	83(83%)	02(11.1%)	16(88.9%)
Ciprofloxacin(5µg)	09(9%)	91(91%)	01(5.5%)	17(94.5%)
Norfloxacin(10µg)	06(6%)	94(94%)	01(5.5%)	17(94.5%)
Nalidixic acid(30µg)	05(5%)	95(95%)	02(11.1%)	16(88.9%)
Levofloxacin(5µg)	46(46%)	54(54%)	05(27.7%)	13(72.3%)

Table II: Susceptibility pattern of ESBL nonproducing *E.coli* and Klebsiella spp.

Drugs	*E.coli* (n=216)		Klebsiella spp. (n=70)	
	Susceptible	Resistant	Susceptible	Resistant
Imipenem(10µg)	212(98%)	04(2%)	63(90%)	07(10%)
Nitrofurantoin(10µg)	200(92.6%)	16(7.4%)	25(35.7%)	45(64.3%)
Amikacin(30µg)	210 (97.2%)	06(2.8%)	67(95.7%)	03(4.3%)
Netilmicin(30µg)	210 (97.2%)	06(2.8%)	68 (97.1%)	02(2.9%)
Gentamicin(10 µg)	186 (86.1%)	30(13.9%)	65 (92.8%)	05(7.2%)
Cefotaxime(30 µg)	114 (52.8%)	102(47.2%)	21 (30%)	49(70%)
Ceftazidime(30 µg)	122 (56.5%)	94(43.5%)	21 (30%)	49 (70%)
Cotrimoxazole(1.25/23.75µg)	71(32.8%)	145(67.2%)	42 (60%)	28(40%)
Ciprofloxacin(5 µg)	99 (45.8%)	117(54.2%)	56(80%)	14(20%)
Norfloxacin(10 µg)	77(35.8%)	139(64.3%)	49(70%)	21(30%)
Nalidixic acid(30 µg)	35(16.2%)	181(83.8%)	20 (28.6%)	50(71.4%)
Levofloxacin(5 µg)	127 (58.8%)	89(41.2%)	49 (70%)	21(30%)

Table III: MICs for the different resistance phenotypes observed in *K. pneumoniae* and *E. coli*

Species and resistance phenotype	No. of strains	MIC range of cefotaxime(µgm/ml)	MIC range of ceftazidime(µgm/ml)	MIC range of ceftriaxone (µgm/ml)
Klebsiella spp.				
Kp 1	5	>32	32-128	>32
Kp 2	11	32-64	32-128	32-64
Kp 3	2	32-128	32	64-128
E. coli				
Ec 1	33	>64	32-128	>32
Ec 2	42	32	32-64	>32
Ec 3	25	32-64	64-128	64

Kp=Klebsiella spp., Ec= *E. coli*

In all ESBL producers, MIC was found to be between 32-128 µg/ml (Table-III). MIC was done to overcome false positive results which may be seen in double disk synergy test.

Discussion:

ESBL producing strains are gradually increasing, especially in nosocomial infections throughout the world. The occurrence of ESBL producers among clinical isolates vary greatly worldwide and are rapidly changing over time. The ESBL producers in urinary isolates in our study were 31.6% *E.coli* and 20.45% Klebsiella spp. This is much higher than the reported results from USA (*E. coli* 2.2% and Klebsiella spp.6.6%) and Canada (*E.coli* 2.7% and Klebsiella spp. 6.2%).[8] In India, Mathur *et al*[11] observed much higher rates(58%). Most of the patients in their study were hospitalized and therefore susceptible to nosocomial infections with organisms surviving in a hostile environment with pressure of antibiotics. Similarly, in the current study, as the majority of samples were obtained from inpatients where the strains were under pressure of different antibiotics, the MICs of such strains were high leading to true resistance to a particular drug. Reports from JNMC, Wardha, India, showed that the proportion of ESBL positive organisms were 42% of which 41.3% were *E. coli* and 44.7% *Klebsiella pneumoniae*.[9]

ESBL production coexists with resistance to several other antibiotics.[2] ESBLs are encoded by plasmids, which also carry resistance genes for other antibiotics. Multi-drug resistance was 90.5% in ESBL producers, whereas it was 68.9% in nonproducers.[10] Initially ESBL producers were restricted to hospital –acquired infections only, but they have now also been isolated from outpatient departments. Major outbreaks involving ESBL producing strains have also been reported from all over the world.[2] As the total number of ESBL producing *E. coli* and Klebsiella spp. from OPD patients were too low (5 and 2 respectively), no conclusion could be derived from this study on community acquired infection.

The routine susceptibility tests done by clinical laboratories fail to detect ESBL producer strains and can sometimes erroneously report such isolates as sensitive to the broad-spectrum cephalosporins such as Cefotaxime, Ceftazidime and Ceftriaxone.[11] With the spread of ESBL producers in hospitals all over the world, it is necessary to know the prevalence of ESBL producer strains in a particular hospital so as to formulate a policy of empirical therapy in high

risk units where infection due to resistant organisms is much higher.[11] A knowledge of the resistance patterns of bacterial strains in a community helps to guide appropriate and judicious antibiotic use. In our study, high prevalence rate of ESBL producing bacteria may be due to long term antibiotic exposure, prolonged ICU stay, hospital acquired strains and prolonged catheterization. It is prudent in these situations to use non beta lactam drugs initially or use Beta lactam drugs in combination with an beta-lactamase inhibitor. As carbapenem resistance is low, these drugs are the only choice for treatment for severe or life threatening infections caused by ESBL producing organisms. The control measures include judicious use of antibiotics and implementation of appropriate infection control programme to prevent spread of these strains in the hospital.

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