

Research Paper

## Incidence and transferability of antibiotic resistance in the enteric bacteria isolated from hospital wastewater

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### Abstract

This study reports the occurrence of antibiotic resistance and production of  $\beta$ -lactamases including extended spectrum beta-lactamases (ES $\beta$ BL) in enteric bacteria isolated from hospital wastewater. Among sixty-nine isolates, tested for antibiotic sensitivity, 73.9% strains were resistant to ampicillin followed by nalidixic acid (72.5%), penicillin (63.8%), co-trimoxazole (55.1%), norfloxacin (53.6%), methicillin (52.7%), cefuroxime (39.1%), cefotaxime (23.2%) and cefixime (20.3%). Resistance to streptomycin, chloramphenicol, nitrofurantoin, tetracycline, and doxycycline was recorded in less than 13% of the strains. The minimum inhibitory concentration (MIC) showed a high level of resistance (800-1600  $\mu$ g/mL) to one or more antibiotics. Sixty three (91%) isolates produced  $\beta$ -lactamases as determined by rapid iodometric test. Multiple antibiotic resistances were noted in both among ES $\beta$ BL and non-ES $\beta$ BL producers. The  $\beta$ -lactamases hydrolyzed multiple substrates including penicillin (78.8% isolates), ampicillin (62.3%), cefodroxil (52.2%), cefotaxime (21.7%) and cefuroxime (18.8%). Fifteen isolates producing ES $\beta$ BLs were found multidrug resistant. Four ES $\beta$ BL producing isolates could transfer their R-plasmid to the recipient strain *E. coli* K-12 with conjugation frequency ranging from  $7.0 \times 10^{-3}$  to  $8.8 \times 10^{-4}$ . The findings indicated that ES $\beta$ BL producing enteric bacteria are common in the waste water. Such isolates may disseminate the multiple antibiotic resistance traits among bacterial community through genetic exchange mechanisms and thus requires immediate attention.

**Key words:** beta-lactamase, ES $\beta$ BL, Antibiotic resistance, Enteric bacteria, plasmid transfer.

### Introduction

Bacterial resistance to antimicrobial drugs, especially in nosocomial, commensal and zoonotic bacterial pathogens, has emerged as a global concern (Tanvir and Khiyani, 2011). As zoonotic agents, resistant bacteria within livestock are proposed to be a significant risk to human health (Mathew *et al.*, 2007). Fueling this concern is the widespread detection of multidrug resistant enteric pathogens. Antibiotics are intensively used in human, veterinary and agriculture and considered as the most important factor promoting the emergence, selection, and dissemination of resistant organisms (Catry *et al.*, 2033; Houghton, 2002).

$\beta$ -Lactamases are major determinants of resistance to  $\beta$ -lactam antibiotics. Coliform bacteria, isolated from human free environment, may induce a wide variety of resistant species by exposure to  $\beta$ -lactam drugs (Ash *et al.*, 2002; Boon and Cattanaach, 1999). Extended spectrum  $\beta$ -lactamase (ES $\beta$ BL) mediated resistance has been seen in the early years of 1980s in nosocomial enterobacteriaceae strains (Datta *et al.*, 2004; Livemore, 1995).

The ES $\beta$ BLs are plasmid mediated enzymes and capable of hydrolyzing and inactivating a wide variety of  $\beta$ -lactams, including third generation cephalosporins and aztreo-

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nam. Most of these are derivatives of TEM and SHV  $\beta$ -lactamases (Bush, 1989). On the other hand, plasmid mediated  $\beta$ -lactamases showed resistance to  $\alpha$ -methoxy  $\beta$ -lactam (cefoxitin, cefotetan) as well as oxyimino- $\beta$ -lactams (cefotaxime, ceftriaxone and ceftazidime) (Papanicolaou *et al.*, 1990; Rosenau *et al.*, 2000). The plasmid mediated antibiotic resistance facilitate easy spread between species and most likely to occur in the gastrointestinal tract (Kruse and Sorum, 1994; Silva *et al.*, 2006). Moreover, plasmids encoded ES $\beta$ LS often showed co-resistance to aminoglycosides, sulfonamides and quinolones. Therefore infections caused by such strains may be difficult to treat due to diminish choices of antibiotics (Philippon *et al.*, 2002).

Hospitals contain a concentrated reservoir of resistant coliform bacteria which are less common in community. The path between the hospital and the community runs both ways (Kunz and Brook, 2010). Therefore escape of such ES $\beta$ LS producing pathogenic strains from hospital environment to natural water bodies through wastewater discharge will be of much environmental and public health importance (Mathew *et al.*, 2007). Lack of systematic study in this direction has prompted us to investigate the wastewater released from hospitals in the city of Aligarh, India. Wastewater discharge of JN Medical College and City Hospital are quantitatively tested both for *E. coli* and non *E. coli* enteric drug resistant bacteria and their potential to transfer R-plasmids to *E. coli* K-12.

## Materials and Methods

### Bacterial strains

Wastewater from hospital was collected during the winter period of 2004-05 and tested for viable enteric bacteria on selective plates (*Salmonella-Shigella* agar, Luria agar, EMB agar and MacConkey agar plates). Bacterial isolates obtained from selective plates were submitted to biochemical tests and results in sixty nine isolates representing different enteric bacteria. These isolates were differentiated into *E. coli* and non *E. coli* enteric bacteria. The latter group was identified presumptively, having bacteria from *Shigella*, *Salmonella*, *Citrobacter*, *Enterobacter* and *Klebsiella*, on the basis of standard biochemical tests as described by Cappuccino and Sherman (1995). These tests include Gram-staining, starch and gelatin hydrolysis, production of H<sub>2</sub>S and acids, indole, methyl red and Voges-Proskauer test, nitrate, citrate utilization, catalase and oxidase test and production of acid and gas from carbohydrates.

### Identification

Bacterial isolates were selected on eosin methylene blue agar plates and colonies producing metallic shin were picked using sterile tooth-picks. The strains were further identified by biochemical tests as described by Cappuccino

and Sherman (1995). *E. coli* isolates were confirmed serologically by Central Research Institute, Kasualy, India and grouped separately from non *E. coli* enteric bacteria in the present investigation. Non *E. coli* enteric bacteria were further identified tentatively as *Shigella*, *Citrobacter* and *Klebsiella* and those which cannot be identified were grouped as miscellaneous enteric bacteria including four *Salmonella* spp.

### Determination of antibiotic susceptibility

Antibiotic discs/powders namely penicillin, ampicillin, cefuroxime, cefixime, cefotaxime, methicillin, nalidixic acid, cotrimoxazole, norfloxacin, doxycycline, tetracycline, nitrofurantoin, chloramphenicol, and streptomycin were purchased from Hi-Media Laboratory Ltd., Mumbai (India). Antibiotic sensitivity behavior of the isolated strains was determined by disc diffusion method as described by Bauer *et al.* (1996) as adopted by Ahmad *et al.* (1994).

### Determination of minimum inhibitory concentration (MIC) of antibiotics

Minimum inhibitory concentrations were determined by agar spot method against test strains as per recommendation of WHO as adopted by Ahmad *et al.* (1994). The MIC was considered the lowest concentration of the antibiotic at which no visible growth of test strain occurred.

### Assay for the detection of $\beta$ -lactamase production

$\beta$ -lactamase production in the test strains was detected by rapid iodometric method as described by Catlin (1975) and adopted by Ahmad and Aqil (2007). Briefly, the test strains were grown overnight on nutrient agar plates. Several colonies of the test strain were taken from the plates to make a dense suspension in 0.1 mL of penicillin solution (6 mg/ml) in a small test tube. After 30 min incubation at room temperature, 2 drops of starch solution and 1 drop of iodine reagent were added in reaction mixture leading to development of blue color due to starch-iodine reaction. The test tubes were shaken for 1 min at room temperature. Rapid decolorization of blue color indicated the presence of  $\beta$ -lactamase. No change in blue color for 10 min indicated negative results. Substrate specificity of  $\beta$ -lactamases was similarly tested against other  $\beta$ -lactam drugs (ampicillin, cloxacillin and cefixime) replacing penicillin-G in the test system. Strains of *E. coli* harboring transferable plasmids (*E. coli* MTCC 391 and *E. coli* 38) were used as positive controls.

### Isolation of plasmid DNA and agarose gel electrophoresis

The plasmids were isolated from drug resistant enteric bacteria by the method of Kado and Liu (1981) and characterized by agarose gel electrophoresis (Maniatis *et al.*, 1989). The molecular weight estimates of the isolated

plasmids were determined by comparison of their relative mobilities on 1% agarose gels with standard DNA molecular weight markers as well as *E. coli* V517 plasmids as reference. The molecular weights were calculated by VersaDoc imaging system, model 5000 (Bio-Rad, Hercules, CA, USA).

#### Detection of ESβLs by DDST and on the basis of substrate specificity

The extended spectrum β-lactamases were detected by double disc synergy test (DDST) as described previously (Ahmad and Aqil, 2007). In the DDST, synergy was determined between augmentin (20 mg amoxicillin and 10 mg clavulanic acid) and a 30 mg disc of each third generation cephalosporins test antibiotic placed at a distance of 30 mm apart on Mueller-Hinton agar (Hi-Media Pvt. Ltd. Mumbai, India). The test organism was considered to produce ESβL, if the zone size around the test antibiotic disc increased towards the augmentin disc. This increase occurs because the clavulanic acid present in the augmentin disc inactivates the ESβL produced by the test organism. ESβL production was further confirmed by hydrolysis of β-lactams of first, second and third generations as described by Catlin (1975). β-Lactamases hydrolyzing third generation of β-lactam antibiotics in addition to first and second were designated as ESβLs producer.

#### Detection of transferable antibiotic resistance and β-lactamase production by conjugation

Drug resistance transfer studies were carried out using broth mating technique as described earlier (Ahmad *et al.*, 1994). Briefly the donor and recipient (*E. coli* K-12) strains were grown in 5 mL nutrient broth, mixed in 1:2 ratios in 7 mL fresh nutrient broth and incubated overnight at 37 °C. Mix culture was spread evenly onto double antibiotics selection plates. Plates were incubated at 37 °C for overnight. Colonies grown onto double antibiotic plates were supposed to be transconjugants and tested for confirmation.

**(a) Confirmation of transconjugant Colonies:** Colonies from double antibiotic nutrient agar plates were transferred to double antibiotic MacConkey agar plates. Colorless colonies indicating non lactose fermenting were observed on the selection plates and confirmed as recipient *E. coli* K-12 Sm<sup>r</sup> strains on the basis of their cultural, morphological and nutritional characters.

**(b) Screening of transconjugants:** Transconjugants observed on double antibiotic plates were further screened to possess antibiotic resistance markers and their ability to produce β-lactamases as described earlier.

**(c) Percent of Transfer:** Percent transfer of drug resistant marker was calculated as follows:

$$\% \text{ transfer} = \frac{\text{Colonial count of recipient strain on double antibiotic plate which acquire resistance from donor}}{\text{Colonial count of recipient strain on streptomycin plate}} \times 100$$

## Results and Discussion

Indiscriminate use of antibiotics has led to an increasing incidence of antibiotic resistance among strains of enterobacteriaceae, worldwide (French, 2010; Hiramatsu *et al.*, 1997). In the last two decades, ESβLs have emerged as a major contributor of drug resistance. In this study a total of 69 isolates of enteric bacteria were isolated from 50 samples of hospital wastewater in the vicinity of Aligarh city, India. On the basis of morphological, cultural and biochemical characteristics, these isolates were grouped into four different genera, *Escherichia* (18 isolates), *Citrobacter* (07), *Shigella* (11), and *Klebsiella* (04). Other twenty nine isolates could not be differentiated on the basis of biochemical tests and grouped as unidentified (miscellaneous) enteric bacteria. These characteristics of enteric bacteria were similar to those reported in literature (Ananthnarayan and Paniker, 1986). Further molecular characterization of these isolates is needed to explore their genetic linkages. The findings revealed that the wastewater discharged from hospital is heavily loaded with multiple drug resistant enteric bacteria and can be a plausible source for contamination to the other natural water bodies.

The multidrug resistant strains are common in the random population screened. The acquisition of antimicrobial resistance provides a mechanism for survival to bacterium in an otherwise hostile environment. Resistance is generally plasmid encoded or transposons mediated (Mather *et al.*, 2007; Carattoli, 2003). These plasmids are free to mutate without lethal effects on the host cell. They can also move between species and even genera, accumulating genes by conjugation or recombination. Transfer of resistance R-factors to other members of the enterobacteriaceae especially *E. coli*, *Salmonella*, and *Shigella* etc. makes the problem an even greater concern to clinicians, particularly in controlling diseases like typhoid, dysentery, diarrhoea and other gastro-intestinal infections both in humans and animals (Ahmad *et al.*, 1994; Silva *et al.*, 2006).

The resistance patterns against 14 antibiotics tested are presented in Table 1. Majority of the isolates showed resistance to one or more antibiotics. Such multiple antibiotic resistances is known to arise by acquisition of resistance genes through genetic exchange and mutation and physiological mechanisms such as possession of specific proteins and efflux pump (Hermansson *et al.*, 1987; Herwig *et al.*, 1997). Based on the disc diffusion test, incidence of antibiotic resistance was found maximum (73.9%) to ampicillin

**Table 1** - Incidence of antibiotic resistance in the isolates of enteric bacteria.

Antibiotics	Total no. of resistant isolates (%)					
	<i>E. coli</i> (n = 18)	<i>Shigella</i> (n = 11)	<i>Citrobacter</i> (n = 7)	<i>Klebsiella</i> (n = 4)	Miscellaneous (n = 29)	Total (n = 69)
Penicillin	7 (38.9)	5 (45.5)	5 (71.4)	4 (100.0)	22 (75.7)	43 (63.8)
Ampicillin	15 (83.3)	9 (81.8)	4 (57.1)	3 (75.0)	20 (69.0)	51 (73.9)
Cefuroxime	4 (22.2)	5 (45.5)	3 (42.9)	2 (50.0)	12 (41.4)	26 (39.1)
Cefixime	3 (16.7)	1 (9.1)	1 (14.2)	2 (50.0)	7 (24.1)	14 (20.3)
Cefotaxime	3 (16.7)	2 (18.2)	3 (42.9)	0 (00.0)	9 (31.0)	17 (23.2)
Methicillin	6 (33.3)	6 (54.5)	4 (57.1)	4 (100.0)	15 (51.7)	35 (52.7)
Nalidixic Acid	12 (66.7)	10 (90.9)	5 (71.4)	4 (100.0)	22 (75.9)	53 (72.6)
Cotrimoxazole	12 (66.7)	6 (54.5)	4 (57.1)	3 (75.0)	13 (44.8)	34 (55.7)
Norfloxacin	9 (50.0)	5 (45.5)	4 (57.1)	2 (50.0)	17 (58.6)	37 (53.6)
Doxycycline	4 (22.2)	0 (00.0)	1 (14.3)	0 (00.0)	4 (13.8)	9 (13.0)
Tetracycline	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	4 (13.8)	4 (5.8)
Nitrofurantoin	0 (00.0)	2 (18.2)	1 (14.3)	3 (75.0)	3 (10.3)	9 (13.0)
Chloramphenicol	2 (11.1)	0 (00.0)	0 (00.0)	2 (50.0)	5 (17.2)	9 (13.0)
Streptomycin	0 (00.0)	0 (00.0)	0 (00.0)	0 (00.0)	3 (10.3)	3 (4.4)

n, number of isolates.

(A), followed by nalidixic acid (Na), penicillin (Pn), co-trimoxazole (Co), norfloxacin (Nx), methicillin (M), cefuroxime (Ce), cefotaxime (Cef), cefixime (Cfx), doxycycline (Do), nitrofurantoin (Nf), chloramphenicol (C), tetracycline (Tc) and least (4.4%) to streptomycin (Sm). Bacteria were more sensitive to cefuroxime and cefotaxime among the  $\beta$ -lactams. Similarly, less resistance was observed against protein synthesis inhibiting antibiotics such as tetracycline, doxycycline, chloramphenicol and streptomycin.

The resistance profile might be the results of excessive use of antibiotics in the treatment of bacterial infections. It is expected that these isolates have received antibiotic pressure in hospital environment during treatment. High resistance to  $\beta$ -lactams among enteric bacteria from clinical and environmental origin has been reported worldwide (Ash *et al.*, 2002; Jain and Mondal, 2007; Rollinson, 1989). Resistance to nalidixic acid among clinical isolates of *Pseudomonas* and *Staphylococcus* is a common problem, but high incidence against enteric bacteria is less frequently reported (Murray, 1986; Rao *et al.*, 2010).

Increased incidence of ampicillin resistance was observed in *E. coli* (83.3%) followed by *Shigella* *Klebsiella* and *Citrobacter* sp. While miscellaneous constitute about 69.0% ampicillin resistant isolates. *Klebsiella* and *Shigella* were relatively more resistant to nalidixic acid compared to *E. coli*. The MIC data revealed that maximum number of isolates had higher level of resistance against ampicillin followed by nalidixic acid, penicillin, cefuroxime and cefotaxime (Table 2). The higher level of MIC and multiple antibiotic resistances in the enteric bacteria indicated the involvement of R-plasmids as the bacterial chromosome could not bear such an extra load of additional antibiotic re-

**Table 2** - Range of MIC values of antibiotics against test strains

Antibiotic	% of isolate showing different range of MIC values ( $\mu\text{g/mL}$ )			
	$\leq 25$	50-100	200-400	800-1600
Penicillin	36.2	31.9	1.5	30.4
Ampicillin	26.1	13.0	5.6	55.1
Cloxacillin	0.0	43.5	13.0	43.5
Cefuroxime	60.8	2.9	17.4	18.8
Cefotaxime	78.3	5.8	7.3	8.7
Nalidixic acid	27.5	8.7	14.5	49.3

sistance genes (Ahmad *et al.*, 1994, Livermore and Williams, 1982).

To ascertain the possible role of  $\beta$ -lactamases in the resistance against  $\beta$ -lactams, all the 69 isolates were tested for  $\beta$ -lactamase production using five substrates (penicillin, ampicillin, cefuroxime, cefodroxil, and cefotaxime). Of these, 63 isolates produced  $\beta$ -lactamase hydrolyzing one or more substrates (Table 3).  $\beta$ -Lactamases of these enteric bacteria hydrolyzed penicillin (76.8%) followed by ampicillin (62.3%), cefodroxil (52.2%), cefotaxime (21.7%) and cefuroxime (18.8%). The differential hydrolysis of  $\beta$ -lactam antibiotics by these enzymes is due to their substrate specificity (Bush, 1989). The data revealed that  $\beta$ -lactamase(s) production is the major mechanism of resistance against  $\beta$ -lactam antibiotics. Similar reports were also made by several workers among clinical isolates on enteric bacteria (Datta *et al.*, 2004). However, environmental isolates of wastewater are less frequently screened for production of  $\beta$ -lactamases (Aubron *et al.*, 2005).

On the basis of hydrolysis of different generation of  $\beta$ -lactam antibiotics, 15 isolates of enteric bacteria were found to produce extended spectrum  $\beta$ -lactamases, a group of enzymes that confer resistance to oxyimino cephalosporins and monobactams (Jacoby and Medeiros, 1991).

ES $\beta$ LS are modification of the ubiquitous TEM and SHV class A enzymes, which have undergone a series of mutation at distinct sites in the enzyme that either increase the size of the active site or alter its binding characteristics to allow the larger cephalosporins to enter (Bush, 1989). The

**Table 3** - Production of  $\beta$ -lactamase against individual  $\beta$ -lactam substrates.

Antibiotic hydrolyzed	No. of isolates producing $\beta$ -lactamase (%)					Total (n = 69)
	<i>E. coli</i> (n = 18)	<i>Shigella</i> (n = 11)	<i>Klebsiella</i> (n = 4)	<i>Citrobacter</i> (n = 7)	Miscellaneous (n = 29)	
Penicillin	14 (77.8)	10 (90.9)	4 (100.0)	6 (85.7)	19(65.5)	53(76.8)
Ampicillin	8 (44.4)	9 (81.8)	2 (50.0)	6 (85.7)	18(62.1)	43(62.3)
Cefuroxime	2 (11.1)	2 (18.2)	0 (0.0)	2 (28.6)	7(24.1)	13(18.8)
Cefodroxil	6 (33.3)	6 (54.5)	4 (100.0)	2 (28.6)	18(62.1)	36(52.2)
Cefotaxime	3 (16.7)	2 (18.2)	0 (0.0)	3 (42.9)	7(24.1)	15(21.7)

n, number of isolates.

**Table 4** - ES $\beta$ L production by drug resistant enteric bacteria.

Strain designation	Resistance pattern	$\beta$ -Lactam substrate hydrolyzed				
		Pn	A	Ce	Ced	Cef
<i>E. coli</i> (EC 1)	Pn, A, Ce, Cef, Co, Na	+	+	+	+	+
<i>E. coli</i> (EC 2)	Pn, A, Ce, Cef, M, Co, Cfx, Na	+	+	-	+	+
<i>E. coli</i> (EC 3)	Pn, A, M, Ce, Cef	+	+	+	+	+
<i>E. coli</i> (EC 4)	Pn, A, M, Ce, Co, Cef, Do, Cfx, Nx, Na	+	+	+	+	+
<i>Shigella</i> sp1.	Pn, A, M, Co, Nx, Na	+	+	-	-	+
<i>Shigella sonnei</i>	Pn, A, M, Co, Ce, T, Cef, Do, C, Nx, Na	+	+	+	+	+
<i>Salmonella</i> sp1	Pn, A, Co, Ce, Cef, Cfx, Nx, Na	+	+	+	+	+
<i>Salmonella</i> sp2	Pn, A, Co, M, Ce, Cef, Cfx, Nx, Na	+	+	+	-	+
<i>Shigella dysenteriae</i>	Pn, A, M, Ce, Cef, Co, Cfx, Nx, Na	+	+	+	+	+
<i>Salmonella</i> sp.3	Pn, A, M, Ce, Cef, T, C, Do, Cfx, Nx, Na	-	+	+	+	+
<i>Salmonella</i> sp.4	Pn, A, M, Ce, Co, C, Cef, Do, T, Cfx, Nx, Nf, Na	+	+	+	+	+
<i>Citrobacter</i> sp.1	Pn, A, M, Ce, Co, T, Cef, C, Do, Cfx, Nx, Nf, Na	+	+	+	+	+
<i>Citrobacter</i> sp2	Pn, A, M, Ce, cef, Co, C, Cfx, Na	+	+	+	+	+
<i>Salmonella</i> sp.5	Pn, A, M, Ce, Cef, Co, Na	+	+	+	+	+
<i>Shigella</i> sp.2	Pn, A, M, Ce, Cef, Co, Na	+	+	+	+	+

+ = Hydrolysis; - = No hydrolysis.

Pn, penicillin; A, ampicillin; Ce, cefuroxime; M, methicillin; Co, co-trimoxazole; Cef, cefotaxime; Nx, norfloxacin; Cfx, cefixime; Do, doxycycline; Nf, nitrofurantoin; C, chloramphenicol; T, tetracycline; and Na, nalidixic acid.

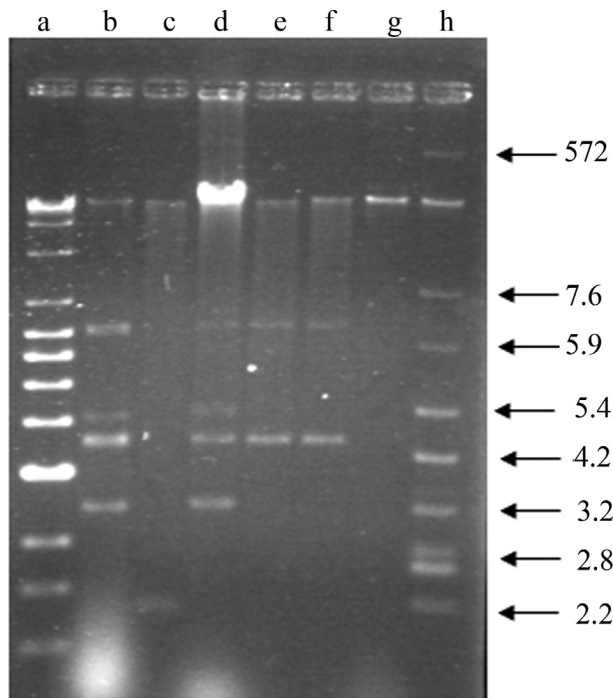
**Table 5** - Plasmid profiling of selected bacteria of enterobacteriaceae.

Stains	Number of plasmids	Plasmid mol. wt.
<i>E. coli</i>	5	43.79, 8.17, 3.14, 2.61, 1.74
<i>Shigella sonnei</i>	2	38.30, 0.84
<i>Citrobacter</i> sp.	5	57.24, 6.29, 3.34, 2.63, 1.77
<i>Salmonella typhimurium</i>	3	41.88, 6.32, 2.63
<i>Shigella dysenteriae</i>	3	45.79, 6.32, 2.63
<i>Shigella</i> sp.	1	47.88

incidence of ESβL production was found maximum in the isolates of *Citrobacter* sp (42.86%) followed by the enteric bacteria of miscellaneous unidentified group including *Salmonella* spp. (24.14%), *Shigella* sp (18.18%) and *E. coli* (16.67%), while none of the *Klebsiella* isolate could produce ESβL (Table 4). Among clinical isolates of enteric bacteria ESβL production was reported with varying frequency (68-80%) by other workers (Mathur et al., 2002; Sahin et al., 2003). However, production of ESβL by enteric bacteria of environmental isolates is less frequently known.

Plasmid profiling of these 15 multiple drug resistant isolates revealed the presence of more than one plasmid or the different forms of a single plasmid. The molecular weight of these plasmids ranged from few kb to 57.24 kb. Multiple bands with close molecular weight in a single bacterial strain may indicate the different forms of a single plasmid (Figure 1; Table 5). ESβL production among *E. coli* and other enteric bacteria are commonly encoded by plasmids (French, 2010; Livermore, 1982).

To investigate the transferable nature of these plasmids, all 15 ESβL producing strains were conjugated with *E. coli* K-12 (Sm<sup>r</sup> recipient) strain. Interestingly, of these, 4 isolates could transfer ampicillin marker to the recipient with transfer frequency ranged from  $2.6 \times 10^{-4}$  to  $1.8 \times 10^{-5}$ . In addition, two standard donor strains for β-lactamase, *E.*



**Figure 1** - Agarose gel electrophoresis profiling of plasmids isolated from ESβL producing drug resistant enteric bacteria. Lane a, Super mix lambda DNA; lane b, *E. coli*; c, *Shigella sonnei*; d, *Citrobacter* sp.; e, *Salmonella typhimurium*; f, *Shigella dysenteriae* and g, *Shigella* sp. (plasmid DNA isolated from the enteric bacteria) and lane h, *E. coli* V 517 reference plasmids. Values marked with arrows show the molecular wt of *E. coli* V 517 reference plasmids.

**Table 6** - Transferable nature of antibiotic resistance markers and β-lactamase production among 15 ESβL producing isolates of enteric bacteria

β-lactamase producing <i>E. coli</i> donor strains	Resistance pattern	Characteristics of trans conjugants of <i>E. coli</i> K-12 Sm <sup>r</sup> .					
		B-lactamase production against		Transfered marker		Transfer frequency of Amp marker	
		Ph	A	A	A	Ph	A
<i>E. coli</i>	Pn, A, M, Ce, Co, Do, Nx, Na	+	+	+	+	$2.6 \times 10^{-4}$	+
<i>Shigella</i> sp.	Pn, A, M, Co, Nx, Na	+	+	+	+	$1.5 \times 10^{-5}$	+
<i>Salmonella</i> sp.	Pn, A, M, Ce, Co, C, Cef, Do, T, Cfx, Nx, Nf, Na	+	+	+	+	$1.8 \times 10^{-5}$	+
<i>Shigella</i> sp.	Pn, A, M, Ce, Co, Nf, Na	+	+	+	+	ND	+
<i>E. coli</i> (EC-38)	Pn, A, Co	+	+	+	+	$7.0 \times 10^{-3}$	+
<i>E. coli</i> (Rp 4)	Pn, A, K	+	+	+	+	$8.8 \times 10^{-4}$	+

ND = Not determined. Pn, penicillin; A, ampicillin; Ce, cefuroxime; M, methicillin; Co, co-trimoxazole; Cef, cefotaxime; Nx, norfloxacin; Cfx, ceftaxime; Do, doxycycline; Nf, nitrofurantoin; C, chloramphenicol; T, tetracycline; K, kanamycin and Na, nalidixic acid.

*coli* EC-38 and *E. coli* RP4 were also used as control. The frequency of conjugation of ampicillin marker for control strains ranged from  $7.0 \times 10^{-3}$  to  $8.8 \times 10^{-4}$  (Table 6). Our findings on transferable nature of  $\beta$ -lactamses encoding plasmids including ESBL are in agreement with the reports available in literature (Galani *et al.*, 2002; Tait, 1993).

The finding of this investigation highlights the importance of safe disposal and better hospital wastewater treatment strategies to check the spread of drug resistant pathogenic bacteria in the environment as these may pose the public health on greater risk.

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