Incidence of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* from faecal samples in patients and healthy subjects

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Abstract
Faecal carrier rates of extended spectrum beta lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* were analysed in 138 inpatients, 126 outpatients and 216 healthy individuals. Overall faecal carriage of these ESBL producing organisms was found to be 24.2% (116 out of 480 samples). The frequency of carriage was higher among inpatients (42.03%) followed by outpatients (29.37%) and healthy subjects (9.72%). Among ESBL producers *E.coli* was the predominant isolate. The present investigation suggests circulation of ESBL producing organisms both in the hospital environment and community.

Keywords: *Escherichia coli, Klebsiella pneumoniae,* faecal carriage, extended spectrum beta lactamase (ESBL) producers.

Introduction
Drug resistance in Gram negative bacilli to several beta lactam antibiotics is mainly due to production of inactivating enzymes especially extended spectrum beta lactamases (ESBLs). ESBLs are plasmid mediated enzymes which are capable of hydrolyzing penicillins, first, second and third generation cephalosporins and Aztreonam.[1] They can be inhibited by beta lactamase inhibitors like clavulanic acid.

Being plasmid mediated ESBLs can spread among patients in hospital and community causing several outbreaks.[2,3] Intestinal colonization and faecal carriage of ESBL producing Enterobacteriaceae, especially *Escherichia coli* and *Klebsiella pneumoniae* by asymptomatic healthy individuals is a potential risk factor for their transmission and infection in the community.[4]

Infections caused by ESBL producing enterobacteriaceae pose major challenges in treatment and are associated with increased mortality and morbidity in hospitalized patients.[5] ESBL coding plasmids also carry genes that code for drug -resistance to other non beta lactam antibiotics like Quinolones. In our laboratory we routinely screen for ESBL production in *E.coli* and *Klebsiella pneumoniae* isolated from clinical samples. Faecal isolates are not routinely tested for ESBL production. Hence we investigated faecal carriage of ESBL *E.coli* and *Klebsiella pneumoniae* from both out patients and inpatients suffering with diarrhoea and healthy asymptomatic subjects in the community.

Materials and Methods
A total of 480 stool samples were collected from 138 inpatients (83 adults + 55 patients), 126 outpatients and 216 healthy subjects who accompanied the patients to the hospital. Faecal samples were obtained from both adult and paediatric patients suffering with diarrhoeal disease, with a minimum period of 4 days stay in the hospital and are on treatment with broad spectrum antimicrobials. Stool samples were collected from outpatients complaining of diarrhoea and also from asymptomatic healthy adults. All the study groups were questioned for prior usage of antibiotics and relevant data was documented.

Faecal samples were transported to the clinical laboratory and immediately inoculated on to two plates of MacConckey agar – one supplemented with 1µg/ml of ceftazidime and other with 1µg/ml of cefotaxime. They are incubated at 35°C in ambient air and examined after 24hrs. Plates without any growth were further incubated for another 48 hours[6]. *E.coli* and *Klebsiella pneumoniae* colonies were identified by Gram staining, morphology on MacConckey agar, motility and biochemical reactions. *E.coli* and *Klebsiella pneumoniae* isolates obtained from faecal samples were further tested for ESBL production by standard combined disc method according to CLSI guidelines. Ceftazidime and cefotaxime with and without inhibitor clavulanic acid (30µg disk) were used for confirmation of ESBL production.[7]

An increase of ≥5 mm in the zone of inhibition in the presence of clavulanic acid indicates ESBL production. *E.coli* ATCC 25922 was used as positive control and *Klebsiella pneumoniae* ATCC 700603 as negative control for the test.

Antimicrobials susceptibility profiles of all ESBL positive faecal isolates were assessed with respect to other classes of antimicrobials like piperacillin – tazobactam, fluoroquinolones, aminoglycosides and carbapenems.

Results
Among various bacterial isolates obtained on culturing faecal samples only *E.coli* and *Klebsiella pneumoniae* strains were considered. Out of 480 stool samples processed 116 (24.29%) yeilded ESBL producing bacteria. A high rate of fecal carriage of ESBL

producing organisms was noticed among patients-35.98% (both inpatients and outpatients) compared to healthy subjects-9.72% (Table 1)

Table 1: Distribution of ESBL producers between patients and healthy subjects

Study group	No. of persons	No. of isolates	
		ESBL producers	Non-ESBL producers
Patients	264	95(35.98%)	169(64.02%)
Healthy subjects	216	21(9.72%)	195(90.28%)
Total	480	116	364

Probability=0.0001

ESBL producing isolates were obtained from 58(42.03%) of 138 inpatients (83 adult inpatients and 55 paediatric inpatients).Among 58 ESBL stains recovered 32(55.17%) were *E.coli* and 26(44.83%) were *Klebsiella pneumoniae*. (Table 3).

Table 2: Distribution of ESBL producing isolates from different study groups

Study group	No. of persons	No. of isolates	
		ESBL producers	Non-ESBL ESBL Producers
Inpatients	138	58(42.03%)	80(57.97%)
Outpatients	126	37(29.37%)	89(70.63%)
Healthy subjects	216	21(9.72%)	195(90.28%)

Probability=0.0001

Out of 126 outpatient samples tested 37 (29.3%) were found to be ESBL producers; 28(75.68%) isolates being *E.coli* and 9(24.32%) were *Klebsiella pneumoniae* (table 2, 3).ESBL producers were recovered from 21(9.72%) of 216 Healthy subjects with *E.coli* as predominant isolate (table 2, 3). The incidence of ESBL producing isolates was observed to be significantly higher in inpatients (42.03%) rather than outpatients (27.37%) and healthy subjects (9.72%)[p< .0001; table 2] . Overall ESBL production was higher among *E.coli* isolates (79 out of 116 i.e 68.10%).

Table 3: Distribution of ESBL producers among *E.coli* and *Klebsiella pneumoniae* in different study groups

Study group	No. of ESBL producers	No. of isolates	
		E.coli	*Klebsiella pneumoniae*
Inpatients	58	32(55.17%)	26(44.83%)
Outpatients	37	28(75.68%)	9(24.32%)
Healthy subjects	21	19(90.48%)	2(9.52%)
Total	116	79(68.10%)	37(31.9%)

Majority of ESBL producers recovered from different study groups were susceptible to meropenem (100%), amikacin (82.76%) and piperacillin-tazobactum (60.34%) [Table 4]. Overall resistance was noted to cefepime followed by ciprofloxacin among ESBL positive isolates recovered from various study groups. Increased rate of resistance to other non beta lactam antibiotics was exhibited by strains recovered from inpatients compared to outpatients and healthy subjects.

Table 4: Antibiogram of ESBL producing faecal isolates in various study groups

Study group	No. Of ESBL positive isolates	No. of Sensitive isolates					
		Pip- taz	Cef	Cipro	Gen	Ami	Mero
Inpatients	58	32(55.17%)	5(8.62%)	14(24.14%)	28(48.28%)	43(74.14%)	58(100%)
Outpatients	37	27(56.76%)	9(24.32%)	17(45.95%)	25(67.57%)	32(86.49%)	37(100%)
Healthy subjects	21	17(80.95%)	14(66.67%)	19(90.48%)	15(71.43%)	21(100%)	21(100%)
Total	116	28(24.14%)	28(24.14%)	50(43.10%)	68(58.62%)	96(82.76%)	116(100%)

Pip – taz - piperacillin-tazobactum; Cef – Cefepime; Cipro – ciprofloxacin; Gen – Gentamicin; Ami – Amikacin; Mero – Meropenem.

Discussion

Eventhough *E.coli* and *Klebsiella pneumoniae* are normal inhabitants of the Gut, they are possible endogenous sources for several infections, particularly complicated urinary tract infections. Present study showed an overall 24.2% faecal carrier rate of ESBL positive *E.coli* and *Klebsiella pneumoniae* among patients (inpatients and outpatients) and asymptomatic healthy subjects.

Higher incidence of ESBL producing bacteria was noted among inpatients (42.03%), than outpatients (29.37%) or asymptomatic healthy persons(9.72%). These finding correlate with Saudi Arabian study which reported higher carriers rates of 26.1% among inpatients compared to outpatients (15.4%) and asymptomatic healthy subjects (13.1%).[6] Increased frequency in feacal carriage of ESBL organisms in hospitalised patients could be explained due to prior therapy with cephalosporins and other non beta lactam antibiotics received by many (112 out of 138) inpatient population. Increased rate of resistance to other non beta lactam antibiotics like fluoroquinolones and aminoglycosides could be due to excessive use of such antibiotics. High fluoroquinolone resistant rates were observed in both inpatients (75.86%) and outpatients (54.05%). Similar pattern in resistance was noted with piperacillin - tazobactam and gentamicin also. Meropenam was active against all the ESBL producing faecal isolates.

Dissemination of ESBL producers to the community may be related to prolonged feacal carriage of these organisms by persons with history of previous hospitalization[8]. Among 126 outpatients, usage of over-the-counter brought antibiotics especially penicillins, fluoroquinolones and cephalosporins was documented from 88 patients while no clear history regarding prior antibiotic usage could be obtained from others. None of the healthy subjects (216) had given any history regarding prior antibiotic therapy in the past 3 months. Unprohibited over the counter sale of antibiotics and lack of awareness among the public might have contributed to reservoir of resistant organisms in the general population. Many oral formulations were brought and used from pharmacies without any prescriptions[9].

Of all ESBL producers obtained from different study groups *E.coli* was the major isolate as it was the predominant faecal isolate (68.10%) recovered from all the 3 groups compared to *Klebsiella pneumoniae* (31.9%). Similar carrier rates of *E.coli* (80.5%) and *Klebsiella pneumoniae* (13.6%) were reported by a study in Lebanon.[10]

Even though routine screening of clinical samples for ESBL production includes individuals with community acquired infections, this may not represent their entire prevalence in the community as asymptomatic carriers are not tested on a regular basis. As gut colonization with ESBL producers precedes infection by them, clinical microbiologists and infection control physicians need to be aware of the hidden pool of these organisms circulating in the community as well.[11]

Call for adequate measures to curb their propagation and prevent growing infections by restricting the inappropriate usage of antimicrobials is the need of the hour.[12-14]

Conclusion

Present study revealed high faecal carrier rate of ESBL producing *E.coli* and *K.pneumoniae* among patients. Faecal carriage seen among some healthy individuals acts as a reservoir of infections in the community.

Infections caused by ESBL producers cause problems due to narrowing of available treatment options. The potential measures to prevent these infections include restricting excessive use of antibiotics along with strict adherence to infection control policies. Prohibition of over the counter sale of antimicrobials without prescription and educating the public regarding the hazards associated with inappropriate use of antibiotics could prove effective in reducing faecal carriage of these organisms in both inpatients and community.

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