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# FAECAL CARRIAGE OF ESBL PRODUCING ORGANISMS FROM HOSPITALIZED PATIENTS

Microbiology

**Girish Chaudhari** PG Student, Dept. of Microbiology, MGM Medical College, Sector 1, Kamothe, Kalamboli, Navi Mumbai-410209.

**Dr. Sharvari Samant*** Associate Professor, Dept. of Microbiology, MGM Medical College, Sector 1, Kamothe, Kalamboli, Navi Mumbai-410209. *Corresponding Author

## ABSTRACT

**Background:** The gastrointestinal tract of humans is a major reservoir of ESBL producers and it serves as a site for the horizontal spread of resistant genes. The carriage of these species is related to the hospitalization and antibiotic consumption.
**Aim:** To detect prevalence of faecal carriage of ESBL producing bacteria from hospitalized patients.
**Methodology:** 100 faecal samples were processed to culture and identify the normal flora of gut and were subjected to antibiotic susceptibility test. ESBL detection was carried out by screening test and phenotypic confirmatory double disc diffusion test.
**Results:** ESBL carriage rate in *E. coli* and *Klebsiella* were 31.5% and 29.03% respectively. High degree of resistance to antibiotics and high ESBL carriage rate was observed in strains isolated from patients with prior antibiotic treatment.
**Conclusion:** Increased prevalence of high resistance and ESBL carriage in Enterobacteriaceae.

## KEYWORDS

Faecal carriage, ESBL, hospitalized patients.

## INTRODUCTION

The widespread and inappropriate use of antibiotics has resulted in a significant increase in antibiotic resistant bacteria worldwide. A major contributor to this increasing resistance in Gram Negative Bacteria (GNB) is the production of inactivating enzymes, in particular Extended Spectrum Beta Lactamases (ESBLs). Extended Spectrum Beta-Lactamases (ESBLs) are enzymes capable of hydrolysing many beta-lactam antibiotics (penicillin, cephalosporins and monobactams) and thereby protect ESBL-producing bacteria from the action of these drugs.

ESBLs are encoded by transferrable conjugative plasmids, which facilitate widespread dissemination, not only between the same species of bacteria, but also across different species. The antibiotic resistance can further spread to the pathogenic bacteria by gene transfer. Infections caused by ESBL producing organisms have been described as an emerging public health problem worldwide. Hence, the present study was planned to detect the prevalence of ESBL producing Gram Negative bacteria (GNBs) isolated from stool samples of hospitalized patients and to study their antibiotic susceptibility pattern.

## MATERIAL AND METHODS

This study was carried out during October 2017 to March 2019 in a tertiary care hospital. The study protocol was reviewed and approved by Institute's Ethical Committee.

A total of 100 stool samples from hospitalized patients, with no evidence of infection of gut were included in study. Stool samples were collected aseptically in a sterile container and history of prior antibiotic treatment of patients in last 30 days was recorded. All the stool samples were examined microscopically for the presence of pus cells, RBCs, ova and cysts. They were inoculated on MacConkey agar and Xylose Lysine Deoxycholate agar(XLD agar) for the growth of bacteria. Different colonial morphotypes resembling GNB were confirmed by Gram's stain and identified using standard biochemical tests.[1] They were then subjected to antibiotic sensitivity test by Kirby Bauer Disc Diffusion method for oral antibiotics, viz., Levofloxacin, Prulifloxacin, Cefotaxim/Clavulanic acid, Cefepime/Clavulanic acid, Tetracycline, Ofloxacin, Ciprofloxacin, Cefuroxime, Amoxyclav, Cefotaxime and also tested for ESBL production by Double Disc Synergy test as recommended by CLSI guidelines.[2]

### Test for ESBL Detection :-

### 1) Screening test

Screening test was done by using Cefotaxim and Ceftazidime discs on Mueller Hinton agar.Zone diameter less than 20mm for Cefotaxim and 21mm for ceftazidime were screen test positive. Strains which are screen test positive were subjected to phenotypic confirmatory test.[2]

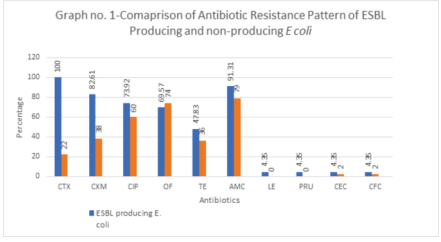
### 2)Phenotypic confirmatory disc diffusion test

It was done by using disc of Ceftazidime (CAZ, 30µg) and Ceftazidime with clavulanic acid (CAC, 30/10µg) and Cefotaxime (CTX, 30µg) and Cefotaxime-Clavulanic acid (CTX/C, 30/10µg) and were placed at a distance of 24mm on an MHA plate inoculated with the lawn culture of the GNBs isolate screened positive for ESBL production and incubated aerobically at 37$^0$C overnight. If there is an increase in zone size ≥5mm with Ceftazidime/clavulanic acid and Cefotaxime-Clavulanic acid in comparison to Ceftazidime or Cefotaxime alone, then the strain of GNBs were confirmed to be an ESBL producer.[2]

## RESULTS

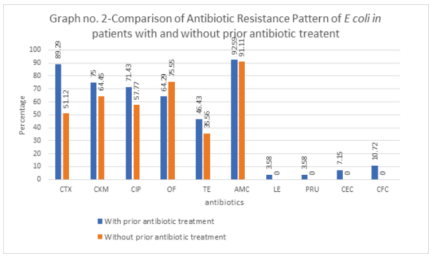
*E. coli* was the most predominant (60.83%) gut flora followed by *Klebsiella spp* (25.83%). Since *Enterobacter spp* (5.83%), *Citrobacter spp* (5%), and *Proteus spp* (2.5%) were isolated in statistically insignificant numbers,, further tests were carried out on *E. coli* and *Klebsiella species* only.

All the isolates of *E. coli* (73) were subjected to test for ESBL production. It was observed that 31.5% strains of *E. coli*were ESBL producers. ESBL producing *E. coli* isolates showed much more resistance to antibiotics like Amoxicillin (91.31%), Cefuroxime (82.61%), Ciprofloxacin (73.92%), Cefotaxime (100%), Tetracycline (47.83%) as compared to ESBL non-producing *E. coli*which were comparatively less resistant to majority of antibiotics like Cefotaxime (22%), Cefuroxime (38%), Ciprofloxacin (60%), Tetracycline (36%), Amoxycillin (79%).

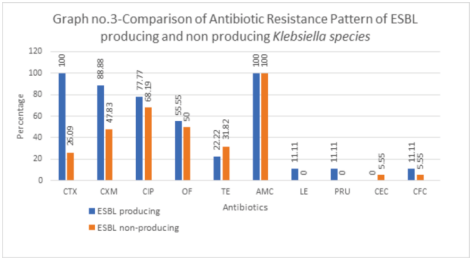



Out of 73 *E. coli* isolated, 28 (38.35%) were isolated from patients with prior antibiotic treatment.Out of 28 such strains 18(64.28%) were ESBL producers. Amongst 73 *E. coli* isolated, 45(61.65%) were isolated from patients without prior antibiotic treatment and out of these 45 strains, 10(22.23%)strains were ESBL producers.

*E. coli* isolated from patients with prior antibiotic treatment were significantly more resistant to antibiotics like Amoxicillin (92.59%), Cefuroxime (75%), Ciprofloxacin (71.43%), Cefotaxime (89.29%), Tetracycline (46.43%) as compared to strains isolated from patients with no prior antibiotic treatment which were resistant to Amoxicillin (91.11%), Cefuroxime (64.45%), Ciprofloxacin (57.77%), Cefotaxime (51.12%), Tetracycline (35.56%).

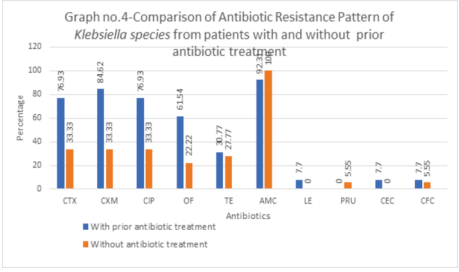



Amongst 31 Klebsiella isolates 9 (29.03%) strains were ESBL producers. Similarly in Klebsiella, ESBL producing strains were more resistant to antibiotics like Cefuroxime (88.88%), Ciprofloxacin (77.77%), Cefotaxime (100%), Ofloxacin (55.55%) as compared to ESBL non-producing strains which were resistant to Cefuroxime (47.83%), Ciprofloxacin (68.19%), Cefotaxime (26.09%), Ofloxacin (50%).




Out of 31 *Klebsiella species* isolated 13(41.93%) were isolated from patients with prior antibiotic treatment.Out of these 13 *Klebsiella species,* 7(53.84%) were ESBL producers. And 18 (58.06%) strains were isolated from patients with no prior antibiotic treatment out of which 2 (11.12%) were ESBL producers.

*Klebsiella species* isolated from patients with prior antibiotic treatment were significantly more resistant to antibiotics like Cefuroxime (84.62%), Ciprofloxacin (76.93%), Cefotaxime (76.93%), Ofloxacin (61.94%), Tetracycline (30.77%) as compared to strains isolated from patients with no prior antibiotic treatment which were resistant to Cefuroxime (33.33%), Ciprofloxacin (33.33%), Cefotaxime (33.33%), Ofloxacin (22.22%), Tetracycline (27.77%).




## DISCUSSION:

*E. coli* and *Klebsiella species* are normal inhabitants of the gut but they are possible endogenous sources for several infections such as urinary tract infection, bacteremia, septicemia etc. Present study showed faecal carrier rate of *E. coli* was 60.83% and *Klebsiella species* was 25.83% which were the most common organisms isolated among hospitalized patients.

This study showed that 31.5%(23/73) were ESBL producing *E. coli*. These 23 ESBL producing *E. coli* isolates were subjected to antibiotic susceptibility testing against oral antibiotics. It showed that 100% strains were resistant to Cefotaxime. A total of 69.57% strains were resistant to Ofloxacin, 73.98% to Ciprofloxacin, 82.61% to Cefuroxime, 91.31% to Amoxiclav.Amoxiclav and Cefotaxime are the most commonly used antibiotics and the gut flora is exposed to these antibiotics. This could be one of the reasons for developing resistance to these antibiotics.

Out of 73 *E. coli*, 28 were isolated from patients with history of prior oral antibiotic treatment within one month of sample taken. The antibiotic susceptibility test of these isolates showed that most of the them were resistant to Cefotaxime, Amoxyclav, Cefuroxime and Ciprofloxacin. Also there were 64.28% ESBL producing strain among these isolates. Increased frequency of Feacal ESBL carriers could be due to prior antibiotic treatment.

Similarly *in Klebsiella species* 29.03% of strains were ESBL producers Among these, 55.55% strains were resistant to Ofloxacin, 77.77% to Ciprofloxacin, 88.88% to Cefuroxime. They were all resistant to Cefotaxime and Amoxyclav. Out of 31 isolates of *Klebsiella species*, 13 were isolated from patients with history of prior oral antibiotic treatment within one month of sample taken out of which 53.84% were ESBL producers. Majority of these strains were resistant to Cefotaxime, Ciprofloxacin, Cefuroxime and all were resistant to Amoxyclav.

In a study by Kassu Desta, et al., *E. coli* (79.7%) and *Klebsiella pneumoniae* (19.5%) were the most significant organisms isolated from hospitalized patients and reported 45% (106/235) of ESBL producing *E. coli* from 267 patients. This shows that the prevalence rate of ESBL producers in our set up was less as compared to the percentage reported in other parts.In all strains of *E.coli*, 88% strains wereresistant to Ciprofloxacin, 93% resistant to Amoxiclav, 99% to Cefotaxime, 99% to Ceftazidime, was reported which is corresponding to present study. Their study also showed 16.9% ESBL producing Klebsiella. In all strains of Klebsiella, 80% resistivity to Amoxyclav, 95% to Cefotaxime, 100% to Ceftazidime, 48% to Ciprofloxacin was reported.[3]

Similarly a study by Siddabathuni Aruna, *et. al*., have reported 42.03% of ESBL producing *E. coli* in their study carried out on 138 patients. In their another study they showed 88.6% (39/44) of ESBL producing *E. coli* isolated from patients with definite history of prior antibiotic use.[4,5]

It was observed that the normal gut flora especially *E. coli* and *Klebsiella species* show moderate to high resistance to majority of oral antibiotics and also higher prevalence of ESBL producing strains. This finding is important because colonization with MDR Gram-negative bacteria, especially ESBL producers can act as a source of infection in these patients.

Recurrent infections and cross transmission of ESBL genes can occur due to colonization of ESBL producers. The cross-transmission of resistant strains in hospital might be attributed to poor hand hygiene practice, infection control measures, multibed rooms and crowded patients in a single room. Identification of patients carrying ESBL producing Enterobacteriaceae in hospitalized patients and adoption of subsequent preventive measures is suggested to prevent cross-transmission and reduce morbidity and healthcare cost.

## CONCLUSION

The selective antibiotic pressure leads to amplification of carriers carrying resistance genes. Admissions to intensive care units and high dependency areas may lead to colonization by such organisms. Use of invasive methods for treatment and diagnosis may increase the risk of colonization. This can lead to further spread of bacteria to other patients. It can be prevented by taking measures like hand hygiene by hospital personnel, isolation of patient in single room.

Measures can be taken to reduce the rate of carriage of ESBL by avoiding the inappropriate use of antibiotics, strict infection control policy, prohibiting sale of antibiotics without prescription and guiding people about the hazards of overuse of antibiotics. Carriage of such ESBL producing organism is a potential risk for transmission and spread of infection particularly in healthcare settings in developing countries where infection control is inadequate.

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