

Prevalence and phenotypic characterization of ESBL producing *E.coli* and *Klebsiella* among the fecal isolates of normal population

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Abstract

Introduction: Prevalence of extended spectrum beta lactamase producing bacteria has been increasing worldwide despite of outbreak situations. ESBLs occupying the gastrointestinal tract are spread across other bacteria and humans through the environment. The present study is a report on the prevalence of ESBL producers among normal individuals of a rural population in south India.

Materials and Methods: 1000 normal individuals were selected from the villages of Salem district. Stool samples were collected and processed in MacConkey agar plates supplemented with Cefotaxime 5ug/ml. Morphological and biochemical methods were used to identify the colonies initially. ESBL producing organisms were identified by Double Disk Synergy Test – DDST and Phenotypic Confirmatory Disk Diffusion test- PCDDT.

Results: From the total number of samples, 37.9% has shown bacterial growth. The rate of ESBL producing Enterobacteriaceae was found to be 33.8% with *E.coli* (29.3%) being the predominant species followed by *klebsiella* (8.6%).

Conclusion: The results of our study gives a brief information about the multidrug resistance and also the prevalence of ESBL producing bacteria in the community faecal flora. Phenotypic methods were successfully applied to screen large number of samples. The rate of ESBL producers in the community is notably high. Routine surveillance programmes and awareness of hygienic practices among the rural population are highly recommended to monitor and control the spread.

Keywords: Beta lactams, E.coli, Enterobacteriaceae, Klebsiella.

Introduction

Bacteria causes a wide variety of infections ranging from minor wounds to life threatening diseases. The invention of Penicillin- a beta lactam antibiotic, reduced the rate of morbidity and mortality rates due to bacterial infectious diseases has been greatly reduced. Beta lactam antibiotics prevents the synthesis of the bacterial cell wall by binding to a transpeptidase enzyme [Penicillin Binding Protein (PBP)] which is required for peptidoglycan cross linking.¹ The most effective way for bacteria to counteract the effect of beta lactams is by producing beta lactamases- enzymes that inactivate the drugs by hydrolyzing the beta lactam ring of the antibiotic.² This led to the introduction of broad spectrum penicillins and first generation cephalosporins to overcome the problem of resistance.³ However, the beta lactamase have been mutated from a narrow spectrum to an extended spectrum, which can hydrolyze many types of beta lactam antibiotics including third generation cephalosporins.⁴ Extended-Spectrum – Beta Lactamases (ESBLs) have spread across many regions of the world and comprising over 300 variants. The Plasmid borne beta lactamases capable of hydrolysing penicillins provide the most common mechanism of resistance to beta lactam antimicrobial agents among gram negative bacteria. Beta lactamases encoded in plasmids are transferred between different species of bacteria which facilitates the emergence of more ESBL

strains.⁵ Most common ESBL enzymes are mutants of TEM, SHV and CTX genes, whereas other ESBL coding genes are also reported, viz., PER and VEB. TEM and SHV are plasmid mediated beta lactamases and CTX belongs to another class of chromosomal origin which shares only 40 of homology with TEM and SHV.⁶⁻⁸ TEM-1 enzyme has been detected in various bacterial species including *Escherichia coli* and other species of Enterobacteriaceae such as *Klebsiella pneumoniae*.⁹ TEM-1 is able to hydrolyse penicillins and early cephalosporins such as cephalothin and cephaloridine. There are many derivatives of TEM found with single amino acid substitutions.¹⁰ SHV beta lactamases are most commonly found in *K.pneumoniae* and responsible for the plasmid mediated resistance in this species. Unlike the TEM type beta lactamases, there are relatively few derivatives of SHV-1.¹¹ CTXM with ceftoximase activity has many variants and drastically spread in the recent years. The choice to overcome beta lactamases leads to the development of beta lactam inhibitors. These compounds structurally resembles beta lactam antibiotics. They can bind to beta lactam antibiotics either reversibly or irreversibly protecting the antibiotics from destruction, they serve as suicide bombers utilizing all available enzymes, these compounds have weak antibacterial activity but are potent inhibitors of many plasmid encoded and some chromosomal beta lactamases. The three important beta

lactamase inhibitors are clavulanic acid, sulbactam and tazobactam.¹²

Risk factors for the spread of ESBL infection includes, hospitalization, surgeries, drainage, drinking water and prior administration of antibiotics. Initially ESBLs outbreaks were associated with nosocomial infections caused by single enzyme-producing strains, but recently a significant increase in community isolates has been observed. Nosocomial and community based spread of ESBL producing bacteria leads to therapeutic failure of beta lactam antibiotics.¹³

Currently, the appropriate methods for ESBL detection are seriously concerned because of the failure in treatment of the 3rd generation cephalosporins and aztreonam. The phenotypic detection is done to detect the resistance of bacteria to the 3rd generation cephalosporins together with beta lactamase inhibitor, clavulanic acid. There are many phenotypic detection methods used in both primary screening and confirmation test to detect ESBLs production in Enterobacteriaceae.^{14,15} The CLSI advocates use of cefotaxime (30µg) or ceftazidime disks (30µg) with or without clavulanate (10µg) for phenotypic confirmation of the presence of ESBLs in *klebsiellae* and *Escherichia coli*.¹⁶

The present work is a community based study aimed to detect the prevalence of ESBL producing enterobacteriaceae among normal individuals of the rural population in south India.

Materials and Methods

Study subjects: A sum of 1000 healthy individuals of both sexes aged 12 to 60 were included in the study. The study was conducted from January 2014 to June 2015 in the Department of Microbiology, Vinayaka Missions Kirupanandha Variyar Medical College, Salem. Malnourished individuals, cases with extremities of age, patients on antibiotics and obvious infectious diseases and immunocompromised patients were excluded from the study.

Sample collection and processing: Stool sample was collected, inoculated into Mueller Hinton broth incorporated with Cefotaxime 5ug/ml and incubated at 37°C for 24 hours. Tubes which showed turbidity was plated on to MacConkey agar supplemented with Cefotaxime 5ug/ml and incubated at 37°C for 24 hours.

Identification of pathogen: Colony morphology and gram staining were used to initially identify the bacteria. Motile long slender Gram negative bacilli on gram staining with large, pink coloured lactose fermenting moist colonies on MacConkey agar plate were suggestive of *Escherichia coli*. Non motile stout Gram negative bacilli on gram staining with large, pink coloured mucoid lactose fermenting colonies were suggestive of *Klebsiella species*. All samples were subjected to catalase, oxidase, motility, TSI, indole, citrate and urease tests for biochemical characterization.

Antibiotic sensitivity test: Antimicrobial susceptibility testing was done on Mueller Hinton agar plates by Kirby-Bauer's disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.

The antibiotic discs namely Amikacin (30ug), Amoxycillin / Clavulanic acid (20/10ug), Cotrimazole (25ug), Ampicillin (10ug), Cefotaxime (30ug), Ceftazidime (30ug), Cefotixin (30ug), Cefepime (30ug), Gentamicin (30ug), Imipenem (10ug) were used with *Escherichia coli* ATCC 25922 and *klebsiella* ATCC 13883 as a controls. The measured zone of inhibition (mm) was interpreted as sensitive/resistant according to the CLSI guidelines

Double disk synergy test – DDST: The isolates which showed resistance or intermediate resistance to any of the third generation cephalosporins such as cefotaxime or ceftazidime were subjected to ESBL screening by the Double Disk synergy test.

Discs of ceftazidime 30ug, cefotaxime 30ug and amoxycylav (20ug amoxicillin and 10ug clavulanic acid) were placed at a distance of 15 mm from center of center in a straight line, with the amoxycylav disc in the middle on a plate of MH agar. The plates were incubated at 37°C overnight. Isolates which showed an enhancement of the zone of inhibition as greater than 5mm on the amoxycylav side of the disc as compared to that which was seen on the side without amoxycylav, were confirmed as ESBL producers.

Phenotypic confirmatory Disk Diffusion test (PCDDT): Phenotypic confirmatory test was done with sensitivity discs containing third generation cephalosporins with and without clavulanic acid as follows: cefotaxime 30ug and cefotaxime 30ug + clavulanic acid 10ug. The zone of inhibition of the antibiotic alone was compared with the zone of inhibition in combination with clavulanic acid. A difference of \geq 5mm increase in zone diameter between cefotaxime and cefotaxime with clavulanic acid confirms the presence of ESBLs.

Results

Age and sex distribution: Majority of the study population falls in the age of 11 to 40 with a male predominance of 65% (Table 1) (Fig. 1). The male: female ratio among the study sample was 1.81:1. The antibiotic sensitivity tests shown that all the isolates were resistant to Cefotaxime, Cefoxitin and Ceftazidime, whereas all are sensitive to Amoxycylav.

Identification of organisms: Among the 1000 samples, organisms were detected in 379 samples in MacConkey medium incorporated with cefotaxime 5ug/ml. Based on colony morphology and biochemical tests, it has been found that majority of the samples had shown the presence of *E.coli* (29.3%). 62.1% of the stool sample from the community had not shown any organism (Table 2 & 3). The antibiotic sensitivity tests shown that all the isolates were resistant to cefotaxime,

cefoxitin and ceftazidime, whereas all are sensitive to amoxyclav.

Confirmatory tests for ESBL production: The DDST and PCDDT tests were performed only on the samples

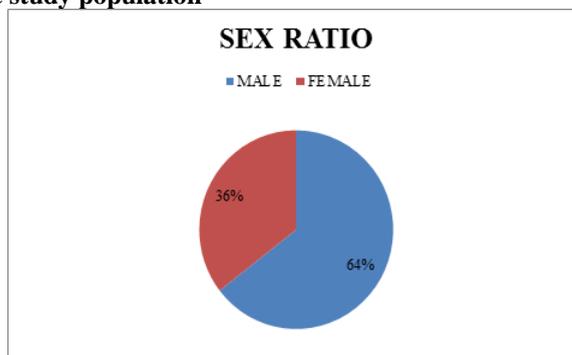
in which the *E.coli* and *klebsiella* were detected (379). It is seen from the table that the DDS test and PCDDT had shown positive for 89.1% of the population (Table 4).

Table 1: Age distribution of the study population

Age group	Frequency	Percentage
0-10	64	6.4%
11 – 20	305	30.5%
21 – 30	529	52.9%
31 – 40	61	6.1%
41 – 50	41	4.1%
Total	1000	100%

Majority of the study population were in the age group between 11 – 30 years and the mean age among them was 23.18 years.

Fig. 1: Sex distribution of the study population



The male: female ratio among the study sample was 1.81: 1.

Table 2: List of organisms isolated

Organism detected	Frequency	Percentage
No organism	621	62.1%
<i>E. coli</i>	293	29.3%
<i>Klebsiella</i>	86	8.6%
Total	1000	100%

Table 3: Biochemical properties of *Escherchia coli* and *Klebsiella species*

Test	<i>Escherchia coli</i>	<i>Klebsiella species</i>
Catalase	Positive	Positive
Oxidase	Negative	Negative
Motility	Motile	Non motile
TSI	A/A + _gas	A/A +gas
Indole	Positive	Negative
Citrate	Not utilized	Utilized
Urease	Negative	Positive

Table 4: Detection of ESBL producers by DDST and PCDDT

Result	DDST	PCDDT
Positive	338	338
Negative	41	41
Total	379	379

DDST: Double Disc Synergy Test. PCDDT: Phenotypic Confirmatory Disc Diffusion Test

Discussion

In hospitals and also in the community, ESBL producing gram-negative bacilli especially *Escherichia coli* and *K. pneumoniae* have emerged drastically during the past decade.¹⁷ The rate of fecal carriage of *E.coli* and *Klebsiella* among the healthy individuals of the rural population of the present study was found to be 37.9%. ESBL producers as confirmed by DDST and phenotypic method was 33.8%. 78% of ESBL producing isolates were *E. coli*, and the remaining 22% were *Klebsiella*. There is a paucity of community based studies revealing the prevalence of enterobacteriaceae in India. However the rate of infection in India is found to be as low as 7% to a higher range of 87% with a maximum of 53% in communities and 85% in nosocomial infections.^{17,18} Despite normally living harmlessly in the gut, *E. coli* can cause various types of infections, especially urinary tract infection. In a study published by Kader et al, 10.2% of the uropathogens isolated from outpatients were ESBL-producing *E. Coli*.¹⁹ In another study it had shown that > 12% of gram-negative uropathogens isolated from community patients were ESBL-producers.²⁰ Some reports from Europe and Canada also suggest that infections caused by ESBL-producing organisms are emerging among community patients.^{21,22}

The presence of ESBL-producing *E. coli* in the gut not only produce extraintestinal infections, but can also result in the transfer of antibiotic-resistance determinants to other strains of *E. coli* and other bacteria within the gastrointestinal tract. Their presence increases the risk of transmission to other individuals as a result of human-to-human transmission or through the environment.²³ In addition, the admission of carriers to hospitals increases the risk of infection for other hospitalized patients.⁹

The rate of ESBL producers as observed by our study is alarmingly high. A study from north India found that the rate of enterobacteria in the gastro intestinal tract was observed to be only 7.7% and subsequent phenotypic detection had confirmed 6.8% ESBL producing organisms.²⁴ A hospital based study in south India in 2010 observed the rate of ESBL producers to be 73%.²⁵

The spread of ESBL-producing organisms to the community could be related to previous hospital acquisition as some hospitalized patients continue to carry ESBL-producing bacteria over prolonged periods, which may contribute to their extrahospital propagation.²⁶ In our study, there was no history of any recent hospitalization or antibiotic consumption among the community patients, many are likely to have been exposed to multiple courses of antibiotics. There are also studies recognizing the potential risk that increasing travel abroad poses to the acquisition of antimicrobial-resistant bacteria.^{27,28} One prospective study from Sweden looking at gut colonization found

an ESBL-producing *E. coli* acquisition rate of 24% in travellers who had been screened before departure.²⁹ Another study focussed on ESBL-producing enterobacteriaceae carriage in patients who had travelled abroad and diagnosed for traveller's diarrhoea on their return. A total carriage rate of 24% was determined in that study.²⁷

Conclusions

The rate of ESBL producers as detected by phenotypic methods in the study population is quite higher and to be taken into serious consideration. Fecal shedding in the community setting in India is an emerging epidemiological problem that requires application of updated control measures. Because of the significant public health implication, infectious disease physicians, microbiologists and community doctors or general practitioners need to be aware that ESBL-producing strains of bacteria are not only circulating in hospital environments but in the community as well and they should deal with them accordingly. Confirmation of community-based transmission of ESBL requires further investigation, including molecular studies, to determine the reservoirs and vehicles for dissemination of ESBL within the community. Laboratory monitoring and detection of ESBL-producing bacteria are important steps in the appropriate treatment of patients and infection control efforts. It is also crucial in the tracking and monitoring of these resistant bacteria in community surveillance programmes. As a control measure, awareness programmes on hygienic practices among the rural population is also advised.

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