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Original Research Article

Prevalence and Phenotypic Characterization of Extended Spectrum of Beta- Lactamase Producing Enterobacteriaceae Isolated from a Tertiary Care Centre, South India

Chadiesh Nagarajan[1], Subashini Panchanathan[1], Shamshad Begum Abdul Khader[1], Pavithraa Suresh[1*]

[1]Microbiologist, Department of Microbiology, Sri Venkateshwaraa Medical College Hospital & Research Institute, Sri Venkateshwaraa Medcity, Nallur-600067, Thiruvallur District, Tamilnadu, India.

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Corresponding Author: Pavithraa Suresh
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Abstract:

Extended spectrum beta lactamase (ESBL) is quite fascinating. ESBL producing Enterobacteriaceae are a group of gram negative bacilli that developed resistance to a wide range of antibiotics, making them a significant public health concern. These can cause infection in various parts of human body, including Urinary tract, Blood stream and Respiratory tract system.

Material and Method: This was a retrospective study carried out in the Department of Microbiology from August 2022 to October 2023. Various clinical samples like, Urine, Sputum, Pus, Wound swab, Blood, Tissue, Sterile body fluids, received in the laboratory from patients belonging to all age groups, both male and female were included in this study.

Results: In this study overall2779 clinical samples was received during the study, out of which 779 (28%) Enterobacteriaceae group organisms were isolated in the microbiology department under sterile condition. Based on the total distribution of ESBL producing Enterobacteriaceae group organisms, the prevalence of ESBL producing organism were estimated to be (221, 42%) and Non ESBL producing organism were estimated to be (308,51%).

Conclusion: A high degree of ESBL producers and carbapenem resistant Enterobacteriaceae are concerning; with emerging resistance to colistin, raising the fear of a return to the PR antibiotic era. An urgent intervention including creating awareness, establishment of robust infection control practices and implementing antibiotic stewardship program are the most important need of the hour.

Keywords: ESBL, Cores stance, Antimicrobial Susceptibility, Gram-negative Bacilli, Nosocomial.

Introduction

Extended spectrum betalactama-se (ESBL)is quit fascinating. ESBL producing Enterobacteriaceae are a group of gram negative bacilli that developed resistance to a wide range of antibiotics, making them a significant public health concern [1]. These can cause infection in various parts of human body, including Urinary tract, Blood stream and Respiratory tract system. The prevalence of ESBL producing Enterobacteriaceae has been increasing globally posing challenges for effective treatment [2]. Additionally, ESBL producing Enterobacteriaceae can spread easily in health care centres, leading to outbreaks and increased transmission rate. This further complicates treatment and infection control measures. By considering the fact, this study details the prevalence of ESBL producing Enterobacteriaceae isolated from clinical specimen in tertiary care centre. Understanding this prevalence study help us to assess the extent of the issue and develop strategies to control their spread. Phenotypic characterization in this study involves their ability to produce ESBL enzymes and their resistant pattern. By understanding the prevalence and phenotypic characterization of ESBL producing Enterobacteriaceae is crucial for developing strategies to combat antibiotic resistance and ensure effective patient care. [1-8]

Aim & Objective:

The aim of this study is to understand the prevalence and phenotypic characterization of ESBL producing Enterobacteriaceae, that gains the understanding of the occurrence and characteristics of the ESBL producing Enterobacteriaceae isolated from specimen in a tertiary care centre.

Material and Method:

Study design: This was a retrospective study carried out in the Department of Microbiology from August 2022 to October 2023. Various clinical samples like, Urine, Sputum, Pus, Wound swab, Blood, Tissue, Sterile body fluids, received in the laboratory from patients belonging to all age groups, both male and female were included in this study. The Clinical samples for this study were received from IPD (In-patient department), OPD (Outpatient department) and Intensive Care Unit (ICU)of tertiary care centre.

Study setting: This study was conducted at Department of Microbiology, Sri Venkateshwaraa Medical College Hospital & Research Institute, Near Chennai, renowned for its cutting-edge facilities and exceptional patient care. With over 500 beds and state of the art infrastructure and houses the region's top consultants and serves more than1000 patients everyday.

Bacteriological study of the clinical sample: A total of 2779 clinical samples were received during the study period like Urine, Sputum, Pus, Wound swab, Blood, Tissue and sterile body fluids were collected in the sterile leak proof screw capped containers and processed for bacterial growth. Sample which yields bacterial growth of the microorganisms that belongs to Enterobacteriaceae family were included for this study. By using conventional method the isolation, identification and antimicrobial susceptibility testing were done by microbiology standard operating procedure. All urine samples were inoculated in CLED (Cystine Lactose Electrolyte Deficient) medium and other samples were inoculated in Blood Agar, Chocolate Agar, Macconkey Agar Under sterile conditions and further incubated at 37◦C overnight. Growth of the bacteria was identified using standard biochemicals tests.

Antimicrobial Susceptibility testing: Antimicrobial susceptibility test was carried out by using Cation adjusted Muller Hinton Agar plates. The MHA plates were inoculated with the standardized inoculums (0.5 Mc Farland standard) of the isolated to farm lawn culture and the antimicrobial susceptibility testing was performed by Kirby Bauer's disc diffusion method and the results were interpreted according to the Clinical Laboratory Standard Institute (CLSI) M100 – 33rd Edition & 34th Edition guidelines 2022 & 2023.

The following antibiotic discs were used Ampicillin (10mcg), Amikacin (10µg), Gentamicin (10 µg), Cefazolin (30µg), Cefotaxime (30 µg), Ceftazidime (30µg), Cefepime (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Cotrimaxozole (25µg), Pipracillin – Tazobactum (100/10µg), Cefoxitin (30µg), Aztreonem (30µg), Imipenem (10 µg), Meropenem (10µg) and Ertapenem (10µg), Additional the urine isolates were tested Against antibiotics such as Norfloxacin (10µg), Nitrofurantoin (300µg) & Fosfomycin (200µg)[1-8]. Fosfomycin was reported only by E.coli strain in urine specimen as per CLSI

Phenotypic Characterization

Method: Double Disc Synergy Test: Enterobacteriaceae isolates which showed resistant to antibiotic disc Cefotaxime (30µg), Ceftzidime (30µg), and Aztreonam (30 µg), were selected for further confirmation of ESBL production by phenotypic screening disc diffusion method.

The Phenotypic characterization was confirmed by Double disc synergy test, in which cefotaxime, ceftazidime individually, and along with the extended combination antibiotic disc of cefotaxime/clavulanicacid(30/10 µg), ceftazidime/ clavulanic acid (30/10 µg) were used for the confirmation of phenotypic characterization of ESBL isolates.

Third generation Cephalosporin antibiotic discs inoculated in Muller Hinton Agar were placed 22mm apart from cephalosporin clavulanicacid combination disc (centre to centre) on lawn culture. Increase in the zone of inhibition more than or equal to 5mm around the combination discs as compared to that of cephalosporin alone was considered to be confirmatory test for ESBL producing isolates.

Klebsiella pneumonia ATCC700603 and Escherichia coli ATCC25922 were used as positive and negative control for the test respectively.

Ethical approval: Institutional ethical committee permission was obtained.

Inclusion criteria: Bacterial isolates of Enterobacteriaceae group in all age groups and ward in the tertiary care centre.

Exclusion criteria: Other group of Bacterial isolates except Entebacteriaceae in tertiary care centre.

Results

In this study total of 2779 clinical samples were received during the study, out of which 779(28%) Enterobacteriaceae group organisms were isolated in the microbiology department under sterile condition – Fig - 1. Based on the total distribution of ESBL producing Enterobacteriaceae group organisms, the prevalence of ESBL producing organism were estimated to be (221, 42%) and Non ESBL producing organism were estimated to be (308,51%)–Fig.2.

ESBL prevalence among female patients is predominantly seen than male patients in this study, 61%of ESBL producing female patients were identified whereas 39% of ESBL producing male patients were identified in the study-Fig.3.

The age wise distribution was segregated in the criteria of 1-10,11-20,21-30,31-40,41-50,51-60,61-70,71-80 and above in the study. Based onthese

criteria the study showed that the highest level of infection occurred in the criteria was 41-50 yrs old patient i.e. (22%), the next down of infection occurred in the criteria was 51-60 yrs old patient ratio i.e.(19%), followed by 61 – 70 yr sold patient ratio estimated to be (16%), 21-30 yrs old patient ratio estimated to be (15%), 31-40 yrs old patient ratio estimated to be (11%), both11-20 & 81–90 years old patient ratio estimated to be (6%) and the least affected age group was 1-10 yrs, i.e.(5%)Fig– 4

Based on the study, ESBL positive samples received from IPD was (141/221, 64%) and OPD was (80/221, 36%) Fig - 5. According to the sample wise distribution showed in this study reveals that maximum number of ESBL producing organism were isolated from urinary sample (106/221, 48%) followed by pus (81/221,37%)–Fig.6.Among the various gram negative bacilli isolated in the study E.coli (120/221, 54%) states the highest level of infection caused by ESBL producing Enterobacteriaceae in the patients, followed by Klebsiella pneumonia (43/221,19%) in the study.

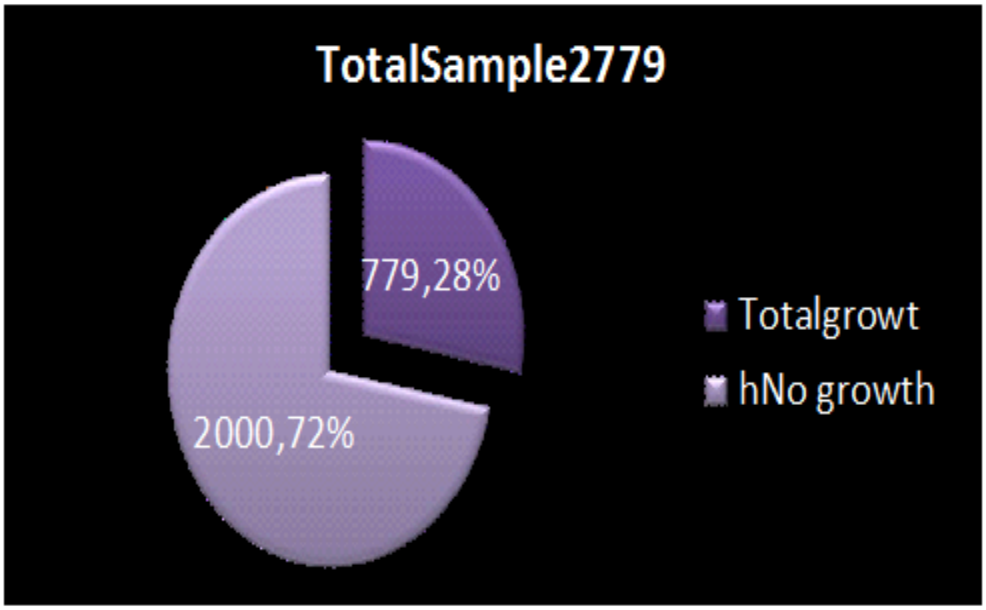

Figure 1: Shows total bacterial isolates during study

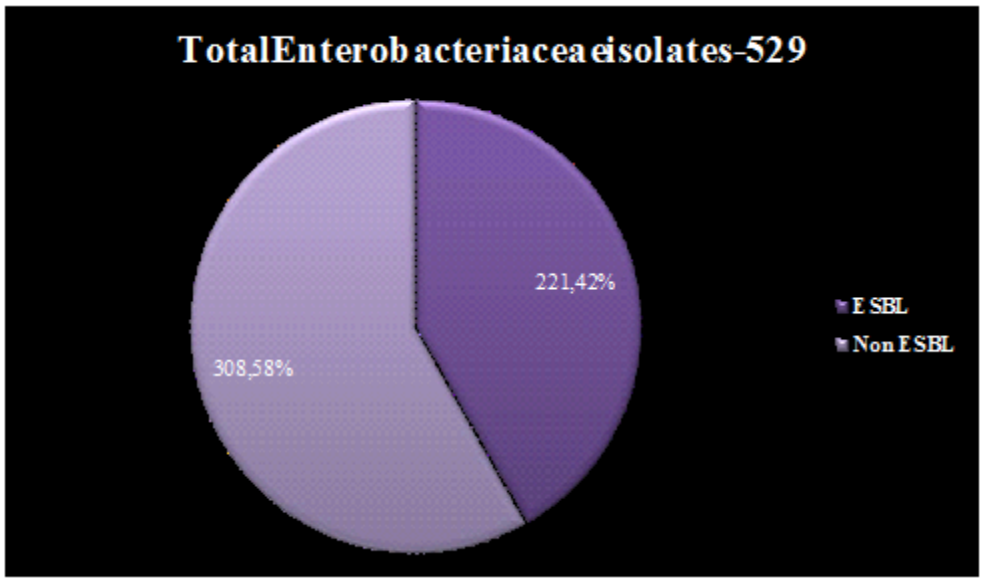

Figure 2: Shows total ESBL producers among Enterobacteriaceae during study

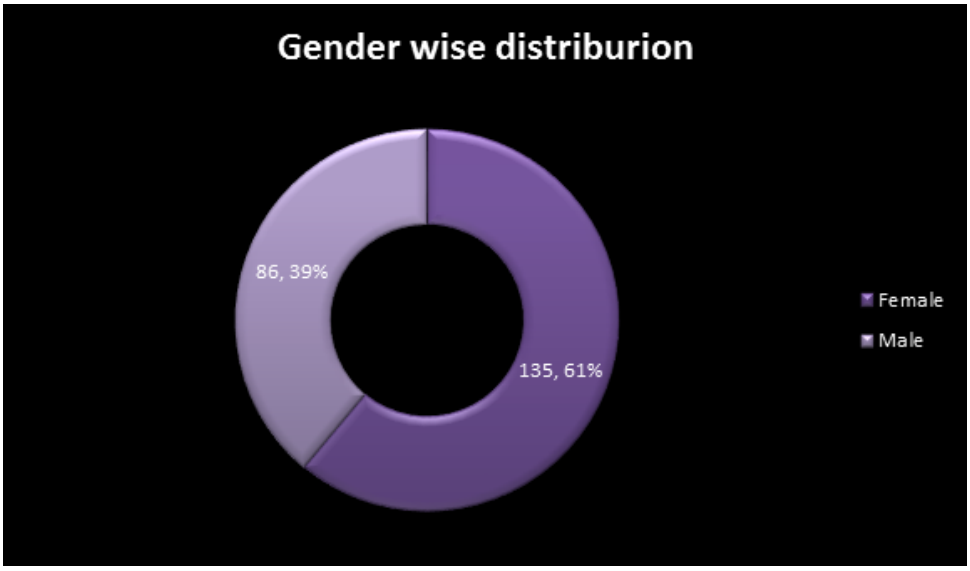

Figure 3: Shows Gender wise prevalence among 221 ESBL producing Enterobacteriaceae during study

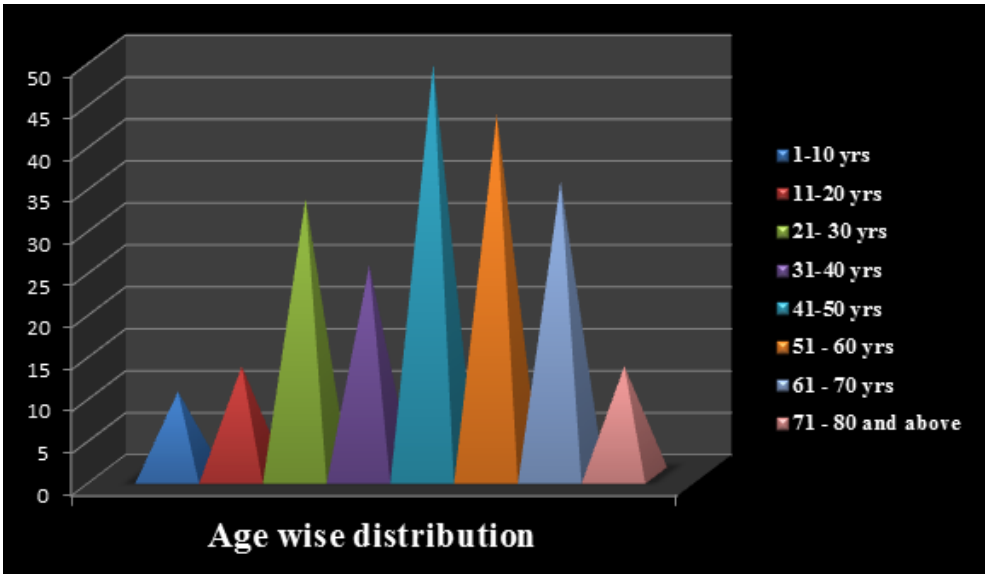

Figure 4: Shows Age wise prevalence among 221 ESBL producing Enterobacteriaceae during study

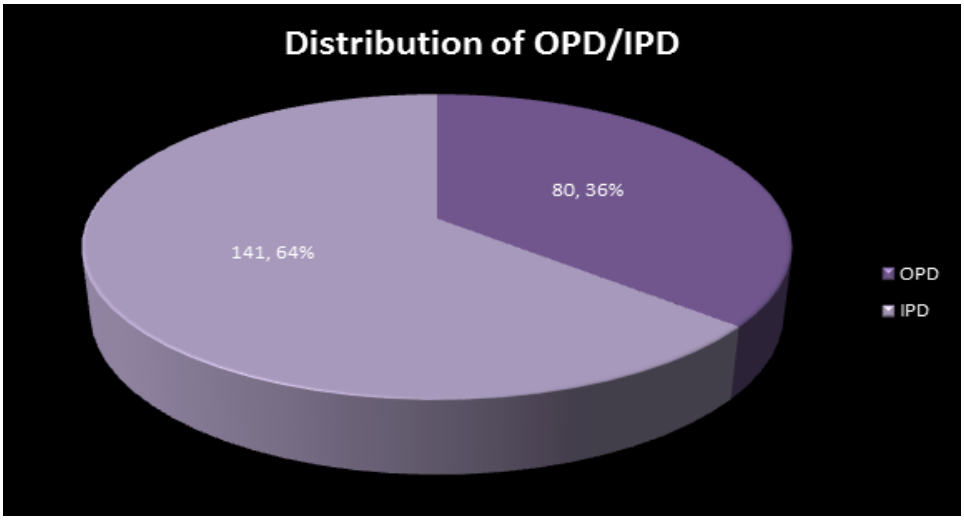

Figure 5: Shows OPD/IPD distribution of ESBL producing Enterobacteriaceae during study

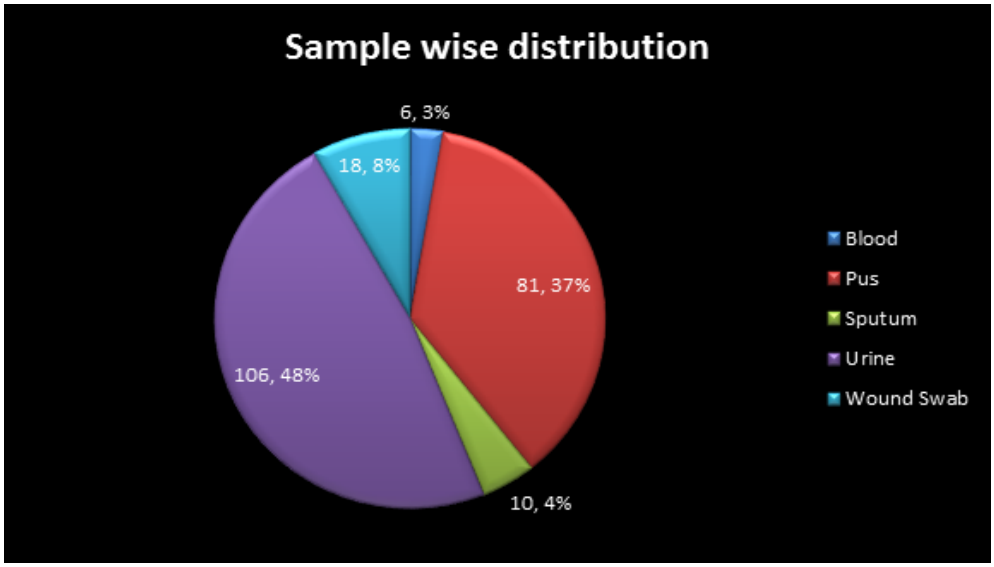

Figure 6: Shows samplewise distribution of ESBL producing Enterobacteriaceae during study

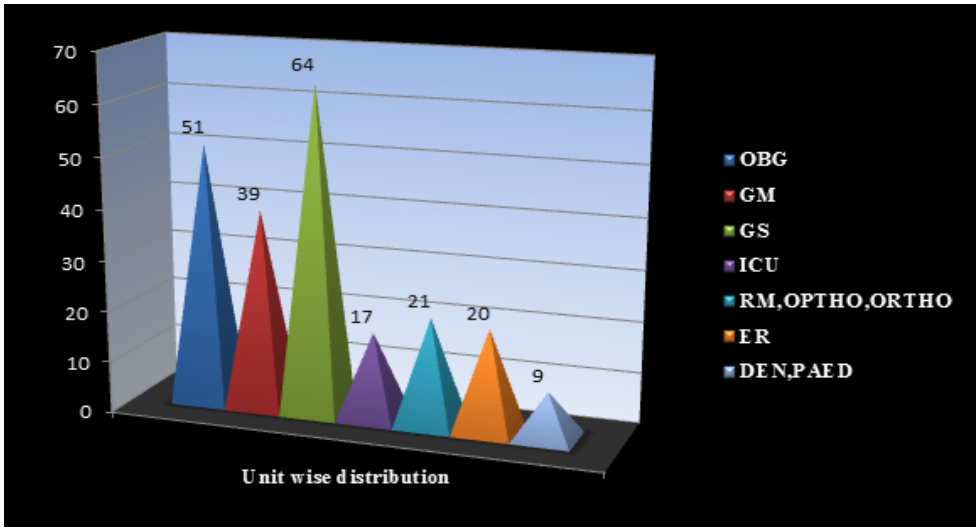

Figure 7: Shows ward wise distribution of ESBL producing Enterobacteriaceae during study

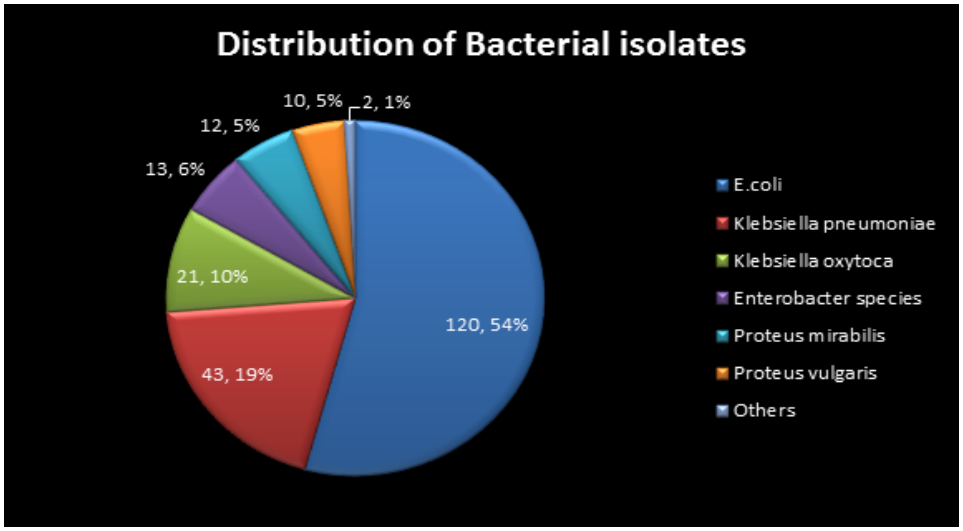

Figure 8: Shows Bacterial Isolates wise distribution of ESBL producing Enterobacteriaceae during study

Discussion

The members of the family Enterobacteriaceae are one of the most important bacterial pathogens isolated from clinical samples [9]. In last few years, bacterial resistance has increased dramatically with plasmid mediated ESBL contributing to this increase world-wide. These plasmids also carry co-resistance genes for othernon-β-lactam antibiotics; limiting the number of effective drugs. To make problems worse, plasmid-mediated ESBL enzymes spread fast among bacteria resulting into nosocomial outbreaks [9]. The prevalence of ESBL producers varies across continents and countries and also within hospitals. [10] In India, no country wide study has been conducted so far for detection of the prevalence of ESBL production [9] the prevalence rate varies in different institutions from 6-87%. [11] Since no data on ESBL prevalence in our institute was available, this study was conducted to look for ESBL prevalence and their antimicrobial susceptibility. The occurrence of ESBL producers among the Enteobacteriaceae in the current study was 25.67% which was similar to a study on urinary isolates from Dibrugarh [14] but higher than a similar study from Hyderabad (19.8%). [12] The prevalence was lower when compared with the studies from Valsad 48%, [13] Bhopal48.27%, Mumbai 53%, [11] Pondicherry (66.7%), [15] Amritsar 45.8 %,[16] Sikkim 34.03%. [10] This clearly indicates that the prevalence of ESBLs varies greatly geographically and rapidly changing overtime. This could be due to difference in the study design, population, associated risk factors, geographical distribution and probably due to differential clonal expansion and drug pressure in the community. [9] The age wise distribution revealed the maximum number (45%) of ESBL producers in the age group 1-20 years as also seen in a similar study from north-west India. [17] In our study ESBL prevalence among female patients is predominantly seen than male patients in this study, 61% of ESBL producing female patients were identified whereas 39% of ESBL producing male and highest level of infection occurred in the criteria was 41-50 yrs old patient i.e. (22%), the next down of infection occurred in the criteria was 51-60 yrs old patient ratio i.e. (19%), followed by 61 – 70 yrs old patient ratio estimated to be (16%), 21-30 yrs old patient ratio estimated to be (15%),31-40 yrs old patient ratio estimated to be (11%),both11-20&81–90 yrs old patient ratio estimated to be(6%) and the least affected age group was1-10 yrs, states the highest level of infection caused by ESBL producing Enterobacteriaceae in the patients, followed by Klebsiella pneumonia(43/221,19%)in the study.

Conclusion

ESBLs were first identified in the 1980s and have gradually spread throughout the world by nosocomial routes. The phenotypic data generated in the current study indicates the high prevalence of ESBL producers in this region of South India. Longitudinal surveillance of the microbial flora and the antibiotic sensitivity pattern should be done in every hospital periodically. Good infection control practices and antimicrobial stewardship program are instrumental in preventing the emergence of outbreaks due to ESBL producing isolates, especially in high risk areas such as the medical ICU, paediatric wards and surgical wards

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