Original Article

PREVALENCE OF ESBL PRODUCING ENTEROBACTERIACEAE FROM PUS SAMPLES AT THE TERTIARY CARE CENTER IN KANPUR

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ABSTRACT: ESBL producing Enterobacteriaceae pose a major problem for clinical therapeutics. The present study was conducted to know the rate of ESBL producing Enterobacteriaceae and their antibiogram among pus samples. **Material and methods:** Pus sample from the patients admitted and / or attending the outpatient department at Rama Medical College, and Research Centre,Hospital Kanpur, with abscess, wound infection, otitis media were included in the study. Duration of the study was June 2014 to May 2015. Pus samples were microbiologically processed by standard techniques and only Enterobacteriaceae isolates were screened for ESBL production by Double disk synergy method. **Results:** Of the 100 isolates, 19 (19%) isolates were ESBL producers. Male to female ratio of ESBL producers is 0.9:1. Maximum number of ESBLs were isolated between the age group of 51-60 yrs. In the present study 24% of *E.coli*, 22% of *Klebsiella,* sp. 17% of *Citrobacter sp.,* 11% of *Enterobacter* sp. and 9% of *Proteus species* were ESBL producers. In the present study, Majority of ESBL producer showed resistance to Ampicillin (100%), Amoxyclav (100%) followed by resistance to cotrimoxazole(78.95%). Resistance to Amikacin was 68%. All the ESBL strains were sensitive to Imipenem (100%). **Conclusion:** The high level of ESBL producers mainly among Enterobacteriaceae isolates is alarming and warrants special attention, both to the clinicians and the microbiologists. Production of ESBLs by clinically important isolates is emerging as a wide spread problem in our set up. Routine detection of these isolates, appropriate infection control and antibiotic management strategies are needed to stop the spread of this emerging form of resistance.

Keywords: Enterobacteriaceae; Drug resistance; ESBL(Extended spectrum beta lactamases) ; DDST(Double disk diffusion synergy test).

INTRODUCTION

Resistant bacteria are emerging worldwide as a threat to the favourable outcome of common infections in community and hospital settings. β-lactamase production by several gram negative and gram positive organisms is perhaps the most important

single mechanism of resistance to penicillins and cephalosporins.[1]

There has been increased incidence and prevalence of extended spectrum β-lactamases (ESBLs), enzymes that hydrolyse & cause resistance to oxyiminocephalosporins & aztreonam.[2] They represent a major group of β-lactamases belonging to Ambler class A penicillinases currently being identified worldwide in large numbers & now found in a significant percentage of *Escherichia coli & Klebsiella pneumoniae strains*. They have also been found in other Enterobacteriaceae strains like *Enterobacter, Citrobacter, Proteus, Morgenella morganii, Serratia marsescens, Shigella dysenteriae, Pseudomonas aeruginosa*.[3] Today over 150 different ESBLs have been described.[3]

Major risk factors for colonization or infection with ESBL producing organism are long term antibiotic exposure, prolonged hospital stay, severe illness, resistance in an institution with high rates of third generation cephalosporin use & instrumentation or catheterization.[1] For detection of β-lactamase production molecular methods are gold standard but technically difficult and lacks facilities. For confirmation of ESBLs, CLSI recommends phenotypic confirmatory disk diffusion test/ combination disk test (PCDDT) using disk containing third generation cephalosporins with and without clavulanic acid.[4] It is necessary to know the prevalence of these strains in a hospital so as to formulate a policy of empirical therapy in high risk units. Equally important is to procure information on an isolate from a patient to avoid misuse of extended spectrum cephalosporins.

The present study is carried out at Rama Medical College Hospital, & Research Centre, Kanpur, to know the prevalence of ESBL producing Enterobacteriaceae from pus samples & to formulate effective antibiotic strategy & plan a proper hospital infection control strategy to prevent the spread of these strains.

MATERIALS AND METHODS

All patients admitting and/or attending the outpatient department in Rama Medical College Hospital with abscess, wound infection, otitis media, were the source of study.Pus collected from such affected sites constituted the material of study. Detailed history and clinical findings were recorded in the proforma.

Inclusion Criteria: All age groups and both sexes having suspected pyogenic infections were included in the present study.Only

those cases yielding growth of Enterobacteriaceae from the cultured pus was included in the study and was further tested for ESBL production.

Exclusion Criteria: Cases of pyogenic infections which did not yield the growth of Enterobacteriaceae, but yielded growth of other bacteria were not included.

Sample collection: Pus samples were collected from the affected site with all aseptic precaution using sterile swab in duplicate or aspirated with the help of sterile syringe and transported immediately to the laboratory. Direct smears were made from the first swab, heat fixed & stained with Grams stain. Smears were screened for pus cells & the presence of Gram negative rods.

The second swabs was used for inoculation on to Blood agar, Mac Conkey agar & Brain heart infusion broth and were incubated aerobically at 37^0 C in ambient air for 24 to 48hours.

Isolates were identified based on colony morphology, motility, relevant biochemical reactions such as Catalase test, Oxidase test, Sugar Fermentation test (glucose,sucrose,maltose,lactose), Hugh Leifsons oxidation fermentation test, Nitrate reduction test, Indole production, Methyl red test, Voges Proskauer test, Citrate utilization test, Urease test, Tripple sugar iron agar test, Phenylalanine deaminase test, Aminoacid decarboxylation test.

Enterobacteriaceae isolates were subjected for antibiotic susceptibility testing by Kirby Bauer disk diffusion technique.

In the present study susceptibility was tested against ampicillin (10µg), Amikacin(30 µg), Amoxyclav (30 µg), Cotrimoxazole (1.25/23.75 µg), Imipenem(10 µg), Ciprofloxacin(5 µg), Netilmicin(10 µg), Ceftazidime (30 µg), Cefotaxime(30 µg), Cefpodoxime(30 µg). These discs were obtained from HiMedia laboratories Pvt. Ltd – Mumbai. The diameter of zone of inhibition was measured and interpreted according to the CLSI guidelines.[5]

All isolates belonging to Enterobacteriaceae were tested for ESBL production by DDST.[6] Ceftazidime (30 µg), Cefotaxime (30 µg), Cefpodoxime (30 µg) and Co-Amoxyclav (Amoxicillin 20 µg + Clavulanic acid 10 µg) (HiMedia Laboratories Ltd. Mumbai) were used for ESBL detection. *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as positive and negative controls respectively.

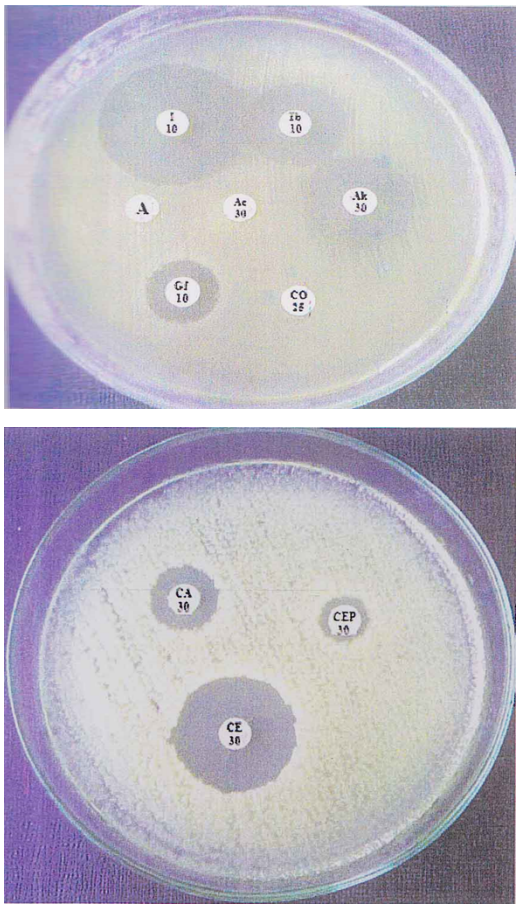


Figure-1: Antibiogram of ESBL producer strain.

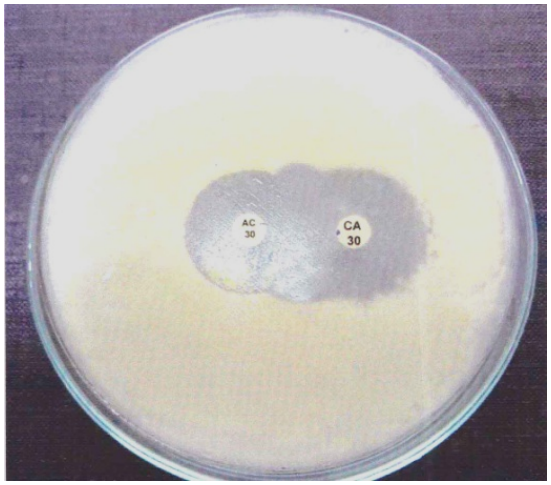


Figure – 2 : Test strain positive on Double Disk diffusion synergy test (DDST).

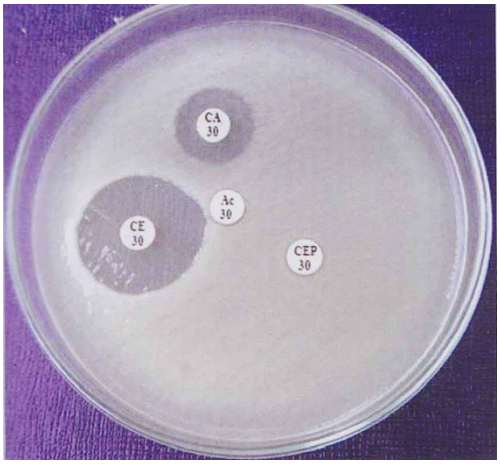


Figure – 3: Positive Control *Klebsiella pneumoniae* ATCC 700603

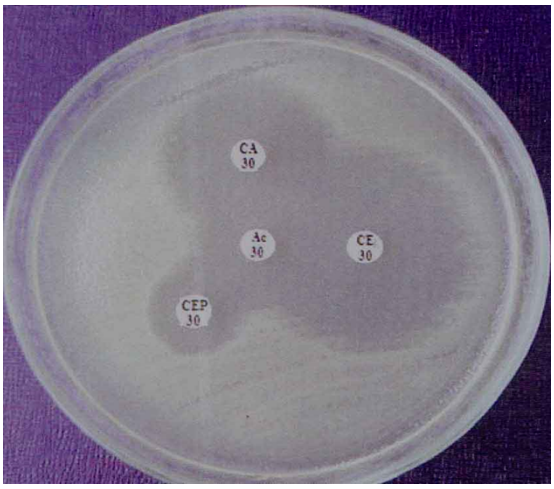


Figure – 4: Negative Control *E.coli* ATCC 25922

RESULTS

The present study was carried out in the Department of Microbiology Rama Medical College, Hospital & Research Centre, between June 2014 to May 2015 to look for the presence of ESBL (Extended spectrum beta lactamases) in Enterobacteriaceae isolated from pus samples. A total of 100

Fig 5: Percentage distribution of Enterobacteriaceae isolated from various pus samples

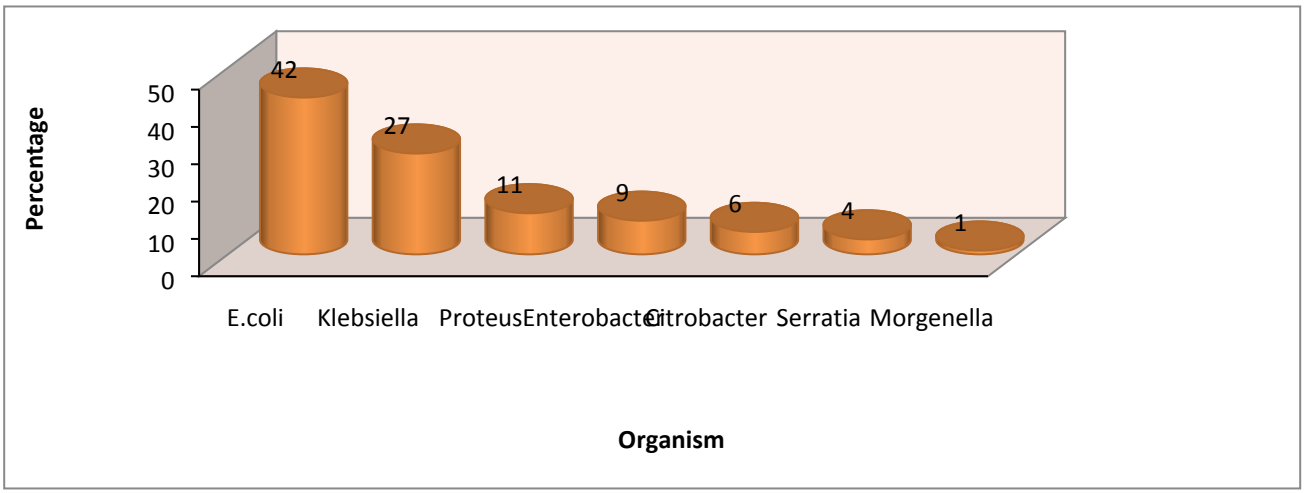


Table-1: Age and sex distribution of ESBL producers.

Age in yrs	Sex				Total	Total Percentage
	Female		Male			
	No.	Percentage	No.	Percentage		
1-10	0	0.0	0	0.0	0	0.0
11-20	0	0.0	0	0.0	0	0.0
21-30	5	26.3	0	0.0	5	26.3
31-40	0	0.0	3	15.8	3	15.8
41-50	2	10.5	0	0.0	2	10.5
51-60	2	10.5	4	21.1	6	31.6
61-70	1	5.3	1	5.3	2	10.5
71-80	0	0.0	1	5.3	1	5.3
81-90	0	0.0	0	0.0	0	0.0
Total	**10**	52.6	**9**	47.4	**19**	100.0

Fig -6: Percentage of ESBL producers among Enterobacteriaceae

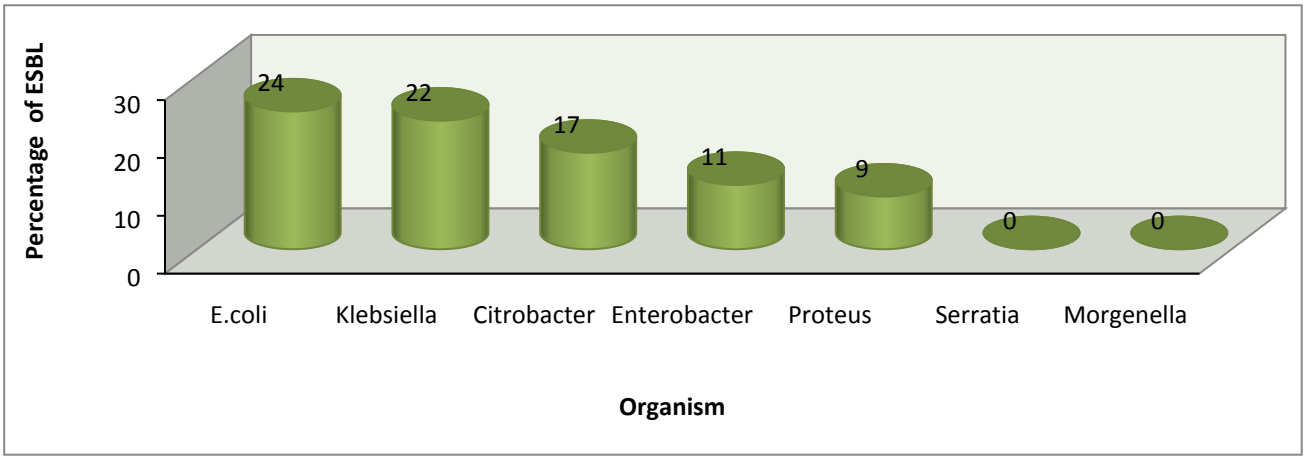


Fig 7: ESBL producers among various clinical conditions

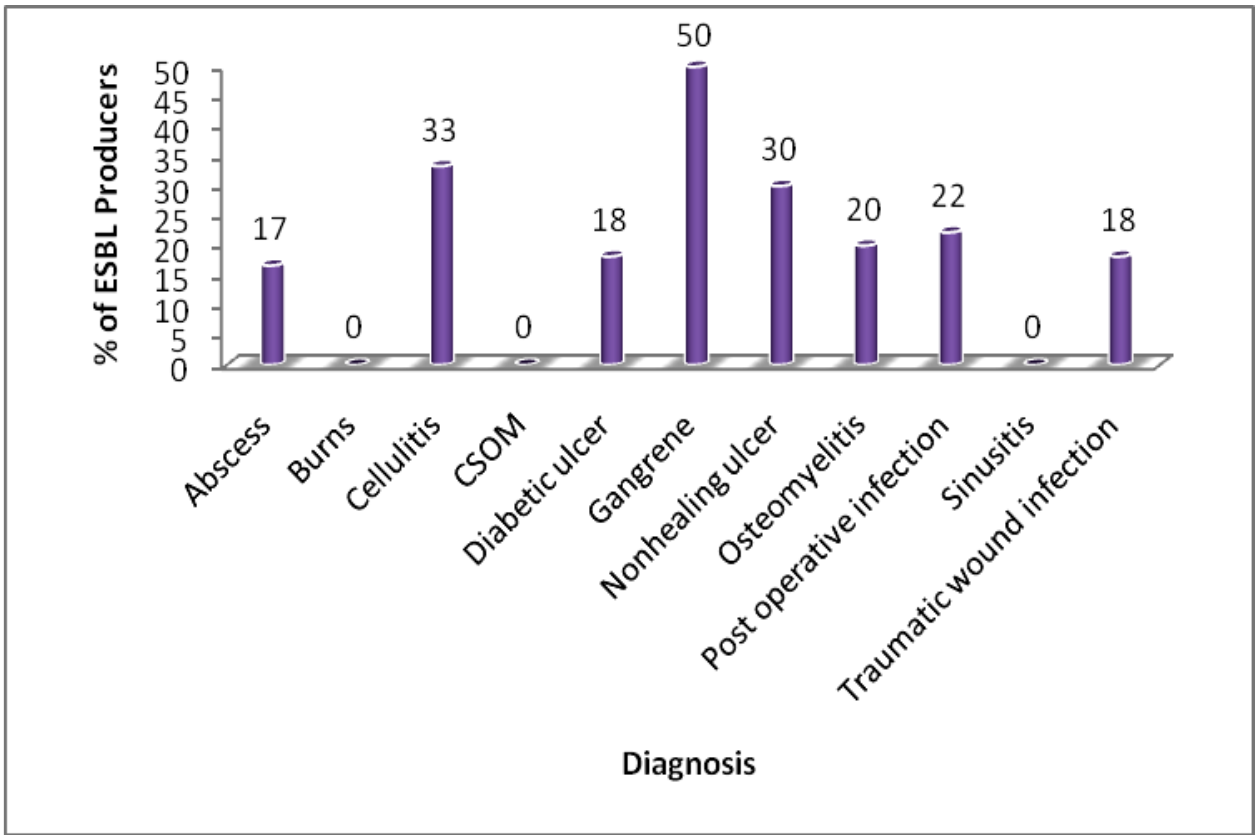
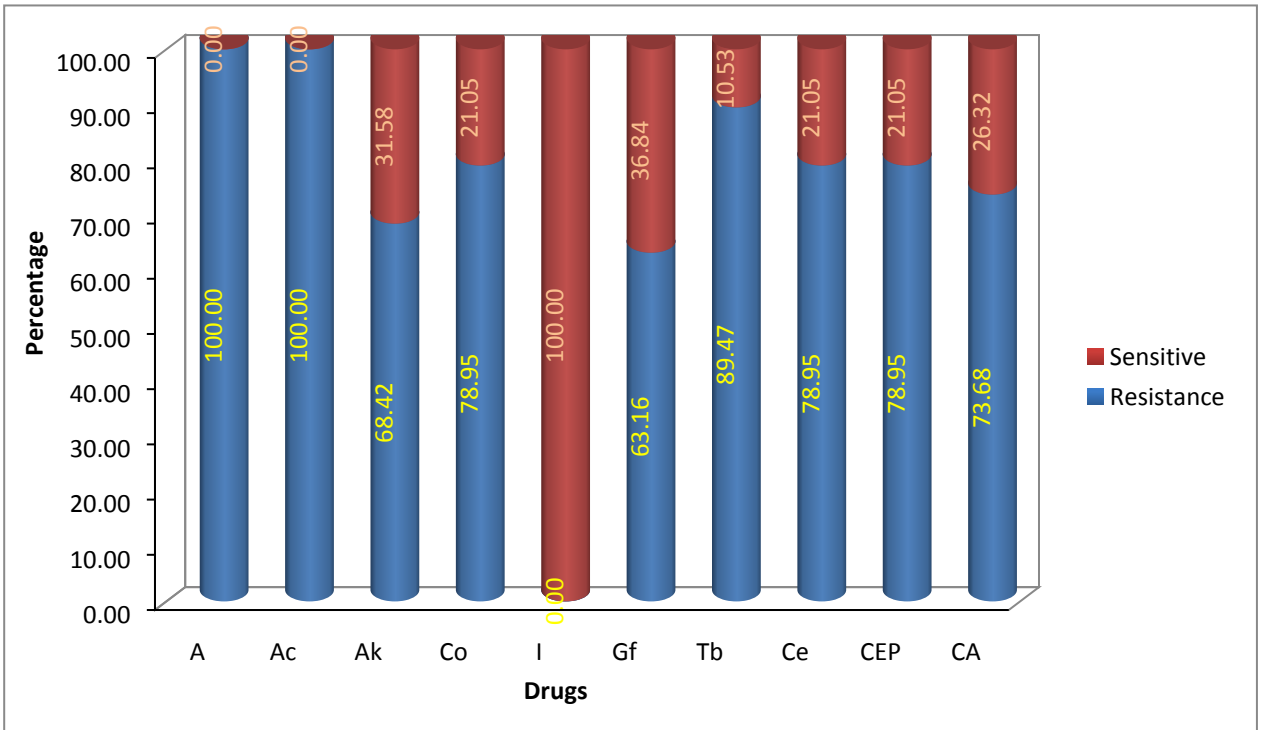


Fig 8: Antibiotic sensitivity pattern of ESBL producers



isolates of Enterobacteriaceae was observed for ESBL production. Out of 100 isolates, commonest isolates were *E.coli 42%, Klebsiella 27%, and Proteus* 11%.**[Fig 5]** ESBLs were most commonly isolated between the age group of 51-60 yrs(around 31.6%) and less number were isolated in both the extremes of age group (Between 1-20yrs and 70-90yrs). The age and sex distribution of the ESBLs is shown in the **table 1**.

Among 100 cases of Enterobacteriaceae, 19 (19%) were ESBL producers. In the present study *E.coli* (24%) was common ESBL producer. Other ESBL producers were *Klebsiella* (22%), *Citrobacter* (17%),*Enterobacter* (11%), and *Proteus species* (9%).No ESBL was isolated from *Serratia and Morganella species*. Percentage of ESBL producers among Enterobacteriaceae is shown in the **Fig 6**.

17-22% of ESBL producers were isolated from abscesses and post-operative infection. 30-50% of ESBL producers were isolated from Gangrene,Cellulitis, Nonhealing ulcer. 20% were isolated from Diabetic ulcer and osteomyelitis. No ESBL isolates were identified from Burns, CSOM and sinusitis . Distribution of ESBL among various clinical conditions is shown in **Fig 7**.

In the present study, all the 19(19%) ESBL producers showed 100% resistance to A (Ampicillin), Ac (Amoxyclav). All the isolates were sensitive to Imipenem. They showed variable resistance pattern to Tobramycin (89.47%), Cotrimoxazole (78.95%), Cefotaxime (78.95%), Cefpodoxime (78.95%), Ceftazidime (73.68%), Amikacin (68.42%), Gatifloxacin (63.16%).The resistance pattern of ESBLs is shown in **Fig 8**.

DISCUSSIONS

ESBL producing Enterobacteriaceae pose a major problem for clinical therapeutics. In the present study, an attempt was made to know the rate of ESBL producing Enterobacteriaceae and to know their antibiogram among pus samples.

Pus samples were collected from 100 patients with suspected pyogenic infections. *E.coli* (42%) was the commonest organism isolated, followed by *Klebsiella*(27%), and *Proteus species*(11%).Similar results were shown by study conducted by Shrestha S et al[7] (35.66% of *E.coli*) and Ananthakrishnan et al [8] (21% of *E.coli*). However, in contrast to our study, in a study by Golia S et al.[9] *Klebsiella* were the major pathogens (63%) but the study group comprised of

children which probably was the reason for the difference.

In the present study, out of 100 cases 19(19%) were ESBL producers. The prevalence of ESBL production among Enterobacteriaceae varies greatly from country to country and among the institutions within the country. In India, the incidence of ESBL mediated resistance was observed among 60-68% of clinical pathogens that were isolated from major hospitals. [10] In our study we have found 19% of ESBL producers which is within the Indian study range. Overall percentage of ESBLs in the present study was 19%. It is consistent with MS Kumar (19.8%)[11], Vinod kumar et al[12] (13.5%). High percentages of ESBLs were reported by Golia S et al (46%%). The high rate in their studies has been attributed to the fact that the study group was from a tertiary care multispeciality centre and indiscriminate use of antibiotics. Jean Pilippe lavigne et al[13] (3.62%) and Subha et al[14] (6.6%) reported low percentage of ESBLs. Hansotia et al[15], Ananthakrishnan et al [8], Neelam Taneja et al[16] have reported ESBLs between 20 – 40%.

The male to female ratio was 0.9:1 .Our results differ from that of Babypadmini et al[17] who observed male to female ratio of 1:1.3.

Thirty percent of ESBLs were isolated from Non healing ulcer and 20% from diabetic ulcer. It is consistent with Ananthakrishna et al (27%).[8]

Most of the past studies have shown ESBL producing *Klebsiella strains* to be more prevalent than ESBL producing *E.coli* strains. However reverse was found to be true in the present study. The percentage of ESBL producing E.coli is 24% that of *Klebsiella* is 22%. Similarly Ananthakrishna et al[8] (E.coli 56.2%, *K pneumoniae* 21.85%), and Blomberg et al [18] (*E.coli 25% and K.pneumoniae* 17%) reported highest percentage of ESBLs among *E.coli.*

In the present study 24% of *E.coli*, 22% of *Klebsiella,* 17% of *Citrobacter,* 11% of *Enterobacter* and 9% of *Proteus species* were ESBL producers.

Vinod Kumar et al. [12] from Gulbarga reported 16.8% and 48.6% of E.coli and K. pneumoniae respectively as the ESBL producers

In the present study, Majority of ESBL producer showed resistance Ampicillin(100%), Amoxyclav(100%) followed by resistance to

cotrimoxazole(78.95%). Resistance to Amikacin was 68%. Similar resistance rates were shown in the studies by Babypadmini et al [17]

In our study, all the ESBL strains were sensitive to Imipenem(100%). Similar results were observed by Vinod kumar et al [12] and Babypadmini et al [17].

In present study, 19 isolates were reported as ESBL producers and hence should be resistance to 3rd generation cephalosporins, but 26.3% were susceptible to Ceftazidime. Similarly Khurana et al [19], and Vinod kumar et al [12], reported approximately 30% of ESBL producers showing false susceptibility to 3rd generation Cephalosporins. Ananthakrishnan et al [8] reported 53% of ESBL producers sensitive to Cefotaxime. Babypadmini et al[17] reported 14% and 12% of ESBL strains showing false susceptibility to Ceftazidime and Cefotaxime. All the above findings suggest the importance of detecting ESBLs in the laboratory, which helps in proper selection of antibiotics. The information regarding ESBL producers, their susceptibility pattern, and risk factors were informed to the consultants who help them to select the proper antibiotics.

CONCLUSION

In the last 20 years ESBLs have gone from being an interesting scientific observation to a reality of great medical importance. The routine susceptibility test done by clinical laboratories fail to detect ESBL positive strains and can erroneously detect isolates sometimes to be sensitive to any of the third generation cephalosporins, hence a special phenotypic confirmatory test(DDST) is indispensable for detecting ESBLs. Among the non betalactum antibiotics, Amikacin was the most effective drug. 26% of the ESBL producers showed false susceptibility to Ceftazidime.Treatment of such strains with Cephalosporins results in therapeutic failure.This highlights the need for all diagnostic laboratories to perform ESBL detection as a routine practice among all the Enterobacteriaceae members.To overcome the problem of emerging ESBLs, combined interaction and cooperation of microbiologists, clinicians and the infection control team are needed.

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