Int.J.Curr.Microbiol.App.Sci (2025) *14*(10): 201-214

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 14 Number 10 (2025)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2025.1410.020

Susceptibility Pattern of Novel Ceftriaxone Sulbactam EDTA by E-Strip Method on Extended Spectrum Beta Lactamase and Carbapenem Resistant Enterobacterales Isolated in a Tertiary Care Hospital, Calicut

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Keywords

Extended Spectrum Beta-Lactamase, Carbapenem-Resistant Enterobacterales, Ceftriaxone-Sulbactam-EDTA, minimum inhibitory concentration, E-Strip

Article Info

Received:
12 August 2025
Accepted:
28 September 2025
Available Online:
10 October 2025

A B S T R A C T

The increasing prevalence of Extended-spectrum beta-lactamase (ESBL) producers and Carbapenem-resistant Enterobacterales, poses a significant threat to public health and challenges in antimicrobial therapy. These resistant organisms are commonly encountered in hospital settings and are associated with high morbidity, mortality, and healthcare costs. Ceftriaxone-sulbactam-EDTA (CSE), a novel beta-lactam/beta-lactamase inhibitor combination with an added metal ion chelator, has shown promising activity against these resistant pathogens. Evaluating the in-vitro susceptibility pattern of CSE using the E-strip method provides crucial data for its potential role in the management of infections caused by ESBL and carbapenem-resistant Enterobacterales. To study the proportion of Extended-spectrum beta-lactamase production and carbapenem resistant Enterobacterals among culture isolates and to determine the minimum inhibitory concentration of ceftriaxone sulbactam EDTA in extended spectrum beta lactamase and carbapenem resistant Enterobacterals by E strip method. Samples are examined using standard bacteriological methods to identify the causative organisms. Antimicrobial susceptibility is tested using Kirby-Bauer disc diffusion method, with results interpreted according to CLSI guidelines. The VITEK® 2 Compact automatic system is employed for testing blood, body fluids and invasive respiratory specimen. Screening of ESBL is done by ceftazidime and ceftazidime clavulanic acid combined disc test. Screening of CRE is done by imipenem (IMP) and meropenem (MEM) disc diffusion and its Phenotypic characterization is done by mCIM and eCIM method. The Minimum inhibitory concentration of ceftriaxone sulbactam EDTA is done by E strip. A total of 183 multidrug-resistant (MDR) Enterobacterales isolates were obtained from diverse clinical specimens, without limiting by age or gender. Out of these, 96 (52.5%) were Extended Spectrum Beta-Lactamase (ESBL) producers, while 87 (47.5%) were classified as Carbapenem-Resistant Enterobacterales (CRE). All ESBL-producing isolates were fully susceptible to ceftriaxone-sulbactam-EDTA, with a predominant minimum inhibitory concentration (MIC) of 1 µg/ml. Among CRE isolates, 87.5% of MBL producers were sensitive to ceftriaxone-sulbactam-EDTA, with a typical MIC of 8 µg/ml, whereas SBL producers exhibited complete resistance to the same. This study identified a high prevalence of Extended Spectrum Beta-Lactamase (52%) and Carbapenem-Resistant Enterobacterales (47%) among clinical Enterobacterales isolates, underscoring the growing challenge of antimicrobial resistance and highlights the significant therapeutic potential of the novel antibiotic combination Ceftriaxone-Sulbactam-EDTA (CSE), evaluated using the E-strip method, in addressing multidrug-resistant Enterobacterale infections. The results demonstrated that CSE exhibited 100% sensitivity against Extended Spectrum Beta-Lactamase (ESBL)-producing Enterobacterales and showed promising efficacy against Carbapenem-Resistant Enterobacterales (CRE), particularly metallo-beta-lactamase (MBL) producers. This would help the clinician to make it as carbapenem sparer and an alternative drug for polymyxins which act against Carbapenem resistant Enterobacterales

Introduction

Since the inception of human civilization, various diseases have afflicted populations, many of which are attributed to members of the Enterobacteriaceae family. These bacteria are implicated in a vast range of microbial diseases and can be isolated from nearly any clinical specimen submitted to a laboratory. Particularly concerning are their roles as significant nosocomial pathogens; infections caused by these organisms can result in substantial morbidity and mortality. This is especially true in settings such as intensive care units (ICUs), internal medicine and surgical wards, and pediatric units, where patients are often immunocompromised and susceptible to severe infections.[1]

Infections caused by Enterobacteriaceae are commonly managed using β-lactam antibiotics, with carbapenems serving as the last line of defense against serious Gram-negative bacterial infections. Nevertheless, the rise of Enterobacterales strains resistant to carbapenems has significantly compromised the efficacy of these treatments. Carbapenemase genes, residing on plasmids, enable horizontal gene transfer among bacteria, leading to the rapid dissemination of resistance traits. This mobility heightens concerns about the potential circulation of resistant strains from healthcare settings into the broader community. Notably, regions like the Indian subcontinent have reported high prevalence rates of carbapenem-resistant Enterobacteriaceae, underscoring the global nature of this public health challenge[2]

Enterobacteriaceae have developed complex mechanisms to counteract the bactericidal effects of β-lactam antibiotics, including alterations in outer membrane proteins, enhanced efflux pump activity, and the production of various β-lactamases. The widespread and often inappropriate use of β-lactams has facilitated the emergence of diverse β-lactamase variants, such as extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and metallo-β-lactamases, contributing to the growing challenge of antimicrobial resistance.[3]

While β-lactam and β-lactamase inhibitor combinations (BL+BLIs) are employed to treat diseases caused by ESBL-producing isolates, carbapenems remain the preferred option for managing these infections. However, the advent of CRE has greatly reduced the effectiveness of treatment. CRE are now classified as critical priority pathogens by the WHO, reflecting their substantial threat to human health.[3]

The therapeutic options for diseases caused by CRE are notably constrained. Polymyxins, specifically colistin and polymyxin B, are frequently designated as the primary treatment modalities. However, the escalating prevalence of resistance to these agents has necessitated the exploration of adjunctive strategies. One promising approach involves the utilization of antibiotic adjuvant compounds that, when added with existing antibiotics, can potentiate their efficacy. These adjuvants function by either overcoming resistance mechanisms in pathogens previously susceptible to antibiotics or by sensitizing intrinsically resistant strains. A notable example is the novel antibiotic adjuvant entity (AAE) comprising ceftriaxone, sulbactam, and disodium EDTA (CSE), which has demonstrated potential in enhancing the activity of ceftriaxone against multidrug-resistant pathogens[4]

This research seeks to evaluate and determine in vitro efficacy of novel, ceftriaxone sulbactam EDTA using E strip against ESBL and carbapenem resistant Enterobacterals.

Materials and Methods

This study included patients with clinically significant multidrug-resistant Enterobacterales isolates from various clinical samples, irrespective of age and gender. The isolates were obtained from diverse specimens, including blood and body fluids, urine, respiratory samples (sputum, bronchial wash, bronchoalveolar lavage, and endotracheal aspirate), pus samples (aspirated pus and pus swabs), tissue, and vaginal swabs. Samples were collected using standard precautions, transported to the Microbiology laboratory in labeled containers, and repeat samples from the same patient were excluded.

Specimens are inoculated into suitable culture media and incubated at 37°C for 48 hours.

For blood and body fluids BACT/ALERT 3D was used. Bacterial identification is carried out by Standard microbiological techniques. Bacterial isolates were subjected to antibiotic susceptibility testing by employing the Kirby Bauer disc diffusion technique according to CLSI Guidelines M100 34thedition.VITEK 2 System is also employed in identification and susceptibility testing

of isolates from blood, body fluids and invasive respiratory specimen. Screening of ESBL is done by ceftazidime and ceftazidime clavulanic acid combined disc test as per CLSI guide lines. Screening of CRE done by imipenem (IMP) and meropenem (MEM) disc diffusion. Phenotypic detection of CRE done by mCIM and eCIM method. Minimum inhibitory concentration (MIC) of ceftriaxone sulbactam EDTA (CSE) is done by E strip

Results and Discussion

The study was conducted in the Department of Microbiology, Aster MIMS Hospital, Calicut. A total of 183 multidrug-resistant (MDR) Enterobacterales isolates (as per the sample size) were obtained from various clinical specimens, with no restriction on age or gender.

Proportion of ESBL and CRE among the Multidrug

Resistant Enterobacterales

183 multi drug resistant Enterobacterales were included in the study out of which 52.5 % (96) were ESBL and 47.5% (87) were CRE

Age Wise Distribution of Patients with Multidrug Resistant Enterobacterales

The incidence of multidrug-resistant Enterobacterales increased significantly with age, reaching the highest level in the > 60 age group, followed by the 51-60 age group, and being lowest in the 11-20 age category.

Location Wise Distribution of Multidrug Resistant Enterobacterales

The location-wise distribution showed that CRE cases were prevalent in Inpatients and the MDICU, whereas ESBL cases were more common in outpatients, followed by Inpatients.

Specimen Wise Incidence of Multi Drug Resistant Enterobacterales

The distribution of isolates by specimen type revealed that urine was the most common source, accounting for 36.8% of CRE and 72.9% of ESBL, followed by blood (17.2% of CRE) and pus (9.4% of ESBL).

Distribution of Multidrug Resistant Enterobacterales

Klebsiella species were the most the predominant organism followed by *E.coli,* among both CRE and ESBL isolates

Phenotypic Characterization of CRE

Phenotypic characterization of the CRE revealed 73.6% of Metallo beta lactamases (MBL), 18.4% Serine beta lactamases (SBL) and 8% of inconclusive results

Sensitivity Pattern of CSE to ESBL

All ESBL producing isolates exhibited 100% sensitivity to ceftriaxone sulbactam EDTA

All ESBL producing isolates were sensitive to CSE with a prevalent MIC of 1 µg/ml
*Resistant- ≥64 µg/ml Intermediate – 16-32 µg/ml Sensitive - ≤ 8µg/ml

Sensitivity Pattern of Ceftriaxone Sulbactam EDTA to CRE

In case of CRE, metallo beta lactamases shows 87.5% sensitivity to ceftriaxone sulbactam EDTA whereas the Serine beta lactamases showed 100% resistance. The metallo beta lactamases shows 87.5% sensitivity to ceftriaxone sulbactam EDTA with a prevalent MIC of 8 µg/ml whereas the Serine beta lactamases showed 100% resistance

Interpretation:
Resistant- ≥64 µg/ml
Intermediate – 16-32 µg/ml
Sensitive - ≤ 8µg/ml

In this study conducted in the Department of Microbiology, Aster MIMS Hospital, Calicut, there was a total of 183 multidrug-resistant (MDR) Enterobacterales isolated. Out of these, 96 (52.5%) were Extended Spectrum Beta-Lactamase (ESBL) producers, while 87 (47.5%) were classified as Carbapenem-Resistant Enterobacterales (CRE). In line with the current findings, a study conducted in Ghana revealed that 49.1% of Enterobacteriaceae isolates exhibited high levels of extended-spectrum beta-lactamase (ESBL) production, while 35.2% were CRE.[5]

Table.1 Proportion of ESBL and CRE among the Multidrug Resistant Enterobacterales

MDR	N=183	Percentage
ESBL	96	52.5%
CRE	87	47.5%

Table.2 Age wise distribution of patients with Multidrug Resistant Enterobacterales

Age	ESBL	CRE
0-10	10	3
11-20	1	2
21-30	14	10
31-40	8	12
41-50	6	13
51-60	22	21
>60	42	29

Table.3 Location wise distribution of multidrug resistant Enterobacterales

Location	ESBL		CRE	
	NO	%	NO	%
IPD	39	40.6	43	49.4
OPD	47	49	15	17.2
ICU	10	10.4	29	33

Table.4 Specimen wise incidence of multi drug resistant Enterobacterales

Specimen	CRE		ESBL	
	NO	**%**	**NO**	**%**
Urine	32	36.8	70	72.9
Blood	15	17.2	4	4.2
Pus	6	6.9	9	9.4
Tissue	9	10.3	6	6.3
BR wash	2	2.3	0	0
BAL	6	6.9	0	0
Wound swab	3	3.4	1	1
E T aspirate	1	1.1	1	1
ET tip	1	1.1	1	1
Body fluids	8	9.2	0	0
Sputum	3	3.4	2	2.1
Vaginal swab	1	1.1	2	2.1

Table.5 Distribution of multidrug resistant Enterobacterales

Organisms	ESBL		CRE	
	NO	%	NO	%
Klebsiella species	60	62.5	60	69.0
Escherichia coli	28	29.2	20	23.0
Enterobacter species	4	4.2	4	4.6
Proteus species	2	2.1	1	1.1
Citrobacter species	2	2.1	0	0.0
Serratia marcescens	0	0.0	2	2.3

Table.6 Phenotypic characterization of CRE

Phenotype	N=87	Percentage
Metallo beta lactamases	64	73.6%
Serine beta lactamases	16	18.4%
Inconclusive	7	8.0%

Table.7 Sensitivity pattern of CSE to ESBL

Sensitivity	*E. coli*		*Klebsiella species*		*Enterobacter species*		*Citrobacter species*		*Proteus species*	
	No. (n=28)	%	No. (n=60)	%	No. (n=4)	%	No. (n=2)	%	No. (n=2)	%
CSE	28	100	60	100	4	100	2	100	2	100

Table.8 MIC of Ceftriaxone Sulbactam EDTA to ESBL

MIC	*Klebsiella* species (n=60)	*E. coli* (n=28)	*Citrobacter* species (n= 2)	*Enterobacter* species (n= 4)	*Proteus* species (n= 2)
0.016	2	3	-	-	1
0.064	1	-	-	-	-
0.094	1	-	-	-	-
0.19	1	-	-	-	-
0.25	3	2	-	-	-
0.38	2	2	-	-	-
0.5	4	2	-	-	-
0.75	7	4	-	1	-
1	16	7	-	2	1
1.5	8	3	2	-	-
2	7	1	-	-	-
4	3	1	-	-	-
6	1	1	-	-	-
8	1	2	-	1	-

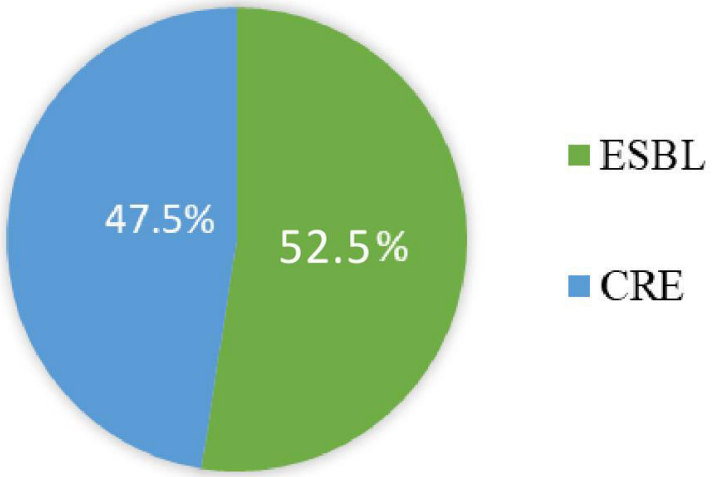
Table.9 Sensitivity pattern of CSE to CRE

Sensitivity	MBL		SBL		Inconclusive	
	No.(n=64)	%	No. (n=16)	%	No. (n=7)	%
Sensitive	56	87.5	0	0	4	57.1
Intermediate	5	7.8	3	18.8	1	14.3
Resistant	3	4.7	13	81.3	2	28.6

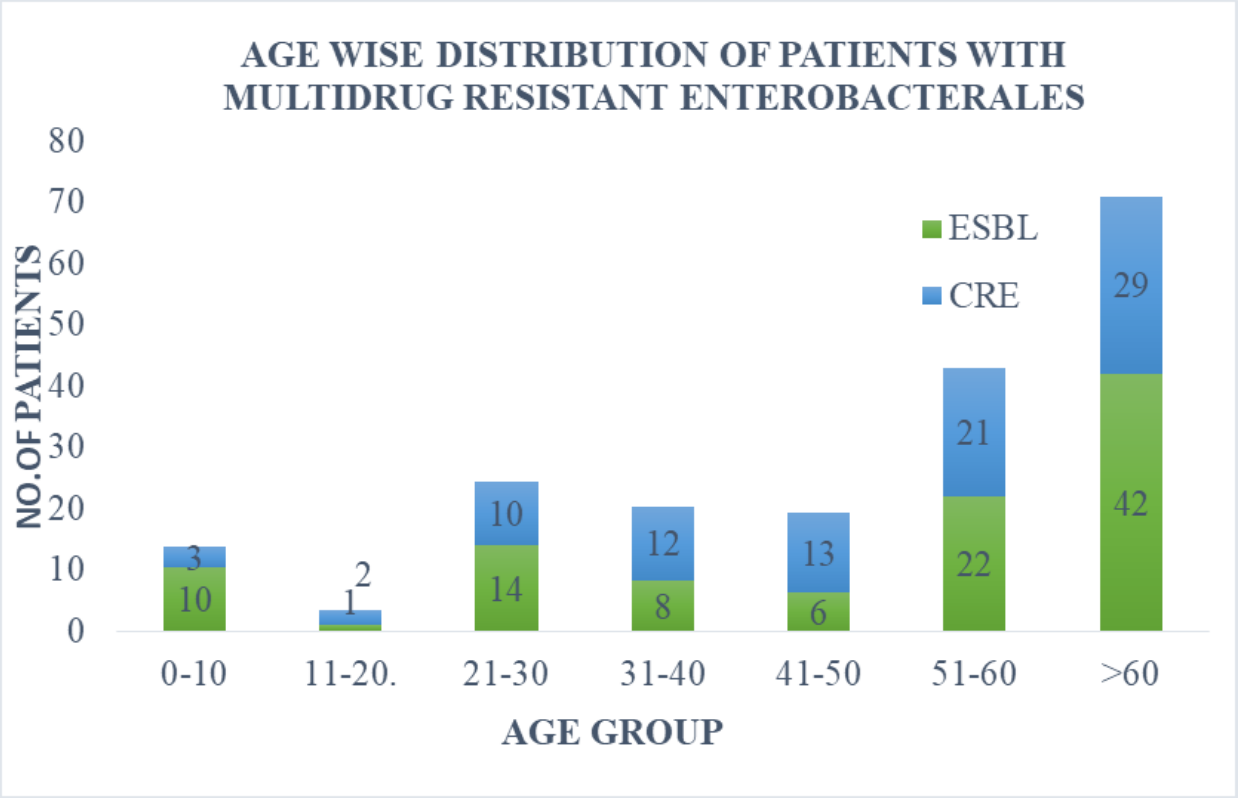
Table.10 MIC of Ceftriaxone Sulbactam EDTA to CRE

MIC	MBL (n=64)	SBL (n=16)	INCONCLUSIVE (n=7)
0.016	-	-	1
0.064	1	-	-
0.25	1	-	-
0.75	3	-	-
1	6	-	-
1.5	4	-	-
2	2	-	-
3	4	-	-
4	9	-	-
6	8	-	-
8	18	-	3
16	2	-	-
24	2	-	-
32	1	3	1
64	-	3	-
256	3	10	2

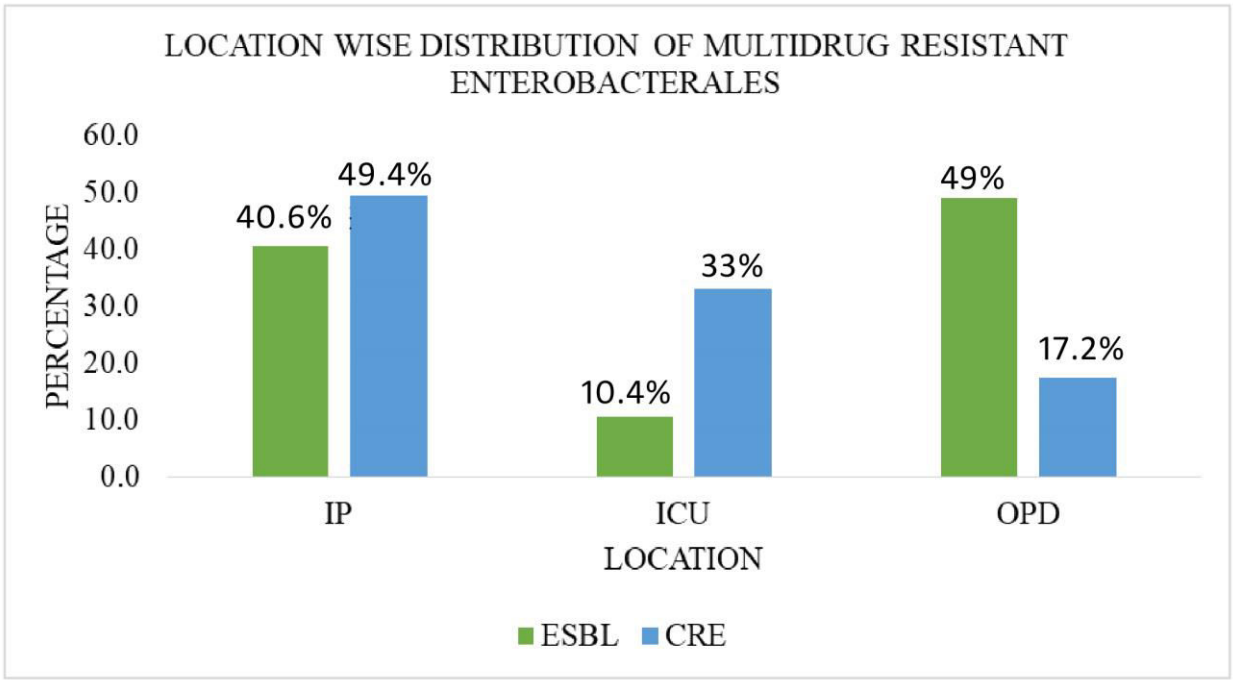
Graph.1 Proportion of ESBL and CRE among the Multidrug Resistant Enterobacterales



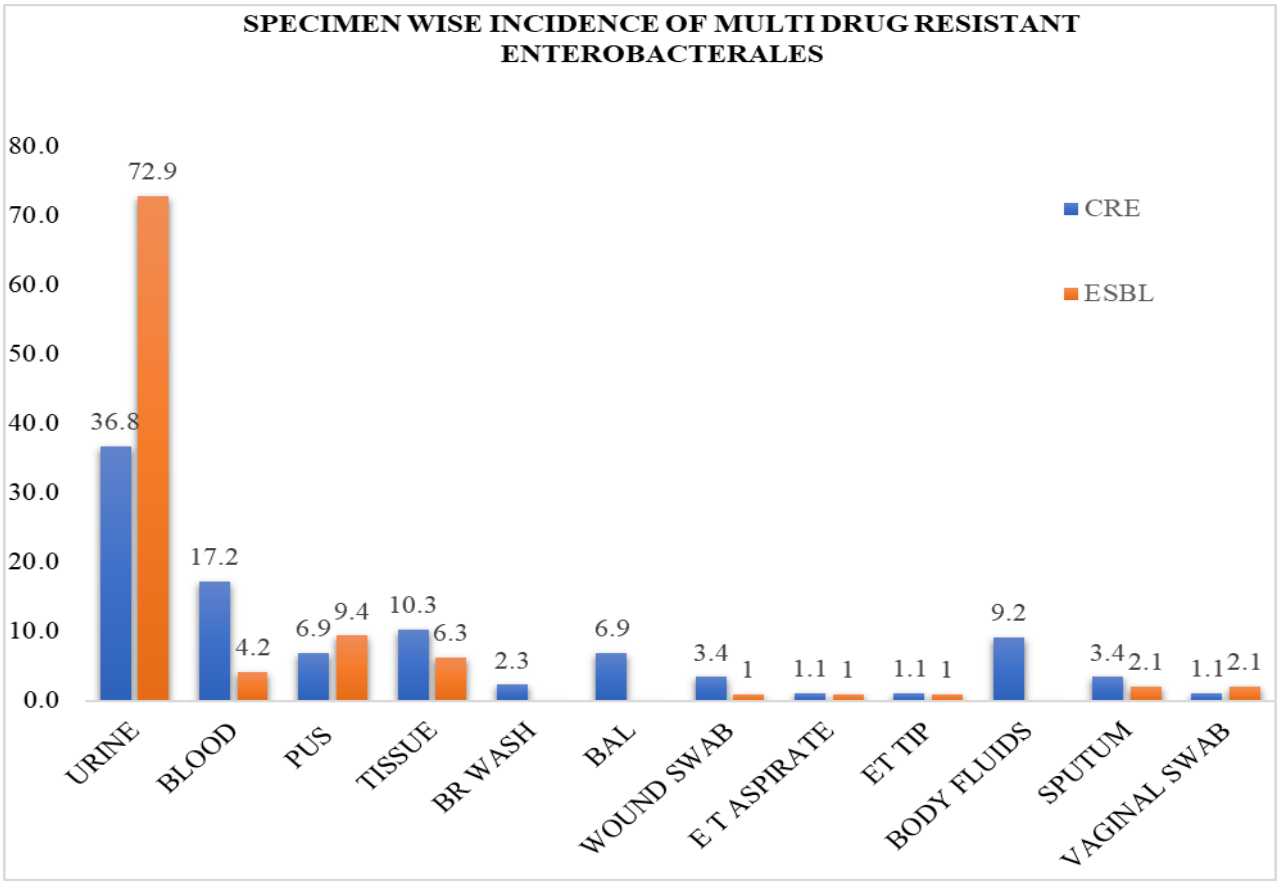
Graph.2 Age wise distribution of patients with Multidrug Resistant Enterobacterales



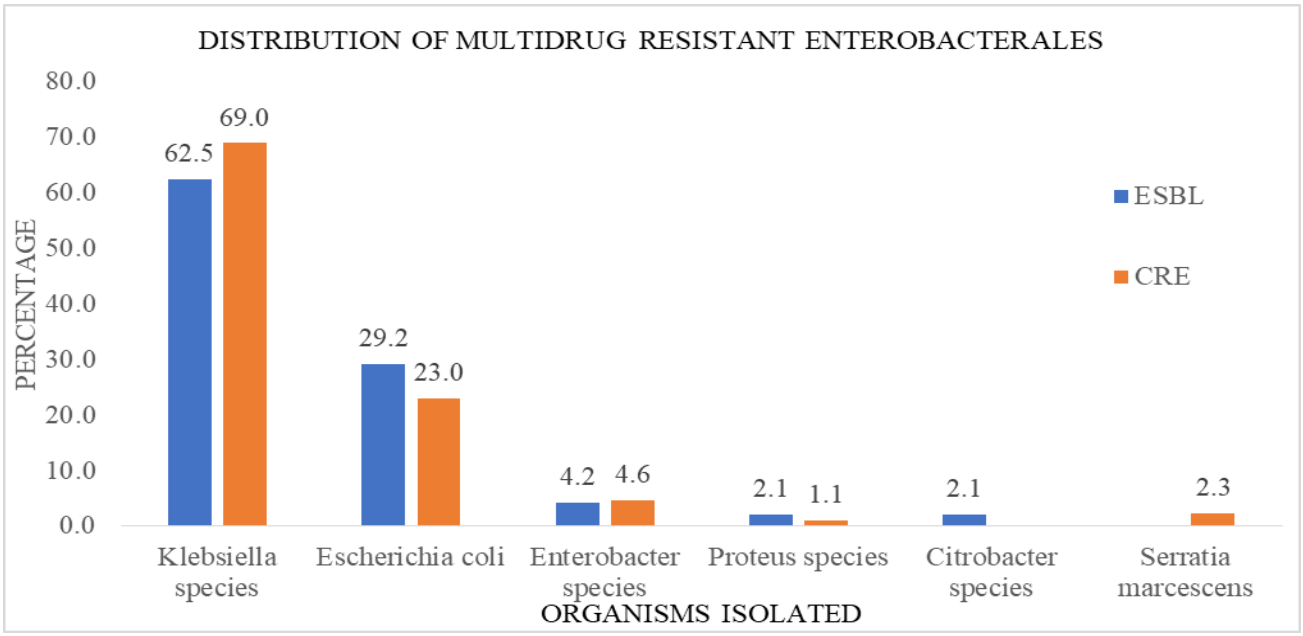
Graph.3 Location wise distribution of multidrug resistant Enterobacterales



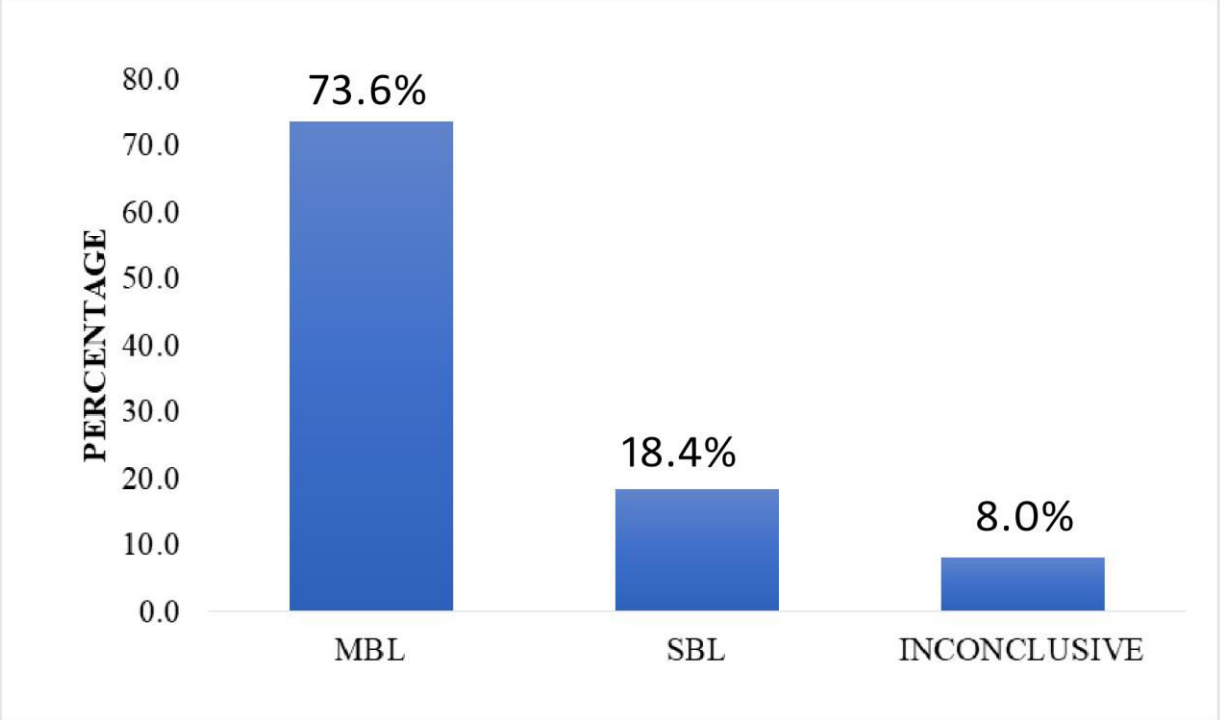
Graph.4 Specimen wise incidence of multi drug resistant Enterobacterales



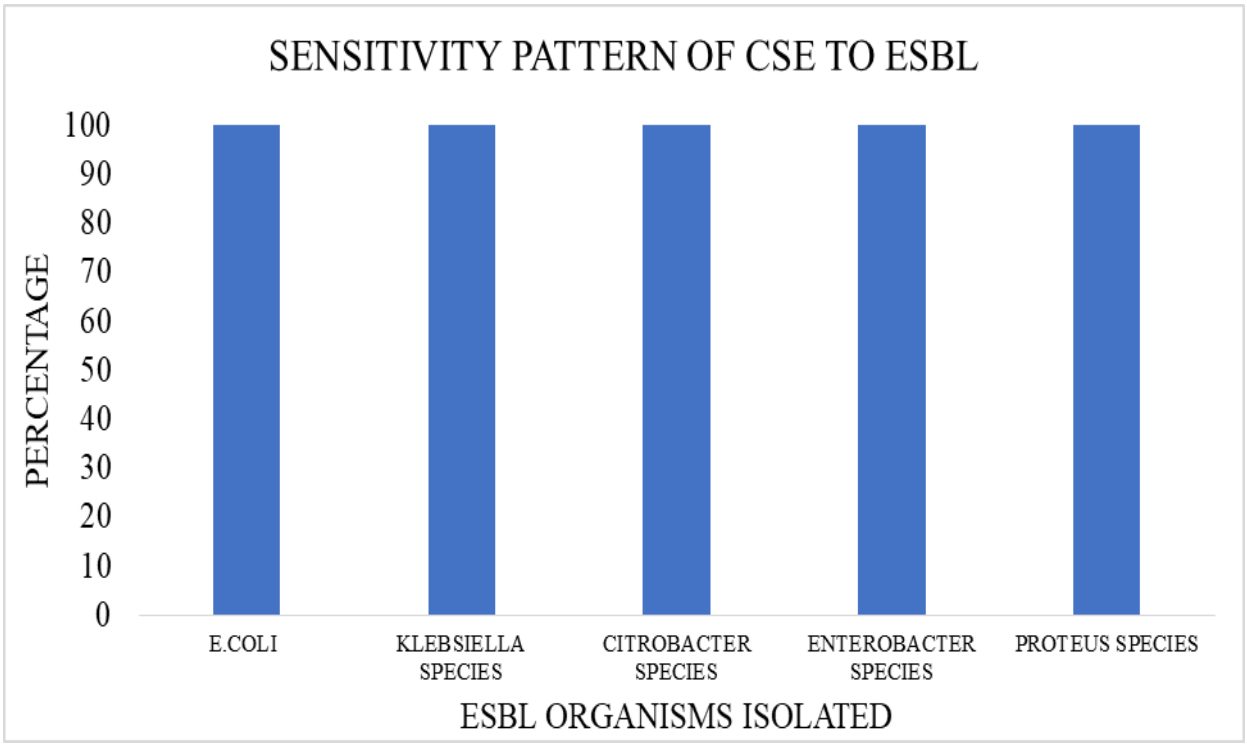
Graph.5 Distribution of multidrug resistant Enterobacterales



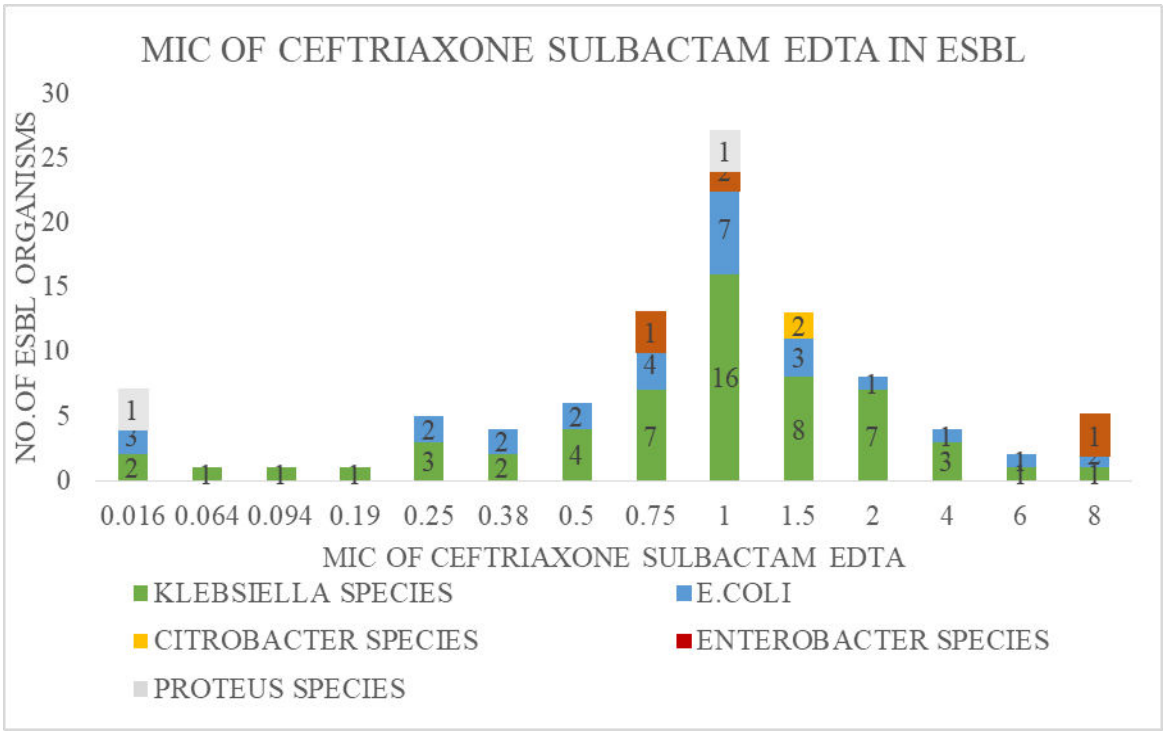
Graph.6 Phenotypic characterization of CRE



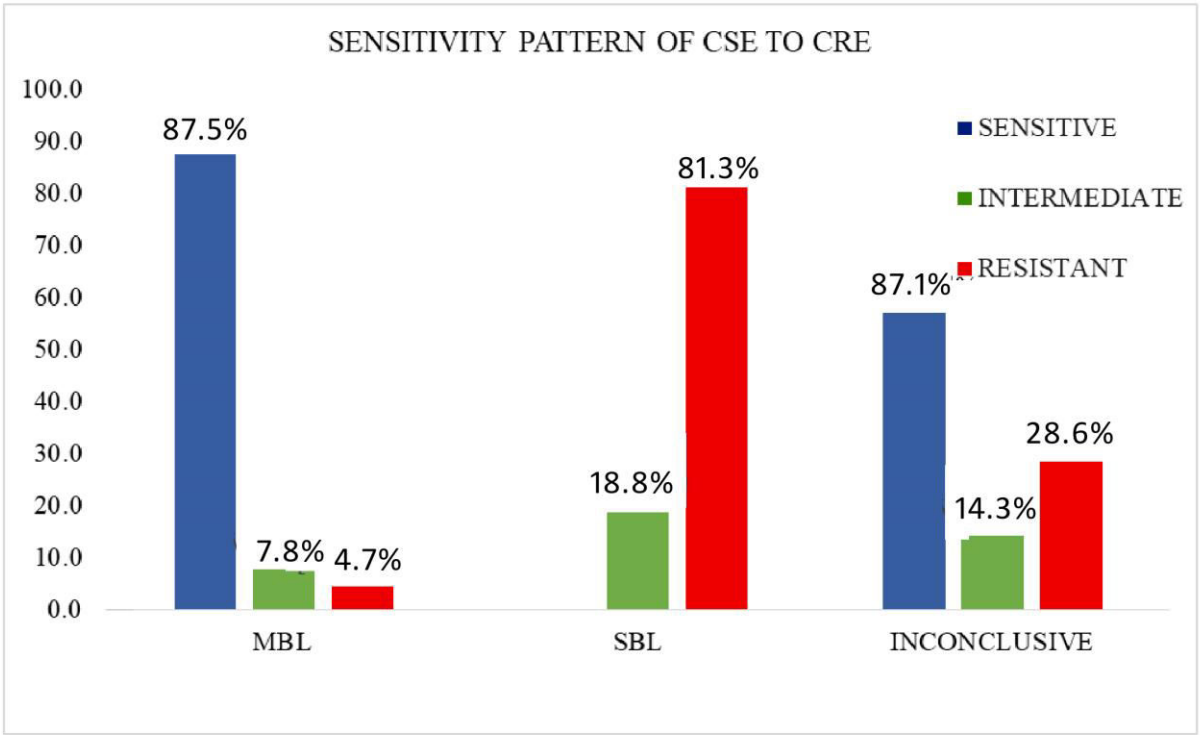
Graph.7 Sensitivity pattern of CSE to ESBL



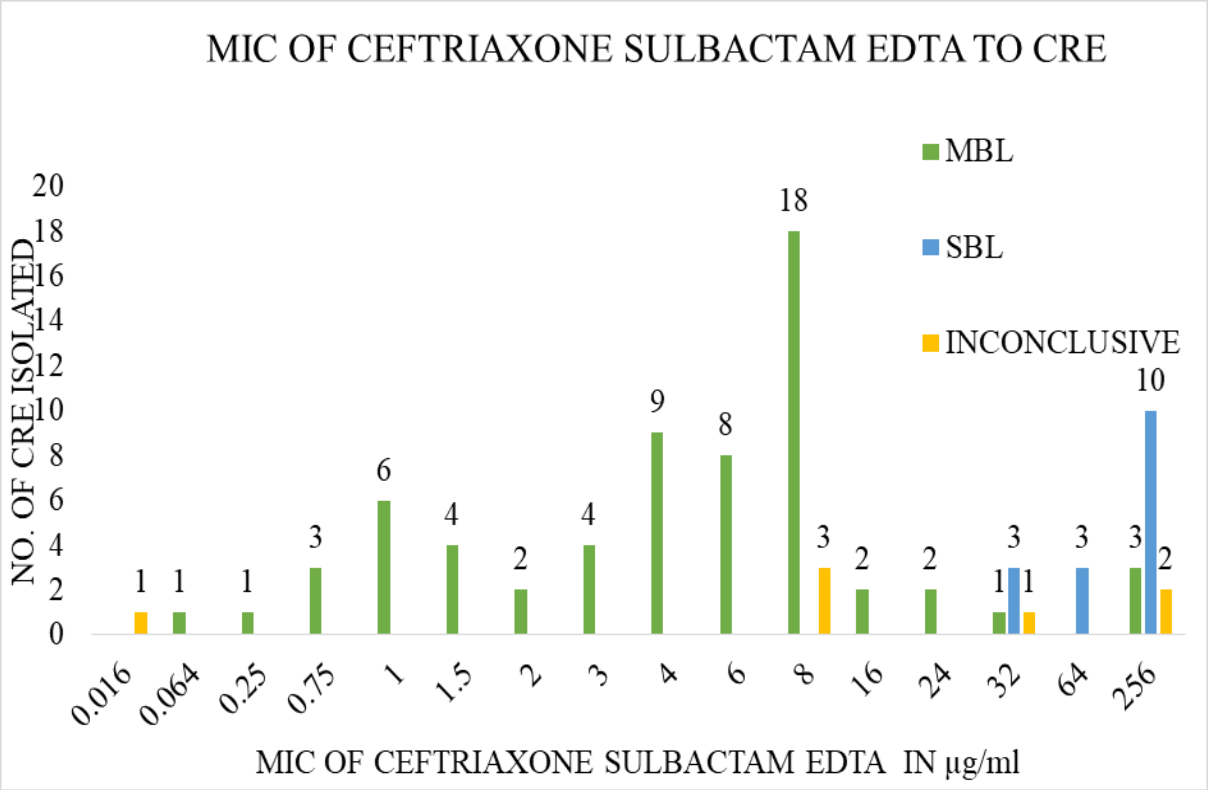
Graph.8 MIC of Ceftriaxone Sulbactam EDTA to ESBL



Graph.9 Sensitivity pattern of CSE to CRE



Graph.10 MIC of Ceftriaxone Sulbactam EDTA to CRE



The incidence of both Carbapenem-Resistant Enterobacteriaceae (CRE) and Extended-Spectrum Beta-Lactamase (ESBL) production showed a marked increase with age, reaching its highest in individuals over 60 years (29 and 42 cases, respectively), and was lowest in the 11–20 age group (2 and 1 cases, respectively). Similar findings were reported by Shilpakar *et al.,* where the majority of CRE isolates were from patients over 60 years (24 cases), with the lowest number found in the 11–20 age group (3 cases)[6]. Likewise, Achiraya Siriphap *et al.,* observed the maximum frequency of ESBL producers among patients aged over 60, accounting for 57% of cases.

The present location wise distribution showed that CRE cases were prevalent in Inpatients (49.4%), a similar result was observed in the study conducted by Pramurtajyoti Deb Barma *et al.,* which shows a higher prevalence of CRE in inpatient isolates (47.74%) and while ESBL cases were more common in outpatients (47%) followed by Inpatients (39%) and the study by Shilpakar *et al.,* demonstrated a significant increase in the prevalence of ESBL, in outpatients (50%) followed by inpatients (39%)[7]

The distribution of isolates by specimen type showed that urine was the most common source with 72.9% of ESBL and 36.8% of CRE, Ibaideya *et al.,* reported that the majority of samples were urine (60.7%) in multi-drug resistant *Enterobacterales* isolates.[8]

Klebsiella species were the most the predominant organism followed by *E.coli,* among both CRE (69% and 29% respectively) and ESBL isolates (62.5% and 23% respectively). Similarly, in the study by Kayastha K *et al.,* the highest percentage of ESBL production was found among *K. pneumoniae* (33.3%), followed by *E. coli* (27.9%) and *K. oxytoca* (16.7%)[9] and the study by S.Krithika *Klebsiella pneumoniae* (67.6%) was the most frequent CRE isolate, followed by Escherichia coli (29.7%)[10]

Phenotypic analysis of the CRE isolates showed that 73.6% (64 out of 87) produced metallo-β-lactamases (MBLs), 16 isolates (produced serine β-lactamases (SBLs), while 7 isolates (8%) yielded inconclusive results. Similarly, in the research carried out by Aboulela *et al.,* 52% (28 of 53 isolates) produced metallo-β-lactamases, 30% (16/53) produced serine

carbapenemases, and 9.4% (5/53) isolates gave inconclusive outcomes.[11]

In the current study, all ESBL producing isolates exhibited 100% sensitivity to ceftriaxone sulbactam EDTA with a prevalent MIC of 1 µg/ml and in case of CRE, metallo betalactamases showed 87.5% sensitivity,7.8% intermediate and 4.7 % resistance to ceftriaxone sulbactam EDTA with a prevalent MIC of 8 µg/ml whereas the Serine betalactamases showed 100% resistance.

In the current study, all ESBL-producing isolates demonstrated complete (100%) sensitivity to ceftriaxone-sulbactam-EDTA, with the most common minimum inhibitory concentration (MIC) being 1 µg/mL. Among CRE isolates, those producing metallo-β-lactamases (MBLs) showed 87.5% sensitivity, 7.8% intermediate sensitivity, and 4.7% resistance to the same combination, with a predominant MIC of 8 µg/mL. In contrast, isolates producing serine β-lactamases (SBLs) exhibited complete resistance to ceftriaxone-sulbactam-EDTA. Similarly, a study by Sahu M reported that CSE-1034 (a combination of ceftriaxone, sulbactam, and disodium EDTA) showed promising in-vitro activity against ESBL and MBL-producing isolates, with susceptibility ranging from 64% to 100% and intermediate responses between 18% and 22% for ESBL producers. For MBL producers, susceptibility ranged from 42% to 89%, with intermediate responses between 10% and 51%.[12]

Additionally, Chaudhary *et al.,* demonstrated that a formulation of ceftriaxone and sulbactam in a 2:1 ratio combined with 3 mg/mL of disodium EDTA (CSE-1034) significantly reduced MIC values by more than eightfold and showed synergistic effects against most ESBL-producing organisms.[13]

Furthermore, a study by S. Singh *et al.,* found that "CSE" was notably more effective against ESBL-producing Enterobacteriaceae (95% efficacy) compared to colistin (89%)[14]

In conclusion, this study identified a high prevalence of Extended Spectrum Beta-Lactamase (ESBL) (52%) and Carbapenem-Resistant Enterobacterales (CRE) (47%) among clinical Enterobacterales isolates, underscoring the growing challenge of antimicrobial resistance. The detection of such significant proportions brings to light the immediate necessity of need for effective alternative treatment options and enhanced surveillance.

This investigation brings attention to the significant therapeutic potential of the novel antibiotic combination Ceftriaxone-Sulbactam-EDTA (CSE), evaluated using the E-strip method, in addressing multidrug-resistant Enterobacterale infections. The results demonstrated that CSE exhibited 100% sensitivity against Extended Spectrum Beta-Lactamase (ESBL)-producing Enterobacterales and showed promising efficacy against Carbapenem-Resistant Enterobacterales (CRE), particularly metallo-beta-lactamase (MBL) producers.

Phenotypic characterization of CRE isolates further revealed that 70% were MBL producers, 18% were serine-beta-lactamase (SBL) producers, while 8% of the isolates showed inconclusive results. This distribution underscores the high prevalence of MBL-mediated resistance mechanisms in CRE, aligning with the observed high sensitivity of these strains to the EDTA-containing CSE combination.

CSE provides an effective and reliable therapeutic option for the control of ESBL, particularly in situations where treatment options are severely limited. It can serve as a viable alternative to carbapenems, helping to preserve these critical last-line antibiotics and slowing the progression of antimicrobial resistance.

Ceftriaxone-Sulbactam-EDTA holds significant clinical value in addressing infections caused by Carbapenem Resistant Enterobacterales, which are among the most challenging multidrug-resistant pathogens. In clinical settings, where treatment choices for CRE are limited, often expensive, and associated with toxicity (e.g., colistin or tigecycline), CSE offers a safer, more accessible, and cost-effective alternative. Its efficacy against both ESBL and a significant subset of CRE strains makes it a versatile option, especially in resource-limited settings.

Therefore, the implementation of CSE in routine antimicrobial susceptibility testing and its appropriate clinical use can play a pivotal role in improving patient outcomes, slowing resistance development, and preserving the utility of existing last-line antibiotics.

The CSE's effectiveness could contribute to improved treatment outcomes, reduced infection transmission, reduced hospitalization duration, and lower healthcare costs. Furthermore, its use can strengthen antimicrobial stewardship efforts and play a key role in mitigating the spread of antimicrobial resistance. The results confirm

CSE's potential as a crucial intervention in managing multidrug-resistant Enterobacterales infections within both healthcare and public health frameworks

Acknowledgement

First and foremost, I bow in gratitude to God Almighty for His abundant blessings, grace, and guidance that have led me through every step of this journey.

I express my deepest sincere gratitude to my honorable guide Dr. RESHMI GOPALAKRISHNAN MBBS, MD, Microbiology, Consultant Microbiologist, Aster MIMS Calicut, for her expert guidance and unwavering support throughout this study. Her insightful feedback, critical evaluation and timely advice have been invaluable in shaping this dissertation.

I wish to express my sincere gratitude to Mrs. SAVITHA M, Professor, Department of Microbiology, MIMS COAHS, Malappuram, for her constant support, constructive criticism, and encouragement, which greatly enhanced the quality of my work.

It is my pleasure to express genuine gratitude to Mr. SUHAS K T, Principal, MIMS COAHS, Mrs. SWATHY VISHWANATH, Assistant Professor, MIMS COAHS, Mrs. JINCY T.H, Associate Professor, Ms. DRISYA DINESAN, Assistant Professor, MIMS COAHS for their valuable help and friendly advices.

I sincerely thank Mrs. BINCHUPRIYA P, Assistant Librarian, MIMS COAHS for her invaluable assistance throughout the research. I also record my thanks to all Laboratory professionals and staffs of Department of Microbiology, Aster MIMS Calicut for their help and support in finishing this study. Without those blessings this project work would have been impossible.

I am thankful to my dear friends: ANJALI M.P, ATHULYA M.M, GAYATHRI. M & VISMAYA P.P for their companionship and encouragement during both good times and difficult moments. I am very grateful to all those who helped me to complete my project successful.

I owe my sincere gratitude to my family whose valuable support gave me courage and confidence throughout this project. I extend my whole hearted gratefulness for their blessings, prayers, and unfailing supports throughout the whole phase work. I take this opportunity to thank all teaching and non-teaching staff who made my path easier. I am very grateful to all those who helped me to complete my project successful.

Author Contributions Statement

Irfana - Conceived the original idea and designed the model, analysed, Investigated and wrote the manuscript. Dr. Reshmi Gopalakrishnan, Validation, Formal Analysis, Project Administration, Supervision. Prof. Savitha M, Manuscript review & Editing. Swathy Viswanath, Review and Editing. Anjali M P, Data visualization. Athulya M.M, Data validation and verification. Gayathri. M, Literature formatting and referencing. Vismaya P. P, Literature search and citation management.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Irfana, Reshmi Gopalakrishnan, Savitha M., Swathy Viswanath, Anjali M. P., Athulya M. M., Gayathri Meyana and Vismaya P. P. 2025. Susceptibility Pattern of Novel Ceftriaxone Sulbactam EDTA by E-Strip Method on Extended Spectrum Beta Lactamase and Carbapenem Resistant Enterobacterales Isolated in a Tertiary Care Hospital, Calicut. *Int.J.Curr.Microbiol.App.Sci.* 14(10): 201-214. **doi:** https://doi.org/10.20546/ijcmas.2025.1410.020