SSR Institute of International Journal of Life Sciences
ISSN (O): 2581-8740 | ISSN (P): 2581-8732
Das *et al*., 2024
DOI: 10.21276/SSR-IIJLS.2024.10.1.22

Research Article

open access

Prevalence and Antimicrobial Resistance of Extended-Spectrum Beta-Lactamase-Producing Gram-Negative Bacilli in Western Odisha: A Comprehensive Study

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Received: 13 Aug 2023/ Revised: 19 Oct 2023/ Accepted: 17 Dec 2023

ABSTRACT

Background: The escalating incidence of beta-lactam-resistant infections poses a substantial healthcare challenge. This study centres on Extended-Spectrum Beta-Lactamase (ESBL) production in Gram-negative bacilli in Western Odisha, addressing prevalence, antimicrobial susceptibility, and associated factors.

Methods: In a cross-sectional study in Western Odisha, 300 isolates were screened for ESBL production using standard microbiological methods. PCDDT, DDS, and TDT were employed as confirmatory tests to confirm antimicrobial susceptibility patterns. Regional variations were evaluated by comparison with previous studies.

Results: Among 300 isolates, 33% were ESBL producers, with *Escherichia coli* prevailing at 38.33%. This strain resisted beta-lactams (85.3%) and other higher antibiotics. PCDDT demonstrated heightened sensitivity for ESBL detection

Conclusion: ESBL prevalence in Western Odisha aligns with global and Indian studies. Resistance patterns are influenced by antibiotic overreliance and institutional outbreaks. Comparison with existing literature illuminates regional variations. The study underscores the urgent need for vigilance, timely detection, and judicious antibiotic use to counter the rising threat of ESBL-producing Gram-negative bacilli. Strategies emphasizing infection control measures and surveillance are pivotal for preserving antibiotic efficacy.

Key-words: ESBL, Antimicrobial resistance, Gram-negative bacilli, Prevalence, Antibiotic susceptibility

INTRODUCTION

The widespread occurrence of resistant bacteria threatens the efficacy of existing infection therapeutics in community and hospital settings. [1,2]

How to cite this article
Das L, Bhuyan SK, Choudhury S, Acharya DP, Prasad N, et al. Prevalence and Antimicrobial Resistance of Extended-Spectrum Beta-Lactamase-Producing Gram-Negative Bacilli in Western Odisha: A Comprehensive Study. SSR Inst Int J Life Sci., 2024; 10(1): 3599-3605.

Access this article online
https://iijls.com/

The compelling and versatile beta-lactam antibiotic class comprises almost half of all systemic antibiotics. Despite beta-lactam resistance predating the development of this crucial medication, the discovery of penicillin fifty years ago signalled the start of the antibiotic era.[3] The primary cause of bacterial resistance to beta-lactam antibiotics is the manufacturing of beta-lactamase. Although penicillin and second- and third-generation cephalosporins were designed to withstand significant beta-lactamases, the emergence of novel beta-lactamases resistant to every class already identified has resulted in resistance. The most recent development in

this class of enzymes is the emergence of Extended Spectrum Beta-Lactamases (ESBL). Mutations in TEM-1, TEM-2, and SHV-1 produce four ESBLs. These bacteria resist early penicillin at high concentrations and first-generation cephalosporins at low doses [2].

Being plasmid-mediated, they proliferated swiftly among the Enterobacteriaceae and possessed genes that conferred resistance to beta-lactamase and to antibiotics like quinolone and aminoglycosides.[4] These plasmid-mediated mutant β-lactamases provide resistance against all extended-spectrum cephalosporins and aztreonam, except cephamycins and carbapenems. Their origin is in previous broad-spectrum β-lactamases [5]. The first ESBL isolate was discovered in the middle of the 1980s in Western Europe; it relocated to the US in the late 1980s and has since piqued interest worldwide. [1]

Among other Enterobacteriaceae species, *K. pneumoniae, K. oxytoca,* and *E. coli* are the primary producers of ESBLs. Other bacteria, such as *Pseudomonas aeruginosa*, *Serratia marcescens,* and *Enterobacter* sp., also have ESBLs, albeit less commonly. The six main risk factors for colonisation or infection with ESBL-producing organisms are prolonged antibiotic exposure, prolonged stays in intensive care units, nursing home residency, severe illness, living in facilities with high ceftazidime and other third-generation cephalosporin use, instrumentation, and catheterization [6]. There has been a continuous increase in the global prevalence of ESBL-producing bacteria in clinical isolates.[7,8]

Although the prevalence of this beta-lactamase varies from 1.8% to 74% worldwide, it has been detected in 6.6% to 91.7% of cases in India [5]. An extensive examination of bacterial strains' resistance patterns and prevalence in each geographic area is necessary to provide guidelines for empirical therapy and implement preventative measures in hospital settings. This work aims to identify ESBL production in gram-negative clinical isolates from various clinical samples to establish an effective antibiotic strategy.

The study focused on determining the production of extended-spectrum beta-lactamases (ESBLs) by gram-negative bacteria, aiming to understand the frequency of ESBL production in this microbial group. Additionally, the research delved into the antibiotic response patterns exhibited by ESBL-producing gram-negative bacteria, contributing valuable insights into the susceptibility and resistance profiles within this context.

MATERIALS AND METHODS

The 24-month study, conducted by the Department of Microbiology at V.S.S. Medical College, Burla, spanned from October 2012 to October 2014. The study at the Department of Microbiology, VSS Medical College & Hospital, involved clinical samples for culture and sensitivity from outdoor and indoor patients. 300 random, non-repetitive clinical isolates of gram-negative bacilli obtained from diverse samples such as blood, pus, urine, sputum, and body fluids were included. Detailed patient history, encompassing the duration of illness, hospital stay, predisposing conditions, and clinical findings, was recorded in the performa.

Inclusion Criteria- Inclusion criteria comprised samples yielding gram-negative bacilli, reflecting the focus on this specific microbial group in the study.

Exclusion Criteria- Clinical isolates other than gram-negative bacilli were excluded from the study.

Statistical Analysis- Standard procedures for culture were followed, and isolates were identified using established methods. Gram-negative bacilli were preserved in egg saline medium for Enterobacteriaceae and Semisolid nutrient agar for non-enterobacteriacea. The Kirby-Bauer disk diffusion method was employed in adherence to CLSI guidelines M100-S24. Various antibiotics were utilized, and the zone of inhibition was measured and interpreted according to CLSI criteria. ESBL production was determined by specific zone sizes for different antibiotics.

Ethical Approval - The study obtained ethical approval, and consent was provided by the hospital committee overseeing research protocols.

RESULTS

During 24-month study period, 300 Gram-negative bacilli (GNBs) underwent an evaluation to produce Extended Spectrum Beta-Lactamase (ESBL). The study aimed to investigate the prevalence of ESBL production among these bacilli and analyze their antibiotic sensitivity patterns (Table 1).

Table 1: Age-gender distribution

Subjects		Number	Percentage (%)
Age in years	0-10	21	7
	11-20	39	13
	21-30	37	12.34
	31-40	40	13.34
	41-50	49	16.34
	51-60	59	19.67
	>61	55	18.34
Gender	Male	165	55
	Female	135	45
Type	Inpatient	241	80.34
	outpatient	59	19.66
Organisms	*E. coli*	115	38.33
	Klebsiella sp.	51	17
	Pseudomonas aeruginosa	49	16.33
	Citrobacter sp.	34	10.33
	Proteus sp.	28	9.33
	Acinetobacter	26	8.66

The male-to-female ratio was 1.2:1, with 165 (55%) male and 135 (45%) female patients. 241 (80.34%) of the 300 instances involved inpatients, while 59 (19.66%) involved outpatients. The age group 51-60 years old accounted for the most significant percentage (19.67%) of the 300 GNB isolates. The most common organisms were *E. coli* (38.33%), followed by Klebsiella sp. (17%), *P. aeruginosa* (16.33%), Citrobacter sp. (10.33%), Proteus sp. (9.33%), and Acinetobacter sp. (8.66%) (Fig. 2).

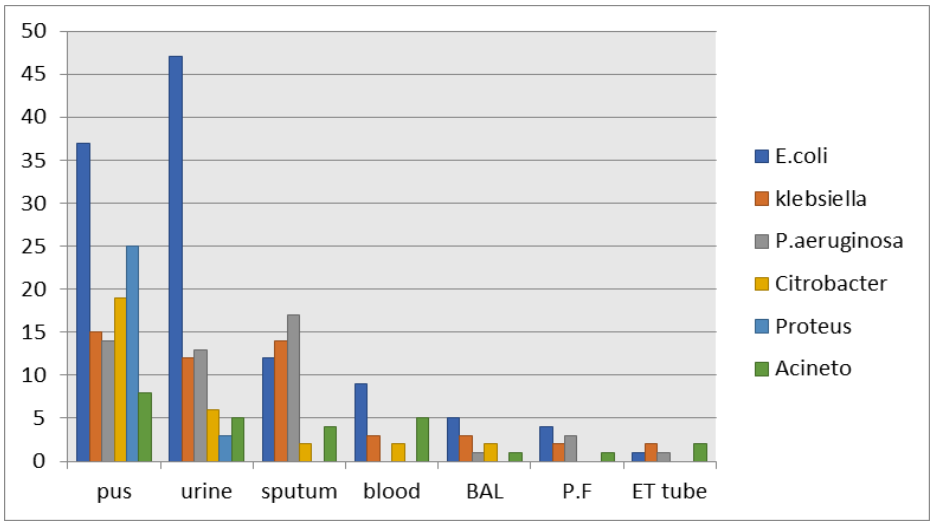

Fig. 1: Distribution of isolates from different clinical specimens

Fig. 1 illustrates the distribution of isolates from different clinical specimens, with pus (39.3%) and urine (28.6%) being the primary sources. Table 2 outlines the resistance of GNBs to 3rd and 4th generation cephalosporins (screening for ESBL). A significant proportion, 256 (85.3%), demonstrated resistance to Cefotaxime, Ceftazidime, and Cefpodoxime.

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Table 2: Gram-negative bacilli resistant to 3rd & 4th generation cephalosporins

Organisms	Cefotaxime		Ceftazidime		Cefpodoxime		Cefpime		Cefpirome		Aztreonam	
	No	%	No	%	No	%	No	%	No	%	No	%
E. coli (n=115)	101	87.8	101	87.8	101	87.8	98	85.2	96	83.4	98	85.2
Klebsiella sp (n=51)	46	90.1	46	90.1	46	90.1	45	88.2	45	88.2	43	84.3
P. aeruginosa (n=49)	38	77.5	38	77.5	38	77.5	32	65.3	32	65.3	33	67.3
Citrobacter sp (n=31)	26	83.8	26	83.8	26	83.8	22	70.9	22	70.9	26	83.8
Proteus sp. (n=28)	21	75	21	75	21	75	18	64.2	18	64.2	18	64.2
Acinetobacter sp. (n=26)	24	92.3	24	92.3	24	92.3	24	92.3	24	92.3	24	92.3
Total (n=300)	256	85.3	256	85.3	256	85.3	239	79.6	237	79	242	80.6

Out of 300 GNBs, 99 (33%) were confirmed as ESBL producers. *E. coli* was the most prevalent ESBL producer (40.6%), followed by Klebsiella spp. (54.9%). The prevalence of ESBL was highest in Klebsiella sp. at 54.9%. Of the 300 isolates, PCDDT (phenotypic confirmatory disc diffusion test) detected 99 (33%), DDS (double-disc synergy) detected 30 (10%), Direct TDT (double disc diffusion test) detected 13 (4.33%), and Indirect TDT detected 75 (25%).

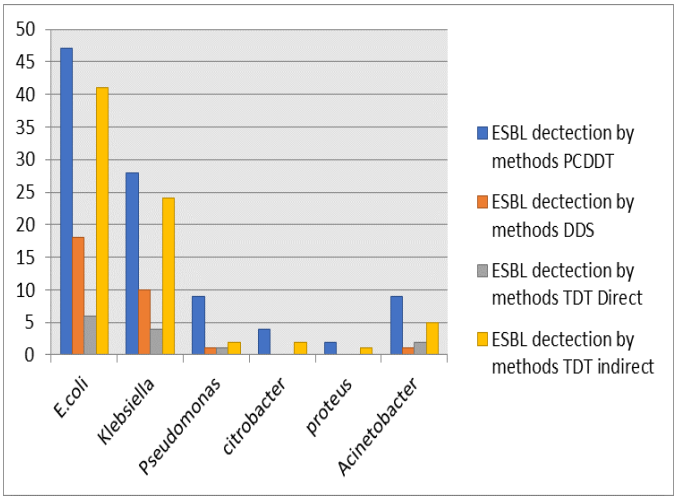

Fig. 2: Detection of ESBL in different methods

Statistical analysis revealed that PCDDT was superior to DDS and TDT (direct and indirect) with a chi-square value of 50.073, degrees of freedom of 2, and a significant p-value of 0.0001.

Table 3: Specimen-wise distribution of ESBL producers

Specimen	No. of isolates	ESBL Producers	Percentage (%)
Pus	118	46	38.98
Urine	86	31	36.04
Sputum	49	14	28.57
Blood	19	4	21.05
BAL	12	2	16.6
Peritoneal fluid	10	1	10
Endotracheal tube Aspirates	6	1	16.66
Total	300	99	33

The maximum number of ESBL producers were isolated from pus (38.98%), followed by urine (36.04%). ESBL producers were more prevalent in inpatients (38.58%) than outpatients (10.16%). Prevalence was highest in the ICU (83.33%) and Medicine ward (55.81%).

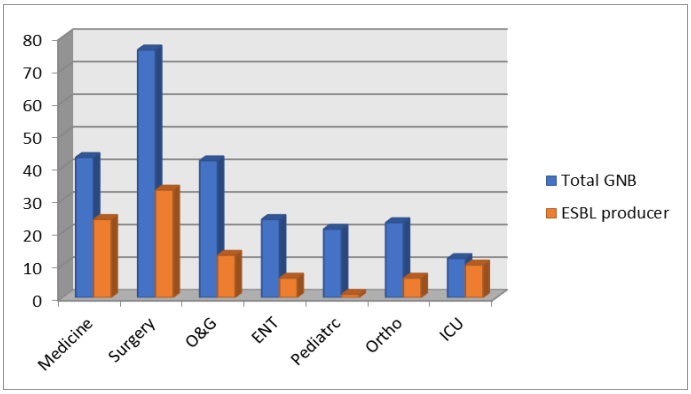

Fig. 3: Department wise distribution

All ESBL producers were 100% sensitive to Imipenem, 65.66% to Amikacin, 46.47% to Piperacillin-Tazobactam, and 43.44% to Ciprofloxacin.

DISCUSSION

The primary source of bacterial resistance to beta-lactam antibiotics is the synthesis of beta-lactamases, of which ESBLs is a well-known variant that is widespread. These plasmid-mediated enzymes are produced by unique strains, frequently linked to multi-drug resistance. The frequency of ESBL producers varies significantly throughout institutions and across regions. This study aims to evaluate the prevalence of ESBL and the antibacterial susceptibility patterns of GNBs, or gram-negative bacteria. [9,10] Thirty-three percent of the 300 isolates under analysis generated ESBL. After contrasting these results with those from previous studies, a discussion follows.

The most common age group for GNB isolation was 51-60 years (19.67%), followed by >60 years, while the least common age group was 0-10 years (7%). These results align with a study by Babypadmini and Appalaraju [11] but contrast with Gupta [12], where the most expected age group was 0-10 years (43%). Males constituted 55% of the total isolates, with females at 45%, resulting in a male-to-female ratio of 1.2:1. This male preponderance is consistent with studies by Menon *et al.* [13]; Mandell *et al.* [14]. A higher percentage of GNBs were isolated from inpatients (80.34%), like Chidambara (90.5%). Among the 300 gram-negative isolates in this study, *E. coli* (38.33%) was the most common, aligning with various studies. Klebsiella sp. constituted 17%, differing from some studies. Other GNBs like Pseudomonas (16%), Citrobacter sp. (10.33%), Proteus sp. (9.33%), and Acinetobacter sp. (8.6%) showed prevalence rates aligned with or higher than certain studies.

The potential dissemination of resistant genes drove the decision to screen ESBL in various GNBs beyond *E. coli* and Klebsiella sp. A significant portion (85.3%) of GNBs showed resistance to specific antibiotics, consistent with findings from Winn *et al*. [15] and Mostatabi *et al.* [16]. Additionally, 80.6% were resistant to Aztreonam, 79.6% to Cefpime, and 79% to Cefpirome. Upon confirmation, 33% were identified as ESBL producers. The prevalence of ESBL producers in this study (33%) aligns with global and Indian studies. In India, the prevalence ranges from 6.6% to 91.7%, placing our findings within the established range. Comparable prevalence rates were noted in studies by Teklu *et al.* [17]; Bush and Fisher [18]; and Siraj and Ali [19].

The maximum prevalence of ESBL was observed among Klebsiella sp. (54.9%), consistent with various studies but lower than others. Like certain studies, *E. coli* was the second most prevalent ESBL-producing organism (40.86%), but lower than others. The prevalence of Acinetobacter sp. in this study (34.6%) was like some studies but lower than others. Prevalence rates for Citrobacter spp. (12.9%) and Proteus sp. (7.14%) were in line with some studies but lower than others. Out of 300 GNBs, 256 were suspected ESBL producers based on the screening test. In the confirmatory tests, 99 (33%) were identified as ESBL producers using PCDDT, DDS, and TDT (direct and indirect). PCDDT demonstrated higher sensitivity for detecting ESBL production than DDS and TDT. [17-20] Among the three tests, PCDDT emerged as the most sensitive, cost-effective procedure for ESBL detection compared to DDST. DDS exhibited lower sensitivity due to issues with optimal disc space and correct storage of clavulanate. [21-24] TDT was more laborious than DDS and PCDDT. These findings are consistent with studies by Khan et al. and Shukla et al., which reported the superiority of PCDDT over DDS. In the present study, TDT outperformed DDS, like Thomson et al, Menon et al, and Datta et al. However, Vercauteren et al. reported DDS as superior to TDT in detecting ESBL.

The study revealed the highest number of GNBs isolated from pus, while Wilkinson *et al.* [24] reported the maximum number of ESBL producers from urine samples. Regarding prevalence among inpatients and outpatients, ESBL producers were found in 38.58% and 10.16%, respectively.

CONCLUSIONS

The rising prevalence of illnesses brought on by organisms resistant to beta-lactam antibiotics has emerged as a severe problem for healthcare in recent years. Identifying Extended-Spectrum Beta-Lactamase (ESBL) production is critical due to the possible widespread distribution of these strains, which pose a significant risk to currently available antibiotics. Institutional outbreaks are on the rise, fueled by selective pressure from expanded-spectrum cephalosporins and lapses in control measures. Vigilance, timely infection recognition, and appropriate antibiotic

therapy are deemed imperative. This study reveals a concerning trend, with 85.3% of isolates displaying resistance to beta-lactams and higher antibiotics, highlighting the impact of overreliance on these drugs for empirical treatment of Gram-negative infections. Addressing this challenge necessitates judicious antibiotic use, stringent hand hygiene practices, and robust infection control measures in hospitals.

The findings underscore the urgent need for a comprehensive approach, emphasizing surveillance, timely detection, and strategic interventions. Collective efforts are crucial to mitigate the spread of ESBL-producing Gram-negative organisms, thereby preserving the efficacy of available antibiotics and safeguarding public health.

CONTRIBUTION OF AUTHORS

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Article editing- Lopamudra Das

Final approval- Lopamudra Das

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