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PREVALENCE AND SENSITIVITY PATTERN OF ESBL PRODUCERS IN DIFFERENT CLINICAL ISOLATES FROM A TERTIARY HEALTHCARE CENTER OF EASTERN INDIA, A CROSS-SECTIONAL STUDY.

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ABSTRACT

Background

Antimicrobial-resistant organisms have led to increased mortality, morbidity, and economic burden throughout the globe. This study focused on measuring the prevalence of antimicrobial resistance bacteria mostly by extended-spectrum β-lactamase (ESBL) producers from several samples in a facility providing tertiary care in Eastern Odisha.

Methods

A cross-sectional study was conducted from February 2021 to January 2022. During that time a total of 2452 culture-positive specimens were processed from different samples. Identification of organisms and antibiotic susceptibility was done manually through Kirby Beuer's disc diffusion method. Phenotypic detection of ESBL producers was performed by a Double disc synergy test.

Results

During the study, E. coli (852) was identified as the most prevalent organism followed by S. aureus (661) and K. pneumoniae (301). Among them, 1571 isolates were ESBL-producing and E. coli was the most prevalent one followed by S. aureus and K. pneumoniae which were 659, 479, and 172 in number respectively. Most of the ESBL producers were isolated from urine samples and the least number from stool samples. We found in this study that the highest population of P.mirabilis and K. oxytoca were resistant to the fluoroquinolones group of antibiotics, Pseudomonas and K. oxytoca are highly resistant to aminoglycosides group of antibiotics, P.mirabilis, Enterobacter, P. vulgaris and Enterococci were showing high resistance towards penicillin group of antibiotics, P.mirabilis was highly resistant towards β-lactamase inhibitor group of antibiotics.

Conclusion

As per the study findings, E. coli is the main producer of ESBLs among members of the Enterobacteriaceae family, and urine is the main source of ESBL-positive isolates. These findings are highly significant from a medical and scientific standpoint and may influence policymakers to better monitor and manage antibiotic resistance.

***Keywords**: Antibiotic resistance, Antibiotic susceptibility, E.coli, ESBL, Gram-negative bacteria, K.pneumoniae E*
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INTRODUCTION

Now a day's World Health Organization (WHO) is facing a serious concern that is Antimicrobial Resistance (AMR). Medical systems and individual patients are struck by this AMR. The rapid increase in morbidity and mortality rates due to nosocomial and community-acquired multidrug-resistant (MDR) bacteria may pose a serious risk to public health throughout the Globe [1-5].

Beta-lactamases are produced by the bacteria itself and by genetic mechanism it leads to the resistance of bacteria to beta-lactam antibiotics [6-8]. Overuse and misuse of broad-spectrum antibiotics catalyze the spreading of ESBL-generating bacteria [9]. Extended-spectrum β-lactamase (ESBL) enzymes can hydrolyze maximum beta-lactam antibiotics such as cephalosporins, penicillins, and aztreonam but not carbapenems which can only be inhibited by clavulanic acid, the beta-lactamase inhibitor [10]. Recently in the Ambler classification; based on

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genotypic categorization ESBLs belong to class A, Metallo-beta-lactamase (MBL) belongs to class B, AmpCs belong to class C and Oxacillianase producers belong to class D. The Bush-Jacoby scheme distinguishes β-lactamase substrates based on phenotypic functional groups [11]. Conventional and automated techniques like Vitek-2, MicroScan WalkAway 96 Plus, and Phoenix 100 are used to detect the ESBL producers and simultaneously gene detection methods like polymerase chain reaction (PCR) are used to detect beta-lactamase genes like CTX-M, TEM, SHV, and OXA [12]. This study focuses on the intensity of ESBL-producing bacteria and their Antibiotic sensitivity pattern in Eastern India.

MATERIALS AND METHODS

Collection and detection of ESBL strains were done over one year (February 2021 to January 2022) in the Department of Microbiology, IMS & SUM Hospital, Bhubaneswar, Odisha, India. The study was approved by the IEC and IRB with approval no. Ref.no/IEC/IMS.SH/SOA/2021/294. Outpatients and inpatients from all departments, and all age groups from different clinical samples were included in the study. A total of 2452 different organisms from different samples i.e Urine (1208), Pus (432), Cerebrospinal fluid (CSF) and other body fluids (362), Sputum (120), Skin swab (110), Tracheal aspiration (60), blood (60), throat swab (46) as well as Stool (30) samples were collected and included in the study. Organism identification, their resistance pattern, and identification of ESBL producers were done by manual method.

Sample Collection and Processing

The patients mostly without prior antibiotic therapy were included in the study. Samples were collected with all aseptic precautions into sterile containers. Urine was collected by *clean catch midstream urine* (CCMSU) or after catheterization. Pus was collected in a sterile needle or through a swab stick from the infection site. The wound swab was collected with a sterile swab stick. CSF was obtained by the lumbar puncture and aspiration. Blood was collected with all aseptic precautions in a blood culture bottle containing Brain heart infusion (BHI) broth. Body fluids such as synovial fluid, pericardial fluid, peritoneal fluid, amniotic fluid, and pleural fluid were collected in a sterile screw cap bottle by needle aspiration. Sputum and Stool were collected in a wide sterile-mouthed container covered with a tightfitting lid [13, 14].

ETHICS STATEMENT

The study was approved by IEC with approval no. Ref.no/IEC/IMS.SH/SOA/2021/294.

Identification of Bacteria

All samples of blood, CSF, sputum, body fluid, pus, swabs, and other clinical specimens were cultured on MacConkey agar, blood agar, and chocolate agar. A urine sample was processed in CLED agar. Identification of bacteria was done based on colony characters, Gram staining, Motility, and biochemical tests [15, 16, 17].

Antibiotic susceptibility testing

For gram negative bacteria; ampicillin (AMP) (10 µg/disc), amoxicillin-clavulanic acid(AMC) (20/10 µg/disc), gentamicin (GEN) (30 µg/disc), amikacin (AK) (30 mcg/disc), norfloxacin (NX) (10 µg/disc), levofloxacin (LV) (10 µg/disc), ciprofloxacin (CIP) (5 µg/disc), nitrofurantoin (NIT) (30 µg/disc), ceftriaxone (CTR) (30 µg/disc), ceftazidime (CAZ) (30 µg/disc), piperacillin tazobactam (PIT) (100/10mcg/disc), cefoperazone (CS) (75 µg/disc), cefoperazone sulbactam (CFS) (75/10 mcg/disc), cefepime (CPM) (30 µg/disc) and For gram positive bacteria; ampicillin (AMP) (10 µg/disc), amoxicillin-clavulanic acid (AMC) (30 µg/disc), gentamicin (GEN) (10 µg/disc), amikacin (AK) (30 µg/disc), ciprofloxacin (CIP) (5 µg/disc), cefotaxime (CTX) (30 µg/disc), linezolid (LZ) (30 µg/disc), azithromycin (AZ) (30 µg/disc), oxacillin (Ox) (1 µg/disc), vancomycin (VA) (30 µg/disc), piperacillin (PI) (10 µg/disc), Penicillin-G(P) (10 µg/disc) were used to perform antimicrobial susceptibility testing by disc diffusion method. Their sensitivity pattern was measured based on CLSI guidelines [18].

Screening for ESBLs production

All isolates were screened for ESBL production by observing the zone of inhibition for the selected discs of cephalosporins i.e cefotaxime (30µg/disc) ≤ 27mm, ceftazidime (30µg/disc) ≤ 22mm, ceftriaxone (30µg/disc) ≤ 25mm and for aztreonam (30µg/disc) ≤ 27mm. Further, it was confirmed by, the increase in the zone size with the addition of an inhibitor (clavulanic acid) by ≥ 5mm.

Confirmatory tests for ESBL enzyme production:

Double disc synergy test

A lawn culture of the test organism (0.5 McFarland turbidity) was made on a Mueller-Hinton agar (MHA) plate using a sterile cotton swab. The disc of ceftazidime(CAZ) (30g) and CAZ (30g) + clavulanic acid (10g) was put with a 15 mm gap. The inoculated plates were incubated for 18–24 hours at 35 °C. The difference in zone diameter of about 5 mm was considered ESBL positive. [19] (fig-1 a,b).

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Fig. 1-a: ESBL Negative

Fig. 1-b: ESBL Positive

RESULT

During the study, a total of 2,452 numbers of organisms were isolated from different clinical samples among them, 1571 i.e. 64% of isolates were found to be ESBL producers. Most of the ESBL producers were from urine samples which 842 (53%) (70%) were isolated from urine followed by pus (65%), and the least no of producers i.e. 30 (1.2%) were from stool (Table-1).

Table -1: Distribution of ESBL producers in different Samples (n=2,452)

Specimen	Isolates number n	ESBL-producers n
Urine	1208	842
Pus	432	282
Blood	120	60
Cerebrospinal fluid and other fluid	326	165
Sputum	120	73
Skin swab	110	69
Tracheal aspiration	60	31
Throat swab	46	21
Stool	30	28
Total	2452	1571

From total 2452 number of isolates E.coli was the most prevalent strain (852/2452)followed by S. Aureus (661/2452), K. Pneumoniae (301/2452), Enterococci (175/2452), Pseudomonas (121/2452), Acinetobacter (110)/2452, Citrobacter (69/2452), K .oxytoca (51/2452), P .vulgaris (43/2452), P.mirabilis (31/2452), Enterobacter (272452) and Morganella (4/2452) (Table-2).

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Table -2: Distribution of Organism

Organism Name	Number
E.coli	852
Klebsiella pneumoniae	301
KKlebsiella .oxytoca	51
Pseudomonas	121
Enterococci	175
proteus	74
Citrobacter	69
Acinetobacter	110
Staphylococcus.aureus	661
Morganella	11
Enterobacter	27
TOTAL	2452

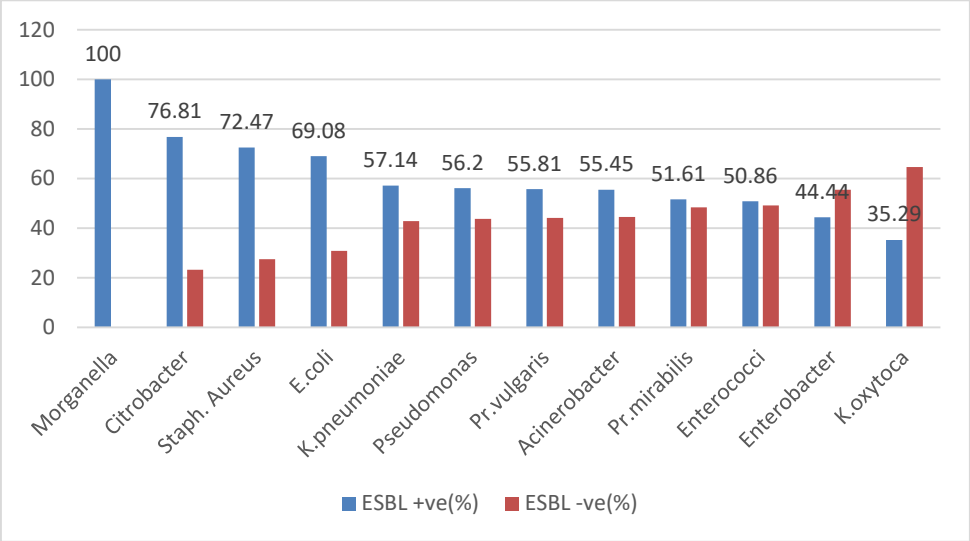

Fig. 2: Comparison of % of ESBL producers with non-producers

Among the ESBL producers, the most prevalent organism was Morganella (100%) followed by Citrobacter spp (76.8%) and the lowest number of producers was K. oxytoca (35.2%) (fig-2).

In the fluoroquinolone group, the highest number of strains of P. Mirabilis (93.54%) and the lowest number of strains of P. vulgaris (20.93%) were resistant to ofloxacin. 77.41% of P. mirabilis and the lowest number of Enterobacter i.e. 4.45% were resistant to gatifloxacin. Also, the highest K. oxytoca (66.67%) and lowest P. vulgaris (6.97%) were resistant to levofloxacin. It was found that E. coli was highly resistant towards gentamicin i.e. 139 from 354. A total number of 320 (33.54%) E. coli were resistant to AK out of which 97 were ESBL producers that are 30.31%. Likewise In the same group, it was found that 97 Pseudomonas were resistant to amikacin, out of which 32 were ESBL positive. The highest number of bacteria were resistant to gentamicin which was a total of 94 (67.62%) for Pseudomonas out of that 56 (59.57%) were ESBL positive. Most of the P.mirabilis (93.54%) strains were resistant to ampicillin whereas Staphylococci spp (18.3%) showed the least resistance towards this drug. It was also seen that the highest number of number Enterobacter (77.78%) and lowest number of Staphylococci (20.27%) were resistant to piperacillin. It was also seen that the highest number 77.78% number Enterobacter and Staphylococci (20.27%) were resistant to piperacillin.

It was seen that the highest number of strains of Morganella and Pseudomonas were resistant to CFM which is 75% and 68.6% respectively. The lowest number of E. coli were resistant to this antibiotic which was 7.75%. Likewise, the highest number of strains resistant to ceftazidime was

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91.3% in Citrobacter, and the lowest in case of 19% in Pseudomonas It was also seen that the highest number was 90.32% of P. mirabilis resistant to ceftriaxone, and the lowest number 14.67% Staphylococci

In the β-lactamase inhibitor group, the highest number of P. mirabilis (90.32%) was resistant to CAC and the lowest number of E. coli (16.14%) was resistant to this (Table 3)

Table 3: Percentage of all clinically isolated bacteria's resistance to different groups of antibiotics

	FLUOROQUINOLONES GROUP			AMINOGLYCOSIDES GROUP			PENICILLIN GROUP		THIRD GENERATION OF CEPHALOSPORIN GROUP.			B-LACTAMASE INHIBITOR GROUP
Bacteria	**OF**	**GAT**	**LV**	**AK**	**GEN**	**NET**	**AMP**	**PT**	**CFM**	**CTR**	**CAZ**	**CAC (CAZ+CA)**
Acinetobacter	29.09	19.09	15.45	22.72	29.09	19.09	31.82	29.09	33.63	42.72	50.9	34.54
Citrobacter	42.02	21.72	26.08	8.69	7.24	5.79	30.43	39.11	52.17	71.01	**91.3**	47.82
E.coli	26.6	18.65	8.7	-	-	-	20.75	24.21	7.75	12.68	33.96	16.14
Enterobacter	55.56	4.45	40.74	77.78	55.56	51.85	92.59	77.78	18.51	29.63	55.56	25.92
Klebsiella oxytoca	68.62	70.58	66.67	66.67	82.35	56.86	50.98	39.01	62.62	47.05	29.41	43.13
Klebsiella pneumoniae	30.23	62.13	30.56	61.46	47.5	11.62	42.56	28.9	32.22	23.9	29.9	32.55
Morganella	50	0	50	50	75	0	50	50	75	75	50	75
Proteusmirabilis	93.54	77.41	35.48	67.74	58.36	45.16	93.54	51.61	67.74	90.32	12.9	90.32
Proteus vulgaris	20.93	30.22	6.97	48.83	53.48	30.23	81.39	67.45	76.74	67.44	37.2	79.06
Pseudomonas	44.63	-	-	80.16	62.8	67.62	55.37	62.8	68.6	76.85	19	28.09
Staphylococcus aureus	34.2	42	41	32.4	21.1	-	18.3	20.27	16	14.67	-	-
Enterococcus	32.2	36.2	26.6	30	12.5	-	20.3	31.1	18	18	-	-

Also, the highest number of P. mirabilis (67.74%) was resistant to CFS. The highest number of strains resistant to AMC was 88.23% in K. oxytoca and the lowest in 9.95% in E. coli. The highest number of Morganella (75%) and the lowest number of E. coli (9.95%) were resistant to PIT(Table 4).

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Table 4: Percentage of resistance of all clinically isolated bacteria to another β-lactamase inhibitor group

Bacteria	CFS (CS+ sulbactam)	AMC (AMX+CA)	PIT (PT+tazobzctam)
Acinetobacter	35.45	38.18	39.09
Citrobacter	53.62	23.18	46.37
E.coli	10.16	9.95	70.86
Enterobacter	18.51	51.85	22.23
K. oxytoca	45.09	88.23	62.7
K.pneumoniae	29.56	76.41	30.89
Morganella	50	75	75
P.mirabilis	67.74	83.87	31.93
P. vulgaris	53.48	74.41	74.41
Pseudomonas	33.88	30.57	32.82

DISCUSSION

Key results and interpretation

E.coli was the most prevalent organism to be isolated and was mostly from urine samples. Morganella was the major ESBL producer in this study. Proteus mirabilis showed the highest resistance towards beta-lactamase inhibitor group drugs.

Comparison of results

Moorthy Kannaiyan et al in their study suggested that when compared to another source, urine (n=111) was the primary source for the ESBL-positive isolates, followed by pus (n=14) and stool (n=5) which is the same as our study where urine was the prime source for ESBL producers [19]. The majority of ESBL-producing strains were found in urine samples (85.38%), pus (10.76%), and stool (3.84%). However, according to a study by Sharma et al. [20], the respiratory tract samples, which had a high incidence of ESBL producers (63.83%), were the main source of ESBL-producing strains, followed by stool samples, urine, bodily fluids, pus, and blood.

Our study found that the prevalence of ESBL producers in different clinical samples was 71.2%. According to a survey from China, the percentage of ESBL manufacturers ranges from 25 to 40% [21, 22]. In India, the reported prevalence ranges from 28% to 84% in various institutions [23], however, according to [24] ESBL producers were 45% in their study.

In this study, From a total 2452 number of isolates, E. coli was the most prevalent (852/2452) strains followed by S. Aureus (661), K. pneumoniae (301), Enterococci (175), Pseudomonas (121), Acinetobacter (110), Citrobacter (69), K .oxytoca (51), P .vulgaris (43), P.mirabilis (31), Enterobacter (27) and organelle (4).

Moorthy Kannaiyan et al [20, 25] isolated 465 Gram-negative organisms from 1279 distinct clinical specimens with E. coli 320 (68.81%), P. aerogenes 119 (25.59%), and K. pneumoniae (26) (5.59%). E. coli (42.4%) and K. pneumoniae (28.5%) were the two most common isolates, according to a prior study by Choudhary et al. [26]. K. pneumoniae was the predominate isolate, according to a study done by Nazneen et al. (47%) [25], Menon et al. (47.14%) [27], Shobha et al. (45.62%) [28], and Nevine et al. [29] (41.17%). According to a study from Egypt, 46% of K. pneumoniae organisms produced ESBLs [30].

The most prevalent Enterobacteriaceae found in clinical samples according to [31] from New Delhi were E. coli (62%), followed by Klebsiella pneumoniae (73%), and these findings were very similar to this study[31].

In this study, it was found that ESBL producers have little action against carbapenem and β-lactamase inhibitors as 55.6% PIT sensitive, and 60.72% CFS sensitive. In our study, IMP showed the highest sensitivity (92.87%) against E. coli. For infections brought on by ESBL-producing isolates, carbapenem is the most effective and dependable antimicrobial drug currently on the market [32]. In that investigation, they found that the majority of the isolates were sensitive to imipenem (96.8%) and piperacillin/tazobactam (69.9%) [33]. But in this study, only 55.6% of isolates were found sensitive to PIT which was different from earlier studies.

Explanation- The varied results that were found in different studies may be due to differences in prevalent organisms and their mechanism of resistance acquired in different hospital environments.

CONCLUSION

According to this study, E. coli is the commonest ESBL producer and urine is the main source of ESBL producers. To stop the spread of these ESBL producers, it is advised to maintain a constant and vigilant watch, use appropriate detection techniques, and follow appropriate treatment protocols. The most effective way to recognize and distinguish between distinct ESBL types, which is necessary to deliver effective treatment, is genotypic identification.

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LIMITATIONS OF THE STUDY

This study could not include all drugs due to financial issues and as all the identification was done by manual method sometimes species level identification was difficult.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

None

ABBREVIATIONS

CLSI-clinical and laboratory standard institute
CLED-cystine lactose electrolyte deficient
CSF-cerebrospinal fluid
ESBL-extended spectrum beta lactamase
OF-ofloxacin
GAT-gatifloxacin
LV-levofloxacin
AK-amikacin
GEN-gentamicin
NET-netilmicin
AMP-ampicillin
PT-piperacillin
CFM-cefepime
CTR-ceftriaxone
CS-cefoperazone
AMX-amoxicillin
CA-clavulanic acid
PIT-piperacillin tazobacctum
CFS-cefoperazone sulbactam
AMC-amoxicillin clavulanic acid
E.COLI-escherichia coli

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