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Original Research Article

Prevalence of ESBL Producing Gram-Negative Bacterial Isolates from Surgical Site Infections in a Tertiary Care Hospital

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Conflict of interest: Nil

Abstract:

Background: Surgical site infection caused by organisms along with ESBL production was increasing from time to time. The purpose of this study was to detect the prevalence of ESBL producing gram negative bacterial isolates from surgical site infections.

Aims & Objectives:

1. To isolate and identify gram negative bacteria from various clinical samples.
2. To determine the antibiotic susceptibility pattern of these isolates.
3. To detect the ESBL production by phenotypic methods.

Materials and Methods: A cross sectional study was conducted in the Dept of Microbiology in Shimoga Institute of Medical Sciences attached to McGann hospital for a period of 6 months and during this period, 112 pus samples from clinically suspected patients with SSIs were collected from various surgical wards. They were identified and antimicrobial susceptibility testing of isolates was done. Detection of ESBL was done by screening test using antibiotics and confirmatory tests such as combined disk potentiation method and E test was done.

Results: A total no of 112 samples were collected of which 92 (82.14%) samples showed bacterial growth whereas remaining 20 (17.85%) samples showed no growth. Among 112 samples, 67 (59.82%) were males and 45 (40.17%) were females. Among 92 culture positive samples, 59 (64.13%) samples were Gram negative and 33 (35.86) were Gram positive isolates of which majority are *Escherichia coli* 25(27.17%) followed by *Staphylococcus aureus* 21(22.82%), *Klebsiella pneumoniae* 16(17.39%), *Coagulase negative staphylococcus* 12(13%), *Pseudomonas aeruginosa* 10(10.86%), *Acinetobacter species* 5(5.43%) and *Proteus species* 3(3.26%). All the Gram-negative isolates were sensitive to Imipenem. Majority of isolates showed resistance to Cefotaxime, Ceftazidime. Out of 59 Gram negative bacterial isolates, 49(83%) isolates were positive for screening test of ESBL. Among 49 positive screening tests, 26(53%) were confirmed by Combined Disk Potentiation method and 32(65.03%) were confirmed by E test. The prevalence of ESBL by confirmatory tests was 59.15%. Majority of ESBL producers were *Escherichia coli* (32.65%), *Klebsiella pneumoniae* (18.18%), *Pseudomonas aeruginosa* (5.1%).

Conclusion: The prevalence of ESBL-producing gram-negative bacteria causing SSI was high. So it is necessary for the microbiologists to routinely detect and report ESBL production in the laboratories which would help clinicians in the treatment. It also prevents the spreads of antimicrobial resistance. Strict infection control policies should be made and established along with the continuous review. Also, the clinical labs should be upgraded with appropriate tools and qualified staffs to identify newer drug resistance pattern or any evolving pattern of resistance among the isolates.

Keywords: SSI, Gram Negative Bacteria, ESBL Screening, Phenotypic Methods.

Introduction

Surgical site infection (SSI) is a type of healthcare-associated infection in which a wound infection occurs after an invasive (surgical) procedure. It has been shown that up to 20% of all infections related

to healthcare are surgical site infections. At least 5% of people who undergoes surgery develop a surgical site infection. Majority of surgical site infections (SSIs) are caused by contamination of the incision site with the patient's normal microbial flora or from exogenous source. Most of the SSIs are preventable by pre-, intra- and postoperative phases of care to reduce the risk of infection.[1]

The beta lactam antibiotics are the commonly prescribed antibiotics especially given as an empirical therapy in intensive care units. Most of these beta lactam antibiotics had developed resistance by producing beta lactamase and it's a major concern in these days. The Extended spectrum beta lactamase (ESBL) are plasmid mediated enzymes that hydrolyze the oxyimino-beta-lactam (3rd generation cephalosporins) and monobactams (aztreonam) but has no effect on cephamycins (cefoxitin and cefotetan).[2] Infections caused by them are multidrug resistant and are very difficult to treat and led to use of an expensive broad spectrum antibiotics. Identification of the causative organism and its antibiotic sensitivity pattern along with ESBL detection can help in timely management of surgical site infections.

Despite improvements in health care system, SSIs pose a major clinical problem as they are associated with increased mortality, morbidity, prolonged hospital stays, antimicrobial resistance and adds the financial burden to the patients.[3] ESBL pose a major problem in clinical therapeutics. Hence it is necessary to identify the prevalence of these strains routinely in the hospitals and to determine the preventive and control strategies.[2] The present study was undertaken to determine the prevalence of ESBL-producing gram-negative bacterial isolates from surgical site infections which would guide the physicians and microbiologists in the selection of the most appropriate antibiotics and also prevent the spread of antimicrobial resistance.

Aims & Objectives

1. To isolate and identify gram negative bacteria from various clinical samples.
2. To determine the antibiotic susceptibility pattern of these isolates.
3. To detect the ESBL production by phenotypic methods.

Methodology

Materials and Methods

A cross sectional study was conducted in the Department of Microbiology in Shimoga Institute of Medical Sciences attached to McGann hospital for a period of 6 months from May 2022 to October 2022. During this period, pus samples from clinically suspected patients with SSIs were collected from various surgical wards like General Surgery ward, Orthopedics, Obstetrics and Gynecology wards etc. Ethical clearance was obtained from the institutional ethical committee before the start of the study. Samples were collected after considering the inclusion and exclusion criteria.

Inclusion Criteria

Pus samples from clinically suspected surgical site infections.

Exclusion Criteria

- Wounds which are primarily healed were excluded.
- Pus swabs from patients who have not undergone surgery.
- Wounds without any clinical suspicion of surgical site infection.

Pus samples from the surgical wounds were collected by using sterile swab stick after cleaning with normal saline from the depth of the wound under aseptic precautions. The care was taken to avoid contamination from the normal microbial flora. Smears were made using the swab on clean glass slide. Gram staining was done and the morphology of the bacteria was noted.[4] The swabs were inoculated on Blood agar, MacConkey agar media, Thioglycolate broth and incubated at 37°C under aerobic conditions. If there was no growth on plates after 24 hours, Thioglycolate broth was checked for turbidity and subculture done if required.[5,6] The organisms was identified and speciated based on colony morphology and biochemical reactions. Antimicrobial susceptibility testing of isolates was done on Mueller Hinton agar (MHA) by Kirby Bauer disk diffusion method according to the CLSI guidelines. The sensitivity of the isolates to various antibiotics such as was Cefotaxime (CTX), Ceftriaxone (CTR), Ceftazidime (CTZ), Imipenem (IPM), Meropenem (MRP), Gentamycin (GEN), Ciprofloxacin (CIP) etc was determined according to CLSI guidelines.

Phenotypic test for screening ESBL production

It was done by Disc diffusion test using Ceftazidime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg) and Aztreonam discs (50µg). Isolates that was found to resistant to one or more of these antibiotics according to the CLSI guidelines were considered to be screening test positive for ESBL production.[7,8]

Phenotypic Confirmatory test for ESBL detection

Isolates that was positive for screening test were subjected to the confirmatory test by Combined Disk Potentiation method and E test

Combined Disk Potentiation method [9]

It was done using Ceftazidime (30µg) alone and in combination with Clavulanic acid (30µg/ 10µg). 0.5 Mc- Farland opacity of test organisms was inoculated into Mueller-Hinton agar (MHA) as lawn culture. The Ceftazidime (CAZ) discs alone and in combination with Clavulanic acid (CAC) were placed on MHA. Isolates showing increase of ≥ 5mm in zone of inhibition of CAC discs in comparison to the CAZ disc alone was considered to be ESBL producer.

E test [10]

The E test ESBL strip carries two gradients: on the one end, Ceftazidime; and on the opposite end, Ceftazidime plus Clavulanic acid. MIC was interpreted as the point of intersection of the inhibition ellipse with the E test strip edge. A ratio of Ceftazidime MIC to Ceftazidime-clavulanic acid MIC equal to or greater than 8 indicates the presence of ESBL.

Data Analysis

Microsoft Excel spreadsheet was used for data entry and analysis was done using statistical software (SPSS).

Data was presented in the form of percentages. Results was expressed using tables and charts.

Results

A total no of 112 samples were collected of which 92 (82.14%) samples showed bacterial growth whereas remaining 20 (17.85%) samples showed no growth. Among 112 samples, 67 (59.82%) were males and 45 (40.17%) were females. Mean age group affected was 40-60 years old.

Table 1: Frequency of samples according to the culture report

Culture	Frequency	Percentage
Growth positive	92	82.14%
Growth negative	20	17.85%
TOTAL	112	100.0

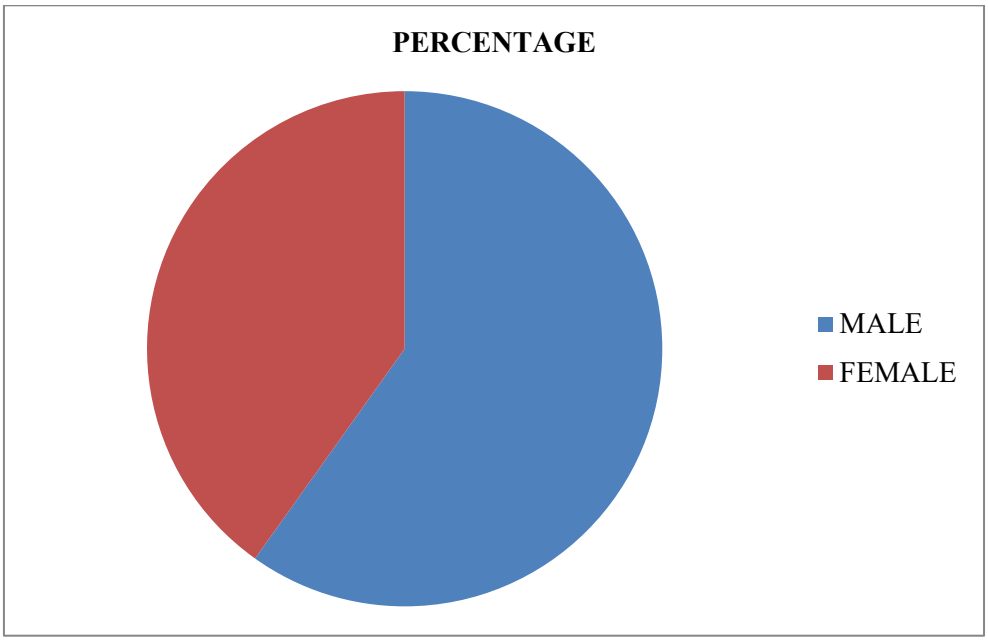

Figure 1: Pie chart showing Sex Distribution

Among 92 culture positive samples, 59 (64.13%) samples were Gram negative and 33 (35.86) were Gram positive isolates of which majority were *Escherichia coli* 25(27.17%) followed by *Staphylococcus aureus* 21(22.82%), *Klebsiella pneumoniae* 16(17.39%), Coagulase negative staphylococcus 12(13%), *Pseudomonas aeruginosa* 10(10.86%), *Acinetobacter species* 5(5.43%) and *Proteus species* 3(3.26%).

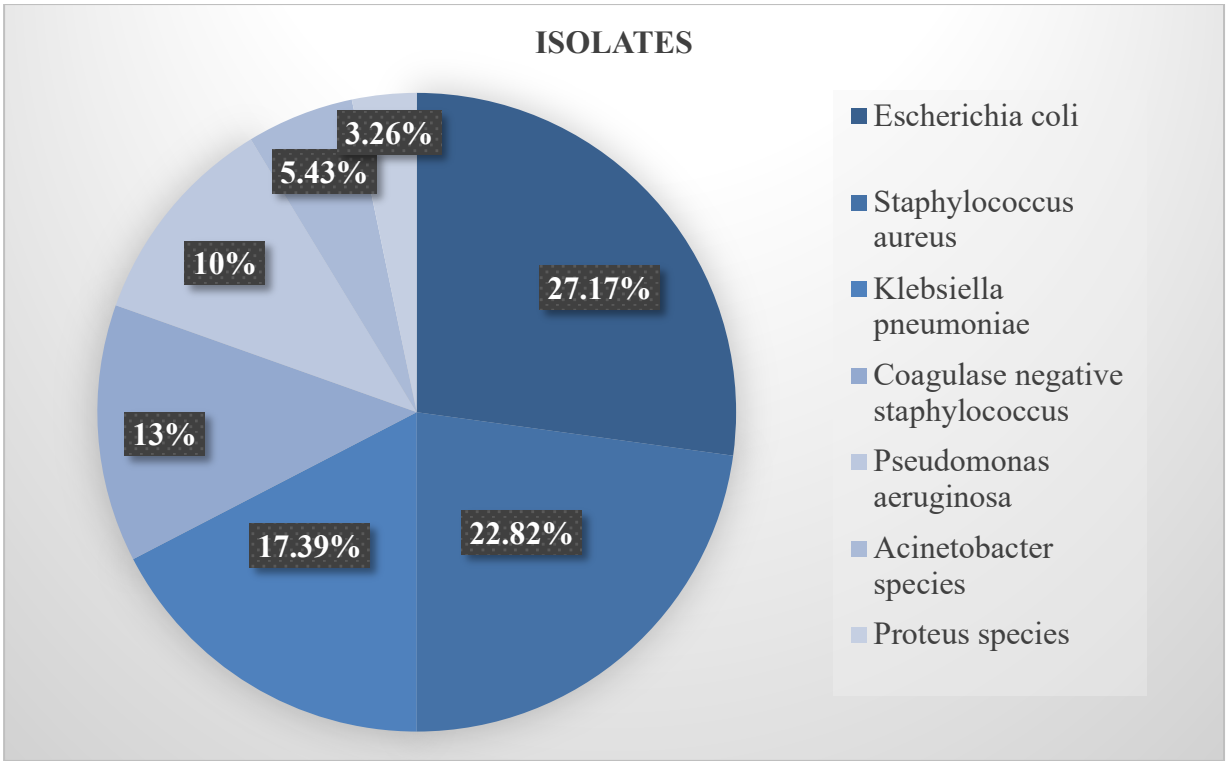

Figure 2: Pie chart showing various isolates

Out of 112 isolates, majority of isolates ie 47(41.96%) was received from General Surgery ward, 31(27.67%) from Ortho ward, 22 (19.64%) from OBG ward, 12(10.71%) from ENT ward.

All the Gram-negative isolates were sensitive to Imipenem. Majority of isolates showed resistance to Cefotaxime, Ceftazidime and it's given in the table below.

Table 2: Antibiotic sensitivity pattern showing % of resistant isolates

Antibiotics	***Escherichia coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Acinetobacter* species**	***Proteus* species**
Piperacillin Tazobactam (PIT)	20%	18.75%	20%	20%	33.33%
Ceftazidime (CAZ)	28%	81.25%	80%	40%	66.66%
Cefotaxime (CTX)	40%	68.75%	90%	20%	66.66%
Ceftriaxone (CTR)	24%	75%	80%	20%	66.66%
Cefepime (CPM)	48%	93.75%	90%	60%	66.66%
Meropenem (MRP)	4%	0%	0%	0%	55.33%
Imipenem (IPM)	0%	0%	0%	0%	0%
Gentamycin (GEN)	12%	12.5%	Not Tested	20%	33.33%
Tobramycin (TOB)	16%	18.75%	20%	20%	0%
Ciprofloxacin (CIP)	28%	37.5%	50%	60%	33.33%
Levofloxacin (LE)	24%	25%	10%	40%	0%
Amikacin (AK)	8%	12.5%	Not Tested	40%	0%
Aztreonam (AT)	8%	6.25%	20%	Not Tested	33.33%

Out of 59 Gram negative bacterial isolates, 49(83%) isolates were positive for screening test of ESBL. Among 49 positive screening tests, 26(53%) were confirmed by Combined Disk Potentiation method and 32(65.03%) were confirmed by E test.

The prevalence of ESBL by confirmatory tests was 59.15%. Majority of ESBL producers were Escherichia coli (32.65%), Klebsiella pneumoniae (18.18%), Pseudomonas aeruginosa (5.1%), Acinetobacter species (2.04%) and Proteus species (2.04%).

Table 3: No and % of ESBL Isolates among Gram Negative Bacteria

Gram negative bacteria	No of isolates tested for ESBL production		Phenotypic test for ESBL production					
			Detected by screening test		Detected by confirmatory tests			
					Combined Disk Potentiation Test		E Test	
	No	%	No	%	No	%	No	%
Escherichia coli	25	42.37%	23	38.98%	15	30.61%	17	34.69%
Klebsiella pneumoniae	16	27.11%	14	23.72%	8	16.32%	10	20.04%
Pseudomonas aeruginosa	10	16.94%	7	11.86%	2	4.08%	3	6.12%
Acinetobacter species	5	8.47%	3	5.08%	-	-	1	2.04%
Proteus species	3	5.08%	2	3.38%	1	2.04%	1	2.04%
Total % Of ESBL Production			49	83.02%	26	53%	32	65%

Discussion

Surgical site infections occurs due to the poor infection control practices which leads to prolonged hospital stay, increased costs and associated with significant mortality and morbidity. Most of the SSIs are uncomplicated involving skin and subcutaneous tissues but rarely can progress to necrotizing infections. It depends upon the patient related factors such as old age, waning immunity, immuno-compromised conditions, nutritional status and surgical factors like poor surgical techniques, prolonged duration of surgery, improper preoperative preparations etc.[11]

ESBL are the enzymes produced by variety of organisms like Enterobacteriaceae, *Pseudomonas aeruginosa* etc.[12] Failure to detect these enzymes leads to uncontrollable spread and therapeutic failure.[13] The prevalence of ESBL varies widely among the hospitals worldwide, geographical location and changing over time to time.

In the present study among 92 culture positive samples, 59(64.13%) samples were Gram negative and 33(35.86) were Gram positive isolates which was consistent with the study done by Madhavi RB *et al* 7 in which 61.6% were Gram negative and 32.6% were Gram positive isolates and also study done by Kanwalpreet Kaur *et* al [14] had similar findings.

In the present study, among 112 samples 67(59.8%) were males and 45(40.1%) were females. Similar observations were present with the study done by Madhavi RB *et al* [7] in which 54.3% were males and 45.7% were female patients.Out of the 92 culture positive isolates, majority were *Escherichia coli* 25(27.17%) followed by *Staphylococcus aureus* 21(22.82%), *Klebsiella pneumoniae* 16(17.39%), Coagulase negative staphylococcus 12(13%), *Pseudomonas aeruginosa* 10(10.86%), *Acinetobacter species* 5(5.43%) and *Proteus species* 3(3.26%). It was consistent with the study done by Islam *et al* [15] in which majority were *Escherichia coli* (29.4%) followed by *Staphylococcus aureus* (22.1%). It was contrary to the study done by Naz *et al* [16] in which majority were *Staphylococcus aureus* 51.1% followed by *Pseudomonas* species (20%) and *Escherichia coli*.

In the present study out of 59 isolates , prevalence of ESBL producing gram negative bacteria causing SSI by confirmatory tests was 59.15% and the majority of the ESBL producer was *Escherichia coli* (32.65%), *Klebsiella pneumoniae* (18.18%), *Pseudomonas aeruginosa* (5.1%), *Acinetobacter species* (2.04%) and *Proteus species* (2.04%). It was consistent with the study done by Ullah *et al* [17] in which the prevalence of ESBL was 58.7% and also study done by Agrawal P *et al* [18] was consistent with the present study in which majority of ESBL producers was *Escherichia coli* (30%) and *Klebsiella pneumoniae* (16%).

Conclusion

In present study, majority of isolates from surgical site infections were *Escherichia coli* 25(27.17%) followed by *Staphylococcus aureus* 21(22.82%), *Klebsiella pneumoniae* 16(17.39%), Coagulase negative staphylococcus 12(13%), *Pseudomonas aeruginosa* 10(10.86%).

Significant no of gram negative bacteria were identified to be ESBL Producers. Prevalence of ESBL among *Escherichia coli* (46.9%) was significantly higher than *Klebsiella pneumoniae* (27.1%).

The prevalence of ESBL producing gram negative bacteria causing SSI by confirmatory tests was 59.15%. Since the prevalence rate was high, it is necessary for the microbiologists to routinely detect and report ESBL production in the laboratories which would help clinicians in the treatment. It also prevents the spreads of antimicrobial resistance.

Strict infection control policies should be made and established along with the continuous review. Also, the clinical labs should be upgraded with appropriate tools and qualified staffs to identify newer drug resistance pattern or any evolving pattern of resistance among the isolates.

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