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Prevalence of Extended Spectrum Beta-Lactamase Producing Gram-Negative Bacilli causing Surgical Site Infections in a Tertiary Care Centre

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Abstract

Hospital-acquired infections (HAIs) are continuing to be a major risk in health care settings. World Health Organization (WHO) describes surgical site infections (SSIs) as one among the major health issue, causing enormous burden to both patients as well as doctors. Multidrug-resistant pathogens that cause SSIs continue to be an ongoing and increasing challenge to health care settings. The objective of the present study was to know the prevalence of extended-spectrum beta-lactamase (ESBL) producing gram-negative bacilli causing SSIs at a tertiary healthcare facility. The present cross-sectional observational study was done for a period of one year. Pus samples from clinically suspected cases of SSIs were collected and subjected to bacterial culture and sensitivity testing. From the total of 140 samples collected, a total of 138 bacterial isolates were isolated. Out of 138 isolates, 85 isolates (61.6%) were identified as gram-negative bacilli of which 33 isolates (38.8%) were identified to be ESBL phenotypes. Majority of the ESBL phenotypes were *Escherichia coli* (25.9%) followed by *Klebsiella pneumoniae* (7%), *Acinetobacter species* (2.4%), *Pseudomonas aeruginosa* (2.4%) and *Proteus species* (1.2%). Regular surveillance of antibiotic sensitivity pattern and screening for beta-lactamase production should be done which helps to know the trends of pathogenic bacteria causing SSI and guides in planning antibiotic therapy.

Keywords: ESBL, Surgical site infection (SSI), Hospital acquired infections (HAI), antimicrobial resistance

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INTRODUCTION

Hospital acquired infections (HAI) are the infections occurring after 48 to 72 hours of hospital admission[1]. They account to significant part of mortality and morbidity in hospitals, and it is estimated that approximately more than 90,000 deaths have occurred following 1.7 million HAIs during 2019[2]. Surgical site infection (SSI) is an infection that occurs after surgery in the part of the body where the surgery took place. SSI can be superficial involving only the skin or it could be more serious involving the tissues under the skin, organs, or implants[3]. Globally SSI is the third most common nosocomial infection after urinary tract infection and pneumonia[4].

Surgical site infection was a major cause of death during 19th century with nearly 80% of the patients undergoing surgery were troubled with "hospital gangrene". Introduction of antisepsis in the field of surgery by Sir Joseph Lister was a breakthrough in prevention of SSI and it turned out to be a boon to mankind[5]. In 20th century with the discovery of antibiotics, the surgical field was further reformed and made reconstructive, complicated and lifesaving surgeries possible. However, with the emergence of drug resistance in bacteria, SSI became one of the major challenges in healthcare system[5,6]. SSI infection is known to increase the burden, by increasing the duration of hospital stay, increasing mortality and adds to the financial burden of the patients[7]. Infections due to drug resistant gram-negative bacilli has narrowed the choice of antibiotics for treatment[8].

Extended spectrum beta-lactamase (ESBL) are the enzymes which inhibit the action of betalactam antibiotics by breaking the betalactam ring. Bacteria producing these enzymes possess resistant to betalactam antibiotics as well as to other class of antibiotics leading to therapeutic challenge to the surgeons[9]. Identification of the causative organism and its antibiotic sensitivity pattern if provided well in time can help in appropriate antibiotic therapy of SSIs. Objective of this study was to know the prevalence of ESBL producing gram-negative bacilli causing SSI and to know their antibiotic sensitivity pattern.

MATERIALS AND METHODS

The present cross-sectional study was done at department of Microbiology, JJM Medical College, Davanagere, Karnataka. Pus samples from clinically suspected patients with SSI were collected from various wards of hospital for a duration of one year (June 2015 to May 2016). Considering prevalence rate of surgical site infections, sample size was calculated by Cogent QC system and estimated sample size was 140. Ethical approval was obtained from Institutional ethical committee before the start of the study. (Ref. No.24/2014-15)

SSIs were considered according to the definition recommended by Centre for Disease Control (CDC) guidelines.3 Samples were collected from patients after considering inclusion and exclusion criteria defined.

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.
- Wound infection formed from cases directly related to skin, subcutaneous tissue, stitch abscesses, infected sebaceous cyst, episiotomy, newborn circumcision site.
- Wounds without any clinical suspicion of surgical site infection.

Sample collection and transportation

Pus samples from the surgical wounds were collected using two sterile cotton swabs after cleaning the wound with sterile normal saline. Samples were collected preferably from depth of the wound under aseptic precaution and care was taken to avoid contamination from normal flora of skin. Samples collected were transferred immediately to the laboratory for further processing. One swab was used for Gram stain and another swab was used for bacterial culture[10].

Microscopy, Culture and Sensitivity Testing

Using the first swab, smears were made on clean glass slides and Gram staining was done. Smears were screened for Gram reaction and morphology of bacteria was noted. Using the second swab, pus samples were inoculated on Blood agar, MacConkey agar and Thioglycolate broth and were incubated aerobically at 37°C for 18-24 hours. In case of no growth on plates

after 24 hours, the respective Thioglycolate broth were examined for turbidity and subcultured if required[11,12]. The bacterial colonies obtained were further processed and identified conventionally, based on colony morphology on culture plates and standard biochemical tests. Gram-negative bacilli were identified using motility test and biochemical reactions such as Indole test, Methyl red test, Voges Proskauer test, Triple sugar iron test and Citrate test. Antibiotic sensitivity testing was done on Muller Hinton agar by Kirby Bauer Disc Diffusion method and interpretation were done as per CLSI guidelines[13,14]. Following antibiotics were used for sensitivity testing of gram-negative bacilli: Ciprofloxacin(5µg), Gentamycin(15µg), Piperacillin-Tazobactam(100/10µg), Meropenem(10µg), Imipenem(10µg), Cefotaxime(30µg), Ceftazidime(30µg), Cefpodoxime(10µg), Ceftriaxone(30µg) and Cotrimoxazole(25µg).

Phenotypic detection of ESBL production

Phenotypic screening test- Phenotypic test for screening ESBL production was done by Disc diffusion test using Ceftazidime(30µg), Cefotaxime(30µg), Ceftriaxone(30µg), Cefpodoxime(10µg) and Aztreonam discs(50µg). A 0.5 McFarland standard suspension of the test organism was lawn cultured on Muller Hinton agar plate and above mentioned discs were placed approximately at a distance of 30 mm apart edge to edge and incubated at 37°C for 18 to 24 hrs. Zone of inhibition were measured carefully and interpretation was done according to CLSI guidelines. Accordingly, the zone diameters for the following antibiotics may indicate ESBL production: Aztreonam (AT 50µg) ≤ 27 mm; Ceftazidime (CAZ 30µg) ≤ 22 mm; Cefotaxime (CTX 30µg) ≤ 27 mm; Ceftriaxone (CTR 30µg) ≤ 25 mm and Cefpodoxime (CPD 10µg) ≤ 22 mm[14].

Phenotypic confirmatory test

Those isolates positive for screening test were subjected to confirmatory test by combined disc diffusion test using Ceftazidime-30µg(CAZ), Ceftazidime clavulanic acid 30/10µg(CAC), Cefotaxime 30µg(CTX) and Cefotaxime clavulanic acid 30/10µg(CEC) discs. A 0.5 McFarland standard suspension of the test organism was prepared, and lawn cultured on Muller Hinton agar plate and above mentioned discs were placed at an approximate distance of 30 mm apart edge to edge. After which plates were incubated at 37°C for 18 to 24 hrs. Isolate showing an increase in the zone diameter of 5 mm with either antimicrobial agent tested in combination with clavulanate versus the zone diameter of the antimicrobial
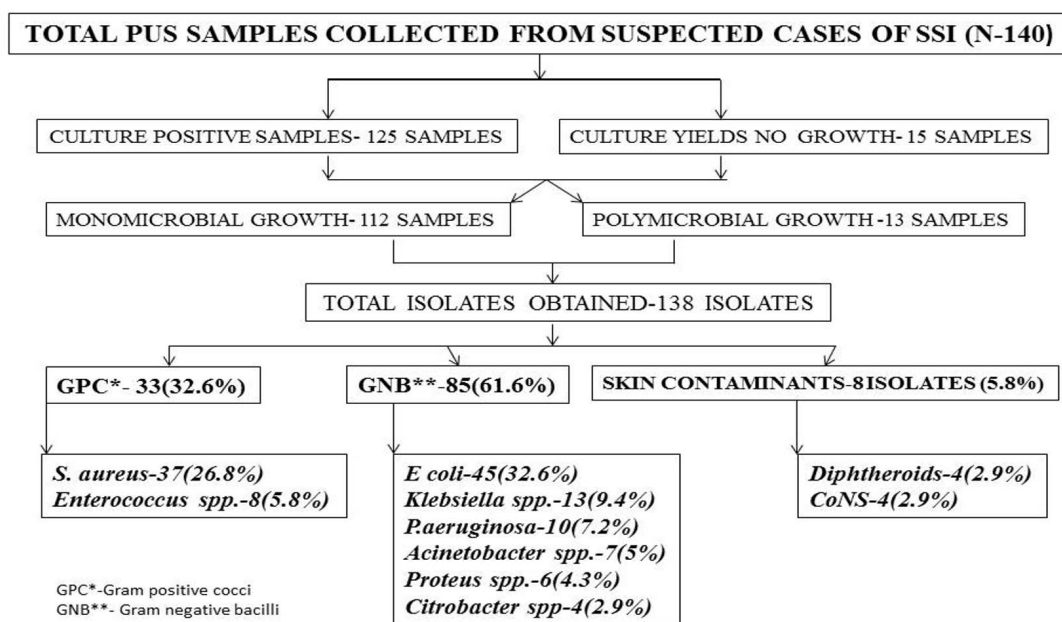
Fig. 1. Bacterial culture of the pus samples from surgical site infection.

Table 1. Antibiotic resistant pattern of gram-negative bacilli

Organism/ Antibiotics	E. coli n=45		Klebsiella pneumoniae n=13		Proteus species n=6		Citrobacter species n=4		P aeruginosa n=10		Acinetobacter species n=7	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ciprofloxacin (5µg)	22	48.9	5	35.5	3	50	3	75	10	100	7	100
Gentamicin (15µg)	15	33.3	4	30.8	1	16.6	3	75	6	60	6	85.7
Piperacillin-Tazobactam (100/10µg)	8	17.7	3	23.1	2	33.3	1	25	1	10	3	42.9
Meropenem (10µg)	2	4.4	1	7.7	0	0	1	25	2	20	2	28.6
Imipenem (10µg)	2	4.4	1	7.7	1	16.6	1	25	2	20	1	14.3
Cefotaxime (30µg)	21	46.6	3	23.1	0	0	3	75	2	20	6	85.7
Ceftazidime (30µg)	23	51.1	6	46.2	2	33.3	2	50	4	40	2	28.6
Cefpodoxime (10µg)	14	31.1	5	38.5	2	33.3	2	50	4	40	1	14.3
Ceftriaxone (30µg)	18	40	4	30.8	1	16.6	3	75	2	20	2	28.6
Ceftazidime-clavulanic acid (30/10µg)	22	48.8	5	38.5	1	16.6	1	25	4	40	2	28.6
Cefotaxime-clavulanic acid (30/10µg)	21	46.6	5	38.5	1	16.6	1	25	2	20	1	14.3
Cotrimoxazole(25µg)	13	28.8	7	53.8	3	50	2	50	-	-	-	-

Table 2. ESBL production among gram-negative bacilli from surgical site infections

Gram-negative Bacteria	No. of isolates tested for ESBL production (n=85)		Phenotypic detection of ESBL production			
			Detected by Screening test		Detected by Confirmatory test	
	No.	%	No.	%	No.	%
E. coli	45	52.9	24	28.2	22	25.9
K pneumoniae	13	15.3	6	7	6	7
Proteus species	6	7	2	2.3	1	1.2
Pseudomonas aeruginosa	10	11.8	4	4.7	2	2.4
Acinetobacter species	7	8.2	2	2.4	2	2.4
Citrobacter species	4	4.7	0	0	0	0
Total % of ESBL production			38	44.7	33	38.8

agent alone were considered to be phenotypic confirmatory test positive[14,15].

Statistical analysis

Data was entered in Microsoft excel data sheet and was analysed using SPSS 22 version software (IBM SPSS Statistics, Somers NY, USA). Categorical data was represented in the form of frequencies and proportions.

RESULTS

From total 140 patients, 76 (54.3%) were male patients and 64 (45.7%) were female patients with male: female ratio of 1.2:1. In the present study a total of 140 samples were collected and subjected for bacterial culture and sensitivity (Fig. 1). Out of 140 samples, 125 samples (89.3%) showed growth and 15 samples (10.7%) showed no growth. Out of 125 samples which showed growth, 13 samples yielded polymicrobial and 112 samples yielded monomicrobial growth, hence a total of 138 isolates were obtained. Out of 125 culture positive cases, 72 samples (57.6%) were collected from wounds over abdominal wall and 40 samples (32%) were collected from wounds over limbs and

few samples from wounds over the groin, thorax, cheek, and neck. In our study 85 isolates (61.6%) were identified as gram-negative bacilli, 45 isolates (32.6%) were gram-positive cocci, and 8 isolates (5.8%) were identified as skin contaminants. The most common bacteria causing SSI in our study is *Escherichia coli* 45(32.6%) followed *Staphylococcus aureus* 36(26%), *Klebsiella pneumoniae* 13(9.4%) and *Pseudomonas aeruginosa* 10(7.2%). Out of 85 gram-negative bacilli causing SSI, 33 isolates (38.8%) were found to be ESBL producers and *E. coli* was the most common ESBL producer, followed by *Klebsiella pneumoniae* 6(7%), *Pseudomonas aeruginosa* 2(2.4%), *Acinetobacter species* 2(2.4%) and *Proteus species* 1(1.2%). Antibiotic resistant pattern and distribution of ESBL producing gram-negative bacilli is shown in Table 1and Table 2, respectively.

DISCUSSION

In the present study out of 85 gram-negative bacilli, 33 (38.8%) were phenotypically identified as ESBL producers. Recent studies have shown that many healthcare setups are facing issues due to ESBL producing bacteria[16,17]. ESBL producing pathogens are difficult to treat and are serious public health concern. Infections due to such resistant pathogens can convert the non-fatal cases to fatal condition making it difficult to treat[18,19]. Genes coding for ESBLs are carried on bacterial chromosomes and can move among bacterial population either by inheritance or by plasmids[20]. Such drug resistant pathogens are of great concern, as they cause fatal infections and increases the duration of hospitalization, thereby increasing the cost of treatment[21]. The prevalence of ESBL producing bacteria varies worldwide. Data from the Tigecycline Evaluation and Surveillance Trial (TEST) global surveillance database says that *K. pneumoniae* showed highest ESBL production rate which were collected in Latin America, followed by those of the Asia/Pacific rim, Europe, and North America (44%,22.4%,13.3% and 7.5%, respectively)[22].

In the present study, prevalence of ESBL producing GNB causing SSI is 38.8%. E coli was the most common ESBL producer with 22 isolates (25.9%), followed by *K. pneumonia* 6(7%), P

aeruginosa 2(2.4%), *Acinetobacter species* 2(2.4%) and *Proteus species* 1(1.2%). In a study done by Akhilesh P.S et al, *E coli* (36.9%) was the most common pathogen causing SSI, 11.66% of gram-negative bacilli were ESBL producers and it was also found that 71.13% of gram-negative bacilli were MDR strains (Multi Drug Resistant strains)[23]. In a study done by Kasukurthy L R et al gram-negative bacilli were the predominant pathogens of SSI, *K pneumoniae* (29%) was the most common isolate followed by *E coli* (22%) and prevalence of ESBL production was 44% of gram-negative bacilli were ESBL producers[24]. ESBL prevalence rates of 35% to 85% have been reported in different places of India, which could be due to different geographic locations, antimicrobial susceptibility pattern and different detection methods[25].

Our study indicates that, tests targeting detection of resistant bacteria and strict hospital infection control programs, investigating ESBL phenotypes causing SSI should be considered with high priority.

Antibiotic resistant pattern of the gram-negative isolates is shown in Table 1. In the present study members of family Enterobacteriaceae showed significant resistance to Ciprofloxacin, third generation Cephalosporin and Cotrimoxazole. However, they were sensitive to Piperacillin-tazobactam, Imipenem and Meropenem. Among the non-fermenters (*Acinetobacter species* and *P. aeruginosa*), isolates showed significant resistance to Ciprofloxacin and Gentamycin, but were sensitive to Imipenem, Meropenem and Betalactam-betalactam inhibitor combination like Piperacillin-tazobactam, Cefotaxime-clavulanic acids. In the present study most of the gram-negative isolates causing surgical site infection were resistant to commonly used antibiotics. However, Carbapenems and Betalactam-betalactam inhibitor combinations remained sensitive which could be considered for treatment. Significant increase in the antimicrobial resistance is reported worldwide as per the third national summary of NHSN (National Healthcare Safety Network) and from a global systematic review[26,27]. Antibiotic resistant pattern may differ in each country, also in different region which could be due to genetic alterations and improper antibiotic usage[2].

CONCLUSION

In the present study *E. coli* [45isolates (32.6%) was the most common pathogen causing surgical site infection. Significant number of gram-negative bacilli were identified to be ESBL producers 33(38.8%). These drug resistant strains can spread and cause cross infections in the hospitalized patients. Antibiotic resistance surveillance should be carried periodically to know the resistant trends and for appropriate antibiotic therapy.

Healthcare setups should protocolize strict infection control practices, follow best practices in management of SSI and educate the healthcare workers regarding rational use of antibiotics.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both the authors have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

DATA AVAILABILITY

All the data sets generated and analyzed during this study are included in the manuscript.

ETHICS STATEMENT

The study was approved by the institutional Ethics committee, JJM Medical College, Davangere, Karnataka, India (Ref. No.24/2014-15)

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