INNSPUB

International Journal of Microbiology and Mycology | IJMM |
pISSN: 2309-4796
http://www.innspub.net
Vol. 14, No. 4, p. 1-9, 2022

Open Access RESEARCH PAPER

Prevalence of extended spectrum beta lactamases and AmpC producing gram negative bacilli among surgical site infections at a Tertiary Care Hospital

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Keywords: Surgical-site infections, Extended spectrum, Beta lactamases, AmpC

Publication date: April 10, 2022

Abstract

Infection control professionals, Clinicians, Microbiologists are concerned about Gram negative bacteria producing Extended spectrum Beta lactamases (ESBL) and AmpC because they pose a challenge of effective antimicrobial therapy resulting in adverse patient outcomes. The objective of the present study is to know the prevalence of ESBL and AmpC thereby making appropriate changes in the choice of antimicrobial therapy and minimizing treatment failures. To know the prevalence of Extended Spectrum Beta Lactamases and AmpC producers among Gram Negative isolates from pus samples suspected of Surgical-Site Infections (SSI). This study is a prospective cross-sectional study. A total of 100 samples from wounds in General Surgery, Obstetrics Obstetrics-Gynaecology, Orthopaedic, ENT and Ophthalmology departments, which were suspected of surgical site infection submitted to the Microbiology Laboratory of Karnataka Institute of Medical Sciences, were included in the study. Out of the total 100 cases, 17 (21.3%) were ESBL producers and *Escherichia coli* was the most common ESBL producing bacteria. 31 (38.8%) were AmpC producers and *Klebsiella species* were the most common AmpC producing bacteria. 27 (33.8%) were both ESBL and AmpC producers/ Co-producers. In the present study, we found an increasing number (21.3%) of ESBL and (38.8%) AmpC producing Gram negative isolates. ESBL and AmpC producing strains were found to show higher rates of resistance to various class of antibiotics when compared to non ESBL and non AmpC producers. The indiscriminate use of cephalosporins should be limited, which helps to minimize the emergence of resistance.

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Introduction

Surgical Site Infection by definition refers to an infection which occurs within 30 days after the surgery or within 1 year when an implant is left in place after the surgery and involving the incision or deep tissues at the operated site or infections involving organ or body space other than the incision, which was opened or manipulated during an operation (Mangram AJ *et al*., 1999). SSIs are classified by the Centre for Disease Control (CDC), USA into superficial incisional SSI, deep incisional SSI, and organ/space SSI (Sattar F *et al*., 2019). Surgical wound infection has plagued surgeons since time immemorial. It is considered as one of the most common form of nosocomial infection (Siguan SS *et al*., 1990). Surgical site infections are the third most commonly reported nosocomial infection and they account for approximately a quarter of all nosocomial infections (Lilani SP *et al*., 2005).

Surgeons are facing a major problem due to surgical-site infections (SSI). In spite of better advances in earlier diagnosis of surgical problems and improved techniques for postoperative surveillance, surgical-site infections continue to occur (Beauchamp, R.D *et al*., 2017). The complications at the surgical site after surgery has brought disgrace to the operating surgeon and also a misfortune to patients due to prolonged recovery time by about 7-10 days increasing the cost of treatment (Siguan SS *et al*., 1990; Hanifah YA, 1990).

Organisms causing surgical wounds can be a part of patient's normal flora or other infected patients or can be acquired from the hospital environment.[7](Anguzu JR, Olila D, 2007) Extended Spectrum beta Lactamases (ESBL) production in Enterobacteriaceae is regarded as one of the important factors for the development of resistance to most of the beta lactam antibiotics (Eliopoulos, G.M. and Bush, K., 2001). The common producers of ESBL are certain strains of Escherichia coli and Klebsiella pneumoniae in many parts of the world (Pena C *et al*., 2006; Rupp ME, Fey PD, 2003). In India, high rates (36.5%) of AmpC β-lactamase (ABL) producing Enterobacteriaceae have been reported (Baral P *et al*., 2013).

The present study was undertaken to provide the surgeons with an up-to-date information on bacteriological species isolated in cases of postoperative wound infections. The study was also undertaken to know the prevalence of ESBL and AmpC producing organisms from surgical site infection which helps the surgeons to treat the patients by giving appropriate and required antibiotics and to reduce the complications after surgery and also to decrease the financial burden on patients by reducing the time of recovery after surgical procedures, duration of hospital stay, morbidity and mortality.

Materials and methods

This is a prospective cross-sectional study. It was conducted in the Department of Microbiology, Karnataka Institute of Medical Sciences, Hubballi for a period of one year. A total of 100 pus samples which were suspected of surgical site wound infection were submitted to the Microbiology laboratory from General Surgery, Obstetrics-Gynecology, Orthopedic, ENT and Ophthalmology departments.

The discharge from the infected site was and were immediately transported to the laboratory for culture and antibiotic sensitivity testing. Received swabs were processed and identified as per standard protocol (ForbesBA *et al*., 2007). Antibiotic susceptibility was performed by Kirby-Bauer disk diffusion method according to standard guidelines (Clinical and Laboratory Standards Institute, 2021).

ESBL

Screening test: (Clinical and Laboratory Standards Institute, 2021; Paterson DL, Bonomo RA, 2005). A zone diameter of ≤27mm for

Cefotaxime 30µg disk and ≤22mm for Ceftazidime 30µg was observed as screening test positive for ESBL. All isolates (*Escherichia coli*, *Klebsiella species* and *Proteus mirabilis*) which were screening positive were subjected to phenotypic confirmatory tests for ESBL detection.

Confirmatory test: (Clinical and Laboratory Standards Institute, 2021; Paterson DL, Bonomo RA, 2005) The MHA plates were lawn cultured with the isolate. In one plate, ceftazidime disk (30µg) alone and in combination with clavulanate (10 µg) were placed at a distance of 20 mm. In another plate Cefotaxime disks (30µg) alone and in combination with clavulanate (10 µg) were placed at a distance of 20 mm. Both the plates were incubated for 24 hours at 37°C. A≥ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone was confirmed as ESBL positive as shown in fig 1 and 2.

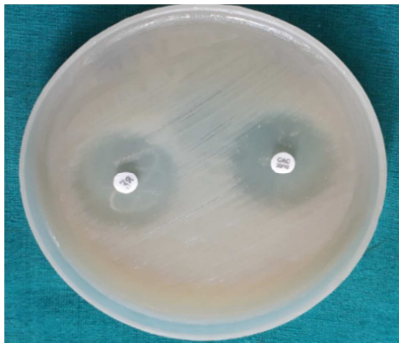

Fig. 1. Phenotypic confirmatory test for ESBL: CaC-Caz<5mm; Isolate - ESBL negative.

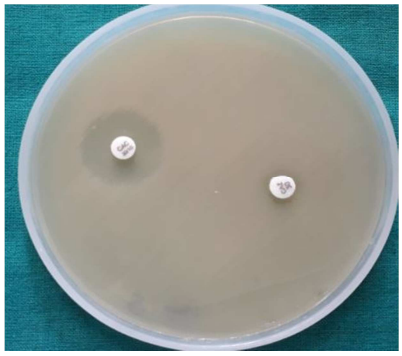

Fig. 2. Phenotypic confirmatory test for ESBL:Cac-Caz>5mm; Isolate - ESBL positive.

Amp C

Screening test: (Singhal S *et al*., 2005). Isolates with cefoxitin zone of <18 mm was considered as screen positives for Amp-C beta-lactamase production.

All the Amp-C screen positive isolates were subjected to Modified three-dimensional test and AmpC disk test for confirmation.

Confirmatory test

*Modified Three-Dimensional Test:*_(Singhal S *et al*., 2005). A pre-weighed sterile microcentrifuge tube was used into which the fresh overnight growth from MHA was transferred. The tube is weighed again to obtain 10^{15} mg of bacterial wet weight. The above bacterial mass was suspended in peptone water and centrifuged at 3000 rpm for 15 minutes to obtain a pellet. Repeated freeze-thawing of the bacterial pellet (10 cycles) was done to get the crude enzyme extract. Lawn culture of Escherichia coli ATCC 25922 was done on MHA plates and cefoxitin (30µg) disks were placed on those plates. Three-centimetre linear slits were cut using sterile surgical blade, 3mm away from cefoxitin disk. 30µl of the enzyme extract was loaded into the slit. The plates were kept upright, dried for 5 to 10 minutes and incubated at 37°C for 24 hours. A clear distortion to the zone at the point where the slit intersected the zone of inhibition of cefoxitin was considered positive three-dimensional test and was interpreted as Amp-C β-lactamase producers. Isolates with no distortion were recorded as non-Amp-C producers as shown in fig 3.

AmpC disk test: MHA plate was lawn culture with *Escherichia coli* ATCC 25922 was prepared on. Sterile disks (6mm) were moistened with sterile saline (20µl) and inoculated with several colonies of test organism. The inoculated disk was then placed beside a cefoxitin disk (almost touching) on the inoculated plate. The plates incubated overnight at 37^0C. Flattening (weak) or indentation (strong) of the cefoxitin inhibition

zone in the vicinity of the test disk was observed as positive test. Isolates with no distortion of zone of inhibition of cefoxitin were considered as negative test (Singhal S *et al*., 2005; Taneja N *et al*., 2007) as shown in fig 4.

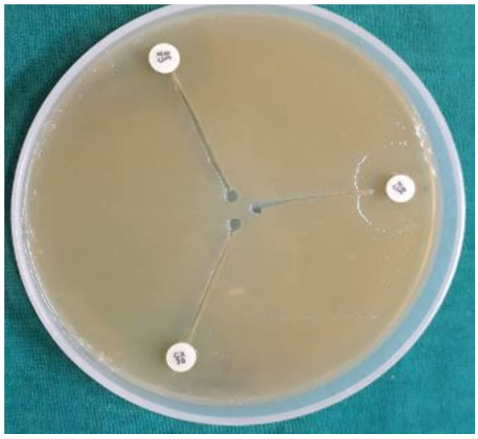

Fig. 3. Modified 3 dimensional test of three isolates: Indentation in the zone of inhibition confirms AmpC producing isolates.

Fig. 4. AmpC disk test of three isolates: Indentation in the zone of inhibition confirms AmpC producing isolates.

Statistical Analysis

Chi-square test, Fisher's exact test and test of proportions were used to analyse the results. Analysis was performed by using newer version of SPSS software.

Results

In this study, 113 bacteria are isolated from 100 samples suspected of surgical site wound infection. Out of 113 aerobic bacteria, 80 (70.8%) isolates are Gram negative bacilli and remaining 33 (29.2%) isolates are Gram positive cocci. *Escherichia coli* (21.2%) is the most common Gram-negative bacilli and Staphylococcus aureus (18.6%) predominating among Gram positive cocci isolated from surgical site wounds. Organisms isolated from surgical site wound infection are seen in Table 1.

In our study, among the 80 Gram negative bacilli, we isolated 17 (21.3%) pure ESBL producers, 30 (37.5%) pure AmpC producer and 27 (33.8%) Co-producers (ESBL+AmpC). The results of ESBL and/or AmpC producers and ESBL and AmpC co-producers are depicted in Table 2.

Table 1. Organisms isolated from surgical site wound infection.

Name of Bacterial Isolate	Number	Percentage (%)
Escherichia coli	24	21.2
NFGNB	23	20.4
Klebsiella species	22	19.5
Staphylococcus aureus	21	18.6
Coagulase Negative Staphylococcus	11	9.7
Pseudomonas species	4	2.6
Citrobacter species	3	3.5
Proteus vulgaris	2	1.8
Proteus mirabilis	1	0.9
Providencia species	1	0.9
Enterococcus species	1	0.9
Total	113	100

Table 2. Results of ESBL and/or AmpC producers and ESBL and AmpC co-producers.

Organism	ESBL positive no (%)	AmpC positive (%)	Co-producers no (%)
Escherichia coli (23)	13(16.3)	12 (15.0)	12 (15.0)
Klebsiella species (21)	4 (5.0)	16 (20)	15 (18.8)
Proteus mirabilis (1)	0 (0.0)	-	-
Citrobacter species (5)	-	2 (2.5)	-
Providencia species (1)	-	0	-
Pseudomonas aeruginosa (4)	-	1 (1.25)	-
Total (50)	17 (21.3)	31 (38.8)	27 (33.8)

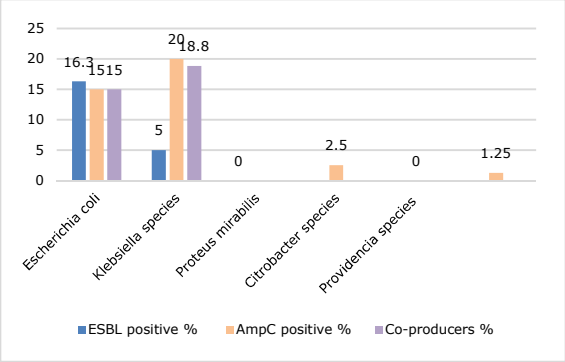

Fig. 5. Prevalence of ESBL, AmpC β-lactamases and co-producers.

Discussion

Many patients after surgery develop SSI which play a great role in morbidity and mortality of the patients (Anathakrishnan AN *et al*., 2000). Most of the causative agents are bacteria. These bacteria gradually develop resistance to beta-Lactam antibiotics due to production of beta-lactamases (Sirot D, 1995). Third generation cephalosporins may be susceptible to isolates in vitro but results in clinical failure when used in vivo (Chaudhary U, Aggarwal R, 2004).

Incidence of ESBL varies among different institutions of the same country (Ahmed I, Salam A, 2002). ESBL producers are the important cause of multi resistance in hospitals (Anathakrishnan AN *et al*., 2000). The increasing incidence of ESBL has resulted in decreased treatment options (Bradford PA, 2001). Beta-Lactamase inhibitors are considered in the treatment of ESBL (Ahmed I, Salam A, 2002).

AmpC beta-lactamases are poorly inhibited by beta-Lactamase inhibitors like clavulanic acid. They can hydrolyze cephamycins (cefoxitin, cefotetan), and other extended-spectrum cephalosporins by which they can be differenciated from other ESBL producers (Jacoby GA, 2009). Modified Three-Dimensional Test and AmpC disk method were performed to detect AmpC beta-lactamases, although the current CLSI guidelines do not describe any method for detection of AmpC beta-lactamases

The trends of antimicrobial resistance among SSI in our hospital will help in guiding clinicians to prescribe appropriate antibiotics. The current study describes detection of ESBL, AmpC β-lactamase and to know their prevalence among surgical site wound infection isolates. Since the incidence of ESBL and AmpC are in a rise, infections by them should be identified and effective measures should be taken to limit the outbreaks.

A total of 100 samples from departments of General Surgery, Orthopedics, OBG, ENT and Ophthalmology samples from patients with SSIs were processed to yield a total of 113 aerobic bacterial isolates. Among them, 44 were females & 56 were males. Out of the 113 isolates, Gram negative bacilli constituted 80 (70.8%) of the isolates with Escherichia coli 24 (21.2%) predominating. The Gram-positive cocci constituted 33 (29.2%), with Staphylococcus aureus 21 (18.6%) predominating.

Comparison of different studies

Study	Year of study	Place	Most common isolate
Agrawal *et al.* (2008)	2003-2004	India	*Escherichia coli* (34.2%)
Giri *et al.* (2008)	2008	India	*Escherichia coli* (33.33%)
Sahu *et al.* (2009)	2008-2009	India	*Escherichia coli* (50.0%)
Rao *et al.* (2013)	2013	India	*Escherichia coli* (20.8%)
Fadnis *et al.* (2014)	2014	India	*Escherichia coli* (38.89%)

All the above studies reviewed, correlated with the findings of the present study. In the present study *Escherichia coli* was responsible for 21.2% cases of surgical site wound infection which was similar to the study conducted by Rao *et al.* (2013) with 20.8% of *Escherichia coli*.

ESBL

The present study indicated that a significant number of gram-negative bacilli harbour ESBL in our hospital. ESBL screening and confirmatory test were done as per CLSI guidelines (Clinical and Laboratory Standards Institute, 2021).

The prevalence of ESBL in our study was 17 (21.3%) which is similar to 20% ESBL producing gram negative bacteria reported by Menon T *et al.* (2006) and Jitendranath A *et al.* (2019) Increased use of third generation cephalosporins to treat gram negative infections and monotherapy with cephalosporins may be responsible for this increased resistance to β-lactams.

Several Indian studies reported prevalence of ESBL producers ranging from 20% to 68% (Menon T *et al*., 2006; Mathur P *et al*., 2002; Agarwal P *et al*. (2008). ESBL production of 20% was reported in Chennai by Menon T *et al.* (2006), 22% by Agarwal P *et al.* (2008) Pune, 68% by Mathur P *et al.* (2002) from tertiary care hospital in North India.

ESBL and AmpC producers have spread worldwide. A survey from 31 centers in 10 European found that the prevalence of ESBL in *Escherichia coli* and *Klebsiella pneumoniae* isolates these ranged from 1.5% to 39-47% (Taneja N *et al*., 2007; Goosens H, 2001).

The prevalence of ESBL production among *Enterobacteriaceae* in this study was observed as highest in *Escherichia coli* 13(16.3%), followed by 4 (5%) in *Klebsiella species*. Highest prevalence of ESBL production in *Escherichia coli* (61.4%) followed by *Klebsiella species* (46.2%) was observed by Rao SPN *et al.* (2014) which is similar to present study.

According to CLSI, *Klebsiellae, Escherichia coli and Proteus mirabilis* were screened for ESBL production by disk diffusion methods. Cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone disks were used for this purpose. Among them cefotaxime and ceftazidime have been proposed as indicators of ESBL production as per CLSI. However, there can be false positive or negative results (Agarwal P *et al*., 2008). False positives occur when there is excess production of TEM-1 or SHV-1 by the isolate but it lacks ESBL (Agarwal P *et al*., 2008). Further, false negatives are due to isolates producing both ESBLs and AmpC β-lactamases. This is because the AmpC β-lactamases resists inhibition by clavulanic (Agarwal P *et al*., 2008; Khan MKR *et al*., 2008) To avoid these errors, Mohanty S *et al.* (2010) used cefepime to detect ESBL and AmpC co-producers, as cefepime is resistant to the action of AmpC enzymes.

AmpC β-lactamases

The gold standard tests for AmpC detection are isoelectric focusing and genotyping characterization as the current CLSI do not specify the screening and confirmatory tests for the detection of AmpC β-lactamases (Coudron PE *et al*., 2000).

Among the 33 isolates screened, 30 (37.5%) were confirmed as AmpC producers by modified three-dimensional test and AmpC disk test. This indicates poor specificity of cefoxitin disk for screening of AmpC production, as the non AmpC producers also shows cefoxitin resistance. Lack of permeation of porin/porin deficient mutants may be the reason for cefoxitin resistance in non AmpC producers (Coudron PE *et al*., 2000).

In the present study, the results of the modified three-dimensional test and AmpC disk test were found to be concordant. The above results were similar to Singhal S *et al.* (2005) and they also concluded that the AmpC disk test was an easier, reliable and rapid method of detection of that AmpC β-lactamases and could be used in diagnostic laboratories.

In the present study, AmpC β-lactamase was mainly seen in *Klebsiella species* 16 (20%), followed by *Escherichia coli* 12 (15%), *Citrobacter species* 2 (2.5%) and *Pseudomonas aeruginosa* 1 (1.25%). Dutta H *et al.* (2014) reported AmpC β-lactamase most commonly in *Klebsiella species* 16 (29.09%), followed by *Escherichia coli* 14 (28.57%) which showed similar findings.

ESBL and AmpC Co-producers

There was a coexistence of both ESBL and AmpC in 27 (33.8%) gram negative isolates in this study. This is because, sometimes in Enterobacteriaceae, plasmid mediated AmpC enzymes disseminate in combination with ESBL (Singhal S *et al*., 2005).

The coexistence was observed in a study conducted by Singhal S *et al.* (2005) with a lower rate of 0.73% and a higher rate of 86.1% was observed in a study conducted by Hemalatha V *et al.* (2007).

Conclusion

Surgical site wound infections seen in the hospitals are only one-dimensional, to get a three-dimensional view, extensive studies are needed. The struggle to minimize surgical site infections will continue. With adequate surveillance and proper co-ordination between clinicians and microbiologists, prevalence of surgical site wound infection can be possibly brought to the negligible level.

Acknowledgement

With deep sense of gratitude I express my sincere thanks to my teachers Dr Asha B Patil and Dr. Maheshkumar S and to my friends Dr. Aaftab, Dr. Shilpa, Dr. Sheethal, Dr. Ganesh, Dr. Pooja, Dr. Akshat and Dr. Smitha for their unconditional support.

Conflict of interest:

The authors have none to declare.

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