



## AEROBIC BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF SURGICAL SITE INFECTIONS

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**ABSTRACT** **INTRODUCTION:** Surgical site infections account for 14% to 16% of all nosocomial infections and antimicrobial resistance is contributing to the increased morbidity and mortality among these patients.

**OBJECTIVES:**

1. To isolate & identify bacteria from pus samples of clinically suspected SSIs.
2. To study the antibiogram of isolated bacteria and to detect MRSA strains, ESBLs & MBLs.

**MATERIALS AND METHODS:** 150 pus samples were collected aseptically from clinically suspected SSIs from various post operative wards (Surgery, Orthopaedic & OBG) at KGH, Visakhapatnam. All the pus samples were processed. Isolation and identification of the organisms was done as per the standard guidelines in the Dept of Microbiology. AST & detection of MRSA strains, ESBL and MBLs was done by Kirby Bauer disc diffusion method as per the CLSI guidelines and confirmed by E-test.

**RESULTS:** Out of 150 pus samples 64% were culture positive and among these 41 (42.7%) were GPC, 46 (47.91%) were GNB & 9 (9.37%) were mixed isolates. Out of total 34 Staph aureus strains 15 (44.12%) were MRSA and out of 55 GNB 16 (29.02%) were ESBLs & 8(14.5%) were MBLs.

**CONCLUSION:** Identification of drug resistant strains like MRSA, ESBLs & MBLs in due time is mandatory not only for proper management of the patient but also to take hospital infection control and preventive measures to avoid their spread.

### KEYWORDS :

#### INTRODUCTION

Surgical site infections are one of the most common causes of nosocomial infections and common complication associated with surgery<sup>[1]</sup>. They account for 14% to 16% of all nosocomial infections according to National Nosocomial Infection Surveillance (NNIS)<sup>[2]</sup>. Centre for Disease Control and prevention (CDC), Atlanta, defines Surgical Site Infection (SSI) as an infection occurring within 30 or 90 days after a surgical operation or within 1 year if an implant is left in place after procedure and affecting either incision or deep tissues at the operation site. These infections may be superficial infections or deep incisional infections involving organ or body space<sup>[3]</sup>. As per CDC, wounds are classified as Class I/Clean, Class II/Clean contaminated, Class III/Contaminated, Class IV/Dirty-infected<sup>[3,4,5]</sup>.

Surgical site infections are responsible for increase in the treatment cost, length of hospital stay and significant morbidity and mortality. Despite the technical advances in infection control and surgical practices, SSI's still continue to be a major problem, even in hospitals with most modern facilities<sup>[6]</sup>.

Although properly administered antibiotics can reduce postoperative Surgical Site Infections (SSI) due to bacterial contamination, widespread use of prophylactic broad spectrum antibiotics can lead to emergence of multi-drug resistant bacteria. Since initial antibiotic therapy is empirical, it is important to know the prevailing antibiotic susceptibility patterns of individual institutions by routine surveillance<sup>[11]</sup>. SSI is one of the quality indicators of the health care system of any hospital. With the increase in incidence of nosocomial infections and multi-drug resistance, a meticulous and periodic surveillance of various hospital acquired infections became mandatory<sup>[12]</sup>. The present study was done to know the status of the SSIs in the hospital as it is one of the main quality indicator to take Hospital infection control and prevention measures.

The aim and objectives of this study includes; to isolate and identify aerobic bacterial isolates of surgical site infections. To study the antibiogram of the isolated organisms causing surgical site infections. And to detect Methicillin Resistant *Staphylococcus aureus* (MRSA) strains among *Staphylococcus aureus*, Extended Spectrum Beta Lactamase (ESBL) and Metallo Beta Lactamase (MBL) strains among Gram negative isolates.

#### MATERIALS AND METHODS

The present study was conducted at King George Hospital (KGH), Visakhapatnam from February 2016 to June 2017. Under strict aseptic precautions 150 samples from various types of surgical sites

suspected to be infected on clinical grounds were collected from the post-operative wards of departments of Surgery, Orthopaedics and Gynaecology & Obstetrics were obtained. and were processed.

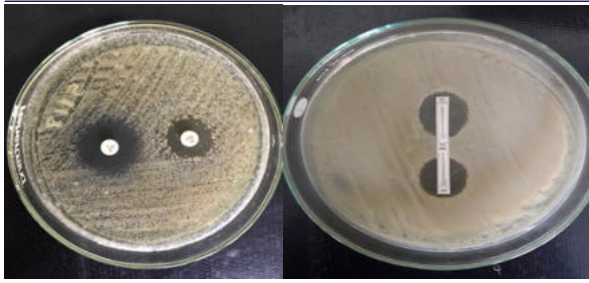
- Organisms were initially isolated on routine culture media like nutrient agar, blood agar and MacConkey agar and were identified by standard microbiological procedures, i.e., by cultural characteristics, Gram's stain, catalase test, oxidase test, motility and other biochemical tests. Antimicrobial susceptibility testing was done by modified Kirby Bauer disc diffusion method as per CLSI guidelines and commercially available antibiotic discs (Hi-media) were used.-
- Cefoxitin 30mcg disks were used to detect Methicillin resistant *Staphylococcus aureus* strains and confirmed by Epsilometer test
- ESBL production and MBL production among Gram negative isolates was done by Combined Disc diffusion test and confirmed by E-test.



- E-test strip of Vancomycin & Cefoxitin
- showing MIC of Vancomycin 1mcg/ml Disc diffusion method - Extended Spectrum Betalactamases



- Etest strip of ceftazidime & ceftazidime / clavulanic - acid showing MIC of 25 mcg/ml & 3 mcg/ml respectively Disc diffusion method - Metallo Beta Lactamases



test strip of Meropenem & Meropenem+EDTA showing MIC of 9 mcg/ml & 1 mcg/ml respectively

**RESULTS**

In the present study, out of 150 samples processed 96 (64%) were culture positive for bacterial isolates and 54 (36%) were culture sterile (Table No.1).

In the present study, out of total 150 cases highest percentage of culture positivity (47.92%) was seen in the age group of 31-45 years .

In the present study, out of a total of 105 male patients, 66.7% showed culture positivity and out of 45 female patients, 33.3% showed culture positivity .

In the present study, out of 150 samples collected, 41% were from patients who underwent elective surgeries and 59% were from emergency surgeries .

In the present study, incidence of surgical site infections was high in surgeries conducted for more than 2 hours (76.04%). Only 23.96% of SSIs were reported from surgeries conducted for less than 2 hours .

In the present study, out of 150 samples processed, majority of them, 70 were collected from clean contaminated surgeries where as only 6 were collected from dirty surgeries. Out of 70 samples collected from clean contaminated surgeries, 45 showed culture positivity. Out of 51 samples collected from contaminated surgeries, 32 samples; 15 out of 23 samples from clean surgeries and 4 out of 6 samples from dirty surgeries showed culture positivity (Table No.2).

Among the Gram positive cocci, *Staphylococcus aureus* (68.29%) was the predominant isolate followed by Coagulase Negative *Staphylococci* (21.95%), *Streptococcus pyogenes* (4.88%) and *Enterococcus faecalis* (4.88%). (Table No.3).

Among the Gram negative bacilli, predominant isolate was *Pseudomonas aeruginosa* (43.48%) followed by *Escherichia coli* (34.78%), *Klebsiella pneumoniae* (15.22%), *Proteus mirabilis* (4.35%) and *Acinetobacter baumannii* (2.17%) (Table No.4).

Among the Gram positive cocci, *Staphylococcus aureus* was 100% sensitive to Teicoplanin followed by Linezolid (94.17%) and Vancomycin (94.17%), (Table No.5).

Among the Gram negative isolates varied antimicrobial susceptibility pattern has been noted. *Pseudomonas aeruginosa* showed 100% sensitivity to Piperacillin and Tazobactam, followed by Cefazidime + Clavulanic acid with 86.95% sensitivity. (Table No.6).

In the present study, out of total 34 *Staphylococcus aureus* isolates, 15 (44.12%) were Methicillin Resistant *Staphylococcus aureus* (MRSA) and 19 (55.88%) were Methicillin Sensitive *Staphylococcus aureus* (MSSA) (Table No.7)

In the present study, out of total 55 Gram negative isolates, Extended Spectrum Beta-lactamase (ESBL) production was seen in 16 (29.09%) isolates (Table No.8).

In the present study, out of total 55 Gram negative isolates, Metallo Beta-lactamase (MBL) production was seen in 8 (14.5%) isolates (Table No.9).

**Table No. 1: Culture positivity among total samples (n=150)**

Culture positivity	Number of cases	Percentage
Culture positive	96	64%
Culture negative	54	36%
Total	150	100%

**Table No.2: Distribution of samples and their culture positivity based on type of surgeries**

Type of surgery	Number of cases	Number of samples showing culture positivity	Percentage of culture positivity
Clean	23	15	15.6%
Clean contaminated	70	45	46.9%
Contaminated	51	32	33 %
Dirty	6	4	4.2 %
Total	150	96	100 %

**Table No.3: Distribution of pure isolates of Gram Positive Cocci (n=41)**

Gram positive isolates	No. of isolates	Percentage
<i>Staphylococcus aureus</i>	28	68.29%
Coagulase Negative <i>Staphylococci</i>	9	21.95%
<i>Streptococcus pyogenes</i>	2	4.88%
<i>Enterococcus faecalis</i>	2	4.88%
Total	41	100%

**Table No.4: Distribution of pure isolates of Gram Negative Bacilli (n=46)**

Gram negative isolates	Total	Percentage
<i>Pseudomonas aeruginosa</i>	20	43.48%
<i>Escherichia coli</i>	16	34.78%
<i>Klebsiella pneumoniae</i>	7	15.22%
<i>Proteus mirabilis</i>	2	4.35%
<i>Acinetobacter baumannii</i>	1	2.17%
Total	46	100%

**Table No.5: Antibiotic susceptibility pattern of Gram positive cocci n=50 (41 from pure culture + 9 from mixed culture)**

	LZ	TEI	VA	CX	AZM	CTX	CTR	AMC	CAZ	LE
<i>Staphylococcus aureus</i> (n=34)	32 (94.17%)	34 (100%)	32 (94.17%)	19 (55.88%)	23 (67.64%)	11 (32.35%)	13 (38.24%)	14 (41.17%)	11 (32.35%)	26 (76.47%)
Coagulase Negative <i>Staphylococcus</i> (n=12)	12 (100%)	12 (100%)	11 (91.67%)	7 (58.33%)	9 (75%)	4 (33%)	7 (53.33%)	4 (33.3%)	6 (50%)	9 (75%)
<i>Streptococcus pyogenes</i> (n=2)	2 (100%)	-	2 (100%)	-	1 (50%)	-	-	1 (50%)	1 (50%)	1 (50%)
<i>Enterococcus faecalis</i> (n=2)	2 (100%)	-	2 (100%)	-	1 (50%)	-	-	-	1 (50%)	1 (50%)

**NOTE:** LZ - Linezolid, TEI - Teicoplanin, VA - Vancomycin, CX - Cefoxitin, AZM

**Table No. 6: Antibiotic susceptibility pattern of Gram negative bacillin=55(46 from pure culture and 9 from mixed culture)**

Isolate	AMC	PIT	CAZ	CAC	CTX	MRP	AK	TOB	AZM	LE
<i>Pseudomonas aeruginosa</i> (n=23)	0 (0%)	23 (100%)	17 (73.9%)	20 (86.9%)	5 (21.7%)	19 (82.6%)	14 (60.8%)	14 (60.8%)	5 (21.7%)	14 (60.8%)
<i>Escherichia coli</i> (n=20)	2 (10%)	20 (100%)	13 (65%)	18 (90%)	13 (65%)	17 (85%)	17 (85%)	16 (80%)	6 (30%)	5 (25%)
<i>Klebsiella pneumoniae</i> (n=9)	1 (11%)	8 (88.9%)	7 (77.8%)	8 (88.9%)	0	8 (88.9%)	7 (77.8%)	7 (77.8%)	2 (22.2%)	3 (33.3%)
<i>Proteus mirabilis</i> (n=2)	0 (0%)	2 (100%)	1 (50%)	2 (100%)	1 (50%)	2 (100%)	1 (50%)	1 (50%)	0	1 (50%)
<i>Acinetobacter baumannii</i> (n=1)	0 (0%)	1 (100%)	0	1 (100%)	0	1 (100%)	0	0	0	0

**NOTE:** AMC - Amoxicillin + Clavulanate, PIT- Piperacillin + Tazobactam, CAZ - Ceftazidime, CAC- Ceftazidime + Clavulanate, MRP - Meropenem, CTX - Cefotaxime, CIP - Ciprofloxacin, TOB - Tobramycin, AK - Amikacin, AZM – Azithromycin, LE - Levofloxacin

**Table no.7: Distribution of MRSA and MSSA isolates among *Staphylococcus aureus***

Organisms	No. of isolates	Percentage
MRSA	15	44.12%
MSSA	19	55.88%
Total	34	100%

**Table No. 8: ESBL producers among various Gram negative isolates**

Organism	Number of isolates	ESBL producers
<i>Pseudomonas aeruginosa</i>	23	6 (26.09%)
<i>Escherichia coli</i>	20	7 (35%)
<i>Klebsiella pneumoniae</i>	9	2 (22.29%)
<i>Proteus mirabilis</i>	2	1 (50%)

**Table No 9: MBL producers among various Gram negative isolates**

Organism	Number of isolates	MBL producers
<i>Pseudomonas aeruginosa</i>	23	4 (17.4%)
<i>Escherichia coli</i>	20	3 (15%)
<i>Klebsiella pneumoniae</i>	9	1 (11.1%)

## DISCUSSION

Post-operative wound infection still remains one of the most important causes of morbidity and is one of the most common nosocomial infection in surgically treated patients. In the present study, an attempt has been made to know the various bacterial flora responsible for surgical site infections and their antibacterial susceptibility pattern. Out of the total number of samples processed, 64% were culture positive and 36% were culture sterile in the present study ,these findings correlated with A. Ramesh, et al., (2012)<sup>[9]</sup> who reported 66% of culture positivity, Jeena Amatya, et al., (2015)<sup>[10]</sup> who reported 60.6%. Sivasankari Selvaraj, et al., (2016)<sup>[11]</sup> reported 76.6% of culture positivity.

Among the total bacterial isolates, *Staphylococcus aureus* was the predominant isolate in the present study with an incidence of 29.16%. This finding correlated with Jyothi Sonawane, et al., (2010)<sup>[12]</sup> who reported an incidence of 29.26% of staphylococcal isolates; Aniruddha, et al., (2017)<sup>[13]</sup> who reported 29%, Bandaru Narasinga Rao, et al., (2016)<sup>[14]</sup> who reported 32.93%

In the present study, Gram positive cocci were mostly sensitive to Teicoplanin, Linezolid and Vancomycin which correlated with Vikrant Negi et al.,<sup>[15]</sup> who reported that Gram positive cocci were sensitive to Vancomycin, Teicoplanin and Linezolid.

In the present study, Gram negative bacilli were sensitive to Piperacillin + Tazobactam, Ceftazidime + Clavulanic acid and Meropenem which correlated with M. Saleem et al.,<sup>[16]</sup> who reported that Gram negative bacilli were sensitive to Amikacin, Imipenem, Piperacillin + Tazobactam and Meropenem.

In the present study, among a total of 34 Staphylococcal isolates, Methicillin resistance was observed in 15 (44.12%) isolates. This finding correlated with Kyathi Jain et al., (2014)<sup>[17]</sup> who reported 48.78% and Rudratej Patil et al., (2015)<sup>[18]</sup> who reported 53.9% of MRSA.

Among the total Gram negative isolates, in the present study, Extended Spectrum Beta-Lactamases (ESBL) production was seen in 29.09%. This finding correlated with Sivasankari Selvaraj, et al., (2016)<sup>[11]</sup> who reported an incidence of 33.3% and Rambabu et al., (2015)<sup>[19]</sup> who reported 35.71%.

- Among the total Gram negative isolates, in the present study, Metallo Beta-Lactamase (MBL) production was seen in 14.5%. This finding correlated with the study of Deepali Shivajirao Kamble, et al., (2015)<sup>[20]</sup> who reported an incidence of 19.19% MBL production in Gram negative isolates and Mita D. Wadekar et al., (2013)<sup>[21]</sup> who reported 18% MBL production among Enterobacteriaceae.

## CONCLUSION

- Hospital infection control committee of the hospitals need to strengthen the surveillance activities of capturing surgical site

infections as it is one of the quality indicator to take corrective and preventive actions to improve the infection control programme.

- Infection by multidrug resistant bacteria enhances the need of antibiotic stewardship and also indicates the need of proper disinfection of hospital environment.

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