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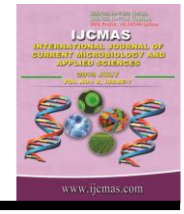

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Aerobic Bacteriological Profile of Surgical Site Infection and their Antimicrobial Resistance Pattern at a Tertiary Care Hospital

A. Venkata Raghavendra Rao*, Reena Rajan and R. Indra Priyadharsini

Department of Microbiology, VMKV Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be university), Salem, Tamilnadu, India

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A B S T R A C T

Surgical site infections are most common nosocomial infection and are associated with prolonged hospital stay, health care cost, morbidity and mortality. The causative agents involved in SSI's may vary with geographical location, between surgeons, various procedures and from hospital to hospital. The present study was done to determine the aerobic bacterial flora from surgical site infections and their antimicrobial resistance pattern. A total of 50 culture positive samples obtained from SSI's were included in this study. Isolates were identified by standard biochemical methods and their antimicrobial resistance pattern was determined by Kirby Bauer Disk Diffusion method. Among the total 65 isolates obtained, Gram negative bacilli 45/65 (69.23%) were the most common isolate. The most predominant organism isolated were *E. coli* (24.62%) followed by *Klebsiella spp* (21.54%), *Proteus spp* (12.31%) and *Pseudomonas spp* (10.77%). Among the Gram positive organism isolated, (21.54%) were *Staphylococcus aureus* followed by *Enterococcus spp* (7.70%) and *Coagulase negative Staphylococcus* (1.54%). All isolates of Gram negative bacilli showed sensitivity to Imipenem. The most effective drug against gram positive organism was found to be Linezolid. For effective control of wound infection, knowledge of causative organisms, their antibiotic sensitivity patterns and administration of judicious therapy are necessary.

Introduction

Surgical site infections (SSI's) are the third most common cause of hospital acquired infections with a reported incidence rate of 14-16% (Hohmann *et al.*, 2012). In India, the overall incidence of wound sepsis ranges from 10%-33% (Bangal *et al.*, 2014). The increased rates of surgical site infection are associated with high morbidity and mortality and are likely to have an important role in the development of antibiotic resistance (Reiye Esayas Mengesha *et al.*, 2014). These infections are usually caused by exogenous and /or endogenous microbial flora that enters post-operative wounds either during surgery or after surgery (Vikrant Negi *et al.*, 2015).

Most SSI's are uncomplicated involving only skin and subcutaneous tissue. Loss of skin integrity by various factors would provide an environment for the colonisation and growth of microbial flora. These infections can sometimes progress to necrotising infections.
Postsurgical wound infection is defined as an

infection in the tissues of the incision and operative area that can commonly occurs between the fifth and 30th days post-surgery.

Most SSI's are caused by the normal flora of patient's skin, mucous membrane or hollow viscera. The most frequently isolated pathogens are *Staphylococcus aureus*, *Enterobacteriaceae*, *Coagulase Negative Staphylococci (CONS)*, *Enterococci* and *Pseudomonas aeruginosa*. The risk of infection depends on various factors such as size of inoculums, virulence and the ability of the organism to invade tissue (Bastola *et al.*, 2017). The development of surgical site infection depends on several factors such as microbial pathogenicity, host defences, local environmental factors and surgical techniques (Mundhada and Tenpe, 2015; Khyati Jain *et al.*, 2014; Arvind Kurhade *et al.*, 2015). Dirty and contaminated wounds reflect the number of pathogens present at the operation site at the time of surgery. Prolonged use of drain may act as a pathway for pathogenic bacteria to enter the wound and thereby increasing the risk of infection.

Etiological agents involved in SSI's may vary with geographical location, between surgeons, various procedures, from hospital to hospital or even in different wards of the same hospital (Arvind Kurhade *et al.*, 2015). Data regarding the causative organisms, their antibiotic sensitivity patterns and administration of judicious therapy are necessary for effective control of wound infection.

Aims and Objectives

To find the aerobic bacterial flora from surgical site infections.

To determine the antibiogram and the antimicrobial resistance pattern of isolates from wound infection.

Materials and Methods

Type of Study: Cross sectional study

Study period: 6 months (March-August 2018)

A total of 50 samples obtained from Surgical site infections (SSI's) received in the Department of Microbiology, VMKVMCH, Salem were included in this study.

Inclusion criteria

Patients of all age group.
Patients undergoing clean surgeries.

Exclusion criteria

Contaminated surgeries.

Infection occurring 30 days after operation if no implant is in place.

Infection on Episiotomy wounds.

Sample collection

Samples were taken from patient before wound cleaning with antiseptic solution. Two wound swabs were collected under aseptic precautions from deep inside wound avoiding contact with skin commensals. For provisional diagnosis, gram staining was done on smear made from one swab. The other swab was inoculated onto Blood agar and MacConkey agar and incubated overnight at 37°C. Bacterial isolates were identified by colony morphology and biochemical characteristics (Collee, 2006).

Detection of antimicrobial resistance

Antimicrobial susceptibility testing was done on these isolates by Kirby Bauer Disk Diffusion method as per CLSI guidelines using the following antibiotics - Amoxycillin / Clavulanic acid (20/10μg), Cefotaxime

(30µg), Gentamicin (10 µg), Ciprofloxacin (10µg), Cotrimoxazole (25µg), Erythromycin (15µg), Clindamycin (2µg), Linezolid (30µg), Teicoplanin (30µg), Ceftazidime (30µg), Cefepime (30µg), Imipenem (10µg) and Piperacillin / Tazobactam (100/10µg). Methicillin resistance is detected by using Cefoxitin disc (30µg) (10). *Staphylococcus aureus* - ATCC 25923, *Escherichia coli* - ATCC 25922 and *Pseudomonas aeruginosa* - ATCC 27853 were used as control strains (CLSI 2016).

Results and Discussion

Out of 50 culture positive samples studied, 40 samples showed monomicrobial growth and 10 showed polymicrobial growth (Fig. 1). A total of 65 isolates were obtained from 50 samples. Gram negative bacilli 45/65 (69.23%) were the most common isolate obtained (Table 1).

The most predominant organism isolated were *E.coli* 16/65 (24.62%) followed by *Klebsiella spp* 14/65 (21.54%), *Proteus spp* 8/65 (12.31%) and *Pseudomonas spp* 7/65 (10.77%).

Among the Gram positive organisms, 14/65 (21.54%) were *Staphylococcus aureus*, followed by *Enterococcus spp* 5/65 (7.70%) and *Coagulase negative Staphylococcus* 1/65 (1.54%).

30 (60%) out of 50 samples were from male patients and 20 (40%) were from female patients.

10/50 (20%) cases were from patients in the age group of 21-40 years.

Majority of the isolates were obtained from patients in the age group of 41-60 years (46%).
Among the gram negative bacilli, 10/16 (63%) *E.coli* were resistant to Cephalosporins, 9/16 (56%) were resistant to Ciprofloxacin and 8/16 (50%) isolates were resistant to Piperacillin / Tazobactam (Fig. 2). The most effective drugs against *E. coli* were Imipenem, Cefoperazone / sulbactam and Aminoglycosides.

10/14 (71%) *Klebsiella spp* were resistant to Cefoperazone / Sulbactam, 11/14 (79%) were resistant to Cephalosporins, 8/14 (57%) were resistant to Piperacillin / Tazobactam and 7/14 (50%) isolates were resistant to Ciprofloxacin (Fig. 3). Imipenem was found to be the most effective antibiotic against multidrug resistant *Klebsiella spp*.

2/8 (25%) isolates of *Proteus spp* were resistant to Cephalosporins, Amoxycillin Clavulanate and Gentamicin (Fig. 4). Isolates of *Proteus* showed 100 % susceptibility to Imipenem and Piperacillin Tazobactam.

100% *Pseudomonas* isolates showed susceptibility to Ciprofloxacin and Imepenem. 4/7 (57%) were resistant to Cephalosporins and 3/7 (43%) were resistant to Cefoperazone / Sulbactam (Fig. 5).

6 (42.86%) out of 14 *Staphylococcus aureus* isolates were resistant to Cefoxitin and were identified as MRSA. All *S. aureus* isolates were resistant to Penicillin. 5/14 (35.71%) were resistant to Clindamycin, 7/14 (50%) to Erythromycin and 13/14 (92.86%) to Ciprofloxacin. All isolates were sensitive to Linezolid (Table 2).

Four (80%) out of 5 isolates of *Enterococcus* were resistant to Penicillin, Erythromycin and Ciprofloxacin and 1/5 (20%) isolates were resistant to Linezolid. A single isolate of CONS obtained from SSI's was found to be sensitive to Erythromycin, Clindamycin, Cefoxitin and Linezolid.

Fig.1 Aerobic bacterial growth pattern from surgical site infections

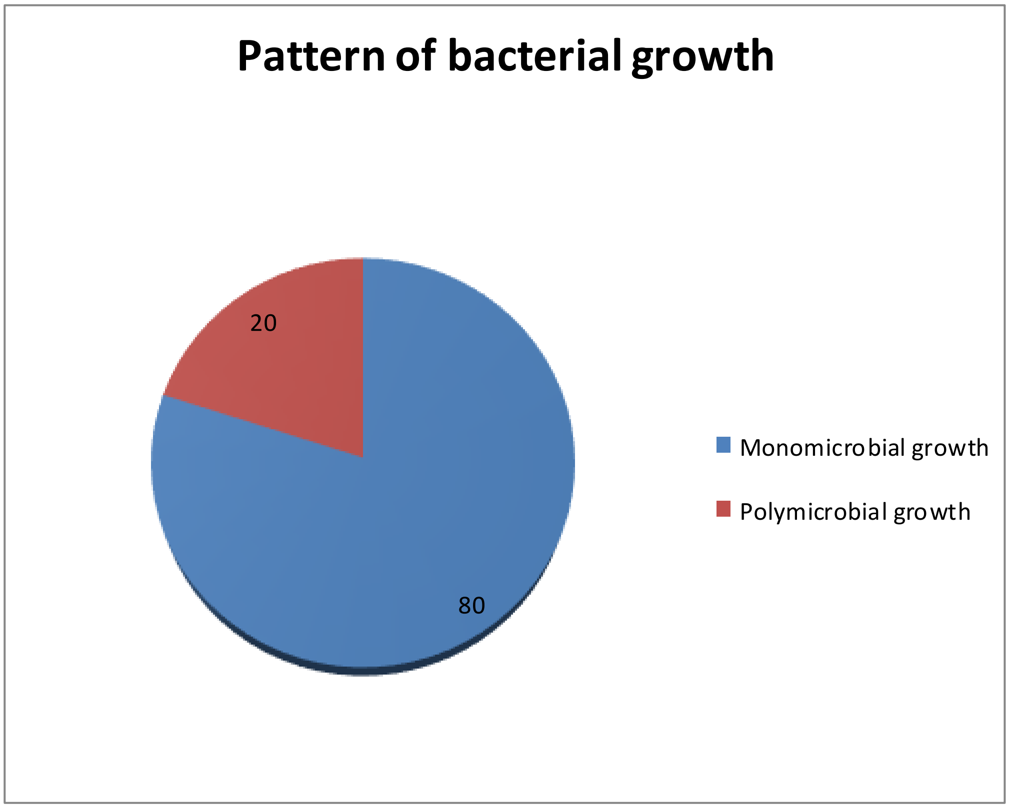

Fig.2 Antimicrobial resistance pattern of *E. coli*

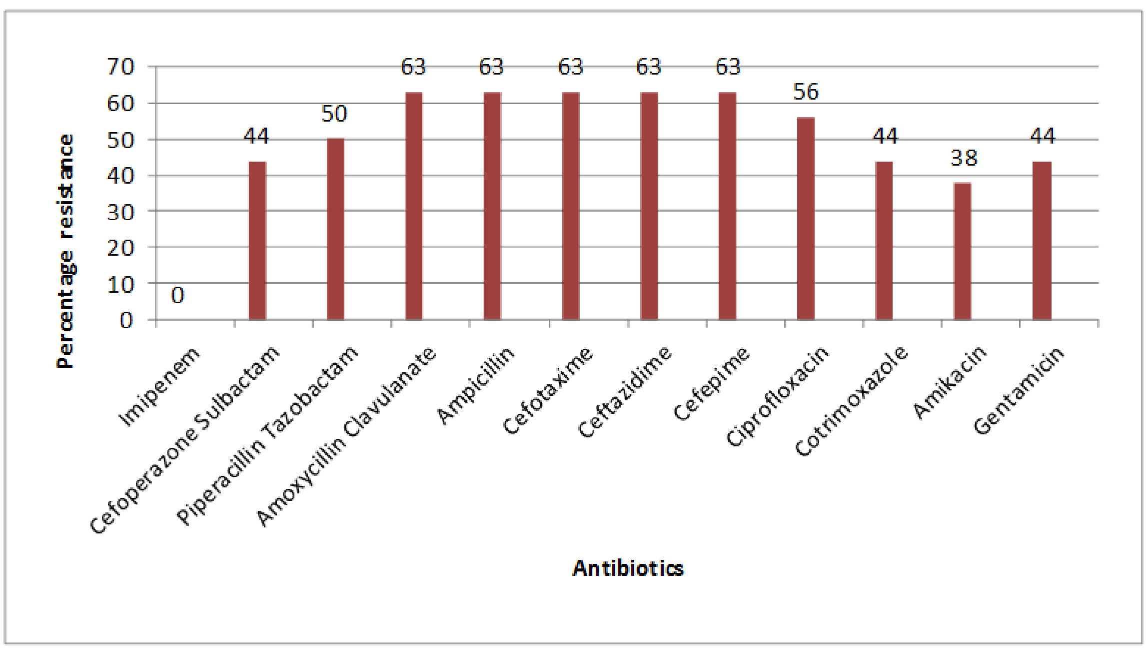

Fig.3 Antimicrobial resistance pattern of *Klebsiella spp*

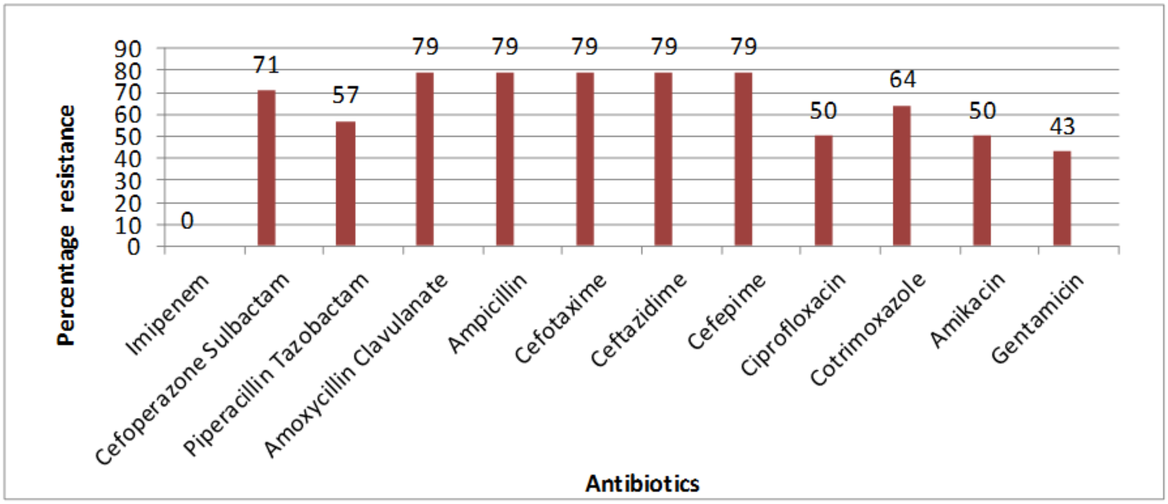

Fig.4 Antimicrobial resistance pattern of *Proteus spp*

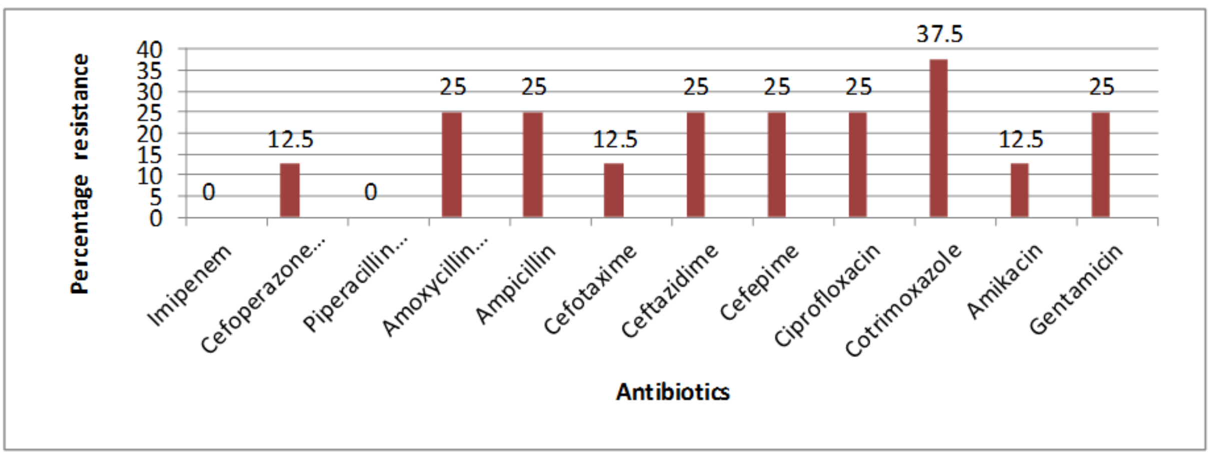

Fig.5 Antimicrobial resistance pattern of *Pseudomonas spp*

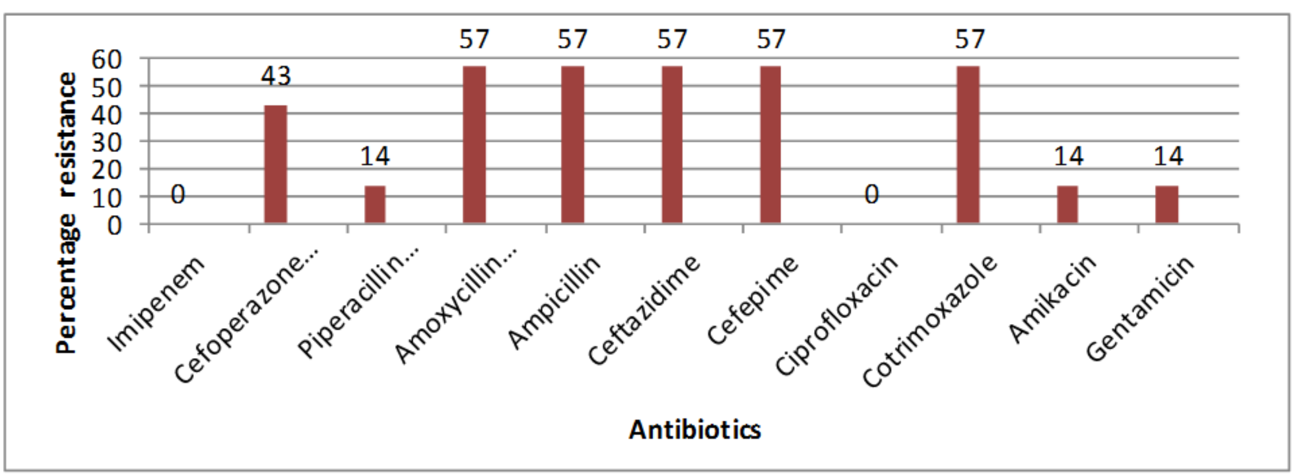

Table.1 Distribution of Polymicrobial growth

Ward	Organisms
Surgery Unit I	*E.coli* & *CONS* *Klebsiella* & *Proteus*
MICU	*E.coli* & *Enterococcus* *Klebsiella, Pseudomonas* & *Proteus*
Surgery Unit II	*E.coli* & *Proteus (2 cases)* *Pseudomonas* & *MRSA* *MRSA* & *Enterococci*
Surgery Unit III	*Klebsiella* & *Pseudomonas*

Table.2 Antimicrobial resistance pattern of Gram positive cocci

Organism	Antibiotics (%)							
	CIP	**CO**	**AK**	**G**	**E**	**CD**	**P**	**LZ**
***MSSA* (8)**	8 (100)	8 (100)	3 (38)	5 (63)	3 (38)	3 (38)	8 (100)	0
***MRSA* (6)**	5 (83)	4 (67)	3 (50)	3 (50)	4 (67)	2 (33)	6 (100)	0
***Enterococcus* (5)**	4 (80)	4 (80)	1 (20)	4 (80)	4 (80)	4 (80)	4 (80)	1 (20)
***CONS* (1)**	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)

CIP- Ciprofloxacin; CO- Cotrimoxazole; AK- Amikacin; G- Gentamicin; E- Erythromycin; CD- Clindamycin; P- Penicillin; LZ- Linezolid

Despite the introduction of meticulous antiseptic regime in surgical practice, SSI is the most common surgical complication in developed and developing countries and is associated with prolonged hospital stay, increased hospital readmissions, health care costs and increased morbidity and mortality.

Prolonged preoperative hospital stay leads to colonization with antimicrobial resistant pathogens. These organism directly affect patient's susceptibility to infection either by lowering host resistance or by providing increased opportunity for ultimate bacterial colonization.

The present study showed predominance of Gram negative bacterial isolates from surgical site infections. Various studies from India have reported the predominance of Gram positive cocci from post-operative wound infection. In a study report by Goswami *et al.*, 2011, 68.85 % isolates were gram negative organisms from SSI's. In our study, 80% samples from SSI s showed monomicrobial growth and 20 % were Polymicrobial. A study conducted by Khyati Jain *et al.*, 2014 has reported 92.30 % isolates as monomicrobial and 7.6 % as polymicrobial from SSI's.

E.coli was the most common isolate obtained in our study; whereas a similar study by Shinde and Kulkarni 2017 have reported *S.aureus* as common organism from SSI's, followed by *E.coli*, *Citrobacter*, *Pseudomonas* and *Acinetobacter*. Kokate *et al.*, (2017) have reported 29.31 % *Pseudomonas aeruginosa* from SSI's followed by 25.86 % *E. coli*. The high incidence of gram negative bacilli in the post-operative wound infection may be attributed to be acquired from normal endogenous flora of patients. Among the Gram positive isolates obtained, *S.aureus* was the common organism. *S.aureus* is most likely associated as normal flora of skin and anterior nares and also with contamination from environment, surgical instrument and from hands of health care workers.

In our study, majority of the isolates were obtained from male patients in the age group of 41-60 years. This is similar to the study findings by Naveen *et al.,* (2014), who have reported the increase in frequency of surgical site infection with age. This may be due to poor immune response, existing co-morbidities in aged patients and reduced compliance with treatment. In another study, Bastola *et al.,* (2017) have shown increased isolation rate from male patients. Pooja patel *et al.,* 2019 have reported maximum number of cases from patients in the age group of 48-58 years. Vikrant Negi *et al.,* (2015) have reported the incidence of SSI among patients above 50 years with *E. coli* as common organism isolated.

All isolates of Gram negative bacilli were sensitive to Imipenem in our study. Least susceptibility was observed against Cephalosporins and Ciprofloxacin. Among the *Enterobacteriaceae*, *Klebsiella* spp was found to be more resistant to antimicrobial agents compared to *E. coli* and *Proteus*. More than 70% isolates of *Klebsiella* were resistant to Cephalosporins and Cefoperazone sulbactam. Isolates of *Klebsiella* showed 100 % susceptibility to Imipenem. The most effective antibiotic against *E. coli* was Imipenem and Cefoperazone sulbactam.

More than 50% isolates of *E.coli* were resistant to fluoroquinolone and Cephalosporins. In a similar study conducted by Pooja Patel *et al.,* (2019), 100 % *E. coli* isolates were sensitive to Imipenem. In a study report by Preethishree *et al.,* (2017), 100 % *E. coli* isolates showed susceptibility to Imipenem and Piperacillin Tazobactam. In our study, isolates of *Pseudomonas aeruginosa* showed 100 % sensitivity to Imipenem and Ciprofloxacin. Kurhade *et al.,* (2015) have reported 48.5% isolates of *Pseudomonas* resistant to Ciprofloxacin.

The prevalence of MRSA from SSI's was found to be 42.86%. Bhave PP *et al.,* 2016 have reported 8.6 % MRSA from post-operative wound infection. In another study report Kokate *et al.,* (2017) have showed 25% MRSA isolates from SSI's and 39.7 % MRSA were reported by Sudhaharan *et al.,* (2018).

All isolates of staphylococcus aureus were resistant to penicillin.100 % isolates were found to be susceptible to Linezolid. This is similar to the study findings by Mundhada and Tenpe (2015).

Among the Gram positive isolates studied, drug resistance was found to be higher among *Enterococcus* isolates. 80% isolates were resistant to Penicillin, Erythromycin, Aminoglycosides and Ciprofloxacin and 20% isolates were resistant to Linezolid. Isolates of *Enterococci* exhibit inherent resistance to antimicrobial agents such as Penicillins, Cephalosporins, Aminoglycosides and Clindamycin.

These organisms also acquire resistance via mobility of resistance genes on plasmids, transposons and chromosomal exchange (Seema Sood *et al.,* 2008).

The present study showed predominance of multidrug resistant *Klebsiella spp* from surgical site infection. SSI's contribute significantly to the cost, the morbidity, and the possible long-term consequences of a surgical procedure. Surveillance of SSI with feedback of appropriate data to surgeons would be desirable to reduce the rate of infection and formulate a proper antibiotic policy for reducing the abuse of antibiotics thereby preventing antimicrobial resistance.

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