



Original Research Article

Bacteriological profile and antibiotic susceptibility patterns of wound infections in a tertiary care hospital in South India

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ARTICLE INFO

Article history:

Received 05-02-2021

Accepted 22-02-2021

Available online 02-04-2021

Keywords:

Anti-bacterial agents

Ciprofloxacin

Methicillin

Staphylococcus aureus

Surgical wound infection

ABSTRACT

Background: Infections caused due to injuries are due to microbial proliferation at the wound site following skin damage. Initial testing of micro-organisms in terms of culturing and sensitivity leads to appropriate antibiotic selection and prevents escalation of antimicrobial resistance.

Aim: To evaluate the bacteriological profile and antibiotic susceptibility patterns of wound infections in this single-centered study.

Materials and Methods: The study included 160 patients suspected to have wound infection. Pus or tissue samples collected from patients were subjected to microbiological processing including Gram staining, culture and antibiotic susceptibility testing. Their demographic data and wound related factors (duration, nature, type) were recorded. The isolated organisms were evaluated for β -lactamase production using Extended spectrum β -lactamase (ESBL) test, Modified Hodge test for Carbapenemase and AmpC β -lactamase enzyme detection tests.

Results: Most participants were 41-60 years old (45.63%). The majority had surgical site infections (SSI, 91.25%), early infected (91.1%) and clean (67.12%) wounds. The bacterial isolation rate was 80% and 45.27% (n=67) were Gram positive isolates, out of which 47 (70.14%) were multidrug resistant. *Staphylococcus aureus* was identified as the predominant organism (n=40), where 33 among 40 were methicillin sensitive, followed by *Escherichia coli* (n=30, 23.43%). Amongst the Gram negative isolates (n=81, 54.73%), 60 (74.07%) were multidrug resistant with majority being susceptible to imipenem, meropenem and amikacin.

Conclusion: The most common pathogen associated with wound infection was Methicillin sensitive *S. aureus* with SSI being the most common type of wound infection.

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1. Introduction

Skin, the largest organ in the human body, plays crucial roles in sustaining life, including water regulation, thermoregulation and most importantly, as the first line of defense against external agents such as micro-organisms.¹ Exposure of the subcutaneous tissue following loss of skin integrity provides an environment that is moist, warm, and nutritive, all of which are conducive for microbial colonization and proliferation.²

Wound injuries are the most common and serious types of trauma and represent a major public health concern. There are many potential factors (age, sex, diabetes, stress, nutrition, oxygenation) involved in the complex wound healing process that can delay healing.³ Wound infections are mainly caused due to proliferation of microorganisms that enter the wound site once the skin is injured. Because of localized inflammation, there is pus formation which consists of white blood cells, damaged cells and dead tissue.⁴ Factors such as age, malnutrition, obesity, endocrine or metabolic disorders, microbial load and host

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defense mechanisms influence the development of wound infection.⁵ In 2015, the overall incidence of wound sepsis in India was 10-33%.⁶

Apart from trauma, other causes for wound infections include surgical site infections (SSI) and diabetic ulcers. Incidence of SSI is a major concern in hospitals, as it increases the length of hospital stay, treatment cost and in a few cases, causes significant morbidity and mortality.⁷

Wound infections are typically polymicrobial, and harbor bacteria, fungi, parasites and viruses that can be aerobic/anaerobic/facultative in nature.² The most common causative agents include *Staphylococcus aureus*, accounting for 20-40% of the infection, methicillin-resistant *S. aureus*, followed by *Pseudomonas aeruginosa* (5-15%), *Escherichia coli*, *Enterococcus* sp., *Proteus* sp. and *Klebsiella* sp.^{8,9}

Appropriate selection of antibiotics depends on the causative agent, the pathophysiology, along with the pharmacokinetics and pharmacodynamics of the drug. The emerging issue of increased antibiotic resistance has escalated the level of difficulty with respect to optimum treatment protocols, especially concerning Gram negative organisms.^{10,11}

Antibiotic resistance being a concern in current times, the protocol of testing of micro-organisms in terms of culturing and sensitivity at the initial stage is vital to provide the appropriate treatment and prevent further complications.¹² Therefore, this study was conducted to study the type of wound infections, the aerobic bacteria associated, along with their antibiograms at our tertiary care centre.

2. Materials and Methods

2.1. Study design

This prospective study was conducted at a tertiary care center in Bengaluru, Karnataka, India, from January 2014 to December 2015. Approval from the Institutional Ethics Committee (STD-1/EC/13-14) and written informed consent from participants were acquired before the commencement of the study.

2.2. Study subjects

The study included pus and tissue samples from 160 patients from outpatient department (OPD)/wards, both surgical and non-surgical, who were suspected to have wound infection (clinical and microbiological). Patients who were very/terminally ill, had bed sores and those who were on antibiotic therapy for more than two weeks at the time of study in case of non-surgical wounds were excluded from the study.

The sample size for the study was calculated based on the formula:

$$n = \frac{p(1-p)Z_{\frac{\alpha}{2}}^2}{E^2}$$

Where, p is the proportion or prevalence, E is the relative error, Z is the value corresponding to level of confidence required.

From the study conducted by Setty et al., the prevalence of SSI patients who underwent various surgeries in the General Surgery department was 21.66%.¹³ Considering the relative error as 25% of prevalence and 90% confidence level, the sample size considered was 160 for this study.

Demographic data (age and gender), department, date of admission, comorbidities, date of SSI event, duration, antibiotic therapy of every patient was recorded. Patients were monitored from the time of inclusion in the study to the date of discharge from hospital.

2.3. Study procedure

Pus or tissue samples collected from patients were subjected to microbiological processing in the laboratory.

2.4. Collection of Pus from swab

Open wounds which had superficial debris was removed by thorough irrigation and cleansing with sterile saline. The swab was gently rolled over the surface of the wound approximately 5 times focusing on area where there was evidence of pus or inflamed tissue. For dry wounds, 2 sterile cotton swabs were used after moistening with sterile saline. Swabs were carried in aerobic transporter tube to the laboratory.

2.5. Collection of aspirates

The skin of affected site was thoroughly cleaned with 70% alcohol, followed with betadine solution and again by 70% alcohol. The surface was allowed to dry and then the pus was aspirated from the deepest portion of the lesion by using a sterile syringe and needle. The aspirate was collected into a sterile wide mouth container and transported immediately to the lab.

2.6. Collection of tissue

The surface of the wound was cleaned through irrigation and cleansing with sterile saline. The tissue samples were collected from areas within and around the infection using sterile scalpels/ blades and were sent to the laboratory in a sterile wide mouth container immediately.

2.7. Microbiological processing

All samples were transported to the laboratory within 30 minutes of collection and processed within an hour of collection. All the samples were processed for Gram stain and Ziehl- Neelsen [ZN] stain (only if the sample was an aspirate) to look for the presence of Gram positive/Gram negative and acid-fast bacteria respectively.¹⁴ Pus cells were graded as occasional (<1/oil immersion field [OIF]), few

[1-5/OIF], moderate [5-10/OIF] and numerous [>10 /OIF]. Organisms were graded in a similar way.

Simultaneously, qualitative culturing was performed on 5% sheep blood agar (SBA) and MacConkey's agar (MA) using standard techniques.¹⁴ Small quantity of the sample was inoculated, using a sterile 4 mm nichrome wire loop (Hi-media), in the thioglycolate broth as a back-up. Samples were incubated at 37°C in an aerobic atmosphere for 24 h. Negative culture plates were discarded and the thioglycolate broth was retained for 72 h, to check for turbidity. In case of turbidity, samples were sub-cultured on SBA and MA plates. The isolate/isolates obtained were identified by Gram stain and standard biochemical reactions.

2.8. Biochemical reactions

The isolated colonies, depending on their Gram reaction were then subjected to biochemical tests (catalase, oxidase, coagulase, nitrate reduction, indole, methyl red, Voges Proskauer, citrate utilization, urease, mannitol, motility and triple sugar iron agar) for identification.¹⁵

2.9. Antibiotic susceptibility testing

The susceptibility test was performed on Mueller-Hinton agar (MHA) by Kirby Bauer disk diffusion method, according to Clinical Laboratory Standards Institute (CLSI) M2-A9 2013 guidelines.¹⁶ The antibiotics (HiMedia Laboratories Pvt Ltd) used for the test were ampicillin (10 µg), amikacin (30 µg), aztreonam (30 µg), amoxicillin-clavulanic acid (20/10 µg), cefotaxime (10 µg), ceftriaxone (10 µg), ceftazidime (30 µg), cefoxitin (30 µg), cefipime (30 µg), cefixime (5 µg), ciprofloxacin (1 µg), clindamycin (2 µg), doxycycline (30 µg), erythromycin (5 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), netilmicin (30 µg), ofloxacin (5 µg), oxacillin (5 µg), penicillin g (10 units), piperacillin (100 µg), piperacillin-tazobactam (100/10 µg), tigecycline (15 µg), tobramycin (10 µg), trimethoprim/sulphamethazole (1.2 µg/23.8 µg), high level gentamicin (120 µg), vancomycin (30 µg) and teicoplanin (30 µg). The diameter of the zone of inhibition was measured (measuring scale) and interpreted according to the CLSI M2-A9 2013 guidelines.

2.10. Extended spectrum β -lactamase (ESBL) detection method

The Gram negative bacilli (*Enterobacteriaceae* family) showing reduced zone of inhibition against ceftazidime, cefotaxime and ceftriaxone, when compared with CLSI M2-A9 2013 guidelines were suspected as ESBL producing organism and were confirmed by combination disc method.

2.11. Combination disk test

The antibiotics used for this test were cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg). Four to five colonies of similar morphology were inoculated to 5 ml peptone water and incubated at 37°C for 4-6 h until turbidity matched to that of McFarland 0.5 turbidity standard (1.5×10^8 CFU/ml). Lawn culture method was adapted on MHA plates and cephalosporin along with cephalosporin with clavulanic acid disks were placed 50 mm apart from each other.¹⁷

The zone of inhibition was measured around the disk. An increase of 5 mm in zone of inhibition in a disk containing clavulanic acid compared to the drug alone was considered as ESBL producer.

2.12. Modified Hodge Test for Carbapenemase detection

Two to three identical colonies of *Escherichia coli* (ATCC 25922) were inoculated into saline and incubated at 37°C for 4 to 6 h, until the optical density matched to that of 0.5 McFarland turbidity standard. This suspension was then diluted 1:10, by adding 0.5 ml of 0.5 McFarland to 4.5 ml of the test suspension. A lawn culture of 1:10 diluted *Escherichia coli* on to the MHA plates with a sterile cotton swab was produced. The plate was left undisturbed for 5 mins at room temperature. An imipenem (10 µg) disc was placed at the centre and the test organism was streaked in a straight line from the edge of the disc to the edge of the plate. The plate was incubated overnight at 35°C in ambient air. The presence of distorted zone of inhibition or clover leaf type of indentation at the intersection of the test organism and *E. coli*, within the zone of inhibition of the imipenem susceptibility disc was interpreted as positive result.¹⁸

2.13. AmpC β -lactamase enzyme detection (AmpC disk test)

Isolates showing reduced susceptibility to ceftazidime, cefotaxime and cefoxitin were considered as "screen positive" and selected for detection of AmpC β -lactamases by AmpC disk test.¹⁹

2.14. AmpC Disk Test

A lawn culture of *Escherichia coli* (ATCC 25922) was produced on MHA plate. Sterile disks (6 mm) were moistened with sterile saline (20 µl) and inoculated with a minimum of 3-4 colonies of test organism. The inoculated disks were then placed close to a cefoxitin disk on the inoculated plate. The plates were incubated overnight at 35°C. Appearance of a flattening or indentation in the cefoxitin inhibition zone in the vicinity of the test disk was considered positive. A negative test had an undistorted zone.

All patients with positive cultures from pus samples were treated as per the antibiogram reports obtained. After the modification of antibiotic drug regimen, these patients were followed up to their discharge and the outcome was noted.

2.15. Statistical analysis

Data were analyzed using statistical software R version 3.6.3 and MS Excel. Categorical variables are represented by number (%). They were compared using chi-square test/Cochran Armitage test. Continuous variables are represented by mean ± standard deviation form. Level of significance was set at p≤0.05.

3. Results

The mean age of participants in this study was 46.47±18.09 years, including 108 (67.5%) males and 52 (32.5%) females. Majority of participants belonged to the age group of 41-60 years (n=73, 45.63%). Many patients were admitted to the general surgery department (n=65, 40.63%) (Table 1).

Majority of the patients exhibited SSI (n=146, 91.25%), early infection (n=133, 91.1%), with most being categorized as a clean wound (n=98, 67.12%). The majority were subjected to surgical prophylaxis (n=136, 93.15%) (Table 2).

With respect to surgical prophylaxis for SSI patients (n=146), 11 (6.88%) received cefazolin-metrogyl, 54 (33.75%) received ceftriaxone, 14 (8.75%) received ceftrioxone-metrogyl (Table 3).

With respect to Gram staining, of the 128 (80%) samples that were positive on culture, 111 (86.71%) showed the presence of organisms on direct smear while 17 (13.28%) samples were smear negative. Gram staining of 14 (10.93%) samples showed the presence of organisms on direct smear but culture yielded no growth. None of the samples collected by aspiration (n=13) showed the presence of acid-fast bacilli. Of the 128 samples that tested culture positive, 107 (83.59%) samples had growth of a single organism, 20 (15.62%) had growth of two organisms and 1 (0.01%) sample had growth of three organisms.

Amongst the positive culture samples (n=128), *Staphylococcus aureus* was identified in majority of the samples (n=40, 31.25%) followed by *Escherichia coli* in 30 (23.43%) and *Pseudomonas aeruginosa* in 16 (12.5%) samples (Table 4).

Majority of the cases in the study with SSI reported the growth of *Staphylococcus aureus* and minimal growth of micro-organisms were observed in cases with diabetic ulcer and traumatic type of wounds (Figure 1).

3.1. Antibiotic susceptibility patterns

Amongst the Gram positive isolates (excluding *Enterococcus*) (n=54), 40 (74.07%) were *Staphylococcus aureus* and 13 (24.07%) were Coagulase negative

Table 1: Representation of patient characteristics based on demographic data, department, sample collection and comorbidities

Variable	Sub-category	No. of Patients (%)
Age group (years)	0-20	13 (8.13)
	21-40	43 (26.88)
	41-60	73 (45.63)
	61-80	28 (17.5)
	81-100	3 (1.88)
Department	General surgery	65 (40.63)
	Orthopedics	47 (29.38)
	OBGYN	15 (9.38)
	Neurosurgery	6 (3.75)
	Pediatric surgery	3 (1.88)
	Vascular surgery	10 (6.25)
	Plastic surgery	8 (5)
	Dermatology	2 (1.25)
	Oncology	1 (0.63)
	ENT	2 (1.25)
Method of pus sample collection	Pediatrics	1 (0.63)
	Swab	139 (86.88)
	Aspirate	13 (8.13)
	Tissue	8 (5)
	DM	28 (17.5)
Comorbidities	HTN	11 (6.87)
	DM/HTN	17 (10.62)
	DM/HTN/BA	1 (0.62)
	Tuberculosis	1 (0.62)
	Hypothyroidism	1 (0.62)
	Hyperthyroidism	1 (0.62)
	HTN/	1 (0.62)
	Hyperthyroidism	1 (0.62)
	DM/hypothyroidism	1 (0.62)
	Malignancy	1 (0.62)

OBGYN: Obstetrics and Gynaecology; ENT – Ear Nose Throat; DM - Diabetes Mellitus; HTN – Hypertension; BA – Bronchial Asthma

Table 2: Distribution of SSI patients based on wound factors

Wound Factor	Sub-category	No. of Patients (Row %)
Nature of wound (n=160)	SSI	146 (91.25)
	Diabetic ulcer	10 (6.25)
	Traumatic	4 (2.5)
Duration (n=146)	Early	133 (91.1)
	Intermediate	10 (6.85)
	Late	3 (2.05)
Type (n=146)	Clean	98 (67.12)
	Clean-contaminated	23 (15.75)
	Contaminated	7 (4.79)
	Dirty infected	18 (12.33)
	Given	136 (93.15)
Surgical Prophylaxis (n=146)	Not given	10 (6.85)

SSI: Surgical Site Infection

Table 3: Distribution of drugs in patients for surgical prophylaxis

Drug	No. of Patients		Total
	Single drug	Combination	
Ceftriaxone	54	18	72
Metrogyl	1	35	36
Cefazolin	3	13	16
Piperacillin-tazobactam	6	3	9
Amikacin	2	6	8
Teicoplanin	5	2	7
Ciprofloxacin	3	2	5
Cefoperazone	3	1	4
Linezolid	3	1	4
Piperacillin	3	0	3
Ornidazole	0	3	3
Cefuroxime	0	2	2
Gentamycin	0	2	2
Cefixime	0	1	1
Cefotaxime	0	1	1
Tazobactam	0	2	2
Clindamycin	0	1	1
Ertapenem	0	1	1

Table 4: Distribution of organisms according to single/mixed growth

Microorganisms	Number of isolates		Total
	Single growth	Mixed growth	
<i>Staphylococcus aureus</i>	37	3	40
<i>Escherichia coli</i>	24	6	30
<i>Klebsiella pneumoniae</i>	5	6	11
<i>Klebsiella oxytoca</i>	2	0	2
<i>Enterococcus faecalis</i>	6	7	13
<i>Pseudomonas aeruginosa</i>	10	6	16
CoNS	10	3	13
<i>Acinetobacter baumannii</i>	4	3	7
<i>Citrobacter freundii</i>	2	3	5
<i>Citrobacter koseri</i>	1	1	2
<i>Proteus mirabilis</i>	3	3	6
<i>Proteus vulgaris</i>	0	1	1
NFGNB	1	0	1
<i>Streptococcus pyogenes</i>	0	1	1
<i>Candida nonalbicans</i>	1	0	1

CoNS: Coagulase-negative Staphylococci; NFGNB: Non-fermenting Gram negative bacilli

Staphylococci (CoNS) and 1 (1.85%) isolate was *Streptococcus pyogenes*. Out of the 40 *S. aureus* isolates, 33 (82.5%) were methicillin sensitive and 7 (17.5%) were methicillin resistant. Overall, 47 (88.68%) Gram positive isolates were resistant to penicillin, 41 (77.35%) to augmentin and 35 (66.03%) to ciprofloxacin, whereas, 52 (98.11%) isolates were sensitive to vancomycin and 51 (96.22%) to teicoplanin and 49 (92.45%) to linezolid

Amongst the Gram negative isolates (excluding nonfermentors) (n=58), 55 (94.82%) isolates were identified as ESBL producers, 50 (86.2%) as AmpC producers and 12 (20.69%) isolates as Carbapenemase producers. Overall, 49 (84.48%) Gram negative isolates were sensitive to

imepenem, 44 (75.86%) to meropenem and 43 (74.13%) to amikacin.

Overall, 47 Gram positive and 60 Gram negative isolates were multidrug resistant in this study.

Overall, 47 Gram positive and 60 Gram negative isolates were multidrug resistant in this study.

Amongst the β -lactamase producing organisms, majority of *Escherichia coli* isolates were ESBL (n=28) and AmpC (n=25) producers. A greater number of *Pseudomonas aeruginosa* isolates were AmpC (n=15) producers (Figure 2).

Amongst the nonfermentors (n=23) i.e. 16 (69.56%) *Pseudomonas aeruginosa* and 7 (30.43%) *Acinetobacter*

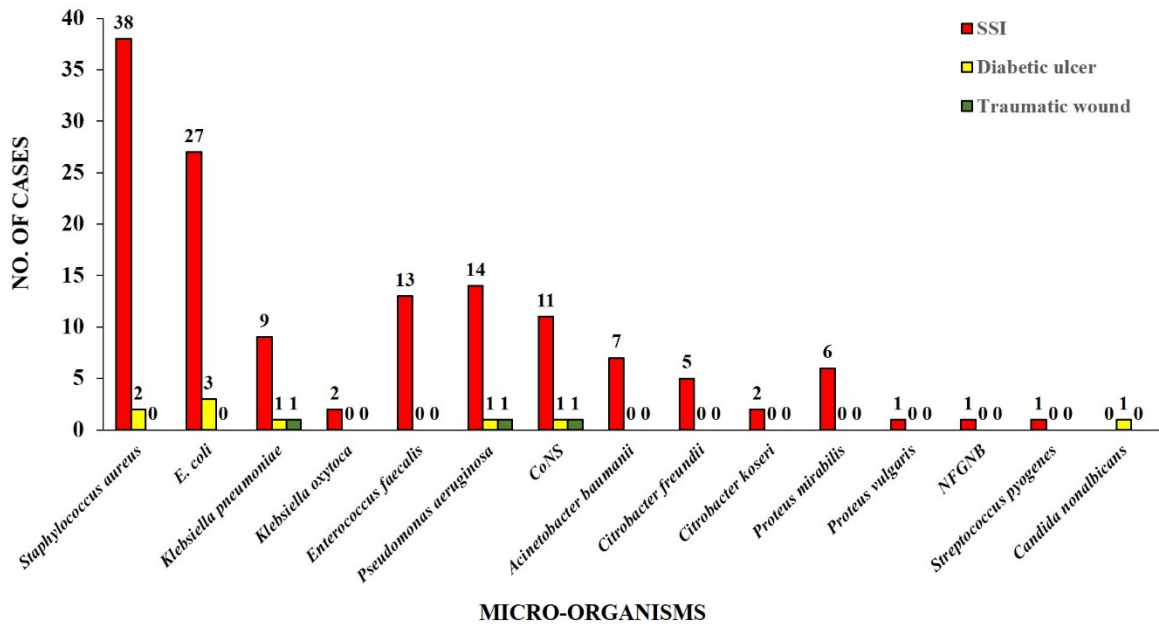


Fig. 1: Distribution of micro-organisms based on the nature of wounds in patients
 Abbreviation: CoNS: Coagulase-negative Staphylococci; NFGNB: Non-fermenting Gram negative bacilli

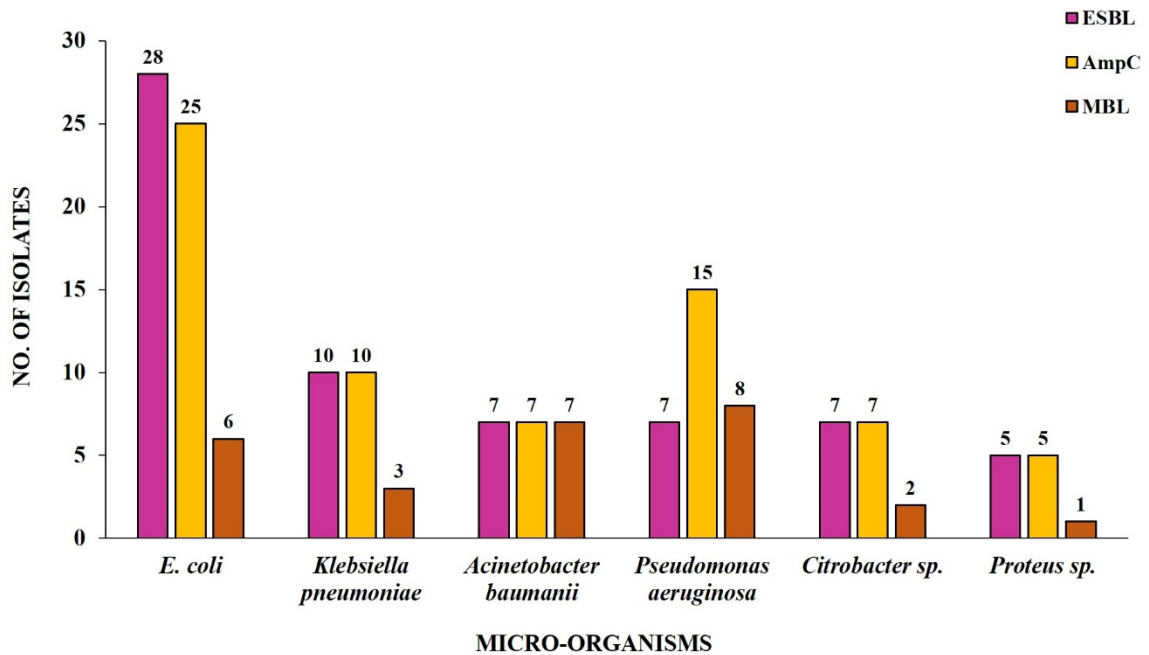


Fig. 2: Representation of β -lactamase producing organisms
 Abbreviations: ESBL: Extended spectrum β -lactamase; MBL: Metallo- β -lactamase

baumanii isolates, 17 (73.91%) were resistant to both aztreonam and ciprofloxacin, 14 (60.86%) to ceftazidime, 13 (56.52%) to both piperacillin and tobramycin along with 12 (52.17%) isolates to amikacin. All isolates of *Acinetobacter baumannii* were sensitive to tigecycline.

Among the 13 isolates belonging to genus *Enterococcus*, 9 (69.23%) were resistant to high level gentamicin (HLG), 10 (76.92%) were resistant to ciprofloxacin and 1 (7.69%) to vancomycin and all the were sensitive to linezolid and teicoplanin.

It was observed that the incidence of clinically suspected SSI was 91.25% (n=146), whereas the incidence of culture confirmed SSI was 72.5% (n=116) in this study. The study variables such as age, gender, type of wound along with comorbidities such as diabetes mellitus and hypertension were insignificantly associated with the incidence of SSI ($p>0.05$) (Table 5).

Overall, 128 (80%) samples showed growth of pathogenic organisms and were treated according to the antibiotic susceptibility report. Patients were followed up regularly, where 121 (75.62%) patients recovered following the wound infection treatment and 7 (4.37%) patients expired.

4. Discussion

The management of infection (mono or polymicrobial) is an important aspect of wound care. Understanding the microbial nature is an imperative part of an efficient treatment strategy. Antibiotic agents are one of the wonder discoveries of the 20th century.²⁰ Therefore, in this study, the bacteriological profile and the antibiotic sensitivity patterns of patients having wound infections were analysed.

Most samples were from the general surgery and orthopaedics department, as many surgeries are undertaken by these departments. The nature of the wound for majority of the patients in this study was SSI. The SSIs are now considered the most common nosocomial infections, representing a major clinical problem with respect to morbidity, mortality, length of hospital stay and the overall costs. They are also considered a global priority because 20-35% of these infections are caused by antibiotic resistant strains.²¹ The proportion of patients with early infection i.e. presenting within 30 days of surgery with SSI was more when compared to intermediate and late infections in this study.

The rate of infection in patients developing SSIs was observed to be higher (67.12%) in clean wounds unlike in other studies where the incidence of infection rates due to clean wounds was very less (3-6%).^{22,23} This finding probably suggests that the factor mainly contributing to the development of infection was in the post-operative period. Surgical prophylaxis was administered to 93.15% patients and the rest who had not received surgical prophylaxis probably underwent emergency surgery as the relevant

information was not recorded.

The most common antibiotic given as surgical prophylaxis was ceftriaxone followed by combination of ceftriaxone-metronidazole and ceftazidime-metronidazole. As observed in the study, there is no uniform guidelines in the hospital for the usage of surgical prophylaxis antibiotics since there were more than twenty different combinations which were given. The exception was in the Obstetrics and Gynaecology (OBGYN) department where the surgical prophylaxis practice was uniform and all patients received ceftazidime-metronidazole as prophylaxis. Though not statistically significant, this could have been the probable reason for lesser number of wound infections in OBGYN department. The use of cephalosporins along with metronidazole is preferred for surgical prophylaxis in hospitals globally because of its effectiveness in alleviating the infection rates.²⁴

In this study, majority of patients had Diabetes mellitus (DM), hypertension (HTN) or DM/HTN as a comorbidity. High blood sugar can increase infection rate and impair wound healing. Poorly controlled diabetes adversely affects the ability of leukocytes to destroy invading bacteria and prevent the harmful proliferation of usually benign bacteria present in the healthy body. Hypertension is a worldwide epidemic and is also common among patients with diabetes.²⁵ However, the association between comorbidities such as DM and HTN with SSI in this study was statistically insignificant ($p>0.05$).

Gram staining yielded 80% positive cultures. As most samples collected were swabs, there could have been the possibility of the organism load being lesser, and hence in case of smear, results were negative for 13.28% of the positive cultures. In a few cases, the culture yielded no growth despite being positive for the presence of organisms on direct smear. There is a chance of them being anaerobic organisms, the isolation of which was not included in the study.

In this study, majority of the isolates were *Staphylococcus aureus*, followed by *Escherichia coli* and *Pseudomonas aeruginosa*. The least common organism to be isolated was *Streptococcus pyogenes* and non-fermenting Gram negative bacilli (NFGNB). The organisms isolated depend on the site which is opened – either skin incision or opening of the gastrointestinal tract. As the number of superficial incisional SSIs and orthopaedic infections were greater in this study as compared to other type of wound infections, *Staphylococcus aureus* can be substantiated for being isolated as the most common pathogen as most of the times the source of infection is patients own endogenous flora.²⁶ Sawdekar et al. also reported *Staphylococcus aureus* being the predominant organism isolated from the infected wounds.²⁷

The antibiotic susceptibility patterns of all isolates were studied. The antibiotic panel for Gram positive cocci, Gram

Table 5: Association of study variables with the incidence of surgical site infection

Variables	Sub-category	SSI – No. of Patients (%)		P value
		Suspected (n=146)	Culture confirmed (n=116)	
Gender	Male	98 (67.12)	78 (67.24)	0.9524
	Female	48 (32.88)	38 (32.75)	
Age group (years)	0-20	11 (7.53)	10 (8.62)	0.8953
	21-40	42 (28.77)	34 (29.31)	
	41-60	64 (43.84)	47 (40.51)	
	61-80	26 (17.81)	22 (18.96)	
	81-100	3 (2.05)	3 (2.58)	
Type of wound	Clean	98 (67.12)	77 (66.37)	0.7381
	Clean-contaminated	23 (15.75)	18 (15.51)	
	Contaminated	7 (4.79)	5 (4.31)	
	Dirty infected	18 (12.33)	16 (13.79)	
Co-morbidities				
DM	Present	47 (32.19)	29 (25)	0.8516
	Absent	99 (67.80)	87 (75)	
HTN	Present	30 (20.54)	26 (22.41)	0.1287
	Absent	116 (79.45)	90 (77.58)	

Abbreviation – SSI: Surgical Site Infection; DM: Diabetes Mellitus; HTN: Hypertension

negative bacilli, *Enterococcus*, *Pseudomonas/Acinetobacter* sp. were selected according to CLSI M2-A9 2013 guidelines. The difference in number of isolates obtained in this study with respect to the organisms being Gram positive or negative in nature could be due to the variations in common nosocomial pathogens inhabiting the hospital setup.

It was observed that of all the *S. aureus* isolates (n=40), 82.5% were methicillin sensitive and 10% were methicillin resistant. A study conducted by Sudhaharan et al. reported 51.9% of *S. aureus* isolates being methicillin sensitive.²⁸ However, the Gram positive cocci were resistant to commonly used antibiotics like amoxicillin with clavulanic acid, ciprofloxacin and penicillin. This difference could be due to better hospital infection control practices followed in our hospital.

Most of the Gram negative bacilli were multidrug resistant with organisms being ESBL, AmpC and carbapenamase producers. This finding alarms the clinicians to be aware of emerging multidrug resistance among Gram negative isolates as the only options for treatment include amikacin and carbapenems. The usage of amikacin in renally compromised patients is questionable and usage of carbapenems for the treatment of wound infections has to be looked upon due to cost factor as most of the patients attending our hospital cannot afford them.²⁹ However, carbapenems are known to possess broad spectrum antibacterial activity, having a unique structure that is defined by a carbapenem coupled to a β -lactam ring that confers protection against most β lactamases such as metallo- β -lactamase (MBL) as well as ESBL and AmpC producers.³⁰ Consequently, carbapenems are considered one of the most reliable drugs for treating bacterial

infections and the emergence and spread of resistance to these antibiotics constitute a major public health concern.³¹

Majority of *P. aeruginosa* isolates showed reduced susceptibility to antibiotics like ciprofloxacin, ofloxacin, and meropenem. Decreased susceptibility of Pseudomonal strains to commonly used antibiotics is a serious threat to the community and may be attributed to inappropriate use of antipseudomonal drugs by the clinicians. Relaxation of general hygienic measures, mass production of low quality antiseptic solutions and medicinal solutions, difficulties in the proper definition of responsibilities among the hospital staff all contribute to development of resistance in *Pseudomonas* sp.³²

Majority of the *Enterococcus* isolates were resistant to high level gentamycin and ciprofloxacin and only one isolate was vancomycin resistant, while all isolates were sensitive to linezolid and teicoplanin. This finding restricted the use of fluoroquinolones, aminoglycosides and the combination of ampicillin and aminoglycoside synergy as a drug of choice for the treatment of wound infections.³³ Isolation of vancomycin resistant *Enterococci* is a warning sign for both clinicians and microbiologists for strict surveillance and more stringent hospital infection control measures. Vancomycin resistance was checked by Kirby Bauer disk diffusion method for *Enterococcus*, approved by CLSI M2-A9 2013 guidelines.

The overall rate of bacterial isolation of wound samples in this study was 80%, comparable to observations reported by Shimokawa et al. (72.6%) and Wadekar et al. (85.5%).^{3,34} On the contrary, studies conducted by Abraham et al. (41%) and Biadlegne et al. (53%) reported lower isolation rates.^{35,36} The difference in these rates could be attributed to the facilities of the hospital

management and implementation of infection prevention and control program. The total number of patients in the hospital along with the environmental conditions could also be contributing factors. All patients were followed up from the date of reporting of wound infection to their discharge/death. Patients who recovered had a longer duration of hospital stay, until their wounds healed, and antibiotic course was completed. Seven patients in our study succumbed to death, where 5 of them expired due to underlying comorbidities such as diabetes and hypertension, along with increased age as an additional risk factor.

Limitations of this study include the small sample size along with the fact that microbiological examination for anaerobes and fungi were not included as a part of this study.

5. Conclusion

The most common pathogen associated with wound infection in our hospital was methicillin sensitive *Staphylococcus aureus* with SSI being the most common type of wound infection. Majority of the SSIs occurred in early postoperative phase and in patients having clean wound at the time of surgery. The most common comorbidity associated with wound infections was diabetes and hypertension. Multidrug resistance pattern exhibited by organisms cause difficulty with respect to selecting appropriate antibiotics for treatment. High number of ESBLs, AmpC and Carbapenamase producing organisms emphasize implementing highly stringent infection control measures. Regular surveillance with Root Cause Analysis (RCA) of all wound infections must be done to check the rate of the same. Strict adherence to the hospital's antibiotic policy by the prescribing clinicians must be worked upon. The formulation of infection control measures and appropriate use of antibiotics must be considered compulsory to alleviate wound infection rates.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that there is no conflict of interest.

References


- Zafar A, Anwar N, Ejaz H. Bacteriology of infected wounds - A study conducted at Children's Hospital Lahore. *Biomedica*. 2008;24:71–4.
- Pushpa M. Bacteriological Profile of Wound Infection and Antibiotic Susceptibility Pattern of the Isolates. *J Microbiol Exp*. 2017;4(5):126. doi:10.15406/jmen.2017.04.00126.
- Shimekaw M, Tigabu A, Tessema B. Bacterial Profile, Antimicrobial Susceptibility Pattern, and Associated Risk Factors Among Patients With Wound Infections at Debre Markos Referral Hospital, Northwest, Ethiopia. *Int J Lower Extremity Wounds*. 2020;doi:10.1177/1534734620933731.
- Bindu D, Chitralakshasaikumar, Kiran M, Praveena R, Illamani V. Bacterial profile and antibiotic resistance pattern of aerobic gram-positive bacteria isolated from pus sample. *Res J Pharma Biol Chem Sci*. 2014;5(5):643–6.
- Gomatheswari SN, Jeyamurugan T. Bacteriological Profile and the Antibiotic Susceptibility Pattern of Microorganisms Isolated from Pus/Wound Swab Isolates in Patients Attending a Tertiary Care Hospital in South India. *Int J Curr Microbiol Appl Sci*. 2017;6(10):1405–13. doi:10.20546/ijemas.2017.610.166.
- Mantravadi H, Chinthaparthi M, Shravani V. Aerobic isolates in pus and their antibiotic sensitivity pattern: a study conducted in a teaching hospital in Andhra Pradesh. *Int J Med Sci Public Health*. 2015;4(8):1076–9. doi:10.5455/ijmsph.2015.12012015225.
- Naz R, Hussain SM, Ain QU. Bacteriological profile of surgical site infections and their antibiotic susceptibility pattern. *Int J Sci*. 2019;5(2):2224–9.
- Cardona AF, Wilson SE. Skin and Soft-Tissue Infections: A Critical Review and the Role of Telavancin in Their Treatment. *Clin Infect Dis*. 2015;61(suppl 2):S69–S78. doi:10.1093/cid/civ528.
- Sida H, Pethani P, Dalal H, Shah N, Shaikh. Current microbial isolates from wound samples and their susceptibility pattern in a tertiary care hospital. *Natl J Integr Res Med*. 2018;9(2):17–21.
- Verma P. Antibiotic sensitivity treatment for gram positive bacteria isolated from pus sample. *Pharmacol Life Sci*. 2012;1(10):3–6.
- Ananthi RM, Kalpanadevi V, Abigail RS, Karthiga L, Victor HK. Aerobic bacteriological profile and antimicrobial susceptibility pattern in postoperative wound infections at a tertiary care hospital. *Int J Medical Sci Clin Invent*. 2017;4(2):2702–6.
- Rahman MS, Chauhan SP, Bawankar S. Bacteriological profile and antibiotic susceptibility profile of wound infection in a tertiary care hospital Chattisgarh. *Indian J Appl Res*. 2018;8(6):29–31.
- Setty NKH, Nagaraja MS, Nagappa DH, Giriyaiah CS, Gowda NR, Naik R. A study on Surgical Site Infections (SSI) and associated factors in a government tertiary care teaching hospital in Mysore, Karnataka. *Int J Med Public Health*. 2014;4(2):171–5. doi:10.4103/2230-8598.133126.
- Ananthanarayan R, Panicker CKJ. Textbook of Microbiology. 7th ed. Hyderabad: Orient Longman; 2005.
- Collee JG, Miles RS, Watt B. Tests for identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney's Practical medical microbiology. Churchill Livingstone; 2006.
- Clinical Laboratory Standards Institute. Performance standard for antimicrobial disk susceptibility tests. Approved standard - 9th edition. CLSI document M2-A9. Wayne, PA: CLSI.
- Thomson KS, Sanders CC. Detection of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae: comparison of the double-disk and three-dimensional tests. *Antimicrob Agents Chemother*. 1992;36(9):1877–82. doi:10.1128/aac.36.9.1877.
- Lee K, Chong Y, Shin HB, Kim Y, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo- β -lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect*. 2001;7(2):88–91. doi:10.1046/j.1469-0691.2001.00204.x.
- Rattan A, Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, et al. Evaluation of Methods for AmpC Beta-Lactamase in Gram Negative Clinical Isolates from Tertiary Care Hospitals. *Indian J Med Microbiol*. 2005;23(2):120–4. doi:10.4103/0255-0857.16053.
- Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev*. 2010;74(3):417–33.
- Ban KA, Minei JP, Laronga C, Harbrecht BG, Jensen EH, Fry DE, et al. American College of Surgeons and Surgical Infection Society: Surgical Site Infection Guidelines, 2016 Update. *J Am Coll Surg*. 2017;224(1):59–74. doi:10.1016/j.jamcollsurg.2016.10.029.
- Lilani SP, Jangale N, Chowdhary A, Daver GB. Surgical site infection in clean and clean-contaminated cases. *Indian J Med Microbiol*. 2005;23(4):249–52.
- Curcio D, Cane A, Fernández F, Correa J. Surgical site infection in elective clean and clean-contaminated surgeries in developing countries. *Int J Infect Dis*. 2019;80:34–45. doi:10.1016/j.ijid.2018.12.013.

24. Dellinger EP. Cephalosporin Plus Metronidazole for Surgical Prophylaxis. *Surg Infect*. 2018;19(4):359–61. doi:10.1089/sur.2018.023.
25. de Boer I, Bangalore S, Benetos A, Davis AM, Michos ED, Muntner P, et al. Diabetes and Hypertension: A Position Statement by the American Diabetes Association. *Diabetes Care*. 2017;40(9):1273–84. doi:10.2337/dci17-0026.
26. Vire FP, Akpaka EP, Unakal C. Prevalent virulent genes among methicillin resistant Staphylococcus aureus isolates from community settings in Trinidad and Tobago. *Int J Infect Dis*. 2018;73:156–7. doi:10.1016/j.ijid.2018.04.3768.
27. Sawdekar H, Sawdekar R, Wasnik V. Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to antibiotic agents at super specialty hospital, Amravati city, India. *Int J Res Med Sci*. 2015;3(2):433–9. doi:10.5455/2320-6012.ijrms20150210.
28. Sudhaharan S, Kanne P, Chavali P, Vemu L. Aerobic bacteriological profile and antimicrobial susceptibility pattern of pus isolates from tertiary care hospital in India. *J Infect in Dev Ctries*. 2018;12(10):842–8. doi:10.3855/jidc.10473.
29. Giri VP, Giri OP, Bajracharya S, Khan FA, Sinha SP, Kanodia S, et al. Risk of Acute Kidney Injury with Amikacin versus Gentamycin both in Combination with Metronidazole for Surgical Prophylaxis. *J Clin Diagn Res*. 2016;10(1):9–12.
30. Codjoe F, Donkor E. Carbapenem Resistance: A Review. *Med Sci*. 2017;6(1):1. doi:10.3390/medsci6010001.
31. Meletis G. Carbapenem resistance: overview of the problem and future perspectives. *Ther Adv Infect Dis*. 2016;3(1):15–21. doi:10.1177/2049936115621709.
32. Goswami NN, Goswami APP, Tripathi CB, Trivedi HR, Patel TK. Antibiotic sensitivity profile of bacterial pathogens in postoperative wound infections at a tertiary care hospital in Gujarat, India. *J Pharmacol Pharmacother*. 2011;2(3):158–64. doi:10.4103/0976-500x.83279.
33. Emameini M, Khoramian B, Jabalameli F, Beigverdi R, Asadollahi K, Taherikalani M. Prevalence of high-level gentamicin-resistant Enterococcus faecalis and Enterococcus faecium in an Iranian hospital. *J Prev Med Hyg*. 2016;57(4):197–200.
34. Wadekar MD, Sathish JV, Jayashree, Pooja C. Bacteriological profile of pus samples and their antibiotic susceptibility pattern. *Indian J Microbiol Res*. 2020;7(1):43–7. doi:10.18231/j.ijmr.2020.010.
35. Abraham Y, Wamisho BL. Microbial susceptibility of bacteria isolated from open fracture wounds presenting to the err of black-lion hospital. *Ethiopia Afr J Microbiol Res*. 2009;3(12):939–51.
36. Biadlegne F, Abera B, Alem A, Anagaw B. Bacterial isolates from wound infection and their antimicrobial susceptibility pattern in Felege Hiwot referral Hospital North West Ethiopia. *Ethiop J Health Sci*. 2009;19(3):173–7.

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Cite this article: Roopashree S, Prathab AG, Sandeep T. Bacteriological profile and antibiotic susceptibility patterns of wound infections in a tertiary care hospital in South India. *Indian J Microbiol Res* 2021;8(1):76–85.