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Original Research Article

Antibiotic resistance pattern of aerobic bacterial isolates from patients with skin and soft tissue infections in Karaikal

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ABSTRACT

Background: Skin and Soft Tissue Infections (SSTIs) are considered as non-fatal burden with significant morbidity and disability. The important challenge of severe SSTIs is choosing a drug for empirical treatment. From this region, only limited local antibiogram data is available.

Aim: To determine the frequency of different aerobic bacteria isolated from patients with SSTIs attending dermatology and surgery departments of GH, Karaikal and to study the antibiotic resistance pattern of the isolates.

Materials and Methods: This is a prospective, cross-sectional study with 100 samples. Standard protocol was followed for collection, processing, identification and antibiotic susceptibility testing. All isolates of *S. aureus* and *Staphylococcus* were screened for methicillin resistance and subsequently subjected to Oxacillin E-strip and Vancomycin E-strip to know the minimum inhibitory concentration (MIC) value. Isolates of Gram negative bacilli resistant to one or more carbapenems were tested for carbapenemase production using Modified Carbapenem Inactivation Method (MCIM) and multi drug resistant (MDR) organisms were identified.

Results: Most effective antibiotic for methicillin sensitive *S. aureus* (MSSA) are Clindamycin (82.75%), Gentamicin (80.95%) and Cotrimoxazole (75%). The methicillin resistant *S. aureus* (MRSA) incidence is 6.89% (2/29). Around 66.67% (4/6) of *Staphylococcus* was Cefoxitin resistant. The carbapenem resistance was found to be 13.88% (5/36). Around 43.13% (22/51) Gram negative bacilli were MDR.

Conclusion: The presence of MRSA and carbapenemase producing Gram negative bacilli are worrisome. Further, routine surveillance is needed to monitor the trends in antibiotic resistant pattern. However, this data paves way for judicious use of antibiotics for treatment and to prevent development of resistance in future.

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1. Introduction

Skin and soft tissue infections (SSTIs) are a common health problem worldwide and mostly presented as a mild infection. They are caused either due to infection (e.g.,

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bacterial, fungal, viral skin diseases and scabies) or non-infection (e.g., dermatitis, urticaria and psoriasis). Bacterial SSTIs may present as purulent type such as carbuncles, furuncles and abscesses or non-purulent type such as cellulitis, erysipelas and necrotizing fasciitis.¹ Among the infectious causes, predominantly Gram-positive cocci such as *Staphylococcus aureus* accounting to 21.5% followed by other Gram-negative bacteria have been reported.² Infections.³ In terms of years lived with disability accounted for 4.02% in India.⁴

In India, epidemiological data of SSTIs is in the form of small case series or surveys from hospitals or communities. It is difficult to know the exact incidence and prevalence due to the variable presentation of SSTIs with short duration (tend to resolve in 7 to 10 days). However, the global burden of disease study reported, that in India, the SSTIs stand 10th in rank according to age standardized years lived with disability.⁴ SSTIs were highlighted as the third most diagnosed disease condition in emergency care settings after chest pain and asthma.⁵ An incidence rate of 18.21/ 1000-person year in patients with SSTIs has also been reported from Tamil Nadu.⁶

Due to temporal and geographic variations in the antibiotic susceptibility pattern, the medical management of severe SSTIs is really challenging. Because, it requires local antibiogram pattern to choose drug for empirical treatment to regulate the infection, prevent morbidity and improve quality of life and avoid the emergence of drug resistance. From this region, there is only limited data on local antibiogram available to choose appropriate empirical antibiotic till the report is available. Considering these facts, the present study was carried out to determine the aerobic bacterial agents from clinical specimens of patients with SSTIs attending Dermatology and Surgery departments in a secondary care hospital. This was followed by performing antibiotic susceptibility testing to know the antibiotic resistance patterns to different groups of locally available antibiotics from this region.

2. Material and Methods

This is a prospective, cross-sectional study with a sample size of 100 within the duration of two months (September-October). The sample size was calculated using data from annual report of Antimicrobial Resistance research and Surveillance Network of ICMR.² This study was approved by Institutional Ethics Committee (JIP/IEC-OS/2022/255).

2.1. Inclusion criteria

All patients (of all ages) with skin and soft tissue infections attending Surgery and Dermatology departments, Government Hospital were included in the study.

2.2. Exclusion criteria

Participants with burn wounds and allergic reactions which are usually not caused by bacteria are prone for greater risk of contamination during specimen collection, and may prove fatal to patients. Hence, they were excluded from the study.

The patients with SSTIs attending the outpatient Departments of Surgery and Dermatology unit were provided with information leaflet and upon their willingness, consent was obtained. The baseline data was collected. After cleaning the wound with sterile saline, pus was collected using sterile separate swabs from same site) and placed in a container and transported immediately to the laboratory. If there is a delay of more than two hours, the collected specimen was stored at 4°C upto 24 hours. The isolation and identification of pus were done using standard procedure.⁷ Antimicrobial susceptibility testing (AST) pathogens were performed manually using Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.⁸ *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 were used as controls for AST. The following antibiotic discs were used for disc diffusion method - Ampicillin (10µg), Gentamicin (10µg), Amikacin (30µg), Ampicillin-Sulbactam (10/10µg), Azithromycin (15µg), Erythromycin (15µg), Clindamycin (2µg), Piperacillin-Tazobactam (100/10µg), Cefoxitin (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Penicillin (10 units), Linezolid (30µg), Tetracycline (30µg), Vancomycin (30µg), Co-trimoxazole (1.25/23.75µg), Imipenem (10µg), Meropenem (10µg). After 24 hours, the observations were noted and result interpretation was based on CLSI as sensitive, intermediate and resistant based on the diameter of zone of inhibition.

2.3. Methicillin resistance determination

Cefoxitin (30 µg) disc was used as a surrogate marker to test for methicillin resistant *Staphylococcus aureus* (MRSA) by Kirby bauer disk diffusion method (CLSI). All the isolates of *S.aureus* and *Staphylococcus* spp that showed resistant to cefoxitin were subjected to Oxacillin E test (OXA: 0.016-256 µg).⁹

2.4. D-test for inducible clindamycin resistance

The test was performed by placing Erythromycin (15 µg) and Clindamycin (2 µg) disc at a distance of 24mm apart adjacent side in standard disc diffusion method for all isolates of *Staphylococcus aureus*, *Staphylococcus* spp and *Streptococcus*.⁸

2.5. Vancomycin resistance detection

Vancomycin (Van: 0.016-256 μg) ezy MIC strip by E test was performed for all the isolates of *Staphylococcus aureus* and *Staphylococcus* spp to detect Vancomycin intermediate *Staphylococcus aureus* (VISA) and Vancomycin resistant *Staphylococcus aureus*(VRSA).⁹

2.6. Carbapenemase detection

All the isolates of the Enterobacterales and *P. aeruginosa* resistant to carbapenems like imipenem or meropenem were subjected to modified carbapenem inactivation method (mCIM) for carbapenemase production.¹⁰

2.7. Multi drug resistant Organism (MDRO) detection

The bacterial isolate resistant to more than one class of antibiotics were noted from the AST reading (Kriby-Bauer method) and are called as multiple drug resistance organisms.^{11,12}

2.8. Statistical analysis

All the data were entered into Microsoft excel sheet and the results were calculated as percentage, frequency/ proportion for recording antibiotic resistance pattern.

3. Results

A total of 110 pus swabs were collected from patients with skin and soft tissue infections who attended Surgery and Dermatology department between the study period of September -October 2022. swabs were excluded due to incomplete questionnaires and withdrawal of consent. In a total of 100 pus swabs, 85 were collected from Surgery department with SSTIs such ulcers, diabetic foot infections, abscesses, necrotising fasciitis, cellulitis, surgical site infection and bite wound infections whereas 15 pus swabs were collected from Dermatology department with erysipelas, pyoderma, folliculitis and cellulitis (Table 1). The SSTIs were predominantly seen in the age group of 41-60 (n= 52), followed by 21-40 (n=20) and >60 (n=20) and in <20 age group (n=8)(Figure 1). There is a male predominance of 78% compared to females (22%) (Figure 2).

On processing the 100 pus swabs for culture, 87 specimen yielded growth and remaining 13, no growth was noted. Of the 87 pus swab with growth, 76 showed the growth of pathogens and 11 pus swabs showed the growth of normal flora like Coagulase negative *Staphylococcus* and Diphtheroids. Out of the 76 pus swab, pathogens were isolated from 63 specimens of Surgery department and 13 of Dermatology department. Among the 76 pus swab with growth, 54 pus swabs showed single organism as pathogen whereas total of 49 Gram positive cocci and 51 Gram negative bacilli were isolated (Table 1). *Staphylococcus*

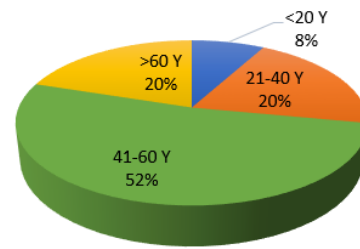


Fig. 1: Age distribution of patients with SSTIs

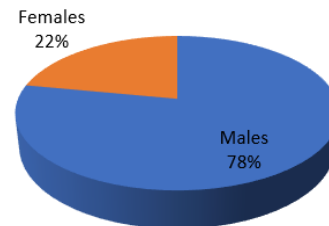


Fig. 2: Gender distribution of patients with SSTIs

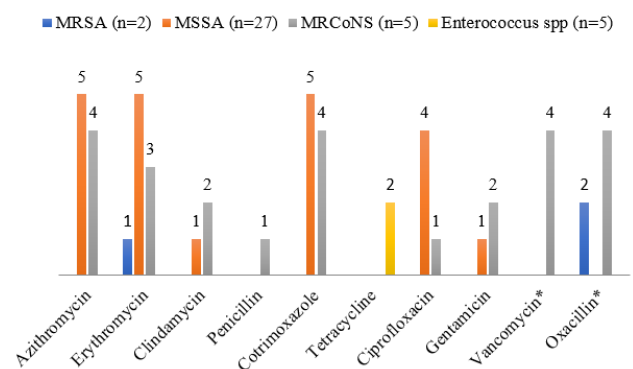


Fig. 3: Antibiotic resistance pattern of Gram Positive organisms {MRSA-Methicillin resistance *Staphylococcus aureus*; MSSA-Methicillin sensitive *Staphylococcus aureus*; MRCoNS-Methicillin resistance Coagulase negative *Staphylococci*; *: Epsilometer test}.

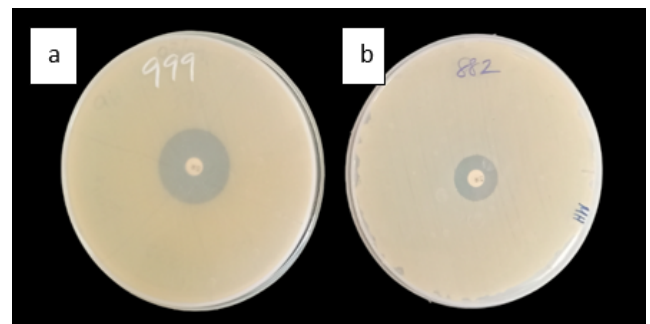


Fig. 4: MRSA detection using cefoxitin (Cx) on MHA using Kriby Bauer disc diffusion method. **a:** Cx sensitive *S. aureus*; **b:** Cx resistance *S. aureus*

Table 1: Bacterial profile of skin and soft tissue infections (Departmentwise)

Total isolate s (n=100)	Surgery (n=85)							Dermatology (n=15)			
	Cellulitis n=4	Abscess n=14	Ulcers n=31	DF n=21	NF n=4	Bites n=2	SSI n=3	Erysipelasy n=2 (13.33)	Pyoderma n=9 (60)	Folliculiti n=1 (6.67)	Cellulitis n=3 (20)
<i>S.aureus</i> (n=29)	1	5	10	2	0	1	1	2	5	1	1
<i>Staphylococcus</i> spp (n=6)	0	0	2	0	1	1	2	0	0	0	0
<i>Streptococcus</i> spp (n=9)	0	0	4	2	0	0	0	0	3	0	0
<i>Enterococcus</i> spp (n=5)	0	0	1	4	0	0	0	0	0	0	0
<i>E.coli</i> (n=12)	0	3	3	4	1	0	0	0	1	0	0
<i>Klebsiella</i> spp (n=8)	0	2	1	3	1	0	0	0	0	0	1
<i>Citrobacter</i> spp (n=6)	1	1	0	3	0	0	0	0	0	0	1
<i>Proteus</i> spp (n=5)	0	0	2	3	0	0	0	0	0	0	0
<i>Providencia</i> spp (n=3)	0	0	3	0	0	0	0	0	0	0	0
<i>Morganella</i> spp (n=2)	0	1	0	1	0	0	0	0	0	0	0
<i>Pseudomonas</i> spp (n=8)	0	2	2	3	1	0	0	0	0	0	0
<i>Acinetobacter</i> spp (n=3)	0	0	2	1	0	0	0	0	0	0	0
NFGNB (n=4)	2	0	1	1	0	0	0	0	0	0	0

(Footnotes: DF- Diabetic foot; NF- necrotising fasciitis; SSI-surgical site infection; NFGNB-nonfermenter GNB)

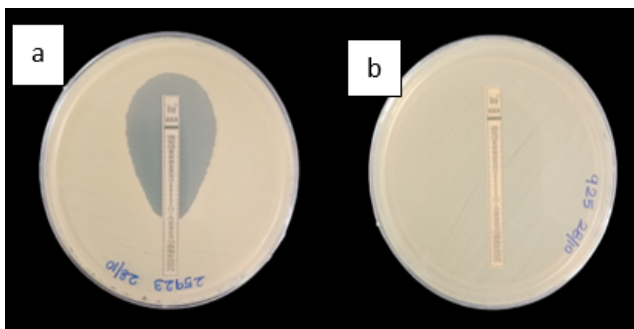


Fig. 5: Oxacillin E strip test; **a:** ATCC Control; **b:** *S.aureus* test isolate showing resistance to oxacillin

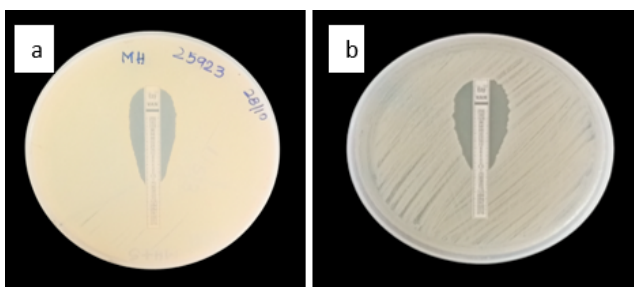


Fig. 6: Vancomycin E strip test; **a:** ATCC Control; **b:** *S.aureus* test isolate showing sensitivity to vancomycin

aureus was predominant cause of SSTIs such as ulcers (n=10; 32.25%), abscesses (n=5; 35.71%) and pyoderma (n=5; 55.56%), diabetic foot (n=2; 7.4%) and cellulitis (n=2; 28.57%) followed by other types (Table 1).

The frequently isolated Gram-positive organisms were *Staphylococcus aureus* (29%) whereas among Gram negative organisms, *Escherichia coli* was the most common (12%) (Table 1). Most of the microorganisms were isolated

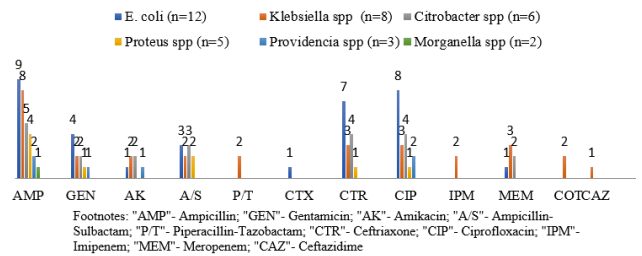


Fig. 7: Antibiotic resistance pattern of members of Enterobacteriales.

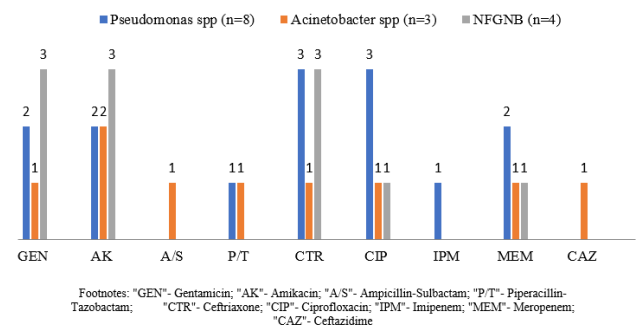


Fig. 8: Antibiotic resistance pattern of *P. aeruginosa*, *Acinetobacter* spp and NFGNB

from specimen collected from outpatient department (n=71; 71%) compared to hospitalised (n=29; 29%).

Antibiotic resistant patterns of *Staphylococcus aureus* and *Staphylococcus* were shown in Figure 3. MRSA prevalence was 6.89% (2/29) isolated from 2 male outpatients reporting to Surgery department, each having an infected non-healing ulcer and a surgical site infection.

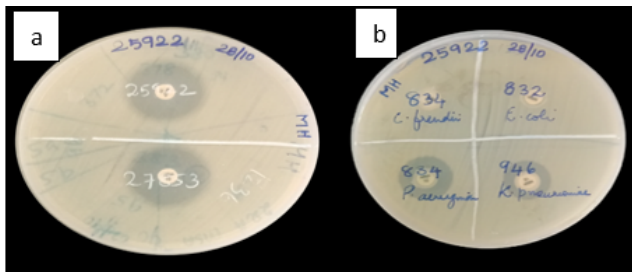


Fig. 9: Carbapenemase detection by mCIM method. **a:** ATCC Controls; **b:** By mCIM shows GNB isolates (834, 832) are carbapenemase producers

These two MRSA identified by disc diffusion method using cefoxitin were subjected to oxacillin E strip test and were found to be resistant (Figures 4 and 5). All the isolates of MRSA and MSSA were negative for inducible clindamycin resistance and were sensitive to vancomycin by Vancomycin E strip test (Figure 6). Low level of resistance was noted for Azithromycin (18.5%), Erythromycin (18.5%), Cotrimoxazole (18.5%) and Ciprofloxacin (14.8%) for MSSA only. There were 5 isolates of Methicillin Resistant coagulase negative *Staphylococcus* (MRCoNS) isolated as single pathogen with pus cells from infected ulcers, necrotising fasciitis, bite wound and surgical site infections. All the *Streptococcus* spp (n=9) were sensitive to Erythromycin, clindamycin, ampicillin, Penicillin, vancomycin and linezolid. In *Enterococcus* spp (n=5), 40% (2/5) were resistant to tetracycline whereas all isolates were sensitive to Penicillin, Ampicillin, Vancomycin, Linezolid and Ciprofloxacin (Figure 3).

The antibiotic resistant pattern of Gram-negative organisms were shown in Figures 7 and 8 . A total of 19.60% (10/51) Gram negative bacilli were resistant to either imipenem or meropenem. Modified carbapenem inactivation (mCIM) method performed with Gram negative bacilli resistant to carbapenem (06/36; 16.66%) detected 66.66% (4/6) GNB as positive for carbapenemase production (Figure 9).

A total of 28% (6 GPC; 22 GNB) of microorganisms were resistant to three or more classes of antibiotic such as Erythromycin, clindamycin, cotrimoxazole, ciprofloxacin, gentamicin, ampicillin and constitute R.

4. Discussion

The antibiotic resistant pattern of various skin and soft tissue infection isolates on routine basis will help to guide clinician in judicious and rationale use of antibiotics. The frequency of aerobic bacteria causing SSTIs and the antibiotic susceptibility testing will help the clinician in choosing an empirical drug of choice for treatment of bacterial infections. In this study, Ramakrishna et al¹³

whereas Sah et al¹⁴ reported from skin and soft tissue infections. The predominance of male patients was seen in this study with male/female ratio of 78/22 and this finding was similar to Sowmya et al.¹⁵ The patients in the age group of 40-60 (n=52; 52%) had higher incidence than 20-40 and >60 age groups, with 20 each and, <20 having 8 patients (n=8; 8%). was observed for patients falling in >30 age group. The advancing age with low healing rate, low immunity, increased catabolic processes, underlying comorbid conditions like diabetes and hypertension the reason for high incidence.¹⁶

The present study shows monomicrobial growth (single pathogen) in 54/76 (71.05%) and polymicrobial growth (more than one pathogen) in 22/76 (28.94%). A study from Telangana¹⁷ reported 93.2% as monomicrobial infections and 6.8% as mixed infections. Few other studies also reported predominantly single pathogen in wound infections.^{15,18,19} In the present study, SSTIs were commonly seen in Surgery department (n=85) than Dermatology department (n=15). In a study from Rajasthan of the SSTIs were contributed by Surgery and departments respectively.²⁰ In the present study, out of 100 isolates, 4951family (44.8%), followed by *Staphylococci* (23.8%).² A study from Chennai also reported Gram negative bacteria twice as that of Gram positive bacteria. Few studies have reported the involvement of Gram positive bacteria more frequently than Gram negative bacteria in superficial infections.^{21,22}

In the present study, *Staphylococcus aureus* (n=29; 59.18%) was the most predominant Gram-positive organism followed by *Escherichia coli* (n=12; 23.52%). Our findings are in accordance with few other studies that reported *Staphylococcus aureus* predominantly followed by *Escherichia coli* in SSTIs.^{15,18,19,23}

In the present study, the prevalence of 6.89% (2/29) MRSA is probably due to a mixed rural and suburban nature of this region. These 2 isolates of MRSA were isolated from OPD cases gives the impression of community acquired MRSA, as they are the most commonly associated type of MRSA with skin and soft tissue infections.^{24,25} The higher prevalence of methicillin sensitive *Staphylococcus aureus* (MSSA) (93%; 27/29) and the AST pattern correlates with few other studies.^{13,26,27}

All beta haemolytic Streptococci (n=9; 18.36%) were susceptible to Clindamycin, Erythromycin, Ampicillin, Penicillin, Vancomycin and Linezolid which is similar to the finding from Ramakrishnan et al.¹³ Enterococcus spp isolates (n=5; 10.2%) were also susceptible to Ampicillin, Penicillin, Ciprofloxacin, Linezolid and Vancomycin but 40% isolates were resistant to Tetracycline. In the present study, members of Enterobacterales (n=36; 70.58%) were more frequently isolated than non-Enterobacterales (n=15; 29.41%) which is similar to a study from South India.²⁸ The Enterobacterales member showed resistance

to ampicillin (29/36; 80.5%), ciprofloxacin (18/36; 50%) followed by ceftriaxone (15/36; 41.6%), gentamicin (10/36; 27.7%), and amikacin, meropenem (6/36; 16.6%) each respectively. In a study from Chennai, <40% susceptibility for ampicillin and <50% susceptibility for ciprofloxacin was reported.¹³ Among the Enterobacterales, 15/36 (41.67%) were resistant to third generation cephalosporins like ceftriaxone. The phenotypic screening for Extended spectrum betalactamases (ESBL), AmpC betalactamases and metallobeta-lactamases (MBL) were not carried out in the present study.

Among the non-fermenters, *Pseudomonas aeruginosa* was the most common organism (n=8; 53.33%), followed by non-fermenter GNB (n=4; 26.67%) and *Acinetobacter* (n=3; 20%). *P. aeruginosa* and nonfermenter GNB exhibited a resistance of 41.66% (5/12) for Amikacin, followed by 33.33% (4/12) each for Gentamicin and Ciprofloxacin. In one study, 48% resistance to amikacin and 62% resistance to ciprofloxacin was reported among the isolates.²⁹ In *Acinetobacter*., OPD isolates showed sensitivity to all the antibiotics whereas ward isolates showed higher resistance towards Amikacin. Similar findings were reported by studies from various parts of India.^{13,30,31}

The Carbapenem are the last resort of drugs needed to treat infections caused by GNB that produce extended spectrum beta-lactamases. In the present study, 6 GNB isolates were resistant to carbapenem. On performing modified carbapenem inactivation method with Gram negative bacilli showed 66.66% (4/6) as positive for carbapenemase production. The carbapenemase production indicates plasmid mediated resistance which is more likely to spread when compared to other mechanism of carbapenem resistance like porin loss or increased efflux pump activity that occur due to chromosomal alterations.¹⁰

In the present study, a total of 28% of microorganisms (6 GPC; 22 GNB) were found to be MDRO showing resistance to Erythromycin, clindamycin, cotrimoxazole, ciprofloxacin, gentamicin, ampicillin. All these resistant organisms were isolated from ulcers, diabetic foot, abscess, cellulitis, necrotising fasciitis and surgical site infection, followed by bite wound and pyoderma. The underlying comorbidities include Diabetes, hypertension and varicose vein. Most of the resistant isolates were obtained from distal part of lower limb compared to other sites and these findings were reported in previous study.³²

5. Conclusions

In this present study, slow emerging antibiotic resistance towards methicillin by *Staphylococcus aureus* and carbapenemase producing Gram negative bacilli are worrisome. With the short duration, choosing the empirical drug for treatment is difficult and need routine surveillance to monitor the trends in antibiotic resistant pattern. However, this data paves way for judicious use of

antibiotics for treatment and to prevent development of resistance in the future.

6. Source of Funding

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7. Conflicts of Interest

There are no conflicts of interest.

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