ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 07 | July - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF DIABETIC FOOT IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL, SOUTH INDIA.

Clinical Microbiology

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ABSTRACT

Background: Diabetic foot infections (DFI) are one of the most common debilitating complications of Type II Diabetic individuals leads to limb amputations. Management of DFI is really challenging owing to delayed wound healing processes and emerging resistance patterns among the pathogenic microbes. **Materials and Methods:** A cross-sectional study was done on 200 diabetic foot infection patients attending a tertiary care hospital in India for a period of 12 months based on Wagner's grading of Diabetic foot wounds. The samples are subjected to standard Gram's staining and CLSI guidelines for identification and antibiogram selection. **Results:** Out of 200 DFI patients studied, the major incidence was observed among males (67%) than females (33%). Major number of cases belonged to Wagner's classification grade 2 (33%). 219 Aerobic bacterial isolates were identified with an average of 1.09 organisms per sample, owing to polymicrobial growth. Gram negative bacilli (74.88%) were the major isolates than the Gram positive cocci (19.63%). Klebsiella pneumoniae (25.57%) being the most predominant followed by Pseudomonas species (20.09%), Proteus mirabilis (10.95%), Escherichia coli (9.13%), Citrobacter species and Enterobacter species (3.65%) each, Proteus vulgaris (1.82%) and Acinetobacter species (0.91%). Staphylococcus aureus (7.30%), Coagulase negative Staphylococcus species (5.47%) and Micrococcus species (12.32%) were among the Gram positive cocci. While 45.9% Klebsiella pneumoniae were predominant ESBL (Extended spectrum beta lactamase inhibitors) producers, 62.5% of Staphylococcus aureus were MRSA (Methicillin resistant Staphylococcus aureus). **Conclusion:** Understanding the regional pathogenic microbial distribution contributes a lot for initiating the empirical antibiotics and to adopt effective management strategies in any hospital.

KEYWORDS

Diabetic foot infections, Wagner's grading, ESBL producers, MRSA.

INTRODUCTION

In the recent decades as the prevalence of diabetes has increased, so too have foot complications, including infections. The development of a foot infection is associated with substantial morbidity, including discomfort, reduced physical and mental quality of life [1], need for healthcare provider visits, wound care, antimicrobial therapy, and often surgical procedures.

The individuals with diabetes have at least a 10-fold greater risk of being hospitalized for soft tissue and bone infections of the foot than individuals without diabetes. The Indian diabetic population is expected to increase to 57 million by the year 2025 [2]. Patients with DM frequently require minor or major amputations of the lower limb (15-27%), and in more than 50% of cases, infection is the preponderant factor [3].

Many studies have reported on bacteriology of Diabetic Foot Infections (DFIs) over the past 25 years, but the results have been varied in different regions. More recently, an increase in the incidence of multidrug resistant (MDR) organisms is threatening the outcome of anti-infectious therapy in the community and in hospitalized patients. Therefore, is urgent need to obtain specimens for culture before initiating empiric antibiotic therapy to help with the selection of a definitive therapy.

Managing infection requires careful attention to properly diagnosing the condition, obtaining appropriate specimens for culture, thoughtfully selecting empirical and then definitive antimicrobial therapy, quickly determining when surgical interventions are needed and providing all other necessary types of wound care. A systematic and, to the extent possible, evidence-based approach to diabetic foot infections (DFIs) should result in better outcomes.

Aim: This study is planned with the aim of determining the bacteriological profile of infected diabetic foot ulcers and the antibiotic pattern of the bacterial isolates.

MATERIALS AND METHODS:

A cross sectional study was conducted in the Department of Microbiology, S.V.Medical College on 200 diabetic foot infection patients attending S.V.R.R.Government General Hospital, Tirupati, a tertiary care hospital in India for a period January 2016 to December 2016 for 12 months. Wagner's classification of wounds was chosen for classifying the diabetic foot wounds [4-6]. The samples were pus and the swabs collected from the depth of the ulcers. They are subjected to Gram's staining and standard CLSI guidelines were used for bacterial culture, identification and the appropriate antibiogram for the isolates. The study was conducted after obtaining the Institutional Ethical Committee approval (IEC).

Inclusion Criteria:

1. Patients with diabetic foot ulcers with age group greater than 20 years. Both males and females were included.
2. All diabetic foot ulcers patients were included irrespective of their treatment history.

Exclusion Criteria:

1. Patients with chronic venostatic change and superficial thrombophlebitis are excluded.
2. Patients with acute osteomyelitis, squamous cell carcinoma and bone tumours are excluded.

***Case history and consent*:** A detailed history of the patients was recorded which included age, sex of the patient, duration of diabetes and foot lesion, earlier treatment (medical & surgical). Informed oral Consent was taken from the patients for the study by explaining the usefulness of the study.

Two swabs were collected from the depth of the ulcers on the feet of each diabetic foot patient. The ulcer was cleaned with normal saline and the surrounding area was cleaned with 70% alcohol. Dead and devitalised tissue overlying the ulcer were removed. One of the swab was used for inoculation into the media for aerobic bacterial growth and the other was used for the preparation of smear for Gram's stain [7]. Nutrient agar, Blood agar, MacConkey agar were used for each sample inoculation. Motility of the organism was identified by Hanging drop preparation. Catalase test and Oxidase test were performed. Organisms were further subjected to relevant biochemical tests as per standards [8,9].

Antibiotic susceptibility testing of the isolates was done on the Muller Hinton agar using Kirby Bauer disc diffusion method [8,9].The

antibiotic discs (obtained from HiMedia) were used for the study were as follows: For Gram positive cocci (GPC): Penicillin G (10 mcg), Amoxycillin clavulanic acid (30 mcg), Clindamycin (2 mcg), Erythromycin (15 mcg), Cotrimaxozole (25 mcg), Ceftriaxone (30 mcg), Ciprofloxacin (5 mcg), Levofloxacin (5 mcg), Piperacillin –tazobactum (100/10 mcg), Imipenem cilastin (10/10 mcg), Linezolid (30 mcg) and Vancomycin (30 mcg) were used.

For Gram negative bacilli (GNB): Amoxycillin clavulanic acid (30 mcg), Ampicillin- sulbactum (10/10 mcg), Amikacin (30 mcg), Aztreonam (30 mcg), Carbenecillin (100 mcg), Ceftazidime (30 mcg), Cotrimoxazole (30 mcg), Ceftriaxone (30 mcg), Ciprofloxacin (5 mcg), Gentamycin (10 mcg), Levofloxacin (5 mcg), Piperacillin –tazobactum (100/10 mcg), Imipenem cilastin (10/10 mcg) were used. Gram-negative bacilli were tested for extended spectrum β-lactamase (ESBL) production by double disk diffusion test and *Staphylococcus* species were tested for methicillin resistance by using 1 µg oxacillin disc. The final identification of the organism and antibiotic susceptibility pattern was reported. All the data was entered simultaneously into excel sheet Master chart for percentage calculation and other analysis. Microsoft word and Excel were used to generate graphs, tables.

RESULTS:

A total of 200 diabetic foot patients were included in the present study. The study included 134 males (67%) and 66 females (33%) between the age group of 20-80 years. Majority of the foot infections were in the range of 51-60 years (28.5%).

Table 1. Distribution of patients based on age

Age distribution	Number (N) of patients	Percentage (%)
20-30	7	3.5
31-40	49	24.5
41-50	47	23.5
51-60	57	28.5
61-70	17	8.5
71-80	3	1.5
Total	200	100

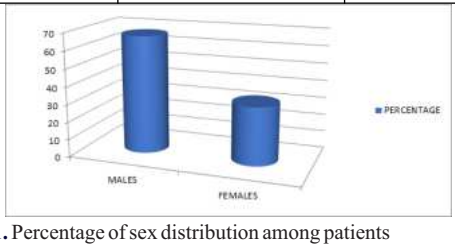

Chart 1. Percentage of sex distribution among patients

Table 2. Distribution of cases based on duration of diabetes mellitus

Duration of illness	Number of patients (N)	Percentage (%)
<1	27	13.5
2-5	38	19
6-10	69	34.5
11-15	21	10.5
>15	25	12.5
Not known	20	10
Total	200	100

Maximum numbers of foot infections were found in patients with duration of Diabetes ranging between 6-10 years.

Table 3. Distribution of ulcers based on Wagner's grading

Grade of ulcer	Number of patients (N)	Percentage (%)
1	25	12.5
2	66	33
3	34	17
4	40	20
5	35	17.5
Total	200	100

33% of diabetic foot ulcer patients belonged to Grade 2 of Wagner's grading of Diabetic foot ulcers.

Table 4. Aerobic organisms isolated in the study group

Aerobic organisms	Number of organisms	Percentage (%)
Klebsiella pneumoniae	56	25.57
Pseudomonas species	44	20.09
Proteus mirabilis	24	10.95
Escherichia coli	20	9.13
Staphylococcus aureus	16	7.30
Citrobacter species	8	3.65
Enterobacter species	8	3.65
Proteus vulgaris	4	1.82
Acinetobacter species	2	0.91
Coagulase negative Staphylococcus species	12	5.47
Micrococcus species	27	12.32
Total	219	100

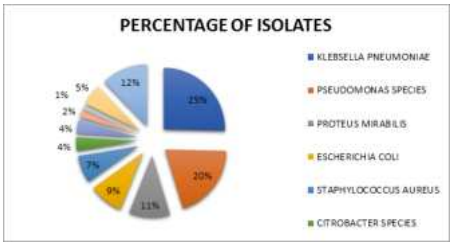

Chart 2. Percentage of aerobic isolates

Klebsiella pneumoniae (25.57%) is the predominant organism isolated followed by *Pseudomonas* spp (20.09%), *Proteus mirabilis* and *Escherichia coli, Staphylococcus aureus*, *Citrobacter* spp., *Enterobacter* spp., *Proteus vulgaris, Acinetobacter* spp., *and Coagulase negative Staphylococcus* species.

Klebsiella pneumoniae (26%) is the predominant monomicrobial organism followed by *Pseudomonas* spp. (18%), *Escherichia coli* (15%), *Micrococci* spp. (6%). *Klebsiella pneumoniae* and *Pseudomonas* species (24.39%) is the commonest polymicrobial growth pattern seen, followed by *Klebsiella pneumoniae* and *Staphylococcus aureus* (9.75%).

Chart 3. Percentage of Polymicrobial growth

Table 5. Antibiotic sensitivity pattern of aerobic isolates

NAME OF THE ANTIBIOTIC	STAPHYLOCOCCUS AUREUS (N=16)	KLEBSIELLA SP (N=56)	PSEUDOMONAS SP (N=44)	PROTEUS SP (N=28)	ESCHERICHIA COLI (N=20)	CITROBACTER SP (N=8)	ENTEROBACTER SP (N=8)	ACINETOBACTER SP (N=2)
AK	-	78.57	75	28	85	50	62.5	50
AMC	81.25	14.28	4.54	17.85	50	25	12.5	50
A/S	-	7.14	31.81	21.42	15	37.5	37.5	50
AZT	-	66	61.36	60.71	55	75	62.5	100
CB	-	-	100	-	-	-	-	-
CD	81.25	-	-	-	-	-	-	-
CIP	25	44.64	88.36	42.85	50	50	37.5	50
COT	14.6	21.42	13.63	32.14	25	37.5	50	50
CTR	25	8.9	13.63	28.57	15	37.5	12.5	0
ERY	37.5	-	-	-	-	-	-	-
GEN	-	12.5	32.14	28.57	30	25	50	50
IMP	-	96.42	75	92.85	80	37.5	37.5	50
IMP/C	-	100	100	100	100	100	100	100
LE	25	82.14	84	96.4	80	87.5	87.5	100
LNZ	100	-	-	-	-	-	-	-
PEN	12.5	-	-	-	-	-	-	-
P/T	100	100	100	100	100	100	100	100

POLY B	-	-	100	-	-	-	-	-
VA	100	-	-	-	-	-	-	-

AK= Amikacin; AMC= Amoxycillin with clavulinic acid; A/S = Ampicillin with sulbactum; AZT= Aztreonam; CB= Carbenecillin; CD= Clindamycin; CIP= Ciprofloxacin; COT= Cotrimoxazole; CTR= Ceftriaxone; ERY= Erythromycin; GEN= Gentamycin; IMP= Imepenem; IMP/C= Imepenem with cilastin; LE= Levofloxacin; LNZ= Lenozolid; PEN= Penicillin; P/T= Penicillin with Tazobactum; POLY B= Polymyxin B; VA= Vancomycin.

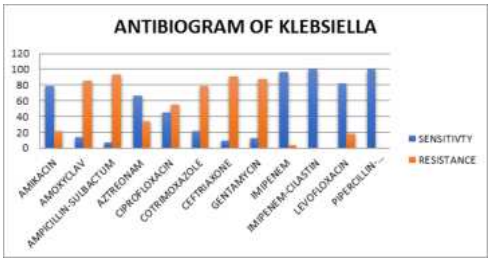

Chart 4. Antibiogram of *Klebsiella pneumoniae*

Klebisella pneumoniae is showing 100% sensitivity to Pipercillin –Tazobactum, Imepenem with Cilastin followed by maximum sensitivity to Imepenem and Amikacin. More than 90% resistance is seen for Ampicillin-sulbactum and Ceftriaxone.

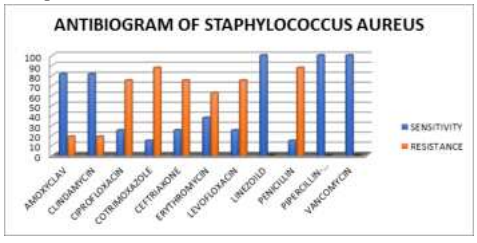

Chart 5. Antibiogram of *Staphylococcus aureus*

Staphylococcus aureus is showing 100% sensitivity to Pipercillin–Tazobactum, Vancomycin and Linezolid. More than 80% sensitivity is seen in cases of Amoxicillin-clavulinic acid and Clindamycin. 87.5% resistance is encountered in cases of Penicillin and Co-trimoxazole.

10 (62.5%) out of 16 isolates of *Staphylococcus aureus* are methicillin resistant (MRSA- Methicillin resistant *Staphylococcus aureus*), while 38% are methicillin sensitive (MSSA).

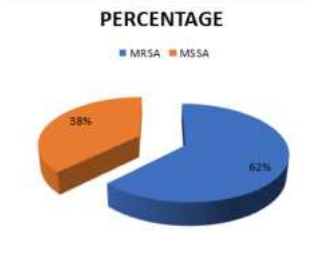

Chart 6. MRSA Percentage

Out of all the Gram negative bacilli, 45% of *Klebsiella pneumoniae*, 30.27% of *Pseudomonas* spp were ESBL producers followed by *Escherichia coli* and *Enterobacter* spp., *Citrobacter* spp. and *Acinetobacter* spp.

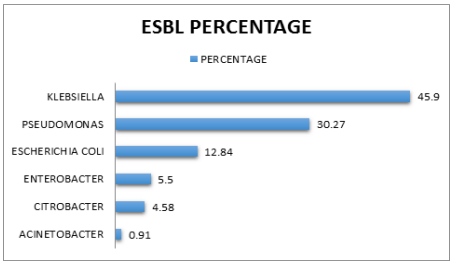

Chart 7. Percentage of ESBL producers

Figure 1: Diabetic foot-wound image

Fig. No. 2: Direct Gram's image of the sample

Fig. No. 3: Growth of *Klebsiella pneumoniae* on MacConkey agar

Fig. No.4: ESBL confirmatory test

DISCUSSION:

Many cases of Bacteriological profile of Diabetic foot infections were studied and proposed throughout the world showing the evolution of new predominance of some bacteria over the other bacteria. Similar study was conducted at our hospital in 2006 by Leela rani et al [10].

Comparative studies regarding incidence of age, sex and duration of DM.

In the present study, diabetic foot infections were more common in the age group of 51-60 years. This coincides with studies of Kavitha et al [11] and Pothati et al [12]. Males (67%) were more prone to diabetic foot infections than females (33%) in the current study. Similar kind of male predominance was observed in the studies of Pothati et al (67.2%) [12] and little high incidence was shown by Kavitha et al (73.21%) [11], Manikandan et al (76.1%) [13]. In the distribution of patients based on duration of diabetes mellitus the present study showed that most of the DFI patients had lesions during 6-10years (34.5%) of diabetes which is co-relating with the study of that of Leela Rani et al [10] and Vimalini Hena et al [14].

Comparative studies regarding wound grading, aerobic isolate growth.

Based on Wagner's Grading of diabetic foot ulcers, in the current study, majority of the patients belonged to grade 2 (33%) followed by grade 4 (20%) which is similar to the study done by Pothati et al (41.8%) [12] and Hefni AH et al [15]. Other studies showed highest incidence of DFI belonging to various grades like Chavan et al (grade 4 - 27.5%)

[16]. In the present study the percentage of monomicrobial growth (52.05%) is more when compared to polymicrobial growth (41.09%). Similar finding was shown by Pothati et al [12] as Monomicrobial growth (68.1%) more than polymicrobial growth (31.8%). Out of the 200 samples tested, the total number of aerobic bacterial isolates was 219 which showed an average of 1.09 organisms per sample. This has almost correlated to the studies of Manikandan et al (1.1) [13] and Kavitha et al (1.1) [11]. The present study was based on only aerobic bacterial isolates excluding the anaerobic bacteria. *Candida* was isolated in the present study (0.91%) which was also isolated in other study by Manikandan et al (3%) [13] and Ozer et al [17].

Comparative studies regarding predominance.

In the present study, out of 219 isolates, the growth of Gram negative bacilli was predominant (74.88%) over the growth of Gram positive cocci (19.63%). Predominance of Gram negative bacilli was quite similar to studies of Jayashree Konar et al (72.36%) [18] and Pothati et al [12] who showed Gram negative bacilli were 72.7% and Gram positive cocci were 27.2%. While studies showed that Gram positive cocci were predominant, shown by Kavitha et al [11] and Al-Mijalli [19] studies on Diabetic foot infections. The present study showed that the most common isolate was *Klebsiella pneumoniae* (25.57%) followed by *Pseudomonas* spp., (20.09%), *Proteus mirabilis* (10.95%), *Escherichia coli* (9.13%). *Klebsiella pneumoniae* predominance in the present study is co-relating with the study of Ozer et al (36.50%) [17]. *Pseudomonas* spp., (20.09%) isolation was similar to studies of Manikandan et al (18%) [13], but some of the studies showed *Pseudomonas* spp. as the most predominant isolate like in Deepa et al (33.33%) [20], Jayashree Konar et al (31.43%) [18]. Among Gram positive cocci, *Staphylococcus aureus* occupied 37.20% and among the total isolates the percentage of isolation was 7.30%. The lower percentage of *Staphylococcus aureus* has also been reported by Leela Rani et al (10.64%) [10], Ozer et al (10.8%) [17] and Pothati et al (12.27%) [12]. Whereas in some studies *Staphylococcus aureus* has been the predominant isolate like Kavitha et al (32.31%) [11].

Table 6. Comparison of the aerobic bacteria isolated in various studies (in percentage, %)

Study	*Klebsiella* spp	*Pseudomonas* spp	*Proteus mirabilis*	*Proteus vulgaris*	*Escherichia coli*	*Citrobacter* spp	*Enterobacter* spp	*Acinetobacter* spp	*Staphylococcus aureus*
Leela Rani et al 2006	12.03	13.42	23.14	6.0	25.42	1.85	3.7	-	10.64
Kavitha et al 2010	15.38	12.31	9.23	6.15	4.62	-	-	-	32.31
Manikandan et al 2010-12	10	18	6	-	20	-	-	3	17
Jayashree Konar et al 2013	2.98	31.43	2.98	-	23.88	-	-	-	22.38
T.Deepa et al 2014	16	33.33	6.6		20	13.5	-	10.6	25
Pothati et al 2018-20	10	31.8	4.5		27.2	-	-	-	12.7
Present study	25.57	20.09	10.95	1.82	9.13	3.65	3.65	0.91	7.30

Comparative studies regarding Antibiogram of the isolates.

Gram negative bacilli being the most predominant isolates in the current study showed 100% sensitivity to Imipenem with cilastin and Piperacillin with tazobactum drug formulations. Levofloxacin also had higher sensitivity percentages indicating higher cure rates in all cases of mild, moderate and severe DFI. In the present study, *Klebsiella pneumoniae* was 96.42% sensitive to Imepenem which indicates avoidance of combination of Impenem with cilastin unless resistance is proved against Impenem alone. They showed good sensitivity to Levofloxacin (82.14%), Amikacin (78.57%) and Aztreonam (66%). High resistance was observed against Amoxycillin with clavulinic acid, Ampicillin with sulbactum, Ceftriaxone, and Gentamycin. *Pseudomonas* species showed 100% sensitivity to Carbenecillin and Polymyxin B. Higher sensitivity of Floroquinolones like Ciprofloxacin (88.36%) and Levofloxacin (84%) was observed which indicates avoidance of usage of second line drugs. Moderate sensitivity was seen in case of Amikacin (75%), Imipenem (75%) and Aztreonam (61.36%). Ceftazidime was only 25% sensitive in the study. As per Pothati et al [12], the most common isolate *Pseudomonas aeruginosa* showed 100% sensitivity to Meropenem and 93% to Imipenem and 80% sensitivity to Piperacillin-tazobactam, 90% to Cefaperazone with sulbactum. Where Otta S et al [21] stated that members of *Enterobacteriaceae* family were mostly sensitive to piperacillin-tazobactam, levofloxacin. Amoxicillin-clavulanic acid and cephalosporins were the most resistant antibiotics. *Pseudomonas* spp. Were usually sensitive to piperacillin-tazobactam (86.6%) and ceftazidime-clavulanic acid (71.4%), whereas *Acinetobacter* spp. was mostly sensitive to netilmicin (60%). They showed a higher degree of resistance to imipenem than those of *Enterobacteriaceae.* In the current study, *Proteus* species showed maximum sensitivity towards Levofloxacin (96.4%) and Imepenem (92.85%). Moderate sensitivity was seen for Aztreonam (60.71%). In the present study, *Escherichia coli* showed maximum sensitivity against Amikacin (85%) followed by Imepenem (80%) and Levofloxacin (80%). Only 50-55% sensitivity was seen against Amoxycillin with clavulinic acid, Ciprofloxacin and Aztreonam. In the present study, *Citrobacter* species showed maximum of 87.5% sensitivity to Levofloxacin followed by 75% sensitivity to Aztreonam. 50% sensitivity was observed in cases of Amikacin and Ciprofloxacin. Among *Enterobacter* species isolated, 87.5% sensitivity was seen for Levofloxacin followed by 62.5% sensitivity for Amikacin and Aztreonam. 50% sensitivity encountered in case of Gentamycin and Co-trimoxazole. Out of 2 isolates of *Acinetobacter* species in the study, 100% sensitivity was seen in Levofloxacin and Aztreonam whereas in rest of other drugs 50% sensitivity was observed.

Present study showed that *Staphylococcus aureus* was 100% sensitive to Piperacillin with tazobactum, Vancomycin and Linezolid. Higher sensitivity (81.25%) was observed for Amoxycillin with Clavulinic acid and Clindamycin. Floroquinolones (25%) have not been very useful against *Staphylococcus* in the present study. Least sensitivity was seen against Penicillin (12.5%). Pothati et al [12] showed all the strains of *Staphylococci* which were isolated were 100% sensitive to Teicoplanin, Linezolid. They were 95% sensitive to Vancomyicin and 80% to Piperacillin with tazobactum. According to Otta S et al [21] *S. aureus* were sensitive to Vancomycin (91.5%), Teicoplanin (91.1%), and Linezolid (90%). They showed 87.5% and 71.8% sensitivity to levofloxacin and piperacillin-tazobactam, respectively.

In the present study, 10 out of 16 isolates of *Staphylococcus aureus* (62.5%) were Methicillin resistant (MRSA) which is co-relating with the study of Deepa et al (66.6%) [20], Mehta VJ et al (60%) [22]. In the present study, the percentage of ESBL producers was 66.46% which is similar to the study of Deepa et al (66.6%) [20] and Mehta VJ et al (69.4%) [22]. Among the ESBL producers, *Klebsiella pneumoniae* (45.87%) is the predominant one similar to the predominance of *Klebsiella* species (50%) in the study of Deepa et al [20] and Pothati et al [12].

CONCLUSION:

The current study showed the changing trend of Gram negative bacilli predominance over the Gram positive cocci in causing DFI. Empirical antibiotic choice towards Gram positive cocci to be restricted. Hence, understanding the regional pathogenic microbial distribution study brings a major revolution for effective management strategies in any hospital.

Limitations:

1. Usage of swab for the sample collection which is of low sensitivity instead of a soft tissue specimen for culture and sensitivity.
2. Anaerobic bacterial study and fungal study has not been done which makes the study incomplete.

Conflict of Interest: The authors declare that there are no conflicts of interest.
Source of Funding: None.

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