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A Study On Bacterial Flora Isolated From Chronic Non-Healing Wound Infections In Patients Of Garhwal Region

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Abstract

Introduction: The chronic non-healing wound infections are generally identified with the presence of bacteria in the wounds. Bacterial presence in the wounds is associated with poor healing. Chronic wounds, with their polymicrobial nature, put a significant burden on health budgets worldwide.

Aim of the study: The present study was conducted to isolate and identify the bacterial flora along with antibiotic sensitivity profiling of the pathogenic isolates against routine antibiotics from patients with chronic non-healing wound infection sat HNB Base Hospital, Srinagar, Garhwal.

Materials & Methodology: A total of 102 specimens (pus, wound exudates, or tissue biopsy) from patients (including 56males and46 females) having chronic non-healing wound infections were studied. Sample collection, Isolation and biochemical identification of aerobic bacteria followed by antibiotic sensitivity profiling of the pathogenic isolates were done using standard protocols.

Result & Conclusion of the study: Staphylococcus aureus were the most prevalent bacteria with 24.6 % of all the isolates. Next to these were Coagulase-negative Staphylococci (CONS) with 11.7% followed by Escherichia coli (9.7%), Enterococcus (8.4%), Klebsiella (8.4%), Acinetobacter (6.5%), Micrococci (6.5%), Diphtheroids (5.8%), Citrobacter (3.9%), Pseudomonas (3.9%) (P. aeruginosa were 66.6%, and 33.3% were P. fluorescens), Neisseria (3.2%), Proteus (3.2%), Streptococci (2.6%) (All were S. pyogenes) and Enterobacter (1.2%). Antibiotic sensitivity profiles of Gram-negative bacterial isolates revealed Acinetobacter and Klebsiella being the most resistant pathogens followed by Enterobacter, Citrobacter, Pseudomonas, E. coli, and Proteus. Antibiotic sensitivity profiles of Gram-positive bacterial isolates revealed Enterococcus and CONS being the most resistant pathogens followed by Streptococci and Staphylococcus aureus.

Keywords: Bacterial flora. Chronic non-healing wound infections. Antibiotic sensitivity profile. Patients of Garhwal region.

Introduction:

A wound which remains for more than six weeks or which does not progress to healing in four weeks is classified as a chronic wound (Frankel et al, 2009). Chronic non-healing ulcers are those that do not respond to the initial treatment or are persistent, despite appropriate care (Souza et al, 2013). These ulcers may be local as well as systemic and comprise a variety of causes including Diabetic foot ulcers (DFU), Buerger's disease (BD), Frost bite, traumatic ulcer, drugs and nutritional deficiencies. According to an estimate by International Diabetes Federation (IDF), 80% of people with diabetes live in low to middle income countries including India (IDF report, 2015).Non-healing ulcers are generally identified with the presence of bacteria in the wounds. Chronic wounds, with their polymicrobial nature, put a significant burden on health budgets worldwide. Bacterial presence in the wound bed has been found to be associated with poor healing (Hussain et al, 2016). A number of Gram positive and Gram negative bacteria are often reported in the clinical specimens from non-healing ulcers. The age standardized prevalence of diabetes & pre-diabetes were 11.2% & 13.2% respectively in a community based study from North India (Ravikumar et al, 2011). DFU does not occur spontaneously, and there are many premonitory signs that may be used to predict those 'at risk' (Joslin, 1934).

Etiologic factors for Buerger's disease include chronic smoking, male preponderance, and low socioeconomic status, genetic and hormonal factors. Apart from that in various studies, a possible role for Rickettsia in this disease has been proposed (Fazeli et al, 2011). In 1987, Bartolo was the first to claim that Rickettsia could be main etiology of Buerger's disease, measuring the titres of antibodies against Rickettsia in BD patients (Fazeli, 2016). Frost bite is also a common cause of chronic non healing ulcers in hilly regions of Uttarakhand. Most of the diabetic foot infections are polymicrobial in nature and mixed organisms are frequently encountered (Bansal et al, 2008; Ramani et al 1991; Viswanathan et al, 2002). The spectrum

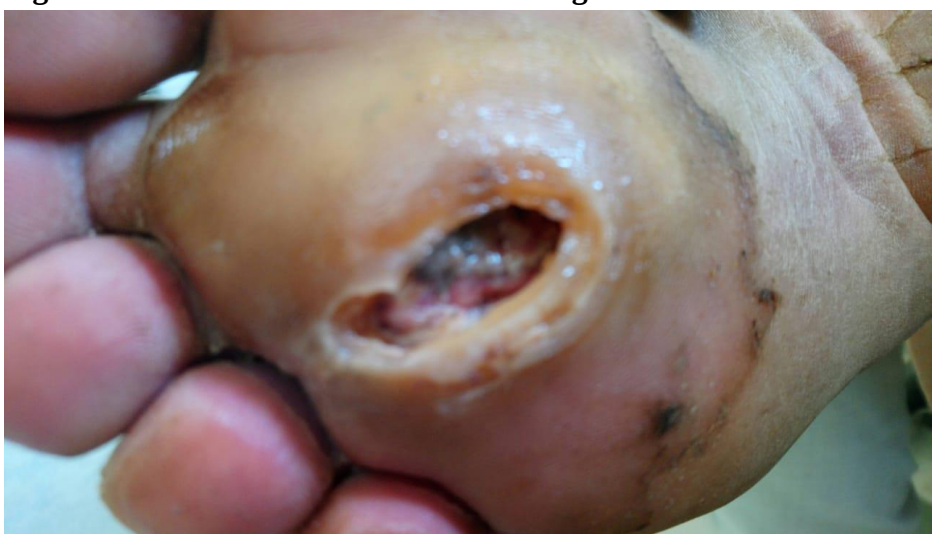
of micro organisms depends mainly on microbial flora of the lower limb, metabolic factors,foot hygiene and the use of antibiotics.

Materials & Methods:

Collection of specimens:
A total of 102 specimens (pus, wound exudates, or tissue biopsy) from patients (including 56 males and 46 females) having chronic non-healing wound infections were taken at HNB Base Hospital, Srinagar, Garhwal for over a period of one year (February 2017 to February 2018).The samples were collected in sterile containers using standard protocols. Ethical clearance was taken from Institutional Ethical Committee (IEC).

Procedure of swabbing: The affected area was disinfected with alcohol to remove commensal flora. Then, sterile swab, moistened with sterile saline to increase adherence of bacteria was used to collect the specimen by rubbing the swab over wound area in a zigzag motion along with twisting the swab so that entire swab surface came in contact with wound surface. Swab was moved from centre to periphery of wound upto the edge of wound (As per the method described by Siddiqui and Bernstein, 2010 & Starr and Macleod, 2003). A representative image of patients with chronic non-healing wound infections is shown as **Figure-1**.

Figure-1: Patient with chronic non-healing wound infection.



Isolation and identification of bacterial isolates:
Aerobic bacterial isolates were identified and isolated using morphological examination and microscopic identification followed by biochemical and aerobic culture methods. The samples were first inoculated on blood agar plates with the help of sterile cotton swabs and “L” shaped spreader. After inoculation, the blood agar plates were subjected to incubation at 37°C for 24 hours. After incubation, all unique colonies were sub-cultured to get isolated colonies and sufficient inoculums were taken for preservation of colonies in BHI agar slants. Blood agar slant were also used for preserving fastidious colonies. Gram’s staining followed by microscopic examination was performed for differentiation of Gram-positive and Gram-

negative bacteria. Biochemical identification of the isolates was done using biochemical tests viz. Catalase test Coagulase test, Oxidation-Fermentation (OF) (Hugh Leifson) test, Lactose fermentation test, Triple Sugar Iron (TSI) agar test, Citrate utilization test, Indole test, Methyl Red (MR) test and Voges-Proskauer (VP) test as per standard methods (Prescott et al, 2003).

Antibiotic sensitivity profiling of the pathogenic isolates:

Pathogenic isolates were subjected to the antibiotic sensitivity testing against routine antibiotics (as given in table-1)using Kirby Bauer disc diffusion method as recommended by the Clinical and Laboratory Standard Institute (CLSI). Inoculum density was kept as approximately 1x108 CFU/ml. The inocula were adjusted to McFarland 0.5 turbidity standard. Mueller Hinton Agar (MHA) was used throughout the testing. The inocula were spreaded on the agar in Petri plates with the help of sterile cotton swab sticks, antibiotic discs were placed on to the surface. Plates were incubated at 37 °C for 24 hours. The inhibition zones were measured in mm.

MRSA isolates were detected by using Cefoxitin (30 µg discs) according to CLSI guidelines, Anand et al, 2009, Furtado et al, 2014. Cefoxitin disc with inhibition zone of < or equal to19 mm was considered as Methicillin resistant while inhibition zone of> or equal to 20 mm zone diameter was considered as Methicillin sensitive.

Multi-drug resistance is defined as resistance to one agent from each 3 or more antibiotics class. (Magiorakos et al, 2012). In this study, we performed D test also, which was application of clindamycin disc 15–24 mm to erythromycin disc from edge to edge (as per CLSI guidelines). Erythromycin (15µg) disc was placed 15 mm edge to edge from clindamycin (2 µg) on Mueller Hinton agar which was plated with Gram Positive Cocci (GPC) isolates. After overnight incubation at 37°C, blunting or flattening of zone (D shape) around Clindamycin in between both discs indicated Clindamycin resistance (Prabhu et al, 2012 and Fiebelkorn et al, 2003).

Vancomycin resistant was assumed by disc diffusion method due to poor resources for MIC. Disc diameter of less than 14 mm or equal to 14mm was considered as resistant (Rebwar et al, 2014).

Table-1: Antibiotics used in the present study

S. No.	ANTIBIOTICS	DISC CONTENT
1	Ampicillin	10µg
2	Amoxicillin-Clavulanic acid	20/10 µg
3	Amikacin	30 µg
4	Cefotaxime	30 µg
5	Chloramphenicol	30 µg
6	Cotrimoxazole	1.25/23.75 µg
7	Cefoxitin	30 µg
8	Ciprofloxacin	5 µg
9	Erythromycin	15 µg
10	Gentamicin	10 µg/120µg
11	Moxifloxacin	5 µg

12	Cefixime	5 µg
13	Aztreonam	30 µg
14	Cefoperazone-sulbactam	75/10 µg
15	Clindamycin	2 µg
16	Meropenem	10 µg
17	Tigecycline	15 µg
18	Linezolid	30 µg
19	Teicoplanin	30 µg
20	Vancomycin	30 µg
21	Azithromycin	15 µg
22	Colistin	10 µg
23	Polymyxin- B	300U
24	Cefipime	30 µg
25	Levofloxacin	5 µg

Result and discussion:

Prevalence of bacteria in the samples:

Out of 102 samples taken in the study, Staphylococcus aureus (S. aureus) were found to be the most prevalent bacteria with 24.6 % of all the isolates. Next to these were Coagulase-negative Staphylococcus (CONS) with 11.7% followed by Escherichia coli (9.7%), Enterococcus (8.4%), Klebsiella (8.4%), Acinetobacter (6.5%), Micrococci (6.5%), Diphtheroids (5.8%), Citrobacter (3.9%), Pseudomonas (3.9%) (P. aeruginosa were 66.6%, and33.3% were P. fluorescens), Neisseria (3.2%), Proteus (3.2%), Streptococci(2.6%) (All were S. pyogenes), and Enterobacter sp (1.2%). Overall prevalence of the bacterial isolates in pus samples is shown in table-2 and depicted in figure-2.

A comparison of prevalence of the bacterial isolatesin present study with few other similar studies is presented in Table-3.

Table-2: Overall prevalence of the bacterial isolates in pus samples

Prevalence of the isolates in pus samples		
Name of Isolates	**Number of isolates**	**Prevalence (%)**
Staphylococcus aureus	38	24.6
Coagulase negative staphylococci (CONS)	18	11.7
Escherichia coli	15	9.7
Enterococcus	13	8.4
Klebsiella	13	8.4
Acinetobacter	10	6.5
Micrococci	10	6.5
Diphtheroids	9	5.8
Citrobacter	6	3.9

Pseudomonas	6	3.9
Neisseria	5	3.2
Proteus	5	3.2
Streptococci	4	2.6
Enterobacter sp	2	1.2

Figure-2: Overall prevalence of the bacterial isolates in pus samples

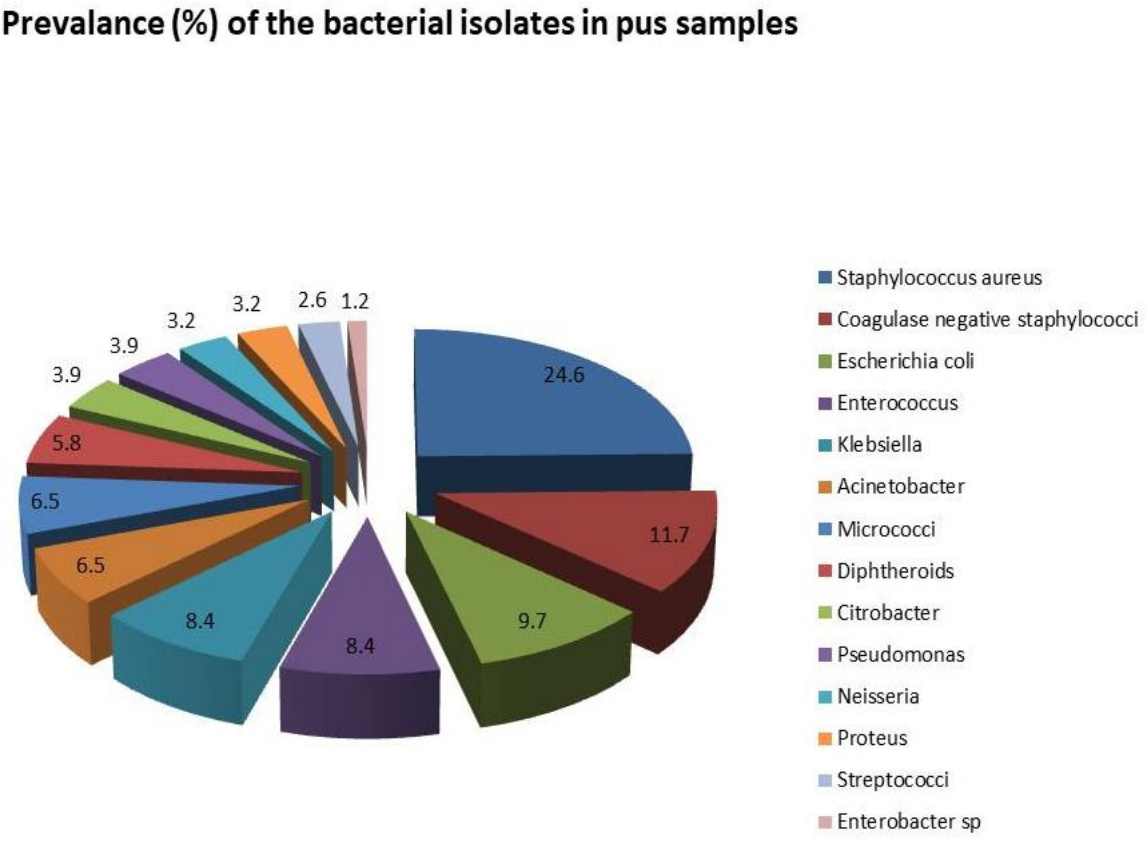


Table-3: A comparison of prevalence of the bacterial isolates in present study with few other similar studies.

S. No.	Study	No. of specimens/patients studied	No. of bacteria isolated	Prevalence of bacteria
1.	Present Study	102	154	S. aureus (24.6 %), CONS (11.7%),Escherichia coli (9.7%), Enterococcus (8.4%), Klebsiella (8.4%), Acinetobacter (6.5%), Micrococci (6.5%), Diphtheroids (5.8%), Citrobacter (3.9%),

				Pseudomonas (3.9%) (P. aeruginosa were 66.6%, and 33.3% wereP. fluorescens), Neisseria (3.2%), Proteus (3.2%), Streptococci (2.6%) (All were S. pyogenes), and Enterobacter sp (1.2%).
2	Meenakshisundaram et al,2016.	75	104	Escherichia coli (22.2%), S. aureus (17.3%), Pseudomonas aeruginosa (17.3 %), Klebsiellaspp.. (10.6%), CONS (10.6 %), Proteusspp. (9.6 %), Streptococcusspp.(5.8 % Corynebacteriumspp.(3.8%), and Enterococcus spp.(2.9%).
3	Yerat and Rangasamy, 2015	104	163	Aerobic (81.66%), anaerobic (14.79%), and fungal (3.55%) isolates were obtained on culture with Gram-negative bacilli (78.98%) being isolated more than the Gram-positive cocci (21.01%). Proteus mirabilis was the most common isolate (26.08%) while Bacteroidesfragilis and Peptococcusspp. were the common anaerobes obtained. 56.73% of patients had polymicrobial infection, and 23.08% of staphylococci were methicillin resistant Staphylococcus aureus.
4	Priyadarshini et al, 2013.	50	75	Gram negative bacilli were more prevalent (65.1%) than gram positive cocci (34.9%). The commonest isolate was Pseudomonas spp. (16%), followed by Escherichia coli

				(14.6%) and Staphylococcus aureus (13.3%).
5	Anandi et al, 2004	107	222	Aerobes: Pseudomonas spp. (11.3%) E.coli (27.7%) Klebsiellaspp. (13.6%) Proteus spp. (16.9%) Enterobacter spp. (9.6%) Enterococcus spp. (7.3%) S. aureus (13.6%) Anaerobes**:** Cl. perfringens(31.1%) Cl. Sporogenes (17.8%) Cl.tetanomorphum (11.1%) Bacteroidesfragilis (20%) Prevotellasp.(13.3%) Peptostreptococcussp. (6.7%)
6	Bessa et al, 2015	213	Not disclosed	A total of 28 different microbial species were isolated; 44·2% were Gram-positive and 55·8% wereGram-negative. The most common bacterial species detected was Staphylococcus aureus (37%), followed by Pseudomonasaeruginosa (17%), Proteus mirabilis (10%), Escherichia coli (6%) and Corynebacterium spp. (5%). The most representative species of Enterococcus was Enterococcuscloacae.

Antibiotic sensitivity profiles of the bacterial isolates:

Among Gram-negative bacterial isolates, **E. coli**isolates showed 100% sensitivity to Polymyxin-B followed by 83.3% sensitivity to Gentamicin and Cefipime each. Sensitivity to Meropenem, Amikacin and Cefoperazone-sulbactam was 78.57%, 73.3% and 72.7% respectively. Sensitivity to Amoxyclav and Moxifloxacinwas 64.28% and 62.5%respectively. Next to these were Tigecycline and Colistinwith 60% sensitivity. The Isolates showed 58.3% sensitivity to Ciprofloxacin, 57.14%to Cefotaxime, 33.3% to Cotrimoxazole, 28.5% to

Cefoxitin and Aztreonam each with least sensitivity to Cefixime (23.06%) and Ampicillin (20%). Overall antibiotic sensitivity profiles of Gram-negative bacterial isolates are detailed in **Table-4** and graphically depicted in **Figure-3.**

Klebsiella isolates showed a lower level of overall sensitivity as compared to other Gram-negative bacteria isolated in this study. In these isolates, maximum sensitivity was 81.81% to Polymyxin-Bfollowed by 70 % sensitivity to Colistin, 64.28% to Meropenem, 61.53% to Amikacin and 60% to Tigecycline. Sensitivity to Aztreonam was 50.54% and 46.15% for Amoxyclav, Gentamicin, Cotrimoxazole each. Cefoperazone-sulbactam with 41.66% sensitivity and Moxifloxacin with 33.3% sensitivity were next to these. The Isolates showed 27.27% sensitivity to Ciprofloxacin and Cefixime each. Lesser levels of sensitivity were found against Cefotaxime (15.38%) and Cefoxitin (10%) while complete resistance was observed against Ampicillin and Cefipime.

Enterobacter isolates showed maximum sensitivity (100%) to Polymyxin-B, Ciprofloxacin, Moxifloxacin, Cefoperazone-sulbactam,Colistin and Aztreonam. Sensitivity to Amikacin, Gentamicin, Cotrimoxazole, Cefoxitin and Tigecycline was 50 % for each. Enterobacter sp are usually reported as intrinsically resistant to Cefipime, the similar was observed in this study. Complete resistance was observed against Amoxyclav, Ampicillin, Cefotaxime, Cefixime, Cefipime and Meropenem.

Acinetobacter isolates showed 100% sensitivity to Polymyxin-B and Colistin each. Sensitivity to Cefopera zone-sulbactam and Tigecycline was 80% each. Next to these were Meropenem, Amikacin, Gentamicin and Amoxyclav with 50%, 44.4%, 42.8% and 30% sensitivity respectively. Lesser levels of sensitivity were found against Ciprofloxacin (28.5%), Aztreonam (25%) Cotrimoxazole, Cefoxitin (20% each), Cefixime (14.28%) and Moxifloxacin (11.11%). Complete resistance was observed against Ampicillin, Cefotaxime and Cefipime.

Proteus isolates showed 100% sensitivity to Cotrimoxazole and Colistin each. Sensitivity to Cefotaxime, Cefixime, Cefipime, Meropenem and Ciprofloxacin was 80% each. Next to these were Polymyxin-B (75% sensitivity), and 66.6% sensitivity to Aztreonam, Gentamicin and Moxifloxacin each. Sensitivity to Amikacin and Cefoperazone-sulbactam was 60% each, while 50% sensitivity was observed against Amoxyclav and Cefoxitin. A lesser level of sensitivity was found against Tigecycline (33.3%). Complete resistance was observed against Ampicillin.

Pseudomonas isolates showed maximum sensitivity (100%) to Polymyxin-B and Colistin each. Sensitivity to Meropenem and Cefoperazone-sulbactam was 83.33% and 80% respectively while 75% sensitivity was observed against Aztreonam and Moxifloxacin each. Next to these were Amikacin(66.66% sensitivity), Gentamicin (60% sensitivity)followed by Ciprofloxacin, Cefoxitin and Cefipime (50% sensitivity for each). Lesser levels of sensitivity were found against Amoxyclav, Cotrimoxazole, Cefixime (33.3%sensitivity for each), and Cefotaxime (25%).Complete resistance was observed against Ampicillin and Tigecycline.

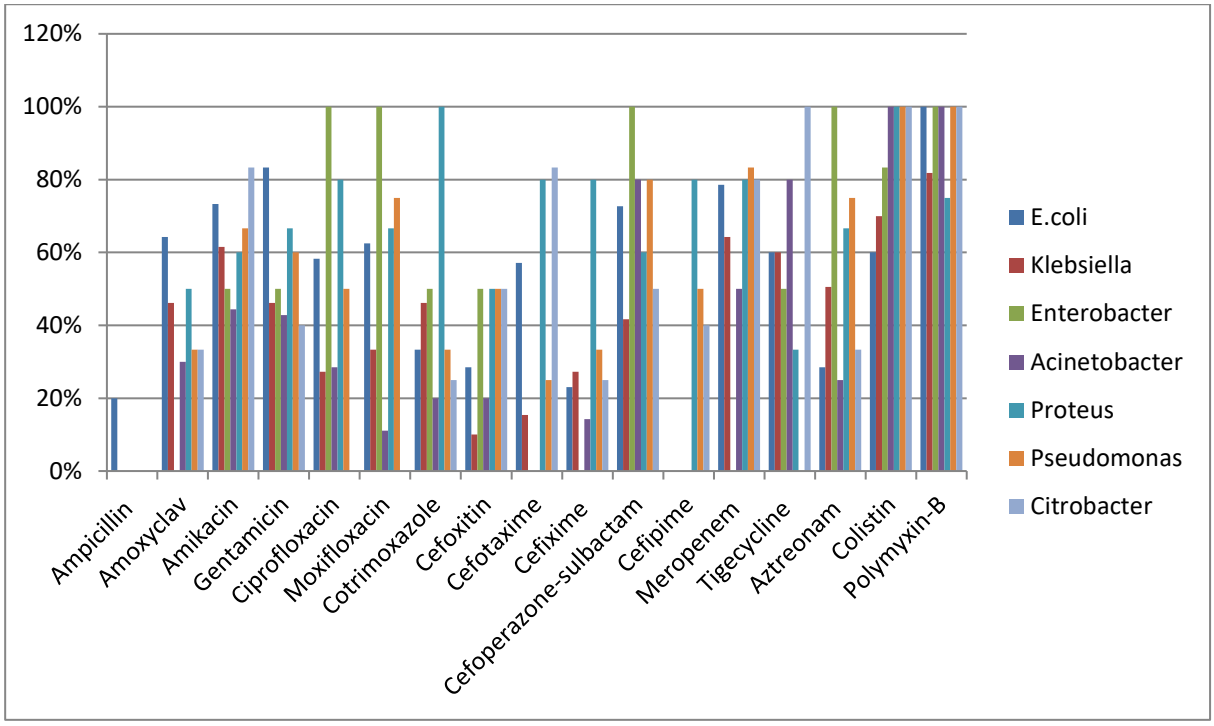
Citrobacter isolates showed 100% sensitivity to Polymyxin-B, Colistin and Tigecycline each. Sensitivity to Amikacin and Cefotaxime was 83.3% each followed by 80% sensitivity to Meropenem. Next to these were Cefoperazone-sulbactam, Cefoxitin (50% sensitivity for each), Gentamicin and Cefipime (40% sensitivity for each), Aztreonam and Amoxyclav (33.3% sensitivity for each) followed by Cotrimoxazole and Cefixime (25% sensitivity for each). Complete resistance was observed against Ampicillin, Ciprofloxacin and Moxifloxacin.

Overall, Antibiotic sensitivity profiles of Gram-negative bacterial isolates revealed Acinetobacter and Klebsiella being the most resistant pathogens with overall sensitivity of 38% and 40% respectively followed by Enterobacter, Citrobacter, Pseudomonas, E. coli, and Proteus with overall sensitivity of 49%, 50%, 54%, 56%, and 66% respectively. Polymyxin-B and Colistin were the most effective antibiotics against Gram-negative bacterial isolates with overall efficacy of 94% and 88% respectively. Next to these were Cefoperazone-sulbactam, Amikacin, Meropenem, Gentamicin, Tigecycline, Moxifloxacin, Ciprofloxacin, Cotrimoxazole with overall efficacy of 69.19%, 62.74%, 62.31%, 55.55%, 55%, 49.79%, 49.15%, 43.96% respectively. A lesser level of antibacterial effect was shown by Cefotaxime, Cefoxitin, Amoxyclav, Cefixime, Cefipime and Ampicillin with 37.26%, 36.93%, 36.72%, 28.99%, 28% and 3% overall efficacy respectively.

Table-4: Overall antibiotic sensitivity (%) in Gram-negative bacterial isolates

Antibiotic	Bacterial Isolates						
	E.coli	Klebsiella	Enterobacter	Acinetobacter	Proteus	Pseudomonas	Citrobacter
Ampicillin	20%	0%	0%	0%	0%	0%	0%
Amoxyclav	64.28%	46.15%	0%	30%	50%	33.3%	33.3%
Amikacin	73.3%	61.53%	50%	44.4%	60%	66.66%	83.3%
Gentamicin	83.3%	46.15%	50%	42.8%	66.6%	60%	40%
Ciprofloxacin	58.3%	27.27%	100%	28.5%	80%	50%	0%
Moxifloxacin	62.5%	33.3%	100%	11.11%	66.6%	75%	0%
Cotrimoxazole	33.3%	46.15%	50%	20%	100%	33.3%	25%
Cefoxitin	28.5%	10%	50%	20%	50%	50%	50%
Cefotaxime	57.14%	15.38%	0%	0%	80%	25%	83.3%
Cefixime	23.06%	27.27%	0%	14.28%	80%	33.3%	25%
Cefoperazone-sulbactam	72.7%	41.66%	100%	80%	60%	80%	50%
Cefipime	83.3%	0%	0%	0%	80%	50%	40%
Meropenem	78.57%	64.28%	0%	50%	80%	83.33%	80%
Tigecycline	60%	60%	50%	80%	33.3%	0%	100%
Aztreonam	28.5%	50.54%	100%	25%	66.66%	75%	33.3%
Colistin	60%	70%	100%	100%	100%	100%	100%
Polymyxin-B	100%	81.81%	100%	100%	75%	100%	100%

Figure-3: Overall antibiotic sensitivity (%) in Gram-negative bacterial isolates



Among Gram-positive bacterial isolates, **Staphylococcus aureus** isolates showed 100% sensitivity to Linezolid followed by 88% sensitivity to Chloramphenicol, 87.5%, sensitivity to Cefotaxime, 85.7% sensitivity to Cefipime and Meropenem each and 81.25% sensitivity to Tigecycline. Sensitivity to Cefoperazone-sulbactamwas 71.42% while 68.96% sensitivity was observed against Vancomycin. Next to these were Gentamicin, Amikacin and Cefoxitin with 66.66%, 64.70% and 63.33% sensitivity respectively. The isolates showed 56% sensitivity to Levofloxacin, 55.8% sensitivity to Clindamycin, 45.45% sensitivity to Azithromycin, 42.3% sensitivity to Amoxyclav, 36.36% sensitivity to Teicoplanin, 31.03% sensitivity to Erythromycin and 30.43%sensitivity to Cotrimoxazole. Lesser levels of sensitivity were found against Ciprofloxacin (14.81%) and Ampicillin (9.6%). Methicillin resistance was observed in 36.84% isolates. Overall antibiotic sensitivity profiles of Gram-positive bacterial isolates are detailed in **Table-5** and graphically depicted in **Figure-4.**

Coagulase negative staphylococci (CONS) showed maximum sensitivity (100%) to Linezolid followed by 90.9% sensitivity to Tigecycline, 86.6% sensitivity to Vancomycin and 81.25%sensitivity to Chloramphenicol. Next to these were Levofloxacin, Amoxyclav, Clindamycin and Gentamicin with69.23%, 57.14%, 55.5% and 53.84% sensitivity respectively. Sensitivity to Teicoplanin, Azithromycin, Amikacin and Ampicillinwas 42.85%, 40%, 36.36% and 35.71% respectively. 28.57% sensitivity was observed against Ciprofloxacin and Erythromycin each while 25% sensitivity was observed against Cotrimoxazole. CONS are usually reported as intrinsically resistant to Cefipime, Cefotaxime, Cefoperazone-sulbactam and Meropenem, the similar was observed in this study.

Enterococci showed 100% sensitivity to Linezolid followed by 72.7% sensitivity to Vancomycin, 71.42%sensitivity to Chloramphenicol and 66.6% Sensitivity to Amikacin. Sensitivity to Tigecycline, Levofloxacin, Erythromycin, Gentamicin (120µg against 10µg in others) and Teicoplanin was62.5%, 50%, 38.5%, 38.4% and 37.5% respectively while 33.3% sensitivity was observed against Cefoperazone-sulbactam and Clindamycin each. Lesser levels of sensitivity were found against Amoxyclav (30%), Ampicillin (27.2%), Ciprofloxacin (20%), Azithromycin, Cefoxitin and Cotrimoxazole(14.2% for each). Enterococciare usually reported as intrinsically resistant to Cefipime, Cefotaxime and Meropenem (Edwards, 1995), the similar was observed in this study.

Streptococci showed maximum (100%) sensitivity to Vancomycin, Linezolid, Cefoperazone-sulbactam, Cefotaxime and Cefoxitin each. Sensitivity to Levofloxacin, Teicoplanin, Amoxyclav and Chloramphenicol was 75% each while 50% sensitivity was observed against Gentamicin, Ciprofloxacin, Tigecycline, Clindamycin and Azithromycin each. Lesser levels of sensitivity were found against Ampicillin, Amikacin, Erythromycin and Cotrimoxazole (25% for each) while complete resistance was observed against Meropenem and Cefipime which is usually seen as intrinsic resistance in S. pyogenes.

Micrococci and **Diphtheroids** were not found to be pathogenic in this study, this may be attributed to the established findings that they are among the common commensals of human skin, and hence they were not included in antibiotic sensitivity testing performed in this study.

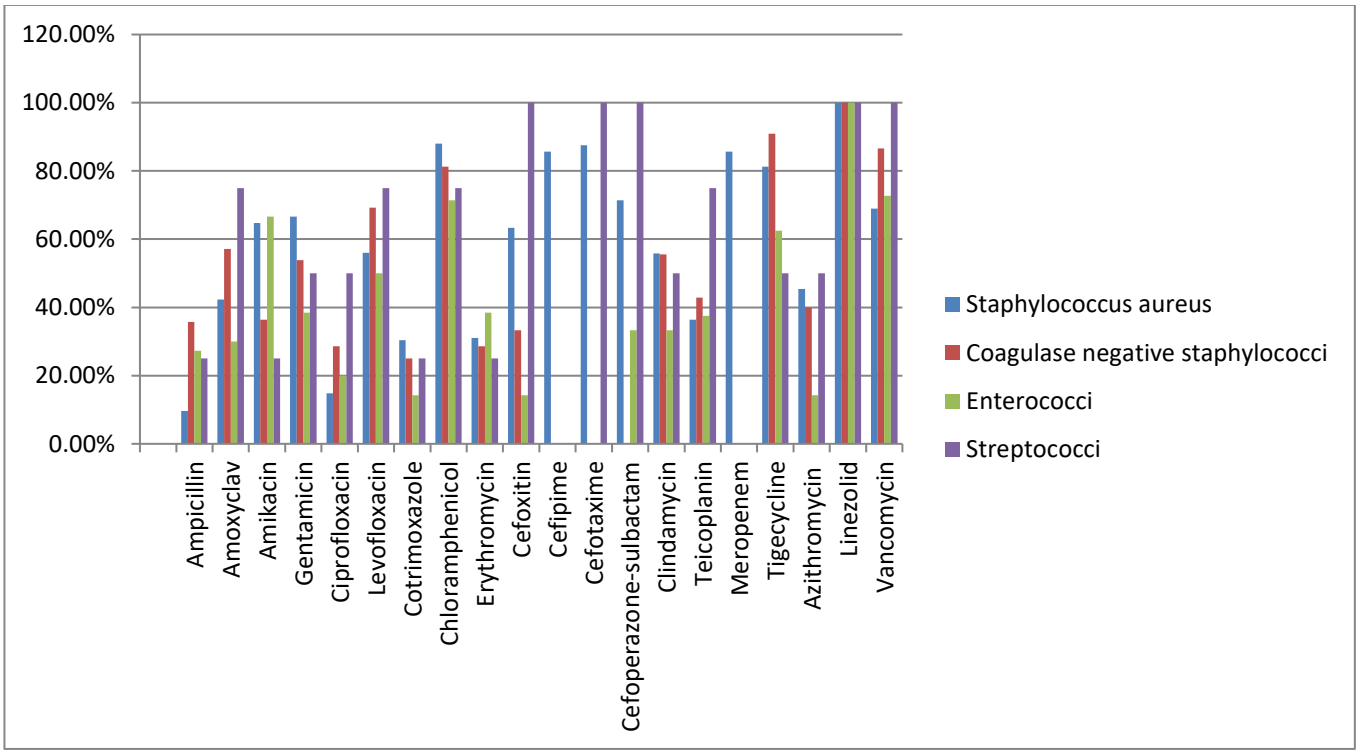
Overall, Antibiotic sensitivity profiles of Gram-positive bacterial isolates revealed Enterococcus and CONS being the most resistant pathogens with overall sensitivity of 36.22 % and 43.24% respectively followed by Streptococci and Staphylococcus aureus with overall sensitivity of 58% and 59.25% respectively. Linezolid, Vancomycin, Chloramphenicol and Tigecycline were the most effective antibiotics against Gram-positive bacterial isolates with overall efficacy of 100%, 82.07%, 79% and 71.16% respectively. Next to these were Levofloxacin, Cefoxitin, Gentamicin, Cefoperazone-sulbactam, Amoxyclav, Clindamycin, Amikacin, Teicoplanin and Cefotaxime with overall efficacy of 63%, 52.74%, 52.24%, 51.18%, 51.11%, 48.65%, 48.18%, 47.93%, and 46.88% respectively. A lesser level of antibacterial effect was shown by Azithromycin, Erythromycin, Ciprofloxacin, Ampicillin and Cotrimoxazole with 37.43%, 30.78%, 28.35%, 24.40% and 23.68% overall efficacy respectively while Cefipime and Meropenem were the least effective with 21.43%overall efficacy for each.

Table-5: Overall antibiotic sensitivity in Gram-positive bacterial isolates

Antibiotic	Bacterial Isolates			
	S. aureus	**Coagulase negative staphylococci (CONS)**	**Enterococci**	**Streptococci**
Ampicillin	9.6%	35.71%	27.27%	25%
Amoxyclav	42.3%	57.14%	30%	75%

Amikacin	64.70%	36.36%	66.66%	25%
Gentamicin	66.66%	53.84%	38.46%	50%
Ciprofloxacin	14.81%	28.57%	20%	50%
Levofloxacin	56%	69.23%	50%	75%
Cotrimoxazole	30.43%	25%	14.28%	25%
Chloramphenicol	88%	81.25%	71.42%	75%
Erythromycin	31.03%	28.57%	38.5%	25%
Cefoxitin	63.33%	33.33%	14.28%	100%
Cefipime	85.7%	0%	0%	0%
Cefotaxime	87.5%	0%	0%	100%
Cefoperazone-sulbactam	71.42%	0%	33.3%	100%
Clindamycin	55.8%	55.5%	33.3%	50%
Teicoplanin	36.36%	42.85%	37.5%	75%
Meropenem	85.7%	0%	0%	0%
Tigecycline	81.25%	90.90%	62.5%	50%
Azithromycin	45.45%	40%	14.28%	50%
Linezolid	100%	100%	100%	100%
Vancomycin	68.96%	86.6%	72.72%	100%

Figure-4: Overall antibiotic sensitivity (%) in Gram-positive bacterial isolates



Overall, with reference to the magnitude of resistance, multi-drug resistant (MDR) bacterial isolates detected in present study were Acinetobacter & Enterobacter (100% each) >Klebsiella(92.3%) > CONS(77.78%) >Staphylococcus aureus (68.42%) >Citrobacter (66.66%) >Proteus(60%) >E.coli(53.3%) >Enterococci(46.15%) >Pseudomonas (33.33%)

>Streptococci(0%). Vancomycin resistance was found in decreasing order Staphylococcus aureus (23.68%) >Enterococcus (23.07%) > CONS (11.11%).Overall magnitude of resistance with reference to Methicillin and Vancomycin resistance in present study and its comparison with other studies is presented in **Table-6**.

Table-6: Overall magnitude of resistance with reference to Methicillin and Vancomycin resistance in present study and its comparison with other studies.

S. No.	Study	Isolates	Methicillin resistance %	Vancomycin resistance %
1.	Present Study	S. aureus: 38 CONS:18 Enterococci: 13	Methicillin resistant S. aureus: 36.84% Methicillin resistant Coagulase Staphylococci: 61.1% Methicillin Resistant Enterococci: 46.15% S. aureus Dtest+ve: 13.15 CONS D test +ve: 22.22% Enterococci D test+ve: 7.69%	Enterococci RVS*: 27.2% S. aureus RVS: 26.08% CONS RVS: 23.80%
2	Mohanty et al, 2019	S. aureus: 284	MRSA**: 44.7% DTEST+VE: 22.8%	RVS: 11.6%
3	Goswami et al, 2011	S. aureus:48 183 tot	MRSA: 29.17 %	VRSA[#]: 48.13%
4	Praharaj et al, 2013	Enterococcus: 367	NA	VRE[##]: 8.7%
5	Hasan et al, 2016	S. aureus:29	MRSA: 72%	VRSA: 52%
6	Sivaradjy et al, 2021	Enterococcus: 427	NA	VRE were 6.12%(in 2018) 13.2% (in 2019) 19.2%(in 2020)
7	Sharma et al, 2010	CONS: 300	Methicillin resistant Coagulase Staphylococcus prevalence: 52%	All isolates were susceptible to Vancomycin

*RVS: Reduced Vancomycin Susceptibility

**MRSA: Methicillin resistant Staphylococcus aureus

[#]VRSA: Vancomycin resistant Staphylococcus aureus

[##]VRE: Vancomycin resistant Enterococci

Conclusion of the study

This study presents a picture of aerobic bacterial flora associated with chronic non-healing wound infections with special reference to antibiotic sensitivity profiling of the pathogenic isolates against routine antibiotics. Out of all the aerobic bacteria, Staphylococcus aureus were found to be the most prevalent bacteria followed by CONS, E. coli, Enterococcus, Klebsiella, Acinetobacter, Micrococci, Diphtheroids, Citrobacter, Pseudomonas, Neisseria, Proteus, Streptococci and Enterobacter.

Antibiotic sensitivity profiles of Gram-negative bacterial isolates revealed Acinetobacter and Klebsiella being the most resistant pathogens followed by Enterobacter, Citrobacter, Pseudomonas, E. coli, and Proteus. Polymyxin-B and Colistin were the most effective antibiotics against Gram-negative bacterial isolates. Next to these were Cefoperazone-sulbactam, Amikacin, Meropenem, Gentamicin, Tigecycline, Moxifloxacin, Ciprofloxacin,and Cotrimoxazole. Lesser levels of antibacterial effect were shown by Cefotaxime, Cefoxitin, Amoxyclav, Cefixime, Cefipime and Ampicillin. Antibiotic sensitivity profiles of Gram-positive bacterial isolates revealed Enterococcus and CONS being the most resistant pathogens followed by Streptococci and Staphylococcus aureus. Linezolid, Vancomycin, Chloramphenicol and Tigecycline were the most effective antibiotics against Gram-positive bacterial isolates. Next to these were Levofloxacin, Cefoxitin, Gentamicin, Cefoperazone-sulbactam, Amoxyclav, Clindamycin, Amikacin, Teicoplanin and Cefotaxime. Lesser levels of antibacterial effect were shown by Azithromycin, Erythromycin, Ciprofloxacin, Ampicillin and Cotrimoxazole while Cefipime and Meropenem were the least effective.

With these findings, this study not only brings forth the spectrum of most efficacious antibiotics in treating the patients with chronic non-healing wound infections but also presents the potentially applicable combinations of routine antibiotics with special attention to antibiotic resistance developing in the course of modern treatment strategies. Further research and insights in this direction will help explore new and better strategies to use the available therapeutics for patient healing and cure.

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