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Chronic Nonhealing Ulcers – Garhwal Region (Etiopathology, Microbiology with Susceptibility, Managements & Outcomes)

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Authors' contributions

This work was carried out in collaboration among all authors. Author KA managed the clinical cases, designed the study, wrote the protocol and wrote the first draft of the manuscript. Author MPB managed the sample and data collection and literature searches. Author SK was involved with the Clinical/Histo- Pathology work and relevant comments. Author PK managed the tabulation and statistical analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Chronic nonhealing ulcers, with varied etio-pathologies, are difficult to manage and warrant meticulous, early and prolonged directed treatment to prevent their development and complications.

Methods: Patients of chronic ulcers (>3 months' duration), having undergone surgical management at our Institute, VCSGGMS&RI-UT, between January 2018 – August 2019, numbering one hundred twenty five (N= 125), were included in this concurrent observational study, aimed at identifying implicated microrganism (s) and their antibiotic susceptibility, for promoting wound healing, along with surgical measures.

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Results: Male patients (M:F :: 87:38; 69.6% males) in the "20-50 year" age-group (74; 59.2%), with diabetic ulcers (35;28.0%), burns etc. (21;16.8%) and traumatic ulcers (18;14.4%) etc. predominated in the chronic non-healing state. Gram positive (68; 54.4%) organisms (including Staphylococcus) were the major isolates from the ulcers; organisms showing higher sensitivity to the newer generations/groups of antibiotics. Uncontrolled Diabetes, other prolonged illnesses &/or under-nutrition were important causative factors, requiring their remediations and also debridements \pm skin/flap coverage (45; 36.0%) with prolonged course of antibiotics and occasional amputations (18; 14.4%) for adequate treatment.

Conclusion: Skilled intensive multidisciplinary effort is essential to achieve satisfactory healing and prevent disfigurement and to limit disability and death (11; 8.8%) among the patients.

Keywords: Antibiotic sensitivity; bacterial profile; chronic wound; diabetic ulcer; nonhealing ulcers.

ABBREVIATIONS

Ant.	: Anterior
Post.	: Posterior
(CE) CT	: (Contrast Enhanced) Computed Tomography
CoNS	: Coagulase negative Staphylococcus aureus
MRSA	: Methicillin Resistant Staph aureus
HPE	: Histo-Pathological Examination
Sex	: M: Males; F: Females
R&A	: Resection and Anastomosis
MDR-TB	: Multi Drug Resistant Tuberculosis
TB	: Tuberculosis
OPD	: Out Patient Department
ATT	: Anti Tubercular Therapy
(H, R, Z, E, S)	: (Isoniazid, Rifampicin, Pyrazinamide, Ethambutol, Streptomycin)
AFB	: Acid-fast Bacilli
PVD	: Peripheral Vascular Disease
VCSGGMS&RI	:Veer Chandra Singh Garhwali Government Medical Sciences & Research Institute (UT)
CRP	: C Reactive Protein
HbA1c	: GlycatedHb
Staph aureus	: Staphylococcus aureus
E coli	: Escherichia coli

1. INTRODUCTION & REVIEW OF LITERATURE

Chronic nonhealing wounds are those that have failed to proceed through an orderly and timely reparative process to produce anatomic and functional integrity over a period of three months; whereas chronic wounds are those that have failed to heal usually within three weeks time [1-6]. These wounds are challenging to treat, significantly burden the health care systems and the economy, and lead to reduced quality of life and social isolation of the patients. Adverse wound healing conditions are promoted by numerous systemic factors (eg. malnutrition, ageing, tissue hypoxia, diabetes, immune suppression, periwound skin damage by exudate and other bodily fluids, etc.) and local factors (eq. repeated trauma, poor perfusion / oxygenation,

bacterial colonisation, excessive persistent inflammation, unresponsiveness to normal wound-healing regulatory signals, etc.), resulting in a hostile wound healing environment, contributing to the pathogenesis [7-11]. Malignant transformation (Marjolin's ulcer), including both squamous and basal cell carcinomas, can also occur in any long-standing wound [1-6,12-14]. Smoking, frostbite also contribute to the development of Peripheral Vascular Disease (PVD) and other types of chronic ulcers, common in rural patients of Uttarakhand, including low to middle income communities, as well as trauma, drugs and nutritional deficiencies [15-17]. Treatment directed against the specific pathology and the colonising organisms is important to effect healing and clinical cure in these patients.

2. AIMS, MATERIALS & METHODS

We aim to outline the present scenario of bacterial flora associated with non-healing ulcers in patients of Garhwal region, in this concurrent observational study, and mentioning the various possible modes of management and outcomes of such patients for their better management henceforth, by (a) specimen collection (of pus / wound exudates / tissue samples), (b) isolation and identification of bacterial flora from the cases with non-healing ulcers, presenting to the Dept. of Surgery Ward/OPD/Emergency [VCSGGMS &RI & HNB Base Teaching Hospital, Srikot, Srinagar, Uttarakhand, India – 246178. (vcsgsrinagar.org)], between January 2018 to August 2019 (N= 125), (c) performing antibacterial susceptibility profiling of the pathogenic isolates against routine antibiotics [using Kirby Bauer disc diffusion method, as recommended by the Clinical and Laboratory Standard Institute (CLSI)], and (d) doing histopathology examination of the respective representative tissue samples from ulcers; with data collection from patient case-records and intra/post-operative findings and lab results [18].

3. RESULTS

Patients of chronic nonhealing ulcers included in our study, between January 2018 - August 2019, numbered one hundred and twenty five (Σ N=125), with most in the 20-50 year age-group (n= 74; 59.2%), followed by those in the 50-70 year group (25; 20.0%), <20 years age (20; 16.0%) and >70 years age (6; 4.8%) and more of male patients seeking surgical attention for their disease (M:F = 87:38) [Table 1] & [Graph. 1 (a, b)]. Diabetic patients were the most prone to development of chronicity and nonhealing state in their ulcers (35; 28.0%), and burns etc taking up the next group (21;16.8%); traumatic (18; 14.4%), vascular (12;9.6%), and surgical site infections (10;8.0%) were also common causes of chronic nonhealing ulcers; rare etiopathologies included those related to decubitus ulcers (9;7.2%), sinus/fistulae (8;6.4%), bites/unclassified (7; 5.6%) and neoplastic (5; 4.0%) [Graph 2 a].

Pus/discharge from the wounds was obtained during the initial visit and subjected to isolation. culture and identification of aerobic organisms in the wounds, using appropriate relevant methodology [Table 2] & [Graph. 3 (a,b)]. Despite our best efforts, no organism growth could be obtained from six of the cases (6; 4.8%), whereas four of the specimens (4; 3.2%) yielded Mycobacteria on microscopy. These AFB showed susceptibility to all the first-line antitubercular drugs (H, R, Z, E, S), except for one of the isolates that showed resistance to Isoniazid (INH), requiring appropriate modification in the ATT regimen.

Most of the rest of the patients showed predominant mono-microbial growth (120 microbial isolates from 115 patients) [Table 2] & [Graph. 3 (a,b)] and classification and antibiotic susceptibility was carried out on those specimens by appropriate lab techniques. The Gram positive bacterial isolates (68;54.4%) included Coagulase negative *Staphylococcus aureus* (CoNS) group (21; 16.8%), Methicillin-Resistant *Staph. Aureus* (MRSA) (17;13.6%), *Staph. Aureus* (19;15.2%), Enterococcus genus (11;8.8%); whereas, the Gram negative isolates (52;41.6%) included Klebsiella (19;15.2%), Acinetobacter (9;7.2%), *Escherichia coli* and



Graph. 1. Demography/epidemiology





a typical *E. coli* (7 & 2;5.6 &1.6% respectively), Proteus (6;4.8%), Enterobacter (4;3.2%), Pseudomonas (3;2.4%), Citrobacter (2;1.6%). Thus, the chronic ulcers showed altered microbial flora due to various modes of wound contamination and growth selectivity owing to the several unsuccessful management strategies attempted and employed in their healing and prior treatment.

Antibiotic susceptibility showed some interesting and intriguing patterns [Table. 2] & [Graph. 3 (a, Co-trimoxazole (Trimethoprim b)]. sulfamethoxazole) was highly active against Proteus, Ciprofloxacin / Ofloxacin against E coli and Enterobacter and Moxifloxacin against CoNS, atypical E Coli and Enterobacter. Ampicillin / Amoxycillin had a low sensitivity among the organisms in clinically active doses, while Piperacillin (+Tazobactam) still worked well against CoNS and Pseudomonas and Ticarcillin against MRSA. Among the Cephalosporins, Cefoxitin / Cefuroxime (IInd generation) showed good activity against Staph aureus and Citrobacter: Cefotaxime / Cefixime (III) against Proteus: Ceftriaxone / Cefoperazone (III) against Acinetobacter and Enterobacter; and Cefepime (IV) against CoNS. E coli. Proteus. Enterobacter. Aztreonam could be used for Pseudomonas, while Carbapenems had high activity against most of the isolated organisms; Tetracycline / Chloramphenicol group was worth trying against all Gram positive organisms, while Tigecycline proved to be another good "broad spectrum" antibiotic, even for multi-drug resistant strains and complicated infections. Aminoglycosides could be used as "add-on" therapy in most cases. while Macrolides could not be recommended as mono-therapy in any case. Clindamycin, Vancomycin and Teicoplanin showed development of high degrees of resistance amongst the organisms, while Linezolid could be considered a good "first choice" against the Gram positive microbes; the Polypeptides (Colistin / Polymyxin) showing preference towards Gram negative aerobes.

Likewise, on the other hand, Staph aureus and Enterococci responded well only to Carbapenems and Linezolid, while MRSA responded to these two above, along with Tetracycline / Chloramphenicol and Tigecycline, and CoNS to most of the antibiotic groups [Table. 2] & [Graph. 3 (a, b)]. The floxacins proved good enough for the E coli, while Pseudomonas needed Aztreonam / Teicoplanin&/or Polypeptide group antibiotics for eradication. Citrobacter and Acinetobacter showed multiple drug resistance, responding only to the higher antibiotic groups (Penems, Tigecycline, newer Cephalosporins),



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whereas, Klebsiella, Proteus and Enterobacter showing variable results. Overall, most of the patients were administered combination antibiotic therapy, according to the respective sensitivities of the isolated organisms for their speedy and definite eradication, along with the requisite treatment protocol.

As the patients were presenting in a chronic state of disease, fever / tachycardia was found in 23 (18.4%), while 29 (23.2%) had shock / septicemia [Table 3] & [Graph. 4]. Diabetes / (54; hypertension were major factors 43.2%) associated with nonhealing wounds; 44 (35.2%) were smokers, while 45 (36.0%) had form of hepato-renal-pulmonary some compromise as contributory factors. Lab evaluation of the blood parameters showed deranged TLC in 28 (22.4%) patients, decreased Hb% in 51 (40.8%), deranged blood sugar / HbA1c in 26 (20.8%), reduced serum proteins in 36 (28.8%), while raised CRP levels in 21 patients: 14 (11.2%) (16.8%) showed abnormalities on Color Doppler imaging due to their ulcers resulting from peripheral vascular pathologies.

On histo-pathology examination of the tissue specimens from the ulcers [Table 3] & [Graph. 2 "chronic non-specific b] & [Graph. 4], inflammation" was the commonest finding (65; 52.0%): others being "acute onaoina inflammation" (12; 9.6%), "necrotic" tissue (20; 16.0%), "vasculitic" (15; 12.0%), "neoplastic" (7; 5.6%), "pseudoepitheliomatous hyperplasia" in 2 (1.6%) and "chronic granulomatous (Tubercular)" in 4 (3.2%) patients. Skin graft / Flap cover procedure was required to cover the defect in 45 (36.0%) patients, while 18 (14.4%) required amputations of some sort to control the chronic pathology and effect healing: debridements and conservative management strategy sufficed in the rest (62; 49.6%). Despite our best efforts and management skills, eleven (8.8%) of the patients could not be saved.

4. DISCUSSION

Chronic nonhealing ulcers are a disfiguring and incapacitating affliction, interfering with productivity of the patient and putting an economic burden upon the family and healthcare system. Our study depicts a high incidence of neglected / poorly managed ulcers, resulting in nonhealing wounds in a significant number of patients in hill / remote areas of Uttarakhand. Chronic infection, exposed and uncovered

wounds, accompanied with sometimes irrational antibiotic use resulting in resistant or atypical organisms, and partially controlled underlying diseases, eg. diabetes, put patients at risk for developing these ulcers.

Other recent similar studies depict the presence of varied genera of bacteria, usually polymicrobial [19-23], both aerobes and anaerobes [24], and also yeasts [11,25-30] in chronic wounds, depending mainly on microbial flora of the lower limb, metabolic factors, foot hygiene and the use of antibiotics, with widely varying susceptibilities loco-regionally. antibiotic Diabetes and its complications were the commonest, while neoplastic lesions the least common causes of chronic ulcers, as in other studies [5,6,9,26]. Relevant combination multidrug antibiotic therapy helped achieve prompt conducive environment for healing in most cases [20,22,26-30]. Histopathology from the ulcers suggested "chronic nonspecific inflammatory infiltrate" to be common and few of the cases needing amputations, resulting in limb loss or deformity; the death rate was still high and could be reduced further with earlier interventions to prevent chronicity as well as prompt and intensive management strategies. Molecular techniques should be preferred over culture methods to obtain a better picture of bacterial diversity in the wound bed. This is particularly important for the identification of bacteria that require special transport and culture conditions such as anaerobic bacteria. Anaerobic bacterial infections need to be treated appropriately as they have been reported to delay healing and are involved in biofilm formation. Furthermore, resistance to antibiotics has been reported in anaerobic bacteria. Regular serial monitoring of wound bed flora should be done and appropriate changes in the antibiotic regimen made as per each patient's requirements, to speed up the healing process [6,24].

The surgeon has a greater role to play not only in timely diagnosing signs and causes of chronicity in an ulcer but also to prevent further progression, institute appropriate sensitivitydirected combination antibiotic regimen, manage the underlying disorders affecting wound healing and also to serially debride and provide for functional means of adequate wound coverage over the involved area. Multidisciplinary involvement from the microbiology / pathology and physician colleagues among others, and soliciting assistance, if needed from plasticsurgeon colleagues is imperative, as is good

Table 1. Demography / etio-pathology (January'18 - July'19)

Age-Groups	Males : Females	Total by age-	Site/Type?etiology of Chronic Ulcer (n / %)													
(years <u>)</u>	M:F	groupsn (%)	Traumatic	Diabetic	Vascular (A/V)	SSI	Sinus (Pilonidal) / Fistula / Others - Chronic	Burns / (Other) Non-Healing Ulcers	Decubitus	Bites / OthersUnclassified	Neoplastic					
<20y	16 : 4	20 (16.0%)	5	1	0	3	4	4	1	2	0					
20-50y	49 : 25	74 (59.2%)	7	23	8	5	4	14	5	5	3					
50-70y	20:5	25 (20.0%)	5	10	3	2	0	2	2	0	1					
>70y	2:4	6 (4.8%)	1	1	1	0	0	1	1	0	1					
<u>Total</u> (∑N= 125)	87 : 38 (69.6% : 30.4%)	. ,	18 (14.4%)	35 (28.0%)	12 (9.6%)	10 (8.0%)	8 (6.4%)	21 (16.8%)	9 (7.2%)	7 (5.6%)	5 (4.0%)					

Table 2. Bacteriology (Aerobic) (G/ZN stain ± Culture) (Predominant / Polymicrobial) / Sensitivities

Bacterial	Cotrimoxazole	Cipro / O –	Moxifloxaci	n Ampi/Amocy -	 Piperacillin 	Ticarcillin	"Cefoxitin /	Cotrimoxazole	Cipro / O	Moxifloxacin	Ampi/Amocy -	· Piperacilli	n Tetracycline /	Tigecycline	Amika /	Erythromycin /	Clindamycin	Vancomycin	Teicoplanin	Linezolid	Colistin /
Susceptibility		floxacin		cillin	-		Cefuroxime		– floxaci	า	cillin	-	Chloramphenicol		Gentamy – cin	Azythromycin	-	-	-		Polymyxin
Staph aureus	31.5	21.1	63.2	5.3	52.6	26.3	94.7	ND	ND	57.9	ND	94.7	57.9	52.6	63.2	26.3	36.8	52.6	21.1	100	ND
MRSA	47.1	11.8	58.8	29.4	ND	65.3	11.8	0	ND	ND	ND	94.1	94.1	88.2	58.8	47.1	47.1	70.6	47.1	100	ND
CoNS	19	28.6	90.5	38.1	85.7	ND	33.3	23.8	ND	85.7	ND	ND	85.7	95.2	42.9	33.3	66.7	85.7	47.6	100	ND
Enterococcus	0	18.2	45.5	18.2	45.5	ND	18.2	0	27.3	18.2	ND	81.8	72.7	63.6	27.3	18.2	36.4	72.7	18.2	100	ND
E coli	0	100	42.8	0	28.6	ND	28.6	14.3	14.3	71.4	28.6	57.1	ND	57.1	71.4	0	ND	ND	ND	ND	57.1
Atypical E. coli	50	100	100	100	50	ND	0	50	100	100	50	100	ND	100	100	ND	ND	ND	ND	ND	100
Pseudomonas	0	0	66.7	0	66.7	0	0	0	66.7	66.7	100	33.3	ND	33.3	0	ND	ND	ND	100	ND	100
Citrobacter	0	0	0	0	0	ND	100	0	0	50	0	0	ND	100	0	ND	ND	ND	ND	ND	ND
Acinetobacter	44.4	44.4	22.2	33.3	33.3	11.1	22.2	11.1	88.9	66.7	44.4	100	ND	77.8	44.4	ND	ND	ND	ND	ND	100
Klebsiella	42.1	31.6	36.8	15.8	36.8	ND	10.5	26.3	26.3	63.2	68.4	63.2	ND	63.2	47.4	ND	ND	ND	ND	ND	94.7
Proteus	100	66.7	66.7	16.7	16.7	ND	16.7	100	66.7	83.3	ND	ND	ND	33.3	83.3	ND	ND	ND	ND	ND	ND
Enterobacter	25	75	100	0	25	ND	25	0	100	100	ND	0	ND	50	25	0	ND	ND	ND	ND	75

Not Done / Applicable / Relevant (ND*)
 One of the four isolates showed resistance to Isoniazid (INH)**

Table 3. Clinical & Lab Parameters / Management & Outcomes in Chronic Nonhealing Ulcers

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Clinical & Lab Parameters / Management & OutcomesIn Chronic Nonhealing Ulcers		Age-Gro	up-Wise Dist	ribution (∑N	= 125)					Etio Pathology-Wise Distribut	Total (∑n , %)				
		<20y (n=20)	20-50y (n=74)	50-70y (n=25)	>70y (n=6)	Traumatic (n=18)	Diabetic (n=35)	Vascular (A/V) (n=12)	SSI (n=10)	Sinus (Pilonidal) / Fistula / Entero-cutaneous / Others - Chronic (n=8)	Burns / (Other) Non-Healing Ulcers (n=21)	Decubitus (n=9)	Bites / Others Unclassified (n=7)	Neoplastic (n=5)	
Clinical Features	Fever / Tachycardia	3	12	6	2	3	12	2	1	2	2	1	0	0	23 (18.4%)
	Shock / Septicemia	2	12	13	2	1	7	6	4	1	5	4	1	0	29 (23.2%)
Predisposing /	DM / Htn	1	35	16	2	5	35	4	1	1	4	2	1	1	54 (43.2%)
Associated Conditions	Smoking	1	29	12	2	8	13	5	2	2	7	2	3	2	44 (35.2%)
/ Factors	Immunocompromised state / Hepato- Renal-Pulmonary Compromise / Others	2	27	10	6	7	23	4	3	2	2	2	1	1	45 (36.0%)
Lab Parameters /	↑/↓ TLC	2	17	6	3	4	12	3	2	1	3	2	1	0	28 (22.4%)
Imaging	Hb%	5	30	12	4	10	16	4	3	3	8	4	2	1	51 (40.8%)
	Deranged F/R- BS / HbA1c	2	12	9	3	2	14	3	1	1	2	2	1	0	26 (20.8%)
	S. Proteins (T/A/G)	6	16	11	3	2	9	3	4	4	7	5	1	1	36 (28.8%)
	↑CRP (C-Reactive Protein)	4	11	4	2	2	7	2	2	2	5	1	0	0	21 (16.8%)
	Abnormalities in Vascular Imaging	0	5	7	2	2	3	7	0	0	1	1	0	0	14 (11.2%)
	(Color Doppler A/V)														
HPE Reports	Acute Inflammatory (ongoing)	3	6	3	0	3	2	0	2	2	1	1	1	0	12 (9.6%)
$(\Sigma n = 125)$	Chronic Inflammatory (non-specific)	15	40	7	3	12	15	1	7	3	16	5	6	0	65 (52.0%)
(2 -)	Granulomatous (chronic) (/Tubercular)	1	2	1	0	0	0	0	1	1	1	1	0	0	4 (3.2%)
	Necrotic	1	11	7	1	2	9	6	0	0	1	2	0	0	20 (16.0%)
	PseudoEpitheliomatous Hyperplasia (PEH)	0	2	0	0	0	0	0	0	1	1	0	0	0	2 (1.6%)
	Vasculitic	0	9	5	1	1	9	5	0	0	0	0	0	0	15 (12.0%)
	Neoplastic / In-Situ Ca	0	4	2	1	0	0	0	0	1	1	0	0	5	7 (5.6%)
M/m Modes (∑n = 125)	Conservative (Dressings, Antibiotics, ±	14	35	11	2	8	17	5	7	6	10	3	5	1	62 (49.6%)
	Cover Procedure (Graft / Flap) after Debridements / Wide-Excision	5	29	8	3	8	10	4	2	2	8	6	2	3	45 (36.0%)
	Amputations / Limb-Member Loss	1	10	6	1	2	8	3	1	0	3	0	0	1	18 (14.4%)
Deaths		1	6	2	2	0	3	3	2	0	1	1	0	1	11 (8.8%)



Graph. 4. Lab parameters, management modes and outcomes

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Photo.1. Major anterior abdominal wall chronic nonhealing wound with exposed underneath mesh, in a diabetic patient (underwent retro-rectus sublay mesh repair of midline abdominal incisional hernia)



Photo. 2. Upper limb multiple chronic nonhealing wounds in various stages



Photo. 3. (a, b). PVD (Arterial) with multiple digit amputations and chronic nonhealing ulcer, with exposed bone (a); another patient split-thickness skin-grafted (b)

physiotherapy support to improve functional contamination among such patients is to be recovery of the involved extremity. Cross- prevented. Patient and population should be



Photo. 4. (a, b, c). Facial trauma resulting in skin necrosis (a) and chronic nonhealing wound (b); Intermediate-thickness skin-grafted (c)

educated regarding proper wound care and early visit to a tertiary care center to prevent functional loss.

Photo. 1 depicts a chronic nonhealing wound in the anterior abdominal wall of a diabetic middleaged female patient having undergone mesh repair of incisional hernia. Wide debridement, prolonged directed antibiotics as per sensitivity reports and secondary suturing was required for healing of the wound. Photo. 2 shows multiple chronic nonhealing wounds in various stages of healing on the left upper limb of an adult labourer male patient, which required multiple repeated debridement's and specific antibiotics and later skin-grafting for proper healing. Photo. 3 (a, b) show cases of Peripheral Vascular Disease (arterial) affecting the foot in (a) and the leg in (b), in elderly smoker male patients. The first patient had undergone multiple toe amputations in the past, while the second one, had a large area of necrosis with non-healing ulcer on the leg; both had to undergo coverage procedure (flap ± graft) for appropriate healing of their exposed bony surfaces, besides lifestyle modifications and de-addiction. Photo. 4 (a, b, c) depict a middle-aged male patient having sustained blunt facial trauma during a road-traffic accident, who presented to us with resulting skin necrosis (a); and chronic nonhealing wound (b) developed in the course of his treatment; intermediate-thickness skin-graft (c) procedure was done after thorough debridement and specific organism combination antibiotic administration.

5. CONCLUSION

Knowledge of bacteriology and their antibiotic sensitivity profile in chronic nonhealing ulcers can help the treating surgeon provide better management and prevent amputations and deformity in their patients, which are very common in poorly managed chronic wounds, especially in diabetics, and among the rural populace.

CONSENT AND ETHICAL APROVAL

Appropriate informed and written consent taken from patients included in this study. Appropriate documentation of all cases done and preserved. Appropriate Ethical Clearance sought from Institutional Ethical Committee / IRB - No ethical issues in this study; (approval vide review letter no. IEC/VCSGGMS&RI/38, dated 29/01/2018; application 2018-01623). Libre Office (libreoffice.org), Linux Mint (linuxmint.com), PSPP (gnu.org/software/pspp) & Google (Office Suite / Drive) (google.com/drive) used for data analysis, tabulation, manipulation, interpretation, graphing & charting, with results as observation numbers / percentages, statistical analyses and relevant comparisons.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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