Bacteriological profile in various types of ulcers - A comparative study from a tertiary care centre in North Kerala

Authors
Dr Akhila Aravind¹, Dr Joana Mary Magdaline², Dr Joseph Francis³
¹Senior Resident, Department of Microbiology, Govt. Medical College, Ernakulam, Kerala, India
²Associate Professor, Department of Microbiology, Govt. Medical College Ernakulam, Kerala, India
³Associate Professor, Department of Surgery, Govt. Medical College, Ernakulam, Kerala, India

Abstract
Ulcers especially chronic ulcers pose a significant health care burden worldwide. The non-healing of such ulcers could be due to multiple factors among which bacterial infections are the forerunners. The present study was undertaken for a period of 1 year from February 2015 to know the prevalence of various types of chronic ulcers in our locality as well as to compare the flora obtained from the various types of ulcers. We had identified diabetic foot ulcers as the most prevalent type among the study population followed by venous ulcer & decubitus ulcers. On comparison of pattern of flora of the above types of ulcers in our study it was observed that all of them had a predominant monomicrobial infection with equal proportions of Gram positive & Gram negative isolates, but there was diversity in the individual isolates from different types of ulcers.

Keywords: Chronic ulcers, diabetic ulcers, venous ulcers, decubitus ulcers, bacterial flora.

Introduction
Skin is body’s largest organ which forms a barrier between internal organs & external environment. It is subjected to frequent trauma and so is at risk of infections. About 15% of all patients who seek medical attention either have some skin disease or skin lesion ¹; many of which are infectious. The infection can be from invasion of certain organisms from external environment through breaks in the skin or through organisms which reach the skin through blood as a part of systemic infections. Among the diseases of the skin, ulcers cause a significant burden to healthcare system as well as morbidity & mortality to the mankind². Ulcers are defined as any breach in the continuity of epithelium. Ulcers can be acute or chronic. In various studies, chronic leg ulcers are defined as those which shows no tendency for healing even after 3 months of appropriate treatment or is not fully healed at 12 months.³-⁵ Chronic ulcers are caused by endogenous mechanisms associated with the predisposing conditions. The conditions include decreased tissue perfusion, impaired venous drainage and metabolic diseases. These conditions compromise the integrity of the dermal & epidermal tissue³.

Decubitus ulcers have a different aetiology; as they are caused by sustained external pressure on skin, seen commonly on sacrum & buttocks. Apart from these predisposing conditions certain other factors exacerbate chronic ulceration like age, obesity, smoking, poor nutrition, immunosup-
pression. Infection & biofilm production by the associated bacterial flora. plays a major role in the non-healing of ulcers. Therefore an idea regarding the predominant flora in various types of ulcers is vital so as to start appropriate empirical therapy.

Several studies have shown a difference in the bacteriological profile of various types of ulcers like Diabetic ulcer, Venous ulcer and decubitus ulcers which mainly included facultative anaerobes. In these studies a region wise variation in the flora was also observed. From our region only few studies are available on the flora of various types of ulcers which emphasises the importance of the present study.

Aims & Objectives
1. To know the prevalence of various types of chronic ulcers in patients admitted in surgical wards
2. To identify the bacterial flora associated with different types of ulcers
3. To compare the bacterial flora of different types of ulcers

Materials & Methods
The study was conducted over a period of one year, from February 2015. All inpatients in the surgery wards with ulcers were recruited to the study with informed consent. A patient with ulcer was included only once during the period of study. Patients with malignant ulcers and ulcers associated with burns and post-operative wound infections were not included in the study. Sampling methodology adopted was Universal sampling. Ethical Clearance was obtained from the Institutional Ethical Committee before commencement of the study (IEC 11/2014).

Sample collection
Surrounding skin of the ulcer was disinfected with alcohol swabs. Ulcer area was thoroughly cleaned with sterile saline. Two sterile swabs were used for each patient. Samples were collected by making firm rotatory movements, covering entire area of the ulcer, holding both the swabs together. The swabs were then transported to the laboratory in sterile test tubes and processed within 2 hours.

Processing
One swab was used for gram staining & Ziehl Neelsen staining. The other swab was then inoculated on to solid culture media (Blood agar, MacConkey agar) & into thioglycollate broth. The media were then incubated at 370 C for 18 – 24 hours. Identification of the isolates up to species level was done as per standard microbiological methods by Gram staining, cultural characteristics of the isolates & biochemical reactions. The isolates which could not be identified up to the species level using standard biochemical reactions were identified using automated identification system (Phoenix (BD)).

Clinical details obtained from each patient was numerically coded and entered into Microsoft excel spread sheet and analysed using SPSS 16.0

Results
A total of 103 samples were collected from patients with different types of ulcers. The male – female ratio in our study was 1.7:1.

56 patients had diabetic ulcers, out of which 10 had smoking & 8 had alcoholism as an additional risk factor. Non-diabetic ulcers were 47 in number which included, 15 Venous ulcers 11 decubitus ulcers, 7 due to PAD, 1 secondary to lymphedema & in 13 cases no specific predisposing factors could be identified. Among the patients with venous ulcers 4 had diabetes & 11 had alcoholism also as predisposing factors. The various types of ulcers found in the study are given in figure 1

We had identified diabetic foot ulcers as the most prevalent type among the study population followed by venous ulcers & decubitus ulcers respectively. Comparison of flora of only diabetic, venous & decubitus ulcer was done as the number of cases in other types of ulcers were less. 66 patients had monomicrobial infection; among mono-microbial infection 50 were due to Gram positive bacteria and 16 were due to Gram negative bacteria. Polymicrobial infection was observed in 36 patients; 32 cases yielded two isolates each and 4 cases had
three isolates. Culture was sterile in 1 patient, in whom the ulcer could not be categorised, for lack of specific cause. The overall proportion of flora pattern obtained from patients in the study is given in figure 2 and flora pattern in the major types of ulcer are given in Figure 3.

A total of 142 isolates were obtained from 103 cases. The list of the isolated bacterial species are given in Table 1

**Table 1: Frequency Table of all the Isolated Organisms**

| Isolate                  | Frequency |
|--------------------------|-----------|
| Staphylococcus aureus    | 38        |
| Pseudomonas aeruginosa   | 31        |
| Enterococcus spp         | 10        |
| Escherichia coli         | 8         |
| Proteus spp              | 9         |
| Klebsiella pneumoniae    | 10        |
| Acinetobacter spp        | 5         |
| Corynebacterium spp      | 12        |
| Serratia spp             | 4         |
| MorganellaMorgagni       | 5         |
| Flavobacterium spp       | 1         |
| Enterobacter spp         | 2         |
| Streptococcus spp        | 4         |
| CoNS                     | 3         |
| **Total**                | **142**   |

Out of the total 142 isolates, 113 bacterial species were isolated from the above major types of ulcers. In all three types of ulcers, Staphylococcus aureus & Pseudomonas aeruginosa were the predominant bacteria isolated and the detailed list of bacteria isolated from each of the major types of ulcer is given in table no :2

**Table 2 :** Table showing the frequency of various isolates obtained from major types of ulcers in the study

| Bacteria                        | Venous ulcer | Diabetic ulcer | Decubitus ulcer |
|---------------------------------|--------------|----------------|-----------------|
| Staphylococcus aureus           | 8            | 20             | 4               |
| Pseudomonas aeruginosa          | 7            | 10             | 4               |
| Enterococcus spp                | 0            | 8              | 0               |
| Escherichia coli                | 1            | 5              | 1               |
| Proteus spp                     | 0            | 6              | 1               |
| Klebsiella pneumoniae           | 1            | 6              | 2               |
| Acinetobacter spp               | 0            | 3              | 1               |
| Corynebacterium spp             | 1            | 8              | 1               |
| Serratia spp                    | 0            | 3              | 0               |
| MorganellaMorgagni              | 2            | 1              | 0               |
| Flavobacterium spp              | 0            | 1              | 0               |
| Enterobacter spp                | 0            | 2              | 0               |
| Streptococcus spp               | 0            | 4              | 0               |
| CoNS                            | 0            | 2              | 0               |
Discussion
Ulcers especially chronic ulcers pose a significant health care burden worldwide. It also causes major social as well as economic problem to the affected individual. The non-healing of such ulcers could be due to multiple factors among which bacterial infections are the forerunners.
We had identified diabetic foot ulcers (55%) as the most prevalent type among the study population followed by venous ulcers & decubitus ulcers. This was in discordance with the studies conducted in the Western countries, where venous ulcers tend to be more prevalent than the other major types of ulcers.
The higher prevalence of diabetic ulcers in our study could be because of the more number of patients with poorly controlled diabetes in our population.
Other types of ulcers were due to PAD & lymphedema. Comparison of flora of only diabetic, venous & decubitus ulcer was done as the number of cases in other types of ulcers were less.
In our study 66% of diabetic ulcers showed monomicrobial infection of which Gram positive bacteria showed slight predominance (51%). Staphylococcus aureus was the commonest among Gram positive organisms. Among the Gram negative isolates (49%), Pseudomonas aeruginosa predominated. The other studies on diabetic ulcers showed equal proportions of polymicrobial & monomicrobial infections. All of them irrespective of being polymicrobial/monomicrobial had a Gram negative predominance which was not in accordance with our study.
In our study, pattern of bacterial flora in venous ulcers showed a monomicrobial predominance with equal proportion of Staphylococcus aureus & Pseudomonas aeruginosa. On the contrary majority of other studies revealed a polymicrobial aetiology with Gram positive predominance.
In case of decubitus ulcers in all the available studies polymicrobial infections were observed & gram positive bacteria predominated. This was in contrast to our study which showed predominant monomicrobial infection with Gram positive & Gram negative isolates in equal proportions.

On comparison of pattern of flora of the above major types of ulcers in our study it was observed that all of them had a predominant monomicrobial infection with equal proportions of Gram positive & Gram negative isolates,
In our study there was diversity in the individual isolates from different types of ulcers. This was found to be true with other studies, including those within the state.
The above observation points to the fact that there is a locality wise difference in the bacterial flora associated with ulcers.

Need For the Study
Epidemiological data available from the studies shows that the prevalence of chronic ulcer in Indian population is much higher than that seen in the Western population. In our institution also chronic ulcers is a significant burden. 44% (377/849) of the total samples from the surgery department is from ulcers. There are only fewer studies showing bacteriological profile of chronic ulcers, especially non diabetic ulcer, in our locality, the knowledge of which is important in empirical therapy.

Conclusion
Most prevalent type of ulcer among the study population was Diabetic foot ulcer followed by venous ulcer and decubitus ulcer respectively. The present study & the other available studies showed that bacterial flora varies widely in different types of ulcers.

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