Original Research Article

Clinico-bacteriological profile of trophic ulcers in leprosy patients: A report from a tertiary care center in Bihar

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Abstract

Abstract: The relative lack of information about the most common aerobic and anaerobic bacteria infecting a trophic ulcer in leprosy in India has prompted us to undertake this study. This study aimed to find the bacterial pathogen (if any) in aerobic and anaerobic isolates from trophic ulcers of leprosy to demonstrate the drug sensitivity of the aerobic isolate(s) so as to start a suitable antibiotic therapy. **Materials and methods:** This was a cross-sectional study done over a period of 6 months that is from March 2021 to August 2021, with a sample size of 38 patients. Patients attending the Out Patient Department of Department of Dermatology of Patna Medical College & Hospital, Patna. Prior ethical clearance was obtained from the Institutional Ethics Committee. The ulcer was cleaned with normal saline, slough was removed and the samples were collected from the deeper part of the ulcer with sterile bacterial loop. For aerobic culture, the material was transported in a sterile test tube, and for anaerobic culture, the material was put in Stuart's transport medium. **Results:** Among total 38 samples obtained, aerobic growth was seen in 86.8% of samples while 36.8% were culture-positive for anaerobic isolates. In samples of 7 patients, no growth was seen, this comprised 18.4% of total sample population. Among the sample where growth was observed, the most common organism in aerobic isolates was Staphylococcus aureus (45.2%), followed by Pseudomonas aeruginosa (25.8%), Proteus mirabilis (16.1%), Escherischia coli (12.9%),Klebsiella (9.1%), Providencia sp (2.9%), Streptococcus haemolyticus (2.1%) and Morgenellamorgani (1.4%). Mixed growth was noted in 12.2% of cases [Figure 1]. Among the culture positive 14 (36.8%) patients for anaerobic isolates, *Peptococcus* was the most common single isolate (17.2%), followed by *Peptostreptococcus* (8.8%) and bacteroides (6.8%), whereas mixed growth was seen in 4% cases. **Conclusion:** Secondary bacterial infection is quite common in leprosy trophic ulcers. Early treatment will ensure maximum limb salvage and prevent further complications, thus improving the quality of life of the patient.

Keywords: Clinico-bacteriological profile, trophic ulcers, leprosy

Introduction

Leprosy is a chronic infectious disease caused by the obligate intracellular pathogen Mycobacterium leprae [1], and still remains a public health problem, mainly in Africa, Asia and Latin America [2]. It has many complications including: leprosy reactions, development of plantar and palmar ulcerations, lagophthalmos (loss of eyelid function) and corneal anesthesia [3]. Trophic ulcers are one of the dreaded complications of leprosy. Owing to complete anesthesia of the affected part, the patient is completely unaware of the damage occurring due to trauma to the vulnerable pressure-prone areas of his body such as bony prominences of lateral malleoli, elbow, and heel of the hand (pisiform bone) leading to chronic nonhealing ulcers. The foot is the most common area of the body which is prone to develop ulcers due to cracks and fissures and trauma from external sources and also due to internal injuries caused by walking[4]. These are highly infected with bacteria, which delays the healing process [5], and furthermore, they usually recur, which in such cases increase the physical disability [5]. In such cases, knowledge of the most common bacteria infecting such an ulcer is useful in a clinical setup and in the field (where culture facilities are not available) in starting a treatment empirically and preventing progression of the condition which may lead even to amputation of the limb.There is little information about the pattern of bacterial isolates and drug sensitivities of infected ulcers in leprosy patients with leprosy, and most studies have been carried out in India [5-10].The relative lack of information about the most common aerobic and anaerobic bacteria infecting a trophic ulcer in leprosy in India has prompted us to undertake this study. This study aimed to find the bacterial pathogen (if any) in aerobic and anaerobic isolates from trophic ulcers of leprosy to demonstrate the drug sensitivity of the aerobic isolate(s) so as to start a suitable antibiotic therapy.

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Materials and Methods

This was a cross-sectional study done over a period of 6 months that is from March 2021 to August 2021, with a sample size of 38 patients. Patients attending the Out Patient Department of Department of Dermatology of Patna Medical College & Hospital, Patna, during the study period who were diagnosed cases of leprosy with trophic ulcers were explained about the purpose of the study. After obtaining informed consent form, they were included in the study. Patients who were already on antibiotics, or had comorbidities such as diabetes or other causes of trophic ulcers, or those with grossly contaminated ulcers were excluded from the study. Prior ethical clearance was obtained from the Institutional Ethics Committee.

Collection of samples: The ulcer was cleaned with normal saline, slough was removed and the samples were collected from the deeper

part of the ulcer with sterile bacterial loop. For aerobic culture, the material was transported in a sterile test tube, and for anaerobic culture, the material was put in Stuart's transport medium.

Culture and antibiotic sensitivity: For aerobic isolates, the sample was inoculated on MacConkey and nutrient agar for culture. Antibiogram was obtained by Kirby–Bauer disc diffusion technique and National Committee of Clinical Laboratory Standards guidelines [11]. For anaerobic culture, the material was put in blood agar with neomycin. It was immediately transferred to gas pack system to maintain anaerobic environment. It was incubated at 37°C for 2–3 days.Antibiotic sensitivity for anaerobic culture could not be done due to insufficient logistic support.

Data analysis: Data were analyzed for descriptive statistics using SPSS version 21 and Microsoft Excel and presented in tables. The results were interpreted in terms of frequencies and percentages.

Results

A total of 38 patients were included based on the selection criteria. 37.8% of the study participants were female. The age of the patients ranged from 18 to 67 years with a mean age of 38.9 years. Clinical data of leprosy patients with ulcers are shown in Table1.

All the patients were screened for aerobic and anaerobic isolates. Among total 38 samples obtained, aerobic growth was seen in 86.8% of samples while 36.8% were culture-positive for anaerobic isolates. In samples of 7 patients, no growth was seen, this comprised 18.4% of total sample population. Among the sample where growth was observed, the most common organism in aerobic isolates was Staphylococcus aureus (45.2%), followed by Pseudomonas aeruginosa (25.8%), Proteus mirabilis (16.1%), Escherischia coli (12.9%),Klebsiella (9.1%), Providencia sp (2.9%), Streptococcus haemolyticus (2.1%) and Morgenellamorgani (1.4%). Mixed growth was noted in 12.2% of cases [Figure 1]. Among the culture positive 14 (36.8%) patients for anaerobic isolates, *Peptococcus* was the most common single isolate (17.2%), followed by *Peptostr-eptococcus* (8.8%) and bacteroides (6.8%), whereas mixed growth was seen in 4% cases.All the isolates of Staphylococcus aureus were sensitive to amikacin, imipenem, linezolid and gentamycin. While 50% of them were resistant to cotrimoxazole and amoxiclav. Pseudomonas aeruginosa was maximally sensitive to piperacillin + tazobactam (100%), cefoprazone + sulbactam (87.5%), amikacin (87.5%), and imipenem (75%). While 75% of Pseudomanas isolated showed resistance to cotrimoxazole and amoxicalv. All isolated Escherischia coli were sensitive to cefoperazone + sulbactam and amikacin while 75% of them showed sensitivity to imipenem and 75% to linezolid. Escherischia coli showed maximum resistance to amoxiclav (75%) and ciprofloxacin (50%). Proteus mirabilis was maximally sensitive to cefoperazone + sulbactam (80%), amikacin (80%), and linezolid (80%) while showing resistance to cotrimoxazole, ciprofloxacin, ceftazidime and cefepime. Maximum overall sensitivity was seen with amikacin and linezolid. Maximum overall resistance was noted with cotrimoxazole (58.1%) and amoxiclav (41.9%).

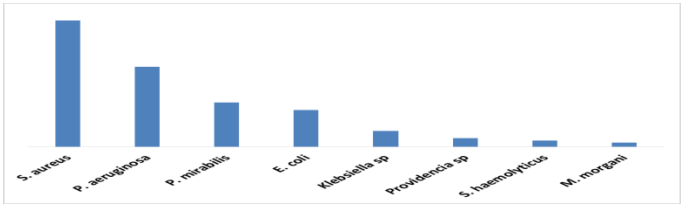

Fig 1: Column showing percentage distribution of samples collected from the study population based on the aerobic isolated obtained (N = 33)

Table 1: Clinical characteristics of the study participants

Clinical characteristics	Number (%)
Duration since diagnosis (< 1 year)	4 (10.5)
Duration of ulcer (< 1 year)	30 (78.9)
Ulcer location	
Lower extremities	35 (92.1)
Upper extremities	3 (7.9)
Osteomyelitis (Yes)	16 (42.1)

Table 2: Sensitivity of common isolated organism to some of the popular antibiotics

Antibiotic	Organism isolated (n)			
	S. aureus (14)	P. auroginosa (8)	P. mirabilis (5)	E. coli (4)
Amikacin	14	7	4	4
Linezolid	14	-	4	3
Imipenam	14	6	-	3
Gentamycin	14	-	-	-
Cefoperazone + sulbactum	-	7	4	4
Piperacillin + Tazobactum	-	8	-	-

Table 2: Resistance of common isolated organism to some of the popular antibiotics

Antibiotic	Organism isolated (n)			
	S. aureus (14)	P. auroginosa (8)	P. mirabilis (5)	E. coli (4)
Cotrimoxazole	7	6	5	-
Amoxiclav	7	6	-	3
Ciprofloxacin	-	-	5	2

Discussion

Consecutive thirty-eight patients of leprosy with trophic ulcer attending the leprosy outpatient department of the Department of Dermatology in a tertiary care center of eastern India were studied. The patients were thoroughly examined according to the predetermined case record form, and the pus obtained from the trophic ulcer site was subjected to aerobic

and anaerobic culture. After the isolation of the organism, a suitable antibiogram was obtained by Kirby–Bauer disc diffusion technique to a set of specific antibiotics for the aerobic isolates. The antibiotic sensitivity for the anaerobic isolates could not be done because of lack of logistical support. Sharma *et al*. found that the most frequent bacterial isolates from trophic ulcers due to diabetes were *Staphylococcus aureus* (38.4%), *Pseudomonas aeruginosa* (17.5%), and Proteus (14%) in their study [12]. Ferreira *et al*. reported that the most frequent isolates were *Staphylococcus aureus* (36.2%), *Proteus mirabilis* (15.5%), Enterobacter aerogenes (8.6%), Escherichia coli, Morganellamorganii, and *Pseudomonas aeruginosa* (13.3%) [13]. Tiendrebeogo *et al*. reported *Staphylococcus aureus* as the most frequent bacterium isolated from such patients [14]. In our study, the most common bacteria to be isolated were *Staphylococcus aureus* (45.2%), *Pseudomonas aeruginosa* (25.8%), *Proteus mirabilis* (16.1%), and *Escherischia coli* (12.9%) which correlated very well with the above studies.Raja concluded that antimicrobial susceptibility results in his study showed that Gram-negative bacteria were sensitive to imipenem and amikacin, while vancomycin showed good activity against Gram-positive bacteria [15].Ramani *et al*. found in their study that the aerobic bacteria were most sensitive to gentamycin [16]. Majumdar *et al*. observed that out of 56 samples studied, aerobic bacterial growth was noted in 54 cases. *Staphylococcus aureus*, *Escherischia coli*, Proteus sp., and Pseudomonas sp. were isolated in 32, 16, 22, and 4 cases, respectively. No growth of organism was found in two cases. Mixed growth (more than one organism) was noticed in 20 (36%) samples. Chloramphenicol and gentamycin were the two drugs that showed efficacy to the extent of 75%–100% and 25%–100%, respectively in *in vitro* studies [10].Our study showed *Staphylococcus aureus* to be equally and maximally sensitive to amikacin, imipenem, gentamycin and linezolid; *Pseudomonas aeruginosa* to be maximally sensitive to piperacillin + tazobactam, cefoprazone + sulbactam, amikacin and imipenem; *Escherischia coli* to be maximally sensitive to cefoperazone + sulbactam, amikacin, imipenem and linezolid. *Proteus mirabilis* was equally sensitive to cefoperazone + sulbactam, amikacin and linezolid.Martínez-Gómez *et al*. found in their study that nearly 30% of *Escherischia coli* strains were resistant to amoxicillin/clavulanic acid and ciprofloxacin [17].Tiendrebeogo *et al*. found that *Staphylococcus aureus* and *Pseudomonas* which were the most common organisms isolated were resistant to many antibiotics such as tetracycline, penicillin, and cotrimoxazole [14].Ebenezer *et al*. concluded that cotrimoxazole and tetracycline were of little value in the treatment of neuropathic plantar ulcers [9]. Our study correlated well with the above studies. *Staphylococcus aureus* showed maximum resistance to cotrimoxazole (50%) and amoxiclav (50%). *Pseudomonas aeruginosa* too showed maximum resistance to cotrimoxazole (75%) and amoxicalv (75%). *Escherischia coli* showed maximum resistance to amoxiclav (75%) and ciprofloxacin (50%). *Proteus mirabilis* showed maximum resistance to cotrimoxazole (100%), ciprofloxacin (100%), ceftazidime (80%), and cefepime (80%).George *et al*. reported that materials from 108 trophic ulcers from leprosy cases were studied bacteriologically. Four cases showed growth of pure anaerobes and 69 showed mixed growth of aerobic and anaerobic bacteria. The predominant anaerobes were fusobacteria (41), anaerobic cocci (30), and bacteroides (25). Clostridia were isolated only in 10 cases [18].

Conclusion

Secondary bacterial infection is quite common in leprosy trophic ulcers. The most common organisms are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*. According to our study, isolates were mostly sensitive to amikacin and linezolid and resistant to cotrimoxazole and amoxiclav. Amikacin and linezolid are the best drugs for empirical therapy at present in areas where culture facilities are not available, so as to curtail the duration of morbidity. Early treatment will ensure maximum limb salvage and prevent further complications, thus improving the quality of life of the patient.

Conflict of Interest: Nil Source of support:Nil

References

1. Rodrigues LC, Lockwood D. Leprosy now: epidemiology, progress, challenges, and research gaps. Lancet Infect Dis. 2011; 11:464-470.
2. World Health Organization. Global leprosy situation. WklyEpidemiol Rec. 2012; 87:317-328.
3. Chauhan VS, Pandey SS, Shukla VK. Management of plantar ulcers in Hansen's disease. Int J Low Extrem Wounds. 2003; 2:164-167.
4. Dharmendra S. Neuropathic ulceration. In: Dharmendra S, editor. *Leprosy*. 1st ed. Vol. 1. Bombay: Kothari Medical Publishing House, 1978, 224–40.
5. Thomson PD. Immunology, microbiology, and the recalcitrant wound. Ostomy Wound Manage. 2000; 46(1A):77S-82S.
6. Palande DD, de Severy C, Rajagopalan MS. Plantar ulcers with osteomyelitis underneath. A bacteriological study. Lepr India. 1977; 49:322-329.
7. Kumar CH, Harikrishnan S, Bhatia VN, Roy RG. Bacteriological study of trophic ulcers in leprosy patients [a preliminary study]. Lepr India. 1983; 55:504-511.
8. Husain A, SreevatsaMalaviya GN, Husain S, Bahadur RR. Characterization of microbial flora of leprous ulcers infested with maggots. ActaLeprol. 1993; 8:143-147.
9. Ebenezer G, Daniel S, Suneetha S, Reuben E, Partheebarajan S, Solomon S. Bacteriological study of pus isolates from neuropathic plantar ulcers associated with acute inflammatory phase. Indian J Lepr. 2000; 72:443-449.
10. Majumdar M, Chakraborty U, Das J, Barbhuiya JN, Mazumdar G, Pal NK. Bacteriological study of aerobic isolates from plantar ulcers of paucibacillary leprosy patients. Indian J Dermatol. 2010; 55:42-43.
11. National Committee of Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement. NCCLS document M100-S14. 1. Vol. 24. Wayne, PA: National Committee of Clinical Laboratory Standards, 2004.
12. Sharma VK, Khadka PB, Joshi A, Sharma R. Common pathogens isolated in diabetic foot infection in Bir Hospital. Kathmandu Univ Med J (KUMJ). 2006; 4:295–301.
13. Ferreira WA, Schettini APM, Nascimento JYD, Ferreira CM, Vasconcelos WDS, Almeida MGD et al. In vitro antimicrobial activity of the antibiotics used for the treatment of aerobic bacteria isolated from plantar ulcers in leprosy patients. Indian J Pract Doctor. 2006; 3:2006.
14. Tiendrebeogo A, Sarr AM. Naturéetsensibilité des bactériess urinfectant les maux performant plantaires d'origenelépruse à l'Institut Marchoux, Bamako, Mali. Acta Leprologica. 1999; 11:153–9.
15. Raja NS. Microbiology of diabetic foot infections in a teaching hospital in Malaysia: A retrospective study of 194 cases. J MicrobiolImmunol Infect. 2007; 40:39–44.
16. Ramani A, Ramani R, Shivananda PG, Kundaje GN. Bacteriology of diabetic foot ulcers. Indian J Pathol Microbiol. 1991; 34:81-7.
17. Martínez-Gómez Dde A, Ramírez-Almagro C, Campillo-Soto A, Morales-Cuenca G, Pagán-Ortiz J, Aguayo-Albasini JL. Diabetic foot infections. Prevalence and antibiotic sensitivity of the causative microorganisms. EnfermInfeccMicrobiolClin. 2009; 27:317–21.
18. George M, Bhatia VN, Kar HK, Roy RG. Anaerobic flora in trophic ulcers in leprosy patients. Indian J Lepr. 1985; 57:334–40.