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The pattern of bacterial isolates and drug sensitivities of infected ulcers in patients with leprosy in ALERT, Kuyera and Gambo Hospitals, Ethiopia

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Summary

Introduction: Leprosy remains a public health problem, mainly in Africa, Asia and Latin America. Leprosy has many complications that include leprosy reactions, development of plantar and hand ulcerations, lagophthalomus and corneal anesthesia.

Objectives: In Ethiopia there is scarce information on the pattern of bacterial isolates and drug sensitivities of infected ulcers in patients with leprosy. This study was undertaken to identify the bacteriology of infected ulcers and to determine their antimicrobial susceptibility pattern.

Study design: A descriptive cross-sectional study was conducted in 245 informed and consented leprosy patients with infected ulcers visiting ALERT, Kuyera and Gambo hospitals during the period August 2006 to May 2007.

Material and methods: Wound aspirate specimens were collected from ulcers of each patient aseptically and inoculated into standard bacteriological media. Antimicrobial susceptibility testing was performed for all isolates according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) by disk diffusion method.

Results: Of the 245 patients investigated, 64·1% were males and 35·9% females ($P < 0{\cdot}05$). The average age of the patients was 50 years (age range 13 to 92 years).

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According to Ridley-Jopling classifications, patients presented with TT (3·7%), BT, (31·4%), BL (44·5%) and LL (15·9%) types of leprosy. Plantar and hand ulcers were observed in 92·2% and 7·8% of patients, respectively. According the patients, the commonest cause of their ulcers was 'spontaneous' (56·7%). There were 44% Gram-positive and 56% Gram negative bacteria ($P > 0{\cdot}05$). *Proteus* spp. accounted for 29·5% of the total isolates followed by *Staphylococcus* spp. (28·8%), β-hemolytic *streptococci* (15·1%) and different types of Gram-negative bacteria (26·2%). Multiple organisms (two or three) were isolated from 19·6% patients. Of the 212 wound samples cultured anaerobically, 5·2% were positive for anaerobic culture. In this study both Gram-positive and Gram negative bacteria showed decreased sensitivity to most antimicrobial agents tested.

Conclusion: *Proteus* spp. was the most common isolate from infected ulcers. Ciprofloxacin, norfloxacin and gentamicin were the most effective drugs against the tested bacteria mainly for Gram-negative bacteria. This refers to the *in vitro*-sensitivity during the study period. The results of this study may help inform clinicians about the selection of an antibiotic in situations where use of an antibiotic may be indicated.

Introduction

Leprosy is a chronic infectious disease caused by the obligate intracellular pathogen *Mycobacterium leprae* (*M. leprae*). The bacteria grow best around 30°C and have preference for the cooler areas of the human body.[1] Leprosy remains a public health problem, mainly in Africa, Asia and Latin America. Early case finding and prompt treatment with MDT remains the cornerstone of leprosy control programmes, which needs to be ensured by strengthening integration with general health services and with critical support from the referral network.[2] The global detection of new cases has continued to show a sharp decline. But in Ethiopia new leprosy cases have been observed from Bale zone in Kokosa woreda since 2002.[3] Leprosy has many complications that include leprosy reactions, development of plantar and palmar ulcerations, lagophthalomus (failure of eyelids function) and corneal anesthesia. Leprosy is not itself directly responsible for most of the complications. It impairs the sensation of pain and thus exposes patients to ulceration and consequently to deformity.[4,5] Chronic ulcers are among the most serious complications of leprosy[6] and these ulcers are highly infected with bacteria, which delay the healing process. The delay is because of competition between host cells and bacterial cells for oxygen and nutrients and increased host cell production of inflammatory cytokines and proteases in response to the bacteria and their associated toxins.[7] In Ethiopia, there were only two studies done in 1970 and 1989, that assessed the bacteriology of infected ulcers and the sensitivity of these organisms to available antimicrobials in leprosy patients who visited ALERT hospital.[8,9] In the hospitals where the study was conducted there are no protocols or guidelines for antimicrobials administration (Personal Communication). The above-mentioned reports from Ethiopia have tried to point out that the burden in leprosy clinic in managing patients with infected ulcers indicated that more attention needs to be given to this problem. Therefore, the present investigation was undertaken to determine the bacterial isolates of infected ulcers in patients with leprosy and the sensitivity of these organisms to available antimicrobials. The study was the first of its kind after 18 years in ALERT hospital and for the first time in Kuyera and Gambo Hospitals. The findings of the study will enable hospitals having ulcer clinics to develop guidelines or protocols for the selection and administration of antimicrobial agents for the treatment of infected ulcers in patients with leprosy.

Materials and Methods

STUDY DESIGN AND AREA

A descriptive cross-sectional prospective study was conducted at leprosy ulcer clinics of ALERT, Kuyera and Gambo leprosy hospitals from August 2006–May 2007.

STUDY POPULATION

One thousand eighteen hundred twenty seven (1827) leprosy patients with ulcers were seen at ALERT ulcer clinic. In addition 100 leprosy patients with ulcers were seen at Kuyera ($n = 80$) and Gambo ($n = 20$) leprosy hospitals during the study. Totally, 1927 patients were screened. Of these, 19/1927 (1·0%) patients had palmar ulcers and 226/1927 (11·7%) had plantar ulcers. Out of 1927, 245 informed and consented patients with ulcers were investigated for bacterial infection. Of these, 205 were from ALERT, 34 from Kuyera and six from Gambo hospital. Patients treated with antimicrobials within the preceding 1-week were excluded from the study. In this study we used these working definitions:

Ulcer is a depressed, well-defined area of excavation, into the deeper layers of an organ, usually resulting from inflammation or ischemia (Landau, 1986).

Infected ulcer is an ulcer associated with purulent drainage or exudates, indurations, erythema or fever that necessitates wound culture (Hess, 2002).

SAMPLE COLLECTION, HANDLING AND TRANSPORT

Aspirated specimens from the infected ulcers were collected from each patient aseptically with sterile syringes by the attending physician. The specimens were kept in Thioglycollate broth (Oxoid Ltd., Hampshire, England) to maintain the viability of microorganisms until processed for aerobic and anaerobic cultures.

CULTURE AND IDENTIFICATION

Aspirated specimens from the infected ulcers collected in ALERT hospital were inoculated directly on Blood, Mannitol salt, and MacConkey agar where as the samples that were collected from Kuyera and Gambo were first inoculated in Thioglycollate broth till they reach the laboratory then they were immediately inoculated into Blood, Mannitol salt, and MacConkey agar since these two hospitals are far from the laboratory (Oxoid Ltd., Hampshire, England). The agar plates were incubated in aerobic atmosphere at 37°C for 24–48 hrs. Specimens for anaerobic culture were inoculated on blood agar and kept in anaerobic Jar containing anaerobic gas generating kits and anaerobic indicators (Genbag anaer, Biomeriux, France) at 37°C for 48 hours. Positivity of the cultures was determined by the reactions obtained from Gram-staining and also the appearance of the growth culture media. The positivity was then confirmed by using standard methods.[10]

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility testing was performed for all isolates according to the criteria of the NCCLS by disk diffusion method.[11] The drugs for disc diffusion testing were: Ampicillin (AMP) (10 μg), Amoxicillin (AML) (25 μg), Amoxicillin-Clavulanic Acid (AMC) (30 μg),

Cephalothin (KF) (30 μg), Ciprofloxacin (CIP) (5 μg), Chloramphenicol (C) (30 μg), Cloxacillin (OB) (5 μg), Erythromycin (E) (15 μg), Gentamicin (CN) (10 μg), Methicillin (MET) (5 μg), Penicillin (P) (10 units), Norfloxacin (NOR) (10 μg), Tetracycline (TE) (30 μg), and Vancomycin (VA) (30 μg) Trimethoprim- Sulphamethoxazole (SXT) (25 μg). Other drugs such as Ampicillin (AMP) (10 μg), Amoxicillin (AML) (25 μg), Cephalothin (KF) (30 μg), Amoxicillin-Clavulanic Acid (AMC) (30 μg), Ciprofloxacin (CIP) (5 μg), Chloramphenicol (C) (30 μg), Gentamicin (CN) (10 μg), Norfloxacin (NOR) (10 μg), Tetracycline (TE) (30 μg), Trimethoprim- Sulphamethoxazole (SXT) (25 μg) were also used in addition to Penicillin, Vancomycin, Erythromycin, Methicillin and Cloxacillin.

American type culture collection strains (ATCC) of *Staphylococcus aureus* (ATCC-25923), *Escherchia coli* (ATCC-25922), *P. mirabilis* (ATCC-43071), and *Pseudomonas aeruginosa* (ATCC-27853), *Streptococcus pyogenes* (ATCC-19615), and *Bacteroides fragilis* (ATCC 25285) were used as a quality control throughout the study for culture and antimicrobial susceptibility testing.

DATA ANALYSIS

Data entry and analysis was done using EPI Info version 3.3.2/STATA version 7 computer software. Comparisons were made using Chi-square test with Fisher exact tests. *P-value* of $<0{\cdot}05$ was considered indicative of a statistically significant difference.

ETHICAL CONSIDERATION

The research project was approved by the Department of Microbiology, Immunology and Parasitology, ethically cleared by the Faculty Research Publications Committee-II (FRPC-II) and endorsed by the Faculty Academic commission, Addis Ababa University. It was also ethically cleared from AHRI/ALERT ethical review committee. Official permission from the ALERT, Kuyera and Gambo hospitals was obtained. Informed consent was sought from all adult patients, while for the children; a written informed consent was obtained from their parents/guardians. A total of 245 patients who agreed to participate and met the inclusion criteria were recruited into the study.

Results

STUDY SUBJECTS

The age and sex distribution of 245 leprosy patients investigated for infected ulcers are shown in Figure 1.

Of the 245 patients, 157 (64·0%) were males and 88 (36·0%) were females ($P < 0{\cdot}05$) resulting in an overall male to female ratio of 1·8:1. The average age of the patients was 50 years (age range 13 to 92 years) and majority of the patients 124 (50·6%) were between the ages of 45–64 years.

CLINICAL FINDINGS

Of the 245 patients, 233 (95·0%) were outpatients and the remaining 12 (5·0%) were inpatients (Table 1).

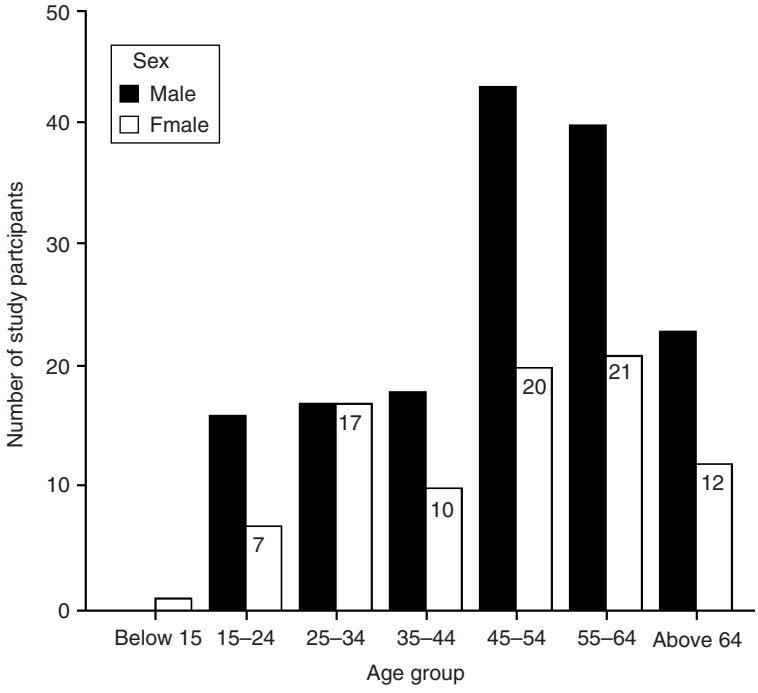

Figure 1. Age and sex distribution of patients investigated for infected leprosy ulcers at ALERT, Kuyera and Gambo Hospitals, Ethiopia *(August 2006–May 2007)*

According to Ridley-Jopling classifications, 9 (3·7%), 77 (31·4%), 109 (44·5%) and 39 (15·9%) patients presented with TT (Polar Tubercluoid), BT (Borderline Tuberculoid), BL (Borderline Lepromatous) and LL (Lepromatous Leprosy) types of leprosy, respectively. Sixty five percent were patients with recurrent ulcers where as the rest presented with new ulcers.

ANTIMICROBIAL SUSCEPTIBILITIES

Bacterial isolates in infected leprosy ulcers

A total of 298 bacterial pathogens were isolated from infected ulcers as shown in Table 1. *Proteus* spp. (*Proteus mirabilis*, *Proteus vulgaris* and *Proteus peeneri*) accounted for 29·5% of the total isolates followed by *Staphylococcus* spp. (*Staphylococcus aureus* and Coagulase negative *Staphylococci*) (28·8%), *β-hemolytic Streptococci* (15·1%) and different types of Gram-negative bacteria (26·2%). The Gram-positive and negative bacteria accounted for 131/298 (44·0%) and 167/298 (56·0%), respectively ($P > 0{\cdot}05$). Multiple organisms (two or three) were isolated from 48 (19·6%) patients.

GRAM-POSITIVE BACTERIA

The susceptibility patterns of Gram-positive bacteria ($n = 131$) isolated from leprosy ulcers against 15 antimicrobial agents are presented in Table 3.

Table 1. Clinical data of leprosy patients with infected ulcers (August 2006–May 2007)

Characteristics	Number	Percentages (%)
Location		
Outpatient	233	95·1
Inpatient	12	4·9
Leprosy classification		
TT	9	3·7
BT	77	31·4
BL	109	44·5
LL	39	15·9
No record	11	4·5
Treatment		
RFT	228	93·1
New case	1	0·4
OPD under MDT	12	4·9
IPD under MDT	2	0·8
Card lost	2	0·8
Ulcer condition		
Recurrent	159	64·9
New	86	35·1
Ulcer location		
Feet	226	92·2
Hand	19	7·8
Foot ulcer site		
Forefoot	190	77·6
Heel and forefoot	55	22·4
Cause of onset of ulcer		
"Spontaneous"	139	56·7
Repetitive walking Stress	62	25·3
Trauma	37	15·1
*Others	7	2·8

* Others: Rat bite, herbal medicine, dryness and medially deviated foot.

BL: Borderline Lepromatous; BT: Borderline Tuberculoid; TT: Polar Tubercluoid; LL: Lepromatous Leprosy; MDT: Multidrug treatment; RFT: Released From Treatment; OPD: Out patient department; IPD: In patient department.

All isolates showed intermediate level of resistance (60–80%) to ampicillin and tetracycline and low level of resistance (<60%) to amoxicillin, amoxicillin-clavulanic acid, cephalothin, ciprofloxacin, chloroamphenicol, cloxacillin, erythromycin, gentamicin, methicillin, norfloxacin, penicillin, trimethoprim-sulphamethoxazole and vancomycin. Most Gram-positive isolates 123/131 (93·9%) showed drug resistance to more than two or more antimicrobial agents.

GRAM-NEGATIVE BACTERIA

The susceptibility patterns of Gram-negative bacteria ($n = 162$) isolated from leprosy ulcers against 10 antimicrobial agents are presented in (Table 4).

High level of resistance (>80·0%) was observed against ampicillin. All isolates showed intermediate level of resistance (60–80%) to amoxicillin, tetracycline and cephalothin and low level of resistance (<60%) to amoxicillin-clavulanic acid, ciprofloxacin, chloroamphenicol, gentamicin, norfloxacin and trimethoprim-sulphamethoxazole. Among the 162

Gram-negative isolates 134/162 (82·7%) strains were also identified as having resistance to more than two drugs.

Discussion

Plantar, palmar and corneal ulcerations are among the complications of leprosy. Ulcers are a consequence of the loss of protective sensation and are caused by repetitive moderate stress, direct trauma, pressure, burns and walking on infected foot.[12] Plantar and palmar ulcers may be infected by the diverse flora of bacteria.[13] Their bacterial etiologies of the infection are not well studied and published information is scarce in Ethiopia.[7,8] Therefore, the present study was undertaken to identify and characterize bacterial etiologies of infected ulcers in patients with leprosy and to determine their susceptibility pattern to the available antimicrobials.

Once the ulcers are formed, secondary bacterial infections usually follow. Wide ranges of aerobic bacteria were reported from several studies.[14–17] In the present study wound aspirates culture from 245 infected ulcers, yielded 80% single organisms and 20% yielded mixed growth (two or more organisms). This is similar with previous Ethiopian study that reported 95% of the culture with single organism and the remaining 5% yielded two organisms.[8] But this is in contrast with studies conducted elsewhere where mixed organisms were dominating.[14–17] These might be due to the difference in sample collection method that helped in avoiding the contamination of the samples by the skin normal flora.

Proteus mirabilis was the most common isolate in this study (Table 2). Similar to other studies reported elsewhere.[9,14,17] Different studies have shown diversified bacterial etiological agents. *Pseudomonas aeruginosa* was the most common isolate in Indian leprosy patients with ulcers.[15] *S. aureus* was the major isolate in studies done in South Africa and Mali. [13,18]

In this study, anaerobic culture was performed for 212 infected ulcers. Of these, 5·2% were positive for anaerobic bacteria. But none of them were identified at species level because of inability to recover them during sub culturing. Gram staining was done and nine out of 11 were Gram-positive cocci where as two were Gram-negative short rods. Goodwin's and Woods[8] and George *et al.*,[16] reported anaerobes as their primary isolates. The presence of anaerobic bacteria as normal flora on the external skin of the human body and internally inside the body is well known. These days, the role of anaerobic bacteria in various infectious processes is recognized increasingly.[16]

In this study, Gram-positive and Gram-negative bacteria showed decreased sensitivity to most of the antimicrobial agents tested (Tables 3 and 4). This is in contrast when compared with previous study done in Ethiopia where most of the isolates were sensitive to ampicillin (73·9%) followed by penicillin (58·7%), Trimethoprim-sulphamethoxaszole (50%), Gentamicin (41·3%), erythromycin (34·8%), chloroamphenicol (26%) and tetracycline (21·7%)[9] Another study conducted in Ethiopia also showed that most strains were sensitive to erythromycin (90%), tetracycline (85%), ampicillin (80%), chloramphenicol (80%), cloxacillin (70%) and penicillin (52%) 7. Similar finding has been reported elsewhere.[15]

In this study male preponderance has been observed (Figure 1).

Similar findings have been reported in other studies.[18–19] Britton and Lockwood[20] described this predominance, as true difference between males and females. This is not because of the under diagnosis in women, although in some countries it was noticed by the delayed presentation of female patients, which results in high deformity.

Table 2. Bacterial etiologic agents of leprosy ulcers among patients who visited ALERT, Kuyera and Gambo Hospitals, Ethiopia (August 2006–May 2007)

Etiologic agents	Number of isolates	Percentages (%)
Proteus spp.		
P. mirabilis	47	15·8
P. vulgaris	38	12·7
P. penneri	3	1·0
Staphylococcus spp.		
S. aureus	68	22·8
Coagulase negative *staphylococci*	18	6·0
β-hemolytic *streptococci*	45	15·1
Morganella morganii	19	6·4
Enterobacter cloacae	8	2·7
Pseudomonas spp.		
P. aeruginosa	7	2·3
P. fluorescens	1	0·3
Escherichia spp.		
E. coli	14	4·7
E. fergusonii	1	0·3
Acinteobacter baumannii	5	1·7
Providencia stuartii	4	1·3
Klebsiella spp.		
K. oxytoca	5	1·7
K. pneumoniae	2	0·7
K. orintholytica	1	0·3
K. terrigena	1	0·3
Citrobacter spp.		
C. koseri	1	0·3
C. freundii	1	0·3
Serratia spp.		
S. ficaria	1	0·3
S. marcescens	1	0·3
Pantoea species	1	0·3
Burkholoderia cepacia	1	0·3
Other Gram-negative rods*	5	1·7
Total	298	100

* Not identified to the species level with the typing method used.

Of the 245 leprosy patients examined, 45% of the patients had borderline leprosy (Table 3). Borderline leprosy can be associated with rapid and severe nerve damage. These groups of patients have high instability and can be complicated by reactions and development of ulcerations.[20]

In this study, more than 90% of the ulcers were located on the plantar surfaces of the feet. This is in agreement with 83·6% reported by Sturm *et al.*[13] The feet remain the major sites of ulcers because they are the site of weight bearing when compared to the hand.[14–17] Fifty-seven percent of the cause of the ulcers in this study was considered as 'spontaneous' by the patients (Table 3). 'Spontaneous' does not mean that there is no reason for the development of ulcers. Since leprosy patients lose the function of sensory nerves early during the development of the disease, they do not sense or feel the pain of injuries even if they are stepping on sharp objects and pebbles are inside the ulcers. These findings are in line with literature that underlines the occurrence of ulcers.[21,22]

In the management of leprosy ulcers using antibiotics, the first rule is 'do not use antibiotics as a routine.' The second general rule is 'do not fail to use appropriate antibiotics

Table 3. Susceptibility patterns of Gram-positive bacteria isolated from leprosy ulcers (August 2006–May 2007)

		Antimicrobial agents No. (%)														
		AMP	AML	TE	C	CN	P	E	SXT	NOR	CIP	AMC	KF	VA	MET	OB
S. aureus	S	8 (11·8)	16 (23·5)	15 (22·1)	50 (73·5)	63 (92·6)	6 (8·8)	17 (25·0)	37 (54·4)	59 (86·8)	47 (69·1)	52 (76·5)	61 (89·7)	68 (100)	60 (88·2)	55 (80·9)
(n = 68)	I	2 (2·9)	7 (10·3)	2 (2·9)	5 (7·4)	–	4 (5·9)	31 (45·6)	8 (11·8)	4 (5·9)	15 (22·1)	8 (11·7)	2 (2·9)	–	4 (5·9)	2 (2·9)
	R	58 (85·3)	45 (66·2)	51 (75·0)	13 (19·1)	5 (7·4)	58 (85·3)	20 (29·4)	23 (33·8)	5 (7·3)	6 (8·8)	8 (11·7)	5 (7·4)	–	4 (5·9)	11 (16·2)
β-hemo	S	12 (26·7)	36 (80·0)	6 (13·3)	27 (60)	–	18 (40·0)	7 (15·6)	10 (22·2)	8 (17·8)	3 (6·7)	41 (91·1)	40 (88·9)	32 (71·1)	ND	35 (77·8)
streptococci	I	10 (22·2)	3 (6·7)	5 (11·1)	12 (26·7)	1 (2·2)	27 (60·0)	28 (62·2)	4 (8·9)	14 (31·1)	18 (40·0)	3 (6·7)	2 (4·4)	3 (6·7)	ND	2 (4·4)
(n = 45)	R	23 (51·1)	6 (13·3)	34 (75·6)	6 (13·3)	44 (97·8)	–	10 (22·2)	31 (68·9)	23 (51·1)	24 (53·3)	1 (2·2)	3 (6·7)	10 (22·2)	ND	8 (17·8)
CoNS	S	6 (33·3)	10 (55·6)	6 (33·3)	11 (61·1)	15 (83·3)	5 (27·8)	7 (38·9)	8 (44·4)	15 (83·3)	16 (88·8)	17 (94·4)	16 (88·9)	17 (94·4)	11 (61·1)	10 (55·6)
(n = 18)	I	–	2 (11·1)	1 (5·5)	2 (11·1)	–	2 (11·1)	2 (11·1)	1 (5·6)	1 (5·6)	1 (5·6)	1 (5·6)	–	–	2 (11·1)	–
	R	12 (66·7)	6 (33·3)	11 (61·1)	5 (27·8)	3 (16·7)	11 (61·1)	9 (50·0)	9 (50·0)	2 (11·1)	1 (5·6)	–	2 (11·1)	1 (5·6)	5 (27·8)	8 (44·4)
Total	S	26 (19·8)	62 (47·3)	27 (20·6)	88 (67·2)	78 (59·5)	29 (22·1)	31 (23·7)	55 (41·9)	82 (62·6)	66 (50·4)	110 (83·9)	117 (89·3)	117 (89·3)	71 (82·5)	100 (76·3)
(n = 131)	I	12 (9·2)	12 (9·2)	8 (6·1)	19 (14·5)	1 (0·8)	33 (25·2)	61 (46·6)	13 (9·92)	19 (14·5)	34 (25·9)	12 (9·2)	4 (3·1)	3 (2·3)	6 (6·9)	4 (3·1)
	R	93 (70·9)	57 (43·5)	96 (73·3)	24 (18·3)	52 (39·7)	69 (52·7)	39 (29·8)	63 (48·1)	30 (22·9)	31 (23·7)	9 (6·9)	10 (7·6)	11 (8·4)	9 (10·5)	27 (20·6)

AMP: ampicillin; AML: amoxicillin; TE: tetracycline; C: chloroamphenicol; CN: gentamicin; P: penicillin; AMC; augumentin; E: erythromycin; SXT: trimethoprim-sulphamethoxazole; NOR: norfloxacin; CIP: ciprofloxacin; KF: cephalothin; VA: vancomycin; MET: methicillin; OB: cloxacillin, CoNS: Coagulase Negative *Staphylococci;* ND: Not Done *S: Sensitive; I: Intermediate; R: Resistant.

Table 4. Susceptibility patterns of Gram–negative bacteria isolated from Leprosy ulcers (August 2006–May 2007)

		Antimicrobial agents No.(%)									
		AMP	*AML*	*TE*	*C*	*CN*	*SXT*	*NOR*	*CIP*	*KF*	AMC
P. mirabilis (n = 47)	S*	16 (34·0)	19 (40·4)	2 (4·3)	18 (38·3)	42 (89·4)	26 (55·3)	41 (87·2)	41 (87·2)	18 (38·3)	24 (51·1)
	I*	–	4 (8·5)	1 (2·1)	6 (12·3)	1 (2·1)	–	2 (4·3)	2 (4·3)	5 (10·6)	3 (6·4)
	R*	31 (65·9)	24 (51·1)	44 (93·6)	23 (48·9)	4 (8·5)	21 (44·7)	4 (8·5)	4 (8·5)	24 (51·1)	20 (42·5)
P. vulgaris (n = 38)	S	3 (7·9)	3 (7·9)	14 (36·8)	23 (60·5)	34 (89·5)	23 (60·5)	37 (97·4)	36 (94·7)	1 (2·6)	7 (18·4)
	I	–	3 (7·9)	1 (2·6)	2 (5·3)	–	1 (2·6)	–	1 (2·6)	1 (2·6)	10 (26·3)
	R	35 (92·1)	32 (84·2)	23 (60·5)	13 (34·2)	4 (10·5)	14 (36·8)	1 (2·6)	1 (2·6)	36 (94·7)	21 (55·3)
M. morganii (n = 19)	S	–	–	3 (15·8)	16 (84·2)	15 (78·9)	3 (15·8)	16 (84·2)	16 (84·2)	19 (100)	19 (100)
	I	1 (5·3)	1 (5·3)	–	1 (5·3)	–	2 (10·5)	2 (10·5)	–	–	–
	R	18 (94·7)	18 (94·7)	16 (84·2)	2 (10·5)	4 (21·1)	14 (73·7)	1 (5·3)	3 (15·8)	–	–
E. coli (n = 14)	S	4 (28·6)	4 (28·6)	1 (7·1)	9 (64·3)	14 (100)	5 (35·7)	14 (100)	14 (100·0)	1 (7·1)	3 (21·4)
	I	1 (7·1)	–	1 (7·1)	1 (7·1)	–	–	–	–	3 (21·4)	4 (28·6)
	R	9 (64·3)	10 (71·4)	12 (85·7)	4 (28·6)	–	9 (64·3)	–	–	10 (71·4)	7 (50·0)
E. cloacae (n = 8)	S	2 (25·0)	2 (25·0)	–	6 (75·0)	8 (100)	7 (87·-5)	8 (100·0)	8 (100·0)	2 (25·0)	2 (25·0)
	I	–	–	3 (37·5)	1 (12·5)	–	–	–	–	–	–
	R	6 (75·0)	6 (75·0)	5 (62·5)	1 (12·5)	–	1 (12·5)	–	–	6 (75·0)	6 (75·0)
P. aeruginosa (n = 7)	S	–	1 (14·3)	–	1 (14·3)	6 (85·7)	–	7 (100)	6 (85·7)	1 (14·3)	–
	I	1 (14·3)	-	–	–	–	–	–	1 (14·3)	–	–
	R	6 (85·7)	6 (85·7)	7 (100·0)	6 (85·7)	1 (14·-3)	7 (100·0)	–	–	6 (85·7)	7 (100)
K. oxytoca (n = 5)	S	–	1 (20·0)	2 (40·0)	4 (80·0)	5 (100)	4 (80·0)	5 (100)	5 (100)	1 (20·0)	–
	I	–	1 (20·0)	1 (20·0)	–	–	–	–	–	1 (20·0)	3 (60·0)
	R	5 (100)	3 (60·0)	2 (40·0)	1 (20·0)	–	1 (20·0)	–	–	3 (60·0)	2 (40·0)
A. baumannii (n = 5)	S	2 (40·0)	3 (60·0)	1 (20·0)	1 (20·0)	5 (100·0)	2 (40·0)	3 (60·0)	4 (80·0)	1 (20·0)	1 (20·0)
	I	–	1 (20·0)	1 (20·0)	–	–	–	1 (20·0)	–	–	3 (60·0)
	R	3 (60·0)	1 (20·0)	3 (60·0)	4 (80·0)	–	3 (60·0)	1 (20·0)	1 (20·0)	4 (80·0)	1 (20·0)
*Others (n = 19)	S	1 (5·2)	1 (5·2)	7 (36·8)	10 (52·6)	17 (89·5)	12 (63·2)	18 (94·8)	18 (94·8)	5 (26·3)	9 (47·4)
	I	–	2 (10·5)	–	–	–	–	–	–	3 (15·7)	1 (5·2)
	R	18 (94·8)	16 (84·3)	12 (63·2)	9 (47·4)	2 (10·5)	7 (36·8)	1 (5·2)	1 (5·2)	11 (57·8)	9 (47·4)
Total (n = 162)	S	28 (17·3)	34 (20·9)	30 (18·5)	88 (54·3)	145 (89·5)	82 (50·6)	149 (91·9)	146 (90·1)	48 (29·6)	63 (38·9)
	I	3 (1·8)	12 (7·4)	8 (4·9)	11 (6·8)	2 (1·2)	3 (1·8)	5 (3·1)	6 (3·7)	14 (8·6)	26 (16·0)
	R	131 (80·9)	116 (71·6)	124 (76·5)	63 (38·9)	15 (9·2)	77 (47·5)	8 (4·9)	10 (6·2)	100 (61·7)	73 (45·1)

[*] *Others: P. stuartii, P. penneri, K. pneumoniae, K. orintholytica, K. terrigena, S. marcescens, S. ficaria, C. freundii, C. koseri, E. fergusonii, Pantoea sp., P. fluorescens and B. cepacia.*

AMP: ampicillin; AML: amoxicillin; TE: tetracycline; C: chloroamphenicol; CN: gentamicin; P: penicillin; E: erythromycin; SXT: trimethoprim-sulphamethoxazole; NOR: norfloxacin; CIP: ciprofloxacin; KF: cephalothin; VA: vancomycin; MET: methicillin; OB: cloxacillin; CoNS: Coagulase Negative *Staphylococci;* ND: Not Done *S: Sensitive; I: Intermediate; R: Resistant.

when needed.' There are absolute and relative indications in the use of antibiotics in leprosy ulcers. The absolute conditions include life threatening infective indications like septicemia and highly virulent bacterial infections such as staphylococci and streptococci. The relative conditions include the presence of complications like cellulites, acute adenopathy of regional lymphnodes, systemic toxaemia and involvement of deeper structures like underlying bones, joints or tendon sheaths.[22] It will be wrong to assume that the antimicrobials sensitive in the past will also be currently effective; several studies have proved the assumption to be wrong. [14- 17]

Antimicrobials resistance in bacteria is increasing, which is a worldwide problem that continues to challenge medical practice.[23–24] In this study, the suggested quinolones (ciprofloxacin and norfloxacin) as treatment options from our findings has shown some resistance. Some indications from my study were documented. There were also some reports that underlined the spread of a plasmid-mediated quinolone resistance determinant in *Enterobacteriaceae*.[25]

Our physicians in ALERT hospital have used the antibiotics to which an aspirated isolate is sensitive, when there was absolute and relative indications stated above and they have found it very helpful in improving the clinical situations (personal communication).

Quinolones resistance usually results from chromosomal mutations, but, plasmid-mediated resistance has been discovered from different bacterial strains, *Klebsiella pneumoniae*, *Citrobacter koseri*, *Enterobacter cloacae*, and *Escherichia coli* isolated in South India and USA.[26] This earlier detection might be a good warning signal to be cautious in the use of these potent and relatively new antimicrobials.

Conclusions and Recommendations

In conclusion, ciprofloxacin, norfloxacin and gentamicin were the most effective drugs against the tested bacteria mainly for the Gram-negative bacteria *in vitro*. Leprosy patients with infected ulcers should be managed by self care education, cleansing, drainage, dressing, debridement, and rest before considering the prescription of antimicrobials. There are absolute and relative indications in the use of antibiotics in infected leprosy ulcers.

In a situation where there is an indication to institute antimicrobial drugs, the above-mentioned agents can be empirically used as first line set of drugs. In a clinical situation where there is no or poor response to these antibiotics, clinicians should consider culture and sensitivity of the ulcer specimen prior to prescribing other antimicrobial agents. The practice of culture and sensitivity may therefore help in selection of the right antimicrobials when use of antimicrobials is indicated.

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