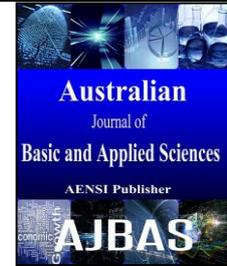




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Drug Sensitivity and the Changing Patterns of Bacterial Isolates of Infected Ulcers of Leprosy Patients in Central Leprosy Institute-Retrospective Analysis

¹Pugazhenthana Thangaraju, ²Senthil kumar sengodan, ³V.C.Giri, ⁴Ulaganathan Aravindan, ⁵R.Veerakumaran, ⁶M. K. Showkath Ali

^{1,2}Division of laboratory, CLTRI, MOHFW, GOI, chengalpattu, Tamilnadu, India.

^{3,4}Divison of epidemiology, CLTRI, MOHFW, GOI, chengalpattu, Tamilnadu, India.

⁵Division of clinical/surgical, CLTRI, MOHFW, GOI, chengalpattu, Tamilnadu, India.

⁶Director, CLTRI, MOHFW, GOI, chengalpattu, Tamilnadu, India.

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ABSTRACT

Objectives: To study the relative frequency of bacterial isolates cultured from infected trophic leprosy ulcers and assesses their comparative in vitro susceptibility to the commonly used antibacterial agents and to plan for the appropriate and rational usage of antibiotics. **Methods:** This is a retrospective study with a review of the bacteriology results of specimens taken from 112 consecutive leprosy treated patients with non healing ulcers at central leprosy teaching and research institute, Tamilnadu during the period February 2013 to February 2014. The specimens were cultured using optimal aerobic microbiologic techniques. Antimicrobial susceptibility testing to different agents was carried out using the disc diffusion method. **Results:** A total of 112 microorganisms were isolated. The main gram negative isolation was *Proteus mirabilis* (39), followed by *proteus vulgaris* (27) and *Pseudomonas aeruginosa* (18) and *Staphylococcus aureus* (5) in gram positive strain. In the total number of the isolated gram negative bacteria, the antibiotics with less resistance were amikacin (55), ceftriaxone (55), cefatoxime (53), ceftazidime (39) ciprofloxacin (33), imipenem (39) and piperacillin tazobactam (33). For gram positive amikacin, ceftriaxone and Chloramphenicol were found sensitive. **Conclusion:** The bacteriological study of plantar ulcers of leprosy patients revealed the occurrence of mainly gram negative organism and proteus species as the main pathogens involved in such infections. The results of this study may guide empirical therapy as well as the pattern of organism and various method of controlling the infections. It also helps for establishing a rational way of prescribing antibiotic in government sectors.

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INTRODUCTION

Leprosy is a chronic granulomatous infectious disease caused by the obligate intracellular acid fast bacilli, *Mycobacterium leprae* (Rodrigues *et al.*,2011) and still it remains a public health problem in Asia (WHO.,2012). It has many consequences of disease including the more difficult leprosy reactions, plantar and palmar ulcerations development, loss of eyelid function and anesthesia of cornea and corneal ulcer (Chauhan *et al.*, 2003). Chronic non healing ulcers are the most serious complications of leprosy which may require repeated admission and management and these are highly infected with bacteria, which delays the healing process (Thomson *et al.*,2000) and which in such cases may increase the physical disability (Thomson *et al.*,2000). There are very few 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 developing country India (Palande *et al.*, 1977; Husain *et al.*, 1993; Ebenezer *et al.*, 2000; Majumdar *et al.*, 2010). This study was planned to observe the changing pattern of bacterial isolates in our setup and to evaluation the sensitive antibiotic for their respective organisms.

After official approval of the CLTRI, we carried out a retrospective study in order to detect the emerging bacterial isolates pattern in trophic ulcers in leprosy patients through analysis of laboratorial data from the medical records.

Study Design, materials and methods:

A descriptive retrospective analysis of laboratory data of pus culture and sensitivity was done at central leprosy training and research institute

Corresponding Author: Dr. Pugazhenthana Thangaraju MD, Department of clinical division, central leprosy teaching and research institute (CLTRI), MOHFW, GOI, Tamilnadu, India.
Tel:+919486279090; E-mail: drpugal23@gmail.com

for year February 2013–February 2014. Only non duplicate isolates were included in the study. Materials used for microbiological evaluation of the pus swab. Swabs were processed for gram stain and culture. For isolation of the aerobes, inoculation was done on nutrient agar, blood agar, MacConkey's agar and Mannitol agar media and incubated overnight at 37°C, and also Sabouraud agar at room temperature. Identification of the isolates was performed using biochemical methods (Cheesbrough *et al.*, 2006) in cases where there was some doubt about identification. The isolates were further tested for antibiotic sensitivity to different classes of antimicrobials on Mueller Hinton agar medium, using Kirby-Bauer disc diffusion method and following the guidelines of the Clinical and Laboratory Standards Institute (Wayne *et al.*, 2012): [a] Cephalosporin class -cefatoxime, ceftriaxone, ceftazidime [b] Aminoglycosides class-Gentamycin, amikacin [c] Fluoroquinolones class -ciprofloxacin, ofloxacin [d] Phenicol class -Chloramphenicol; [e] Penicillin class -oxacillin, Ampicillin, amoxicillin, amoxicillin clavulanic acid, Carbenicillin, piperacillin, imipenem [f] Macrolides class – erythromycin [g] cephalosporins- cefatoxime, ceftriaxone, ceftazidime, Cefoxitin, cefuroxime. The antibiotic discs were of standards Himedia laboratory.

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:

Out of 112 Patients, 73 (65.2%) are Male and 39 (34.8%) are female. The average age of the patients was 58 yrs and the age ranges from 37 to 74 yrs. 68% of the patients are in the age group of 50-65 yrs. (table 1).

The organisms that were isolated from the ulcer are presented in Figure 1 and 2. Only aerobic culture analysis was done, mainly for planning for antibiotic. Among 112 patients, 95 patients showed gram negative organisms. The organisms that include in gram negative spectrum were *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Acinetobacter* sps, *Enterobacter* sps and *Pseudomonas alcaligenes* in decreasing frequencies (Figure 1).

The gram positive cocci and bacillus includes *Neisseria* and *Staphylococcus aureus* (Figure 2). The antibiotic sensitivity of organisms were done by disc diffusion method.

The antibiotic which is highly sensitive against gram negative organisms includes protein synthesis inhibitors (aminoglycosides) as well as the cell wall inhibitors (penicillin derivatives, cephalosporins, and carbapenems) and broad spectrum Fluoroquinolones (Table 2 and Table 3).

Among protein synthesis inhibitors Amikacin is sensitive in 52(49%) gram negative organisms. The cell wall inhibitors namely ceftriaxone is sensitive in 53 (49.43%), cefatoxime 49 (45.8%), ceftazidime 39(36.4%), piperacillin tazobactam 33 (30.84), and Imipenem 39 (36.44%). Ciprofloxacin is sensitive in 33(30.84%) of negative strain.

Among gram positive *Staphylococcus aureus*, amikacin, ceftriaxone and Chloramphenicol are sensitive in 3(60%) of organism.

Table 1: Demographic data.

Sex	Male	Female	Total
Numbers	73 (65.2%)	39 (34.8)	112
Sex ratio Male to female 1.9 : 1			
Age Group	Male	Female	
35-40	1	2	
40-45	1	1	
45-50	7	4	
50-55	18	9	
55-60	17	7	
60-65	13	13	
65-70	6	1	
70-75	10	2	
Total	73	39	

Discussions:

Among the various complications that occur in leprosy are plantar, palmar and corneal ulcerations (Lema *et al.*, 2012; Barreto *et al.*, 2010) and once these ulcers develop, secondary bacterial infections usually follow. Diversified bacteriological agents have been identified in different studies, and in ours the main pathogens were *Proteus vulgaris*, followed by *P. aeruginosa*, being *Proteus mirabilis* the most common pathogen isolated. In a study, by Kumar *et*

al., from our institute CLTRI, India the most common isolate was *P. aeruginosa* (Kumar *et al.*, 1983), which is now not similar to the old bacteriological isolates which were prevailing during 1980s.

The threat of antimicrobial resistance is increasing, which is a worldwide problem that continues to challenge all medical practice (Wright *et al.*, 2007; Godebo *et al.*, 2013), and has become an important concern for the treating clinician, patients

and the drug industries in both the hospital and community environment (Godebo *et al.*, 2013). A review article on antibiotic resistance and prevention of resistance also stressed much on the rational prescription of antibiotic (Harmanjit *et al.*, 2013).

According to a study (Lema *et al.*, 2012) the use of antibiotics follows two main rules as the first is 'do not use antibiotics as a routine' and the second is 'do not fail to use appropriate antibiotics whenever needed'.

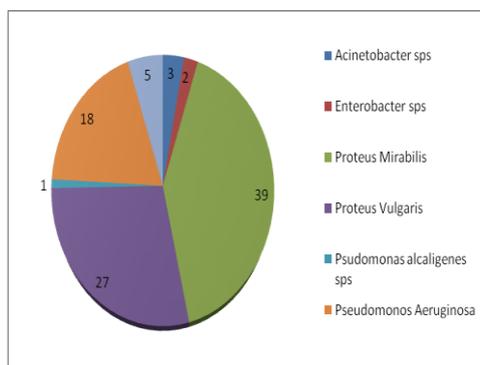


Fig. 1: Distribution of gram negative organisms in numbers.

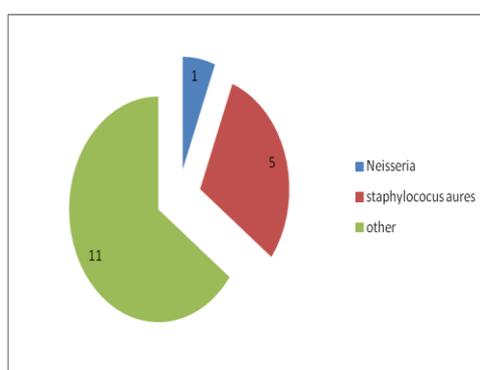


Fig. 2: Distribution of gram positive organisms in numbers.

Table 2: Antibiotic sensitivity chart for gram negative and gram positive organisms.

ORGANISMS	AK	CTR	CTX	CAZ	C
Acinetobacter sps (n=3)	3 (100%)	3 (100%)	3 (100%)	2 (66.7%)	2 (66.7%)
Enterobacter sps (n=2)	1 (50%)	1 (50%)	1 (50%)	0	1 (50%)
Proteus Mirabilis (n=39)	21 (53.8%)	24 (61.5%)	25 (64.1%)	19 (48.7%)	12 (30.8%)
Proteus Vulgaris (n=27)	12 (44.4%)	8 (29.6%)	7 (25.9%)	6 (22.2%)	5 (18.5%)
Pseudomonas alcaligenes sps (n=1)	0	1 (100%)	0	0	1 (100%)
Pseudomonas Aeruginosa (n=18)	7 (38.9%)	10 (55.6%)	7 (38.9%)	4 (22.2%)	6 (33.3%)
others (n=5)	4 (80%)	2 (40%)	4 (80%)	4 (80%)	3 (60%)
Neisseria (n=1)	0	1 (100%)	0	0	0
others (n=11)	4 (36.4%)	3 (27.3%)	2 (18.2%)	(36.4%)	8 (72.7%)
staphylococcus aureus (n=5)	3 (60%)	2 (40%)	3 (60%)	1 (20%)	3 (60%)
TOTAL	55	55	52	40	41

AK-Amikacin, CTR-Ceftriaxone, CTX-Cefatoxime, CAZ-Ceftazidime, C-Chloramphenicol.

Ulcers with osteomyelites and systemic symptoms, however, need antibiotics to recover the affected area from microorganisms and to cure osteomyelites. Even managing the osteomyelites should be done with proper pre culture and planned antibiotic according to the sensitivity.

Antibiotic treatment is then empirical because cultures of infected ulcers and the sensitivity of the

microorganisms are not available in rural areas and as an emergency care.

After the retrospective study in our hospital, we can empirically choose a better antibiotic when it is not possible to perform cultures. According to the results of our study, aminoglycosides (amikacin), quinolones (ciprofloxacin), and cephalosporin of third generation (ceftriaxone, cefatoxime), suggested

as treatment options, show less than 50% resistance (Figure 3). Other ways which were aimed in reducing the antibiotic use and prevention of resistance is the proper hygiene environment condition. Cross infection is the main source of transmission of bacteria from a ulcer of a person to other. So dressing

of ulcer with properly sterilized equipments, disposable materials should be used. The care taker of the ulcer namely the health staffs and nurse attendants should be trained properly in stopping the transmission by maintaining proper hygiene in curing of ulcer.

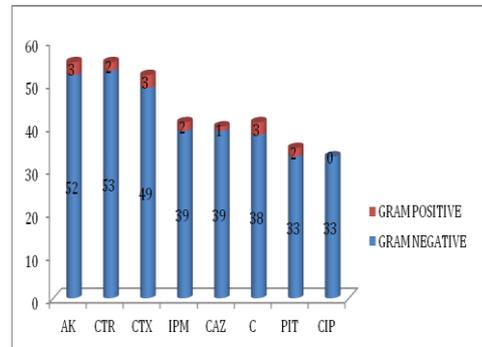


Fig. 3: Major Antibiotics for CLTRI bacteriological isolates.

Table 3: Antibiotic sensitivity chart for gram negative and gram positive organisms.

ORGANISMS	CIP	GEN	IPM	PIT
Acinetobacter sps (n=3)	2 (66.7%)	0	2 (66.7%)	2 (66.7%)
Enterobacter sps (n=2)	0	0		
Proteus Mirabilis (n=39)	15 (38.46%)	10 (25.6%)	13 (33.3%)	12 (30.8%)
Proteus Vulgaris (n=27)	10 (37.0%)	3 (11.1%)	9 (33.3%)	7 (25.9%)
Pseudomonas Alcaligenes sps (n=1)	0			
Pseudomonas Aeruginosa (n=18)	5 (27.8%)	4 (22.2%)	7 (38.9%)	5 (27.8%)
others (n=5)	0	2 (40%)	1 (20%)	1 (20%)
Neisseria (n=1)	1 (100%)	1 (100%)	1 (100%)	
Others (n=11)	0	2 (18.2%)	6 (54.5%)	6 (54.5%)
staphylococcus aureus (n=5)	0	1 (20%)	2 (40%)	2 (40.0%)
TOTAL	33	23	41	35

CIP-Ciprofloxacin; GEN-Gentamycin; IPM-Imipenem; PIT-Piperacillin Tazobactam.

Conclusion:

The bacteriological study of plantar ulcers of leprosy patients revealed the occurrence of mainly gram negative organism and proteus species as the main pathogens involved in such infections. The results of this study may guide empirical therapy as well as the pattern of organism and various method of controlling the infections. It also helps for establishing a rational way of prescribing antibiotic in government sectors.

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