



Original Research Article

Bacteriological Study of Aerobic Isolates from Lesions of Hands and Feet in Leprosy Patients

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A B S T R A C T

Leprosy is a chronic infection of skin and nerves caused by *Mycobacterium leprae* which, although rarely fatal, can lead to disability. The human being is the only known reservoir for this organism. A very high load of *M. leprae* is seen in lepromatous cases, with the two portals of entry being skin and the upper respiratory tract. A total of 260 samples from leprosy patients were selected for the study. Smears were made from hypo/hyper pigmented patches, ear lobes and nares and examined for AFB, Skin biopsy was taken from AFB negative patients for histopathological examination to classify the other types of leprosy. Swabs were collected from the active lesions from all the AFB positive patients and were subjected to culture and antibiogram. Out of the total 260, 188(72.3%) were positive for AFB and all of them were presented with skin ulcers. Of 72(27.1%) AFB negative cases, 3(4.1%) were Indeterminate type, 43(49.1%) Tuberculoid type and 26(36.1%) Borderline tuberculoid type. Of the 188 smear positive cases, 128(68%) were found to be culture positive for single type of organism and 60(31.9%) were culture negative. Major organism isolated was *Staphylococcus aureus* 57(44.5%), *Klebsiella* species 31(24.2%), which were sensitive to antibiotics like Ciprofloxacin, Vancomycin, Cotrimoxazole and Gentamicin and resistant to Erythromycin, Penicillins, Cephalosporins. The percentage of culture positive cases was similar to other studies. Many studies revealed *Staphylococcus aureus* as the frequently isolated organism. The clinical and histopathological results were compared, where we find high degree of concordance. The histopathological assessment and clinical diagnosis coincides in 70.8% of cases. This study shows a high prevalence of secondary bacterial infection among the chronic lesions in lepromatous leprosy patients. This could be due to repeated trauma to this area. Lack of awareness and indiscriminate use of antibiotics, low socio-economic status of the patients resulting in improper or inadequate intake of antibiotics, be reasons for the emergence of the resistant strains.

Keywords

Leprosy lesions, Bacteriological study, Antibiogram, Histopathological examination

Introduction

Leprosy is a chronic infection of skin and nerves caused by *Mycobacterium leprae* which, although rarely fatal, can lead to disability (Jonathan et al, 2nd ed.) Although this organism was first described by Hansen in 1873, it has not been cultivated on non-living bacteriological media (Jawetz, 20th ed.). The human being is the only known reservoir for this organism. A very high load of *M. leprae* is seen in lepromatous cases, with the two portals of entry being skin and the upper respiratory tract (Rees RJ et al, 1977). It appears that inhalation of *M. leprae* in the discharge of nasal secretions of an infected individual is the important mode of transmission (Noordan SK, 1993). As the organism causes loss of sensation, repeated trauma to these areas will lead to chronic non-healing ulcers superficially or deep especially in the lower and upper limbs, which get uninfected by bacteria, leading to discharge of pus etc.

Materials and Methods

A prospective data collected from Rehabilitation centre, Kukatpally, Hyd, during a period of Two years from September 2012 to August 2014. After obtaining institutional ethical clearance, 260 patients suspected of leprosy based on clinical examination with hypopigmented patches, nerve thickenings and/ or lesions were included in the study. Slit skin smear from all of them were subjected to acid fast staining. From the patients who were AFB positive, swabs were taken from open active lesions from hands/feet and subjected to bacteriological study. The sample was collected according to procedure explained in Konemann, 6th ed.

These swabs were inoculated onto MA and BA plates and incubated at 37°C overnight.

The isolated colonies were identified by biochemical reactions as per CLSI guidelines. They were further subjected to antibiotic sensitivity testing on MHA plates; standardizing the inoculums to 0.5 McFarland's standard.

The antibiotics used were Gentamicin (10mcg), Oxacillin (1 mcg), Erythromycin (15 mcg), Ciprofloxacin (5 mcg), Cefaperazone (75mcg), Cefotaxime (30mcg), Penicillin (10 units), Cotrimoxazole (1.25 mcg), Tetracycline (30 mcg), Chloramphenicol (30 mcg), Vancomycin (30 mcg) for Gram positive organisms, and Gentamicin (10 mcg), Amikacin (30 mcg), Ciprofloxacin (5 mcg), Cefaperazone (75 mcg), Ceftaxime (30 mcg), Cotrimoxazole (1.25 mcg), Imepenem (10 mcg), Tobramycin (10 mcg), Piperacillin/Tazobactam (100/10 mcg) for gram negative organisms. Interpretation of the sensitivity test was performed as per CLSI guidelines. The antibiotic control strains used were *Staphylococcus aureus* ATCC 25923, for gram positive bacteria and *Escherichia coli* ATCC 25922 for gram negative bacteria. Through clinical examination at least one peripheral nerve thickening was noted.

Histopathology: All AFB negative patients were subjected to thorough clinical examination and skin biopsy was taken. The site of biopsy was selected and the biopsy specimen was taken with strict aseptic precaution. The area was cleaned with disinfectant (70% alcohol) and 2% xylocaine solution was infiltrated under the skin in selected areas. After the site had become completely anesthetic, incision of required measurement was made, and the skin with subcutaneous tissue was dissected out. This was then fixed with neutral formalin solution, processed and stained with haematoxylin and eosin stain for

histopathological examination to classify the type of leprosy.

Results and Discussion

Of the total 260 patients, 188(72.3%) were slit skin smear positive by AFB staining and presented with open active lesions. These patients were classified under lepromatous type of leprosy and subjected to bacteriological culture. 72 (27.1%) patients were AFB negative and classified under other type of leprosy. Of 188 samples subjected for culture, 128 (68%) were culture positive showing growth of single type of organisms, among which 57 (44.5%) were *Staphylococcus aureus*, 31 (24.2%) *Klebsiella* species, CONS 15 (11.7%), *Pseudomonas aeruginosa* 9 (7.03%), *Proteus* species 8 (6.2%), *Escherichia coli* 8(6.2%). 60 (31.9%) were culture negative. Antibiotic sensitivity of the Gram positive cocci is predicted in the Table 1 and Gram negative bacilli in Table 2.

High sensitivity pattern was seen for Gentamicin(88.8%), and Vancomycin(56%), 87% of *Staphylococcus aureus* was found to be MRSA. Increased resistance was observed for Cephalosporins (99.1%), Penicillin (66.25%), Erythromycin (71.35%), Chloramphenicol (53.4%).

Amikacin (89.1%), Gentamicin (89.1%), were found to be most effective against the gram negative bacteria while most of the other antibiotics showed some resistance.

Of 72(27.1%) AFB negative cases, 3(4.1%) were indeterminate type, 43(59.7%) Tuberculoid type and 26(36.1 %) were Borderlinetuberculoid type.

A total of 128 (68%) from 188 samples from open active lesions of lepromatous leprosy cases were culture positive for growth of

single type of aerobic bacterial isolates, the percentage of which is similar to work done by Chatterjee BD et al, 1985, in which (56%) aerobic bacteria were isolated from trophic ulcers in leprosy

In present work aerobic bacterial isolates comprised of *Staphylococcus aureus* 57(44.5%), *Klebsiella* species 31(24.2%), Coagulase negative *Staphylococcus* 15(11.7%), *Pseudomonas* species 9 (7.03%), *Proteus* species 8 (6.2%) and *Escherichia coli* 8(6.2%).

Many studies revealed *Staphylococcus aureus* as the frequently isolated organism. Majumdar M et al, 2010 reported (59.2%), Tiendrebeogo et al,1999 isolated 68.6% positive cases, William Autuennes et al, 2006 isolated (36.2%) of *Staphylococcus aureus*. Chatterjee et al, 1985 isolated 40% of Micrococci from a total of 20 culture positive cases.

Next to *Staphylococcus aureus*, second most organisms isolated in present study was *Klebsiella* species, followed by Coagulase negative *Staphylococcus*, and other gram negative organisms. Studies carried by Majumdar M et al, 2010, reported *Proteus* 40.7% species as second prominent organism isolated followed by *E. coli* 29.6% *Pseudomonas* species 7.4% from culture positive patients which is similar to present findings in our work. Chatterjee et al, 1985, reported *Proteus* 36% and William Autuennes et al, 2006, reported *Proteus* 15.5% and *E.coli* 13.3% in their work.

In contrast the percentage of the isolation of the organisms is different in studies by Kumar CH et al, 1983, who isolated only 4 *Staphylococcus aureus*, 18 *Pseudomonas* species, 11 *Proteus* spp. from a total of 37 isolates with single type of growth out of 108 samples. Jose M Ramos et al, 2014,

isolated *E. coli* (21%), *Staphylococcus aureus* (18.5%), *Pseudomonas* (9.9%) with *Proteus* species (30.9%) being the most frequently isolated organism from a total of 66 culture positive cases from 68 samples.

Ebnezer et al, 2000, found a high frequency of *Proteus mirabilis* isolated from 53.4% of Plantar ulcers in a series of 86 patients followed by *Pseudomonas aeruginosa*(20.9%), *E. coli*(18.6%).

Abdulkadir et al, 1989, from a total of 70 cases 56(95%) cases were culture positive for single type of organism in which *Proteus* species was the most frequently isolated.

Tiendrebeogo et al, 1999, in his study after *Staphylococcus aureus*, isolated 41 strains of *Pseudomonas* spp.

The type of pathogens isolated in our study corroborated with the work done by Sharma RK et al, 1995, who also isolated similar type of organisms including gram positive cocci, *Staphylococcus aureus*, *Staphylococcus albus*, gram negative bacilli, *Escherichia coli*, *Klebsiella* species, *Proteus* species from scrapings collected from 129

leprosy patients catogerised under Multibacillary type of leprosy.

The bacteria isolated in the present study revealed an increase in resistance to antibiotics used in hospitals such as Erythromycin (71.3%), Penicillins(66.25%), Cephalosporins(99.1%). This results of which are similar to those reported by William Autunes et al, 2006 & Tiendrebeogo et al, 1999 who identified high degree of resistance to penicillins(90.48%), tetracyclins(100%), erythromycin (90.48%), chloramphenicol (71.43%), cefoxitin(75%), and penicillin, tetracycline and erythromycin respectively. In addition to it, Tiendrebeogo et al, 1999, also found resistant strains against sulfamethoxazol & trimethoprim. Gram negative organisms like Enterobacteriaceae members (*Klebsiella*, *E. coli*), *Pseudomonas* species, *Proteus* species have been also revealed resistance to many antibiotics. A study performed by Closkey et al, 1998, in their work reported an alarming increase in resistance to ciprofloxacin in patients with leg ulcers up to 24% of those infected with *Pseudomonas* and 40% in those with *Staphylococcus aureus*.

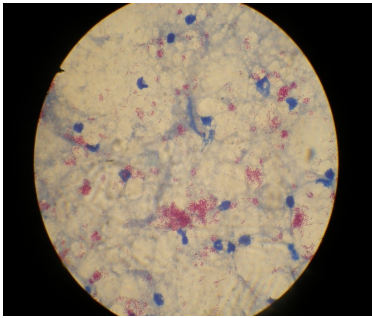
Table.1 Antibiotic sensitivity pattern of Isolated Gram positive organisms

S.No	Antibiotics	Staphylococcus aureus(57)		CONS(15)	
		sensitive	Resistant	Sensitive	Resistant
1	Gentamicin	49(85.%)	8(14.03%)	15(100%)	0
2	Oxacillin	52(91.2%)	5(8.7%)	-	--
3	Erythromycin	25(43.8%)	32(56.1%)	2(13.3%)	13(86.6%)
4	Ciprofloxacin	44(77.1%)	13(22.8%)	1(6.6%)	14(93.3%)
5	Cefaperazone	31(54.3%)	26(45.6%)	7(46.6%)	8(53.3%)
7	Cefotaxime	27(47.3%)	30(52.6%)	8(53.3%)	7(46.6%)
8	Penicillin	8(14.3%)	49(85.9%)	8(53.3%)	7(46.6%)
9	Cotrimoxazole	25(43.8%)	32(56.1%)	7(46.6%)	8(53.3%)
10	Tetracycline	16(28%)	41(71.9%)	11(73.3%)	4(26.6%)
11	Chloramphenicol	15(26.3%)	42(73.6%)	10(66.6%)	5(33.3%)
12	Vancomycin	56(98.2%)	1(1.7%)	-	-

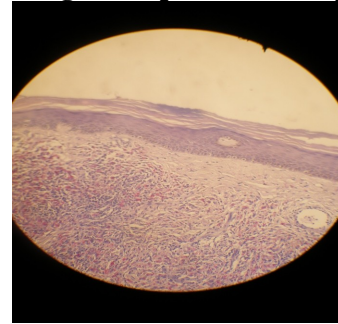
Table.2 Antibiotic sensitivity pattern of Gram negative organisms

S.No	Antibiotic	Klebsiella (31)		E. coli (8)		Pseudomonas (9)		Proteus(8)	
		Sen	Resis	Sens	Resis	Sen	Resis	Sens	Resis
1.	Gentamicin	27(87.1%)	4(12.9%)	8(100%)	0	7(77.7%)	1(11.1%)	7(87.5%)	1(12.5%)
2.	Amikacin	28(90.3%)	3(9.6%)	8(100%)	0	8(88.8%)	1(11.1%)	7(87.5%)	1(12.5%)
3	Ciprofloxacin	20(64.5%)	11(35.4%)	7(87.5%)	1(12.5%)	7(77.7%)	2(22.2%)	6(75%)	2(25%)
4	Cefaperazone	21(67.7%)	10(32.2%)	6(75%)	2(25%)	7(77.7%)	2(22.2%)	5(62.5%)	3(37.5%)
5	Ceftaxime	19(61.2%)	12(38.7%)	6(75%)	2(25%)	7(77.7%)	2(22.2%)	6(75%)	2(25%)
6	Cotrimoxazole	12(38.7%)	19(61.2%)	8(100%)	0	6(66.6%)	3(33.3%)	6(75%)	2(25%)
7	Imepenem	26(83.8%)	5(16.1%)	7(87.5%)	1(12.5%)	6(66.6%)	3(33.3%)	5(62.5%)	3(37.5%)
8	trobramycin	22(70.9%)	9(29.03%)	6(75%)	2(25%)	7(77.7%)	2(22.2%)	6(75%)	2(25%)
9	Piperacillin/ Tazobactam	21(67.7%)	10(32.2%)	6(75%)	2(25%)	7(77.7%)	2(22.2%)	7(87.5%)	1(12.5%)

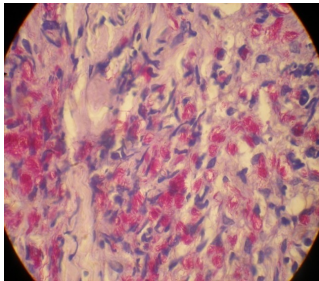
M. leprae in Slit Skin Smear by AFB Staining



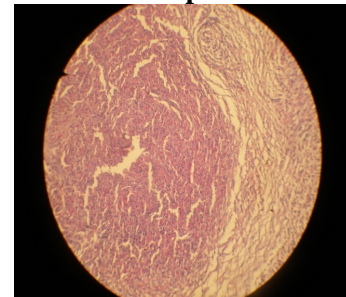
Histopathological image of Lepromatous Leprosy



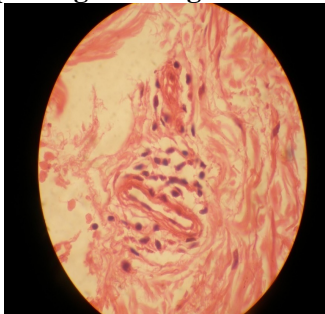
Clump of bacilli in globi



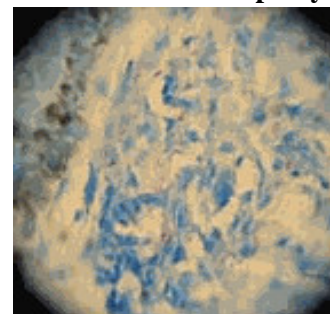
Granuloma in Lepromatous Leprosy



Histopathological image of Tuberculoid Leprosy



AFB of Indeterminate leprosy



All the 72(27.1%) samples were proceeded for histopathological examination for classification of different types of leprosy, on basis of which were classified as 43(59.7%) Tuberculoid type, 26(36.1%) Borderline tuberculoid type, 3(4.1%) Indeterminate type and 0 (0%) borderlineborderline type. The above percentage of types of leprosy indicates lepromatous leprosy (72.3%) and tuberculoid type of leprosy (27.1 %) are two most common and extreme forms of the disease.

The clinical and histopathological results were compared where we find high degree of concordance. The histopathological assessment and clinical diagnosis coincides in 51(70.8 %) cases. In 21(29.1%) there was much clinical and histopathological disagreement. The percentage of correlation of histopathological and clinical diagnosis in our study was similar to the percentage shown by Bhatia As et al, 1993 69% & Nadkarni NS et al, 1999 who found 69% of coincidence in their work.

In other similar study by Dubey GK et al, 1981, reported some disagreement in clinical and histopathological classification who reported 26 TT, 3 BT, 7 BB, 2 BL and 62 LL cases clinically but on basis of histopathological examination there were 20 TT, 9 BT, 7 BB, 6 BL and 58 LL cases.

The work demonstrates the most frequently isolated microbes from lesions from leprosy patients, such as *Staphylococcus aureus*, followed by the Gram negative bacteria of the enterobacteriaceae family such as *Klebsiella*, *Proteus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Antibiotics Penicillin, Erythromycin, Chloramphenicol, Tetracycline, Cefuroxime were ineffective for the treatment of such infections since the isolated bacteria presented high degree of resistance. Cotrimoxazole and Ciprofloxacin

were shown to be effective. This study shows a high prevalence of secondary bacterial infection among the chronic lesions in lepromatous leprosy patients. This could be due to repeated trauma to this area. Lack of awareness and indiscriminate use of antibiotics, low socio-economic status of the patients resulting in improper or inadequate intake of antibiotics, be reasons for the emergence of the resistant strains. Therefore, there is a immediate need for the clinicians to understand the type of bacterial flora that can infect their lesions and their antibiogram. This will help to expedite the correct treatment. Steps can be taken in advance by the clinicians and the patients to follow the regimen for correct treatment. The study suggests the need for development of efficient remedies for the treatment of secondary infections of lesions in such patients as well as strengthening the activities of the hospital infection control committees and also for providing health education almost infection prevention in leprosy patients.

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