Section: Miscellaneous

Original Research Article

# A CLINICO BACTERIAL STUDY OF ISOLATES OF MYCOBACTERIA IN DERMATOLOGICAL INFECTIONS IN A TERTIARY CARE HOSPITAL WEST BENGAL

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ABSTRACT

**Background:** Mycobacterial infections represent an important group of chronic infectious diseases affecting the skin and subcutaneous tissues. These infections are caused by members of the genus Mycobacterium, which include Mycobacterium tuberculosis complex, Mycobacterium leprae, and non-tuberculous mycobacteria (NTM). The aim & objective is to find out the prevalence of Mycobacterial cutaneous infections. To find out the Antibiotic susceptibility of isolates of cutaneous TB. To find out the individual percentage of three member of family of Mycobacteria (M. tuberculosis-complex, atypical Mycobacteria, M.leprae) in respect of their cutaneous manifestations. To compare the distribution of Cutaneous TB & atypical mycobacteria, M.leprae according to the age, sex, microscopy & culture positivity, histological findings & risk factors, comorbid conditions, occupations, vaccination status (BCG). To compare the association of Cutaneous TB with Pulmonary TB. To find out the Morphological Index (MI) & Bacteriological Index (BI) of positive cases of Leprosy & classify Leprosy cases. To find out the association of HIV and Mycobacterial cutaneous infections.

**Materials and Methods:** This hospital-based descriptive cross-sectional study was conducted at Burdwan Medical College and Hospital from March 2012 to February 2013. A total of 100 cases attending the Dermatology department were included in the study as per the predefined inclusion and exclusion criteria.

**Results:** Among 31 Positive Cutaneous Mycobacterial isolates, leprosy was predominant (54.3%) followed by cutaneous TB (38.7%) and NTM (6.5%). Among cutaneous Tuberculosis, LUPUS VULGARIS was most common morphological variant and tuberculide was least Common.

**Conclusion:** We concluded that cutaneous mycobacterial infections, including leprosy, cutaneous tuberculosis, and non-tuberculous mycobacterial infections, constitute an important dermatological health problem. The study demonstrated that these infections are more prevalent among young adults, males, rural populations, and individuals with low socio-economic status.

**Keywords:** Cutaneous mycobacterial infections, Mycobacterium tuberculosis, Non-tuberculous mycobacteria (NTM), Leprosy, Dermatological infections, Clinico-bacteriological study, Acid-fast bacilli (6AFB).

## INTRODUCTION

Mycobacterial infections represent an important group of chronic infectious diseases affecting the skin and subcutaneous tissues. These infections are caused by members of the genus Mycobacterium, which include Mycobacterium tuberculosis complex, Mycobacterium leprae, and non-

tuberculous mycobacteria (NTM). Cutaneous manifestations of mycobacterial diseases are diverse and may range from localized lesions such as nodules, ulcers, plaques, and abscesses to widespread systemic involvement, making clinical diagnosis challenging.[1] Cutaneous tuberculosis (CTB) remains a significant public health concern, particularly in developing countries. It accounts for a small but important proportion of extrapulmonary tuberculosis cases. The clinical presentation of CTB depends on the immune status of the host, route of infection, and bacterial load. Various forms include lupus vulgaris, scrofuloderma, tuberculous verrucosa cutis, papulonecrotic tuberculid, and other less common variants.[2] Accurate diagnosis is essential because cutaneous tuberculosis can mimic several inflammatory, neoplastic, and other infectious dermatological conditions. Leprosy, caused by Mycobacterium leprae, is another major dermatological mycobacterial infection prevalent in India. It primarily affects the skin and peripheral nerves and may lead to significant disability if diagnosis and treatment are delayed. Despite global elimination efforts, India continues to contribute a substantial proportion of the world's leprosy burden. Early identification of cases through clinical examination and bacteriological confirmation plays a vital role in preventing transmission and deformities.[3] In addition to tuberculosis and leprosy, infections caused by non-tuberculous mycobacteria (NTM) have gained increasing recognition. NTM are environmental organisms found in soil and water and can cause opportunistic cutaneous infections, especially following trauma, surgical procedures, injections, or immunosuppression. Common cutaneous NTM pathogens include Mycobacterium marinum, Mycobacterium fortuitum, Mycobacterium chelonae, and Mycobacterium abscessus. These infections often have atypical presentations and may be resistant to conventional anti-tubercular therapy, requiring species-level identification.[4-6]

Tuberculosis continues to be a major public health concern worldwide, with India accounting for a significant proportion of the global burden. Cutaneous tuberculosis represents approximately 1–2% of all cases of extrapulmonary tuberculosis and manifests in several clinical forms depending on the route of infection, host immunity, and bacterial virulence. Similarly, leprosy remains endemic in many parts of India despite substantial progress in control programs. In recent years, there has also been an increasing recognition of infections caused by non-tuberculous mycobacteria, particularly among immunocompromised individuals, post-surgical patients, and those exposed to contaminated environmental sources. The diagnosis of cutaneous mycobacterial infections is often difficult because of their varied clinical presentations and the paucibacillary nature of many lesions. Conventional diagnostic methods such as Ziehl–Neelsen staining, histopathological examination, and culture remain important tools for identification. Culture is considered the gold standard for definitive diagnosis and species identification, although it may require prolonged incubation periods. Advances in microbiological and molecular techniques have improved the detection and characterization of mycobacterial species, enabling more accurate diagnosis and targeted therapy. A thorough clinico-bacteriological evaluation is essential for understanding the epidemiological profile, clinical spectrum, and bacteriological characteristics of mycobacterial infections affecting the skin. Knowledge of the prevalent mycobacterial species and their clinical presentations can facilitate timely diagnosis, appropriate treatment, and improved patient outcomes. Furthermore, regional studies are valuable because the prevalence and distribution of different mycobacterial species may vary according to geographical location, environmental conditions, and population characteristics.

## MATERIALS AND METHODS

**Type of study:** Hospital based descriptive study (Cross-sectional study).

**Study area:** Burdwan Medical College and Hospital.

**Study population:** Patients were selected from Dermatology OPD.

**Inclusion Criteria**

Patients having cutaneous lesions (like skin ulcers, soft tissue ulcers, abscess, pustules, plaques, infected or partially healed wounds, sinuses, fistulas) with a

1. Positive history (present or past) of TB,
2. Contact history to established cases of TB,
3. Positive Tuberculin test,
4. History of BCG vaccination,
5. History of any immunocompromised or immunosuppressed state (like HIV, cancer chemotherapy, corticosteroid users),
6. History of DM & receiving insulin,
7. History of using IV drugs,
8. History of tattooing over the skin(65),
9. History of conversion of any simple cut or wound to non-healing ulcer following a minor surgical procedure, either visiting a catchment area, or related with activities associated with swimming pool, fishery, or farming, and also
10. History of hypopigmented anesthetic, skin patches with or without peripheral neuropathy.

**Exclusion criteria**

1. Established cases of Bacterial & Fungal cutaneous infections,
2. Any Established & Confirmed case of Drug reaction,
3. Known case of Allergic reactions,
4. Known case of autoimmune disorder.

**Study period:** MARCH -2012 to FEB -2013.

**Sample size:** 100 cases.

**Sample Design:** Patients attending in the Department of Dermatology ward that fulfilled the above mentioned inclusion & exclusion criteria were chosen randomly and after taking detailed history and careful clinical examination, samples were taken from them.

**Study Design:** sample or specimen collection-transport- processing (Homogenization digestion & decontamination centrifugation) AFB-microscopy & Culture & Biochemical testing (except M.leprae) Histopathological examination & Tissue staining Identification of Mycobacterial Skin isolates Antibiotic Sensitivity Test of positive isolates (except M.leprae). In case of M.leprae, samples were processed through AFB-microscopy & histopathological examination.

**Study Tools:** Sterile Swab Stick, Tissue cutting knife, Scissors, Scalpel blade for skin scrapings, Grease free Glass Slides, Mortar & pestle, Centrifuge Machine, Distilled water, Sterile normal saline, Z-N stain, L-J media for culture, Inoculating wire loop , Aluminum foil, Safety cabinet (BSF-2), Burner , Incubator, B.O.D, Microscope with oil emersion Lens (100x), Commercially available Biochemical tests kits and reagents, HIV-1&2 rapid kits, Anti-tubercular drugs, Weighing-machine.

**Study Technique:** The identification and isolation of organisms, done according to the standard text book methods and in case of kits and media, according to the manufacturer's guidelines.

**Plan for analysis of data:** collected data was analyzed through statistical software's like-SPSS.

**A. Collection:** Samples were collected in the form of 1.Wounds exudates & pus, 2.Slit skin smear, 3. Tissue biopsy element, 4. Sputum, 5. Serum for HIV screening.

**B. Containers:** 1.Sterile swab stick, 2.Skin biopsy elements in sterile test tube for histopathological study in formalin and another sterile test tube with normal saline for mycobacterial staining and culture, 3.Sputum in sterile, universal, leak-proof, disposable & appropriately labeled containers and placed into bags for the containment of leakage, 4.Sterile disposable syringe for pus and blood collection.

**C. Transport:** transport is done as early as possible to avoid overgrowth organisms other than Mycobacteria. If delay is unavoidable, specimens are stored in a refrigerator (66).

**D. Processing of specimen:** All specimens (except Slit-skin smears, Blood) were processed in the Biological Safety Cabinet through homogenization, decontamination and concentration for optimum recovery of acid-fast bacilli, Specimens collected aseptically, not thought to be contaminated, did not require decontamination.

**Homogenization (2):** Among the specimens mentioned above, only swabs and wound aspirates and tissue material were homogenized before decontamination & centrifugation.

Swabs and wound aspirates were transferred to a sterile 50 ml conical centrifuge tube containing a liquid medium (Dubos Tween albumin broth) at a ratio of 1 part specimen to 5-10 parts liquid medium .The specimens were vortexed vigorously and allowed to stand for 20 minutes. The swab was removed and the resulting suspension was processed by decontamination and centrifugation.

Large pieces of tissue were minced using sterile scalpel and scissors. This material was then homogenized by sterile mortar & pestle with a small amount of sterile saline. This suspension was then processed by decontamination and centrifugation.

Digestion, Decontamination and Concentration (2): Specimens were mixed with equal volume of 4% sodium hydroxide and incubated at 370c with frequent shaking for about 15 minutes. It was then centrifuged at 3000 rpm for 15-20 minutes. The supernatant fluid was poured off and the deposit was neutralized by adding 8% hydrochloric acid in presence of a PH indicator filter paper. The deposit was used for smear, culture.

Detection: By AFB-microscopy followed by Culture & Biochemical reaction

AFB-Microscopy: After preparation of smears, they were stained by Ziehl-Neelsen stain and AFB was identified as per standard procedure. (give the reference number).

Culture: The sediment or deposit after centrifugation and neutralization was inoculated in slants of Lowenstein-Jensen (L-J) medium, prepared manually in the laboratory and incubated at temperatures of 370C and 300C.

Preparation of L-J slants:

Materials required: 1) L-J base, commercially available from HIMEDIA, 2) Glycerol solution, 3) Autoclave, 4) Fresh hen's eggs (24 to 28 eggs per batch of 1000 ml fluid), 5) Glass jar, 6) sterile glass rod, 7) sterile glass stirrer, 8) Round bottom flask (Size-2 lt), 9) sterile 500ml measuring cylinder, 10) sterile gauze, 11) sterile glass funnel, 12)500 ml Methylated spirit, 13) SterileMc-Cartney (28 ml universal containers) bottles, 14) Water bath, 15) Distilled water (600ml)

Lowenstein Jensen Medium is prepared in the laboratory according to the guidelines supplied by the HIMEDIA along with the L-J medium base.

L-J base contains the following ingredients:

| Ingredients | Gms/Liter |
|---|---|
| L-Asparaginase | 3.60 |
| Monopottasium phosphate | 2.4 |
| Magnesium Sulphate | 0.24 |
| Magnesium Citrate | 0.60 |
| Potato starch | 30 |
| Malachite green | 0.40 |

**Method [7]:** One batch of L-J medium is 1600 ml of solution. Quality of media depends on the freshness of the hen's eggs.

a. The eggs (not more than one or two days old) were being soaked in water, scrubbed gently and washed in running water, followed by distilled water and placed in a drain board for draining.

b. The eggs were being soaked in Methylated spirit for 10 min, wiped with sterile gauze and stored in sterile container till use.

37.24 gms L-J base was dissolved completely in 600 ml distilled water containing 12 ml glycerol by heating. The whole solution was autoclaved at 121oC, at 15 Ibs for 15 minutes to sterilize and cooled to room temperature. This solution was stored in the refrigerator.

Meanwhile, 1000 ml whole egg emulsion was prepared aseptically in the following manner:

Individually, the eggs were broken by means of sterile rod on the side of the sterile 1 lit glass jar. Egg yolk and white transferred carefully into the jar. It was ensured that there were no stale eggs. With the help of a sterile glass stirrer, the egg fluid was homogenized within 1-2 minutes. Long breaks were not allowed as froth would be formed in the solution which would affect medium slope. The 1000 ml homogenized egg solution was filtered into sterile 500 ml measuring cylinder through sterile gauze covered glass funnel twice. Filtered homogenized egg solution was transferred in 2 liters round bottom flask.

600 ml of mineral salt solution (L-J base + glycerol + Distilled water) was added to the 1000ml filtered homogenized egg solution in the round bottom flask & mixed thoroughly by gentle agitation for 5 min, till uniform pale green color was obtained.

Then the 5-8 ml of the mixture solution was distributed in sterile Mc-Cartney bottles and coagulation and inspissations of the medium were done in a Water bath, arranging the bottles in a slanted position at 85oC for 45 minutes.

**Sterility Test [7]:**

1. All the slopes of medium prepared in a day were incubated for 48 hours at 37°c.
2. From a batch of medium 10 slopes were randomly selected and incubated at 37°c for 14days.
3. If bacterial and fungal contamination was noted, the entire batch was rejected.

**Storing [7]:** Media slopes were packed in a saran-warp, packed in boxes or iron meshes/racks, labeled with date of preparation, batch number and stored at the 4ºc refrigerator. Although media stored at refrigerator can be used for 2 months.

For optimum growth of Mycobacterium tuberculosis, 5% to 10% carbon dioxide (CO2) and high humidity is required and for optimal recovery of isolates from suspected skin and subcutaneous mycobacterial infections incubation at 300C is necessary. For each sample, inoculation was done in 3 L-J slants. One slant was incubated at 370C in the candle-jar with screw-caps loose, for at least 1 week to allow for evaporation of excess fluid and the entry of CO2. After 7-10 days, L-J slant was removed placed plainly in the incubator. The other 2 L-J Slants were incubated at 300C. Among the two one was covered with aluminum foil.

Cultures were examined first after 4 days (for rapid growing Mycobacteria) and thereafter weekly till 8 weeks for growth. When growth appeared, the rate of growth, pigmentation and colonial morphology were recorded. After 8 weeks of incubation, negative cultures were reported and cultures are discarded.

Rapid-growers produce visible colonies in less than 7 days; Slow-growers require more than 7 days.

For identification of pigment production, when colonies were mature, growth from both foil-wrapped and unwrapped tube were exposed to a bright light, such as a desk lamp, for 2 hours [8]. The cap was being loosed during exposure, because pigment production is an oxygen- dependent reaction [8] The foil-wrapped tube was rewrapped and returned to the incubator, and the cap left loose. The two L-J tubes were examined 24 and 48 hrs after light exposure [8].

## RESULTS

The preliminary identification of mycobacterial isolates based on their colonial morphology, colonial texture; growth rate, pigmentation and repeat AFB/Z-N staining of growth.

In case of positive growth in L-J media, colonies of M. tuberculosis are dry, rough, buff colored, raised, with a wrinkled surface. They were tenacious and easily emulsified and having luxuriant (eugonic) growth in culture. Rapid- growers (M. fortuitum, M. chelonae, M.abscessus) take less than 7 days and Slow-growers (M. tuberculosis, M. bovis, M. avium complex.) take more than 7days to grow.

a) Photochromogens are unpigmented when grown in the dark and develop pigment after light exposure.
b) Scotochromogens are pigmented in the dark; the color does not intensify after exposure to light.
c) Non-Photochromogens are non pigmented when grown in the dark and remain so even after light exposure.

**Biochemical Testing:** Mycobacterial species can be identified by several biochemical tests. But two important biochemical tests (Niacin test, Nitrate test which are discussed in review of literature) were done for identification. In cases of difficulty of identification of strains Real time PCR was done.

**Histopathological Examination And Tissue-Staining (68):** Biopsy samples from skin and subcutaneous tissues were processed in the department of Pathology, BMC for histopathologicatical examination & tissue staining for identification of mycobacterial infection. Haematoxyllin-Eosin (H-E) stain was used for staining of histological slides. Z-N stain was used for tissue- staining. Presence of Granuloma with multi nucleated giant cells in histological-slides and acid-fast organisms in tissue stain indicated the presence of mycobacterial infections.

**Antitubercular -susceptibility testing [7]:** Susceptibility testing of positive-isolates (only for

M. tuberculosis) was done by 1% Proportion method.

**Proportion method:**

**Principle:** All strains of tuberculosis contain some subpopulation of bacilli that are resistant to anti-TB drugs. However, in resistant strains, the proportion of such bacilli is considerably higher than the sensitive strains. The proportion method calculates the proportion of resistant bacilli present in a strain. Two appropriate dilution of the bacilli, 10-2 and 10-4dilutions (undiluted = 106 to 108 CFU/ml), are inoculated on drug-containing and drug-free media, in order to obtain countable colonies on both media. The ratio of number of colonies observed on the drug-containing media to drug-free medium indicates proportion of resistant bacilli present in the strain. Below a certain proportion (critical proportion = 1%), the strain is classified as sensitive; above, as resistant.

**Method:** First line antimycobacterial agents [Isoniazid (I),Rifampicin (R),Ethambutol(E), Streptomycin(S)] against positive isolates of cutaneous tuberculosis (Mycobacterium tuberculosis) added to L-J Media and critical proportion for Interpretation of Proportion Method were.[7]

| Drugs | Media Concentration | Critical proportion to determine resistance |
|---|---|---|
| Streptomycin(dihydro-streptomycin sulfate) | 4 µg/ml | 1% |
| Isoniazid | 0.2 µg/ml | 1% |
| Rifampicin | 40 µg/ml | 1% |
| Ethambutol | 2 µg/ml | 1% |

One set of media bottles for testing one culture consisted of five L-J slope, one for neat, two for 10-2 and two for 10-4; eight L-J drug containing slopes, two each for drugs H, R, E & S (one each for 10-2 and 10-4 suspensions).

**Inoculum preparation,[7]**

1. With a 3mm wire loop (made with 24 SWG nichrome wire, delivers 0.01 ml of inoculum.), a representative sample of approximately 4-5 mg (loop full) was taken from the primary culture and placed on the side wall of a McCartney bottle containing 1 ml SDW and 6 glass beads of diameter 3 mm.
2. The bacterial inoculum was emulsified, (with a loop of water, if required), on to the side wall of McCartney bottle in round rotatory movements with inoculation loop, (this is visible by reduction in the clumpy hydrophobic to aqueous hydrophilic nature of suspension).
3. The emulsified suspension was fully dissolved in the 1ml of Sterile Distilled Water (SDW).
4. The bottle was vortexed for 20–30 seconds; 4 ml of distilled water was added slowly.
5. The coarse particles were allowed to settle down (leave it on stand for approximately 5 min).
6. The supernatant was decanted carefully into another clear, sterile McCartney bottle.
7. The opacity/turbidity of inoculums was matched with McFarland standard no.1, against a black background. This was the neat bacterial suspension, standardized at 1 mg/ml, an equivalent to 107 to 108 CFU/ml.
8. The opacity of the bacterial suspension was then adjusted by the addition of distilled water to obtain a concentration of 1 mg/ml of tubercle bacilli by matching with McFarland's standard 1.
9. Two log dilutions were made further to achieve 10-2 and 10-4 dilutions as given below:

a. The dilution 10–2 was produced by discharging two loopful of the neat bacterial suspension, into a small tube containing 2 ml of distilled water, and shaking.

b. Similarly, the dilution 10–4 was produced by discharging two loopful of the dilution 10–2 into a small tube containing 2 ml of distilled water, and shaking.

Neat: 1 ml SDW with six 3 mm glass beads + 1 loop-full (3 mm loop) of culture

↓

Vortexed for 20 – 30 seconds

↓

4 ml of SDW to the above was added

↓

Turbidity was matched with McFarland std.1 with SDW

↓

S2-10 –2 two loop-full of neat + 2ml of SDW, vortex

↓

S4-10-4 two loop-full of S2 + 2ml of SDW

**Drug susceptibility testing,[7]:**

A loop-full (using 3mm calibrated loop) of each dilution was inoculated on to media slopes uniformly.

**Incubation and Reading**

Incubation of the inoculated slopes was done at 37oC, the growth was observed at 28 days and again at 42days.

Growth was recorded as Confluent growth = 3+; More than 100 colonies = 2+; actual number of colonies was recorded = 1-100 cols.

When the number of colonies on a given dilution was less than 5, the number of colonies with the next larger inoculums was counted.

**Interpretation of the test,[7]**

1. First reading was taken at 28th day after inoculation.
2. The colonies were counted only on the slopes seeded with the inoculum that had produced exact readable counts or actual counts (up to 100 colonies on the slope).

3. The average number of colonies obtained in the drug-containing slopes indicating the number of resistant bacilli contained in the inoculum.
4. The proportion of resistant bacilli existing in the strain was obtained by dividing the number of colonies in drug containing slopes by that in drug free slopes. Below a certain value –the critical proportion the strain was classified as sensitive; above that value, it is classified as resistant. The proportions were reported as percentages.

**Criteria of Resistance,[7]:** Any strain with 1% (the critical proportion) of bacilli resistant to any of the four drugs – Rifampicin, Isoniazid, Ethambultol, and streptomycin – was classified as resistant to that drug. For calculating the proportion of resistant bacilli, the highest count obtained on the drug-free and on the drug-containing medium was taken (regardless of whether both counts were obtained on the 28th day, both on the 42nd day, or one on the 28th day and the other on the 42nd day.)

If, according to the criteria indicated above, the result of the reading on the 28th day was "resistant", no further reading of the test for that drug was done. If the result at the 28th day was "sensitive", a second reading was made on the 42nd day only for the sensitive strain. The final definitive results for all the four drugs were reported on 42nd day. If the strain was resistant for all the four drugs on 28th day, then the report could be given on the same day.

In case growth on the control media was poor even after six weeks (i.e., few or no colonies on the 10-4 bacterial dilution), the test was repeated.

**Blood Samples Processing by Aid of HIV-1&2 Rapid Kit for Screening of HIV.[9]**

Screening of HIV-1&2 was accomplished by means of PAREEKSHAK HIV 1/2 Triline rapid kits.

**Test Method:** 1.Kit &Samples were brought to the room temperature. 2. Test device was placed on a flat surface after removing it from the pouch.3.10µl or1drop of serum or plasma was added into the sample window (S) and allowed to soak in.4.2 drops of diluents provided in the dropper bottle added into the sample window.5. Results were obtained in 20 minutes.

**Interpretation of the Results:** Negative result: The presence of only control line(C) within the result window indicated negative result. Positive results: The presence a band at C and bands at 1and/ or 2 within the result window, indicated a positive result for HIV-1 and HIV-2 respectively. Invalid result: When the control band is not visible at c, the test result was invalid.

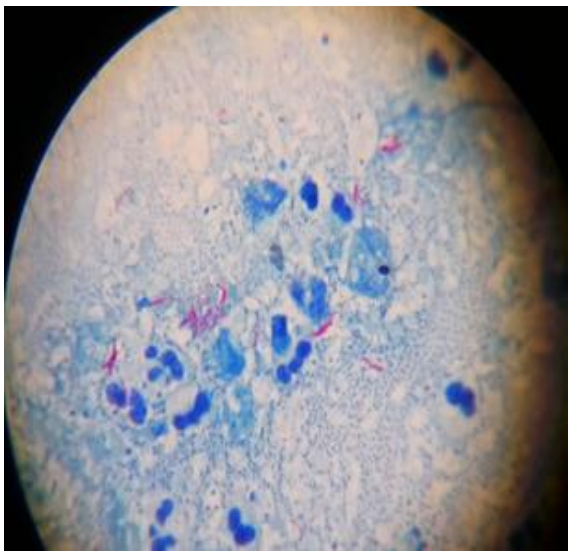


**Figure 1**

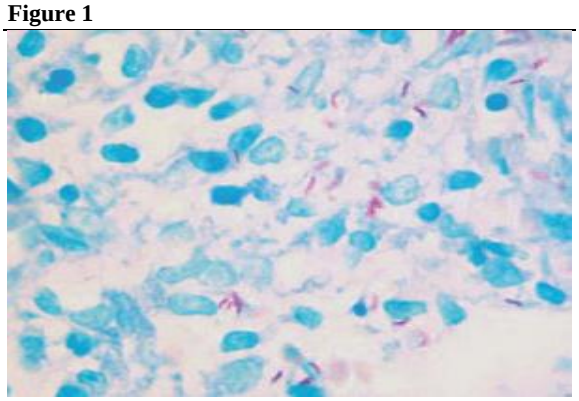


**Figure 2**

Acid-Fast Bacilli on Ziehl-Neelsen stained smear, by Light-Microscopy

**Figure 3**

Manually prepared Lowenstein-Jensen media in the Laboratory

**Figure 4**

Incubation of 2 L-J slants (one is wrapped with aluminum foil) at 300C in BOD incubator.

**Figure 5: (Growth of M. tuberculosis in L-J media)**

**Figure 6: (Growth of M.marinum in L-J media)**

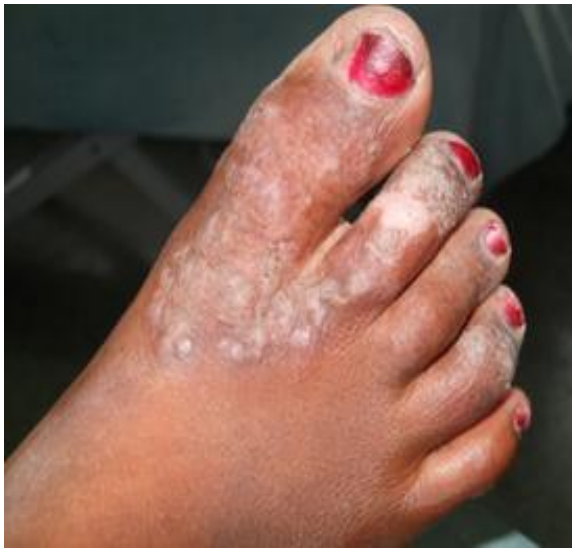


**Figure 7: Specimen of Tuberculosis Verrucosa Cutis**

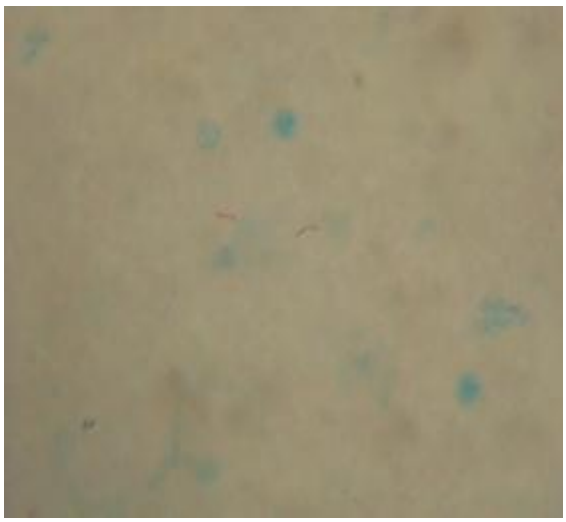


**Figure 8: Tissue Staining Shows AFB from the Specimen of TVC**

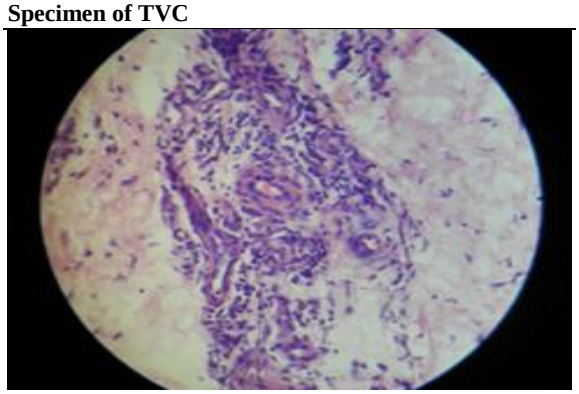


**Figure 9**

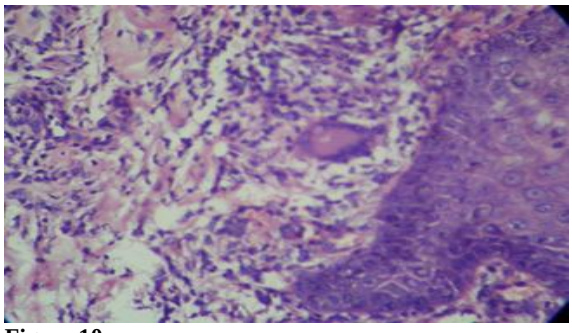


**Figure 10**

Histopathological slides (on Haematoxyllin-Eosin stain) show granuloma with multinucleated giant cells from the specimen of TVC.

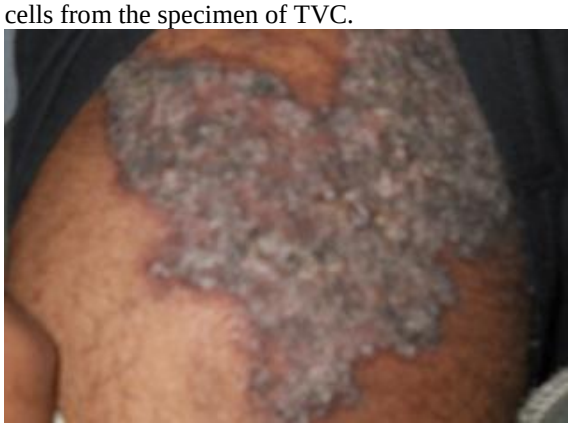


**Figure 11**

Specimen of Lupus Vulgaris

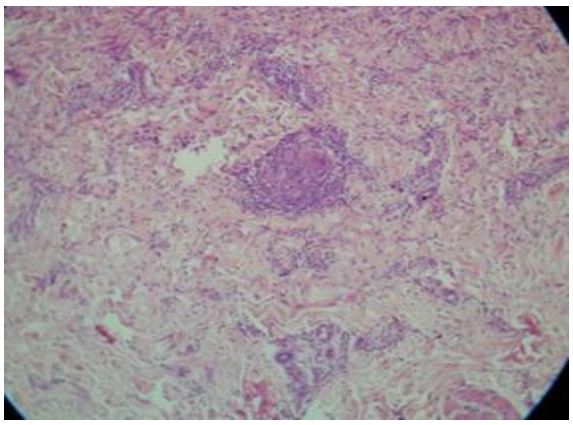


**Figure 12**

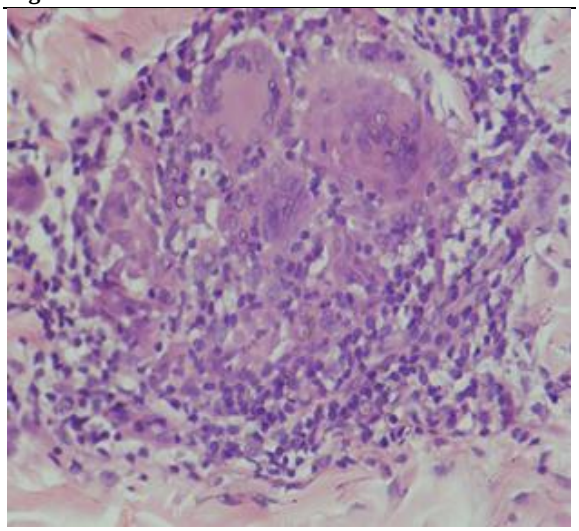
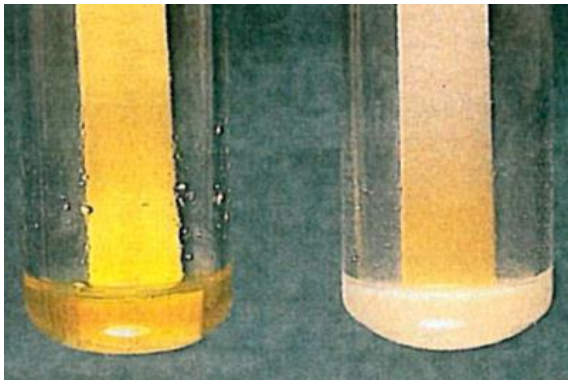


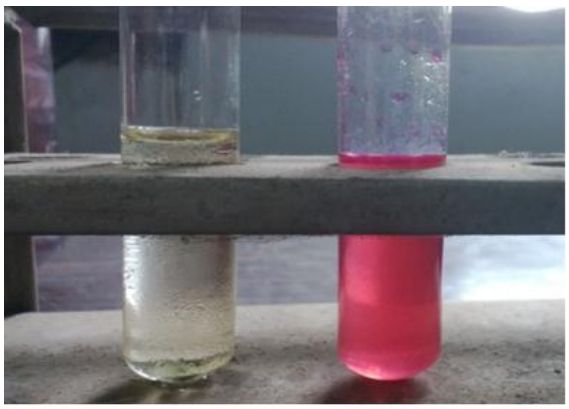
**Figure 13**

Histopathological slides (on Haematoxyllin-Eosin stain) show granuloma with multinucleated giant cells from the specimen of Lupus Vulgaris.



POSITIVE NEGATIVE

**Figure 14: NIACIN TEST**



NEGATIVE POSITIVE

**Figure 15: NITRATE TEST**

**Figure 16: ?**

**Figure 17: ?**

HIV-1&2 Rapid Kit Test shows HIV-1&2 positive in [Figure 16] &only HIV-1 positive but HIV-2 negative in [Figure 17].

**Figure 18: 5 L-J slants inculated with 'neat', '10-2', '10-4' dilution size inoculum.**

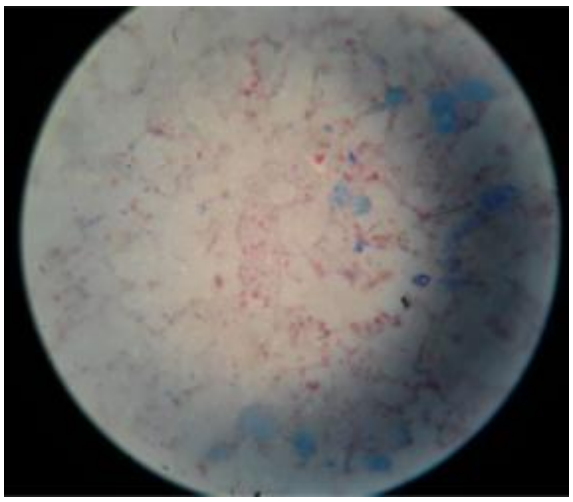


**Figure 19: Slit-Skin smear on modified Z-N stain Show AFB (Lepra-bacilli)**

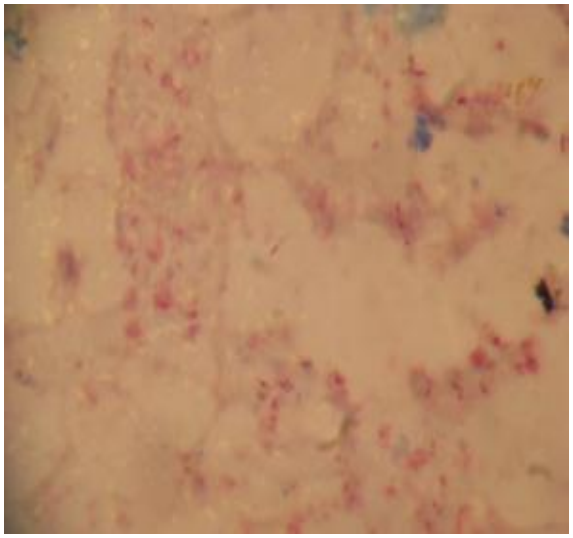


**Figure 20: Lepra bacilli on modified AFB-stain**

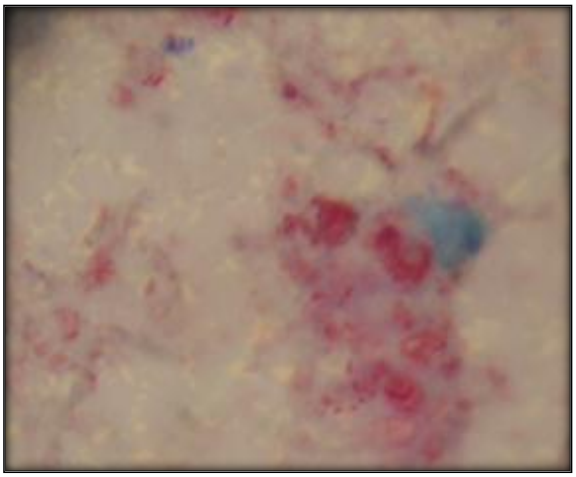


**Figure 21: Slit-skin smear shows Globi**

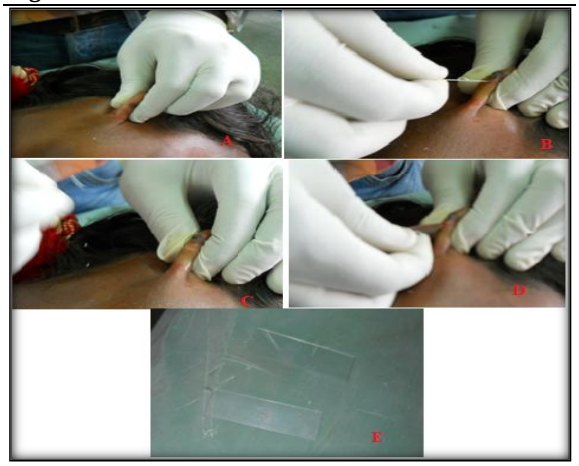


**Figure 22: (A, B, C, D, E) Process of making Slit-Skin smears**

**Table 1: Religion-wise and Residence-wise, and Socio-economic status distribution of Study Population**

| | | Cutaneous tuberculosis | Leprosy | NTM | Total |
|---|---|---|---|---|---|
| Religion | Hindu | 3(25%) | 4(23.5%) | 0 | 7 (22.6%) |
| | Muslim | 9(75%) | 13(76.5%) | 2 | 24 (77.4%) |
| | Total | 12 | 17 | 2 | 31(100.0%) |
| Area of residence | Rural | 10(83.3%) | 15(88.2%) | 1 | 22 (71%) |
| | Urban | 2(16.7%) | 2(11.8%) | 1 | 9 (29%) |
| | Total | 12 | 17 | 2 | 31(100.0%) |
| Socio-economic status | BPL | 8(66.7%) | 11(64.7%) | 0 | 19(61.3%) |
| | APL | 4(33.3%) | 6(35.3%) | 2 | 12(38.7%) |
| | Total | 12 | 17 | 2 | 31(100.0%) |

**Table 2: Distribution of study population according to the proportion of positivity of AFB Microscopy, Culture, Histopathology, and Mantoux-test.**

| Types of cutaneous mycobacterial infections | AFB-Microscopy Positive | Culture positive | Histopathology positive | Mantoux-test positive |
|---|---|---|---|---|
| Cutaneous tuberculosis (12) | 3(25%) | 3(25%) | 12(100%) | 12(100%) |
| Leprosy (17) | 17(100%) | Not done | 17(100%) | Not done |
| Cutaneous infections by NTM (2) | 0 | 2 | 2 | 2 |

**Table 3: Distribution of cutaneous tuberculosis according to morphological variants.**

| Morphological variants | Total no(12) | Percent (%) |
|---|---|---|
| Lupus Vulgaris | 6 | 50 |
| Tubercular verrucosa cutis | 2 | 16.7 |
| Scrofuloderma | 3 | 25 |
| Tuberculide | 1 | 8.3 |

**Table 4: Distribution of Cutaneous tuberculosis according to the sites of lesions**

| Sites of lesions | Lupus Vulgaris | Tuberculous verrucosa cutis | Scrofuloderma | Tuberculide | Total |
|---|---|---|---|---|---|
| Extremities | 4 | 2 | 1 | 0 | 7 (58.3%) |
| Others (Face, buttock) | 2 | 0 | 2 | 1 | 5 (41.7%) |
| Total | 6 | 2 | 3 | 1 | 12(100.0%) |

**Table 5: Co-relation of Cutaneous Tuberculosis & Pulmonary Tuberculosis, Diabetes Mellitus and Status of BCG vaccination, HIV and Use of corticosteroids in the study group.**

| | | Cutaneous Tuberculosis | | Total |
|---|---|---|---|---|
| | | Present | Absent | |
| Pulmonary Tuberculosis | Present | 3 | 10 | 13 |
| | Absent | 9 | 78 | 87 |
| | Total | 12 | 88 | 100 |
| Diabetes Mellitus | Present | 4 | 18 | 22 |
| | Absent | 8 | 70 | 78 |
| | Total | 12 | 88 | 100 |
| Status of BCG vaccination | Vaccinated | 5 | 44 | 49 |
| | Non-vaccinated | 7 | 44 | 51 |
| | Total | 12 | 88 | 100 |
| HIV | Present | 2 | 3 | 5 |
| | Absent | 10 | 85 | 95 |
| | Total | 12 | 88 | 100 |
| Use of corticosteroids | Present | 1 | 3 | 4 |
| | Absent | 11 | 85 | 96 |
| | Total | 12 | 88 | 100 |

**Table 6: Co-relation of Cutaneous infections caused by NTM & Use of coticosteriods in the study group.**

| Use of corticosteroids | NTM | | Total |
|---|---|---|---|
| | Present | Absent | |
| Present | 1 | 3 | 4 |
| Absent | 1 | 86 | 96 |
| Total | 2 | 88 | 100 |

**Table 7: Co-relation of Leprosy & BCG-vaccination in the study group**

| Status of BCG vaccination | Leprosy | | Total |
|---|---|---|---|
| | Present | Absent | |
| Vaccinated | 7 | 43 | 49 |
| Non-vaccinated | 10 | 40 | 51 |
| Total | 17 | 83 | 100 |

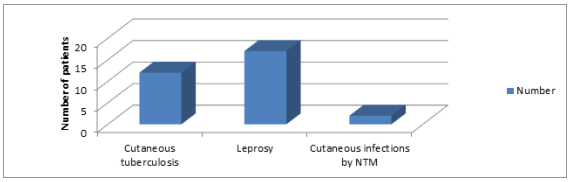



**Figure 1: Distribution of Study population according to the types of cutaneous mycobacterial infection.**

In the present study, a total of 31 patients with cutaneous mycobacterial infections were included. Among the different types of infections, leprosy was the most common condition, accounting for 17 cases (54.8%). Cutaneous tuberculosis was observed in 12 patients (38.7%), whereas cutaneous infections caused by non-tuberculous mycobacteria (NTM) were the least common, reported in only 2 patients (6.5%). Thus, leprosy constituted the major proportion of cutaneous mycobacterial infections in the study population.Among the 31 study participants, the majority belonged to the Muslim community (24 patients, 77.4%), while 7 patients (22.6%) were Hindu. Among patients with cutaneous tuberculosis, 9 cases (75%) were Muslims and 3 cases (25%) were Hindus. Similarly, among leprosy patients, 13 cases (76.5%) were Muslims and 4 cases (23.5%) were Hindus. Both NTM cases were observed among Muslim patients. Regarding the area of residence, most of the patients were from rural areas (22 patients, 71%), whereas 9 patients (29%) belonged to urban areas. Rural predominance was observed among cutaneous tuberculosis cases (83.3%) and leprosy cases (88.2%). Among NTM cases, one patient (50%) belonged to rural and one patient (50%) belonged to urban areas. According to socio-economic status, majority of patients belonged to the below poverty line (BPL) group (19 patients, 61.3%), while 12 patients (38.7%) were from the above poverty line (APL) group. BPL predominance was observed in cutaneous tuberculosis (66.7%) and leprosy (64.7%) cases, whereas both NTM patients belonged to the APL category.Among the 12 cases of cutaneous tuberculosis, AFB microscopy was positive in 3 cases (25%), and culture positivity was also observed in 3 cases (25%). Histopathological examination showed positivity in all cases (100%), and Mantoux test was positive in all patients (100%). All 17 cases of leprosy showed positive AFB microscopy (100%) and histopathological findings (100%). Culture examination was not

performed for leprosy cases. Among the 2 cases of NTM infection, AFB microscopy was negative in both cases, whereas culture and histopathology were positive in both patients. Mantoux test positivity was observed in both NTM cases. Among the 12 patients diagnosed with cutaneous tuberculosis, lupus vulgaris was the most common morphological variant, seen in 6 cases (50%). Scrofuloderma was the second most common presentation, observed in 3 patients (25%). Tubercular verrucosa cutis was reported in 2 patients (16.7%), while tuberculide was the least common variant, found in only 1 patient (8.3%).Among patients with cutaneous tuberculosis, lesions were most commonly located over the extremities, involving 7 patients (58.3%). Lupus vulgaris was the predominant lesion over extremities (4 cases), followed by tubercular verrucosa cutis (2 cases) and scrofuloderma (1 case). Other sites including face and buttock were involved in 5 patients (41.7%). Among these, lupus vulgaris was seen in 2 cases, scrofuloderma in 2 cases and tuberculide in 1 case. Out of 100 study participants, cutaneous tuberculosis was present in 12 patients. Pulmonary tuberculosis was present in 3 patients with cutaneous tuberculosis, whereas 9 patients had no pulmonary involvement. Diabetes mellitus was observed in 4 patients with cutaneous tuberculosis, while 8 patients were non-diabetic. Regarding BCG vaccination status, 5 patients with cutaneous tuberculosis had received BCG vaccination, whereas 7 patients were non-vaccinated. HIV infection was present in 2 patients with cutaneous tuberculosis, while 10 patients were HIV negative. History of corticosteroid use was present in 1 patient with cutaneous tuberculosis, whereas 11 patients had no history of corticosteroid exposure. These findings suggest that associated systemic diseases and immunosuppressive conditions may contribute to the development of cutaneous tuberculosis. Among the 100 study participants, NTM infection was present in 2 patients. Out of these, 1 patient had a history of corticosteroid use, while 1 patient did not have such history. Among patients without NTM infection, corticosteroid use was present in 3 patients, whereas 86 patients had no history of corticosteroid exposure. This indicates that corticosteroid use may be a possible predisposing factor for NTM infection. Among 17 patients diagnosed with leprosy, 7 patients (41.2%) had received BCG vaccination, whereas 10 patients (58.8%) were non-vaccinated. Among patients without leprosy, 43 were vaccinated and 40 were non-vaccinated. The higher proportion of leprosy cases among non-vaccinated individuals suggests that BCG vaccination may provide some protective effect against the development of leprosy.

## DISCUSSION

The study was conducted from March 2012 to February 2013.100 cases were selected from the patients attending the Dermatology ward at Burdwan Medical College and Hospital on the basis of inclusion and exclusion criteria. Samples processing weredone in the Departments of Microbiology & Pathology, Burdwan Medical College. Among them 31 were found to have cutaneous mycobacterial infection. Among 31 positive cases 17 cases (54.8%) had leprosy, 12 cases (38.7%) had cutaneous tuberculosis & 2 cases (6.5%) had cutaneous lesions caused by NTM (Non Tuberculous Mycobacteria). Among the study population, maximum positive cases of cutaneous mycobacterial infections were in the age group of 10-30 years (58.3%), followed by age group above 30 years (25%) and minimum in the age group below 10 years(16.7%). The 16–25 years age group was the most commonly affected, which was noticed by Binayak et al,[10] Satyanarayan,[11] and Wong,[12] So, our study result is matched with the above.

In our study, among the positive cases, the distribution of cutaneous mycobacterial infections was more in male patients (64.5%) in comparison to female (35.5%). Similar findings were reported by Binayak et al,[10] by Pandhi et al,[13] by Kumar et al.[14] Males & Females are equallyaffected reported by Zouhair et al.[12] So, our study is comparable with the Studies of Pandhi, Kumar and Binayak. The incidence of Cutaneous Mycobacterial infections was 3.12 per 1000 patients attending the hospital in this 1yr. The incidence of cutaneous tuberculosis was found to be 1.20 per 1000 patients attending the hospital in this 1yr in our study.Incidence of cutaneous tuberculosis is 0.12% reported from the study by Binayak et al,[10] and 0.1% by Kumar et al.[14] The overall prevalence of Cutaneous TB reported in other Indian studies, respectively 0.59% by Singh,[15] 0.50% by Banerjee,[16] 0.28% by Pandhi et al,[13] 0.24% by Satyanarayan,[11] and 0.26% by Patra et al.[17] So our findings can be corroborated with the studies of Binayaket.al 7 and 0.1% by Kumar et al.[14] The incidence of NTM was 0.20 per 1000 patients attending the hospital in 1 year in our study. Previous studies reported incidence of cutaneous infections by NTM in negligible amount, one study reported 14 per 100,000, other studies reported around 27 per 100,000. So, in our study, we see the incidence of Cutaneous TB & NTM –skin diseases is higher than the previous studies probably due to low BCG vaccination coverage, and high corticosteroid and immunosuppressive drug use in the study population. In our study,among the cutaneous tuberculosis cases, maximum number were of Lupus Vulgaris (50%) followed by Scrofuloderma (25%) followed by Tubercular verrucosa cutis (16.7%) lastly, by Tuberculide (8.3%). Maximum number of lesions of cutaneous tuberculosis was found in extremities (58.3%) in comparison to other sites (41.7%). Tuberculosis verrucosa cutis was the most common type & the most common site of involvement was the limb and the buttock in the

study by Wong et al.[18] Scrofuloderma was commonest type reported by Gopinathan R et al.[19] So, the results of our study in relation to morphological variants of cutaneous TB and its distribution sites are comparable to above mentioned studies. In our study, among the total cases of Cutaneous tuberculosis, Pulmonary tuberculosis was present in 25% cases, Diabetes mellitus present in 33.33% cases, 41.67% cases were BCG vaccinated, HIV was present in 16.67% cases, and 8.33% cases were found to be users of corticosteroids. Among the total no of NTM cases, all were found to be corticosteroid users & Land-workers. A positive co-relation was found between cutaneous tuberculosis and other associated risk-factors like PTB (Pulmonary tuberculosis), DM (Diabetes Mellitus), HIV, use of corticosteroids & negative co-relation found between Cutaneous TB and BCG-Vaccination. Wong and Banerjee,[18] also noticed an association with pulmonary tuberculosiswhile Restrepo 9 noted an association with DM. Lee et al,[20] noticed Tuberculous cellulite-like lesions in a patient who was diabetic and was taking an oral corticosteroid. Decker et al,[21] showed an association of HIV infection with tuberculosis. Zodpey et al,[22] demonstrated BCG vaccination to be moderately effective in adults against skin TB, with an efficacy of 60.9%. Kumar et al,[14] reported that BCG-vaccinated children did not develop disseminated disease. Previous studies showed the positive association of cutaneous NTM infections & immunosuppression, and history of exposure to fish-spine injury, gardening and foresting, aquagenic-trauma and management of aquariums in positive cases of M.marinum infection. So, our study results in relation to associative factors are comparable to previous studies. Among the total cases of Cutaneous TB, 99% cases were found to be histopathologically positive and Mantoux- test positive. 25% case were direct AFB-Microscopy positive & Culture Positive. Among the total cases of NTM, all cases were found to be histopathologicallypositive, Mantoux- test positive& culture positive but no cases were found to be directly AFB positiveon microscopy. Previous studies also reported that apart from PCR, Tissue-culture followed by histopathological study, was the best diagnostic tool for cutaneous TB & NTM-skin lesions, because histopathological reports depend upon duration of the lesion as well as immune status of the host. Our study results could not be corroborated with some of the previous studies becauseof the limitations in the laboratory facility. Our sample size was also very small and in a number of cases it did not yield results that were statistically significant.

## CONCLUSION

The incidence of cutaneous mycobacterial infection in Burdwan Medical College & Hospital, which drains patients from Burdwan and adjacent districts, is not to be neglected. It is important to draw the attention of Clinico-epidemiologists and Microbiologists to its proper prevention and control. Leprosy, cutaneous tuberculosis and NTM are the three types of cutaneous mycobacterial infections. 10-30 years was found to be the most vulnerable age group. Males were affected more than females. Muslims were more susceptible to cutaneous mycobacterial infections than other communities. Residents of rural areas and Persons of Low socio-economic status were comparatively more affected by cutaneous mycobacterial infections. However since the drainage area of the hospital is predominantly rural and the people who attend the OPD are mostly from the lower economic strata the data may be biased.

Lupus-vulgaris followed by Scrofuloderma were the common morphological variants of cutaneous tuberculosis.

Lepromatous was the most common & Tuberculoid the least common type of Leprosy.

Pulmonary tuberculosis, Diabetes Mellitus, HIV, long term use of corticosteroids were found to have significant contributing role in cutaneous tuberculosis. BCG vaccine had Preventive role against cutaneous tuberculosis and leprosy, especially against lepromatous type.

Skin infections due to NTM were common amongst the land-workers and users of corticosteroids.

Histopathological examination, followed by culture, followed by Direct Microscopy can thus, be said to be the best diagnostic tool for Cutaneous TB & NTM-skin lesions, in the absence of PCR.

Mycobacterium tuberculosis followed by Mycobacterium marinumis the commonest organisms of cultivable cutaneous mycobacterial infections.

Incidence of drug-resistance in case of M tuberculosis when they are isolated from dermatological infections is not much common.

More work on the subject involving a larger time span and advanced technology will be necessary for the assessment and control of cutaneous mycobacterial infections.

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