An observational study to assess the adherence to medical therapy, complications associated and outcomes in patient post-surgical debridement for atypical mycobacterial infection from January 2015 to March 2019

A dissertation submitted in partial fulfilment of MS General Surgery Branch I Examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be held on April 2020

CERTIFICATE

This is to certify that the dissertation titled- '**An observational study to assess the adherence to medical therapy, complications associated and outcomes in patient post-surgical debridement for atypical mycobacterial infection from January 2015 to March 2019'** is a bonafide original work done by Dr. Markose Mathew, Registration no. 221711456, during his academic term April 2017-Aril 2020 submitted in partial fulfilment towards M.S. Branch-I (General Surgery) Examination of the Tamil Nadu Dr. M.G.R. University, Chennai to be held in 2020. This work was done under my guidance in the department.

Guide

Head of the department

Principal

DECLARATION CERTIFICATE

This is to certify that the dissertation titled- '**An observational study to assess the adherence to medical therapy, complications associated and outcomes in patient post-surgical debridement for atypical mycobacterial infection from January 2015 to March 2019'** is a bonafide original work done during my academic term April 2017-Aril 2020, submitted in partial fulfilment towards M.S. Branch-I (General Surgery) Examination of the Tamil Nadu Dr. M.G.R. University, Chennai to be held in 2020.

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ANTI-PLAGIARISM CERTIFICATE

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8a6a47f0-acdf-4f85-860d-ae85b21d6d77
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This is to certify that the dissertation titled ‘An observational study to assess the adherence to medical therapy, complications associated and outcomes in patient post-surgical debridement for atypical mycobacterial infection from January 2015 to March 2019’ , submitted by Dr. Markose Mathew with registration number 221711456, has been personally verified by me through URKUND online anti-plagiarism tool. The uploaded document contains the introduction to conclusion pages and the analysis shows 8% plagiarism in this dissertation.

Guide

ACKNOWLEDGEMENTS

I am grateful to God for his abundant blessings and grace which has sustained me. I would like to express my gratitude to my guide Dr. Suchita Chase and co-guide Dr. Beulah Roopavathana for their unwavering support and guidance from the formulation of the research question to the conduct of this study and preparation of this dissertation. I would also like to thank Dr. Priscilla Rupali from the Department of Infectious diseases and Dr. Paul Trinity Stephen for all their guidance and support. Miss Hepsy and Mrs. Poornima from the department of Biostatistics were helpful in technical support and data analysis.

I would also like to acknowledge the support from the Departments of General Surgery and the ward staff of the General Surgery wards for the help in participation and conduct of this study.

I thank my parents and siblings who have stood by me and encouraged me throughout this endeavour.

Last but not the least; I would like to thank the participants for their patience and contribution.

INSTITUTIONAL REVIEW BOARD (IRB) APPROVAL

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November 16, 2017.

Dr. Markose Mathew,
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Sub: **Fluid Research Grant: New Proposal:**
An observational study to assess the adherence to medical therapy, complications associated and outcomes in patients post-surgical debridement for atypical mycobacterial infection from January 2015 to March 2019
Dr. Markose Mathew, PG Registrar/Department of General Surgery/Surgery Unit IV, Employment No.: 21432, General Surgery Unit IV, Dr. Suchita Chase, Associate Professor, Department of General Surgery Unit IV, General Surgery Unit IV, Dr. Priscilla Rupali, Employment No.14371, Infectious Diseases, Dr. Beulah Roopavathana, Assistant Professor, Department of General Surgery Unit IV Employment No. 51601, Dr. Paul Trinity Stephen, Senior Resident, Department Of General Surgery Unit IV Employment No. 29355, Hepsy YS, Senior Demonstrator, Department of Biostatistics, Employment No: 33231, Biostatistics.

Ref: IRB Min. No. 10951 dated 07.11.2017

Dear Dr. Markose Mathew,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "An observational study to assess the adherence to medical therapy, complications associated and outcomes in patients post-surgical debridement for atypical mycobacterial infection from January 2015 to March 2019" on November 07,2017. I am quoting below the minutes of the meeting.

The Committee raises the following queries:

1. Statistical methods – needs elaboration on what methods are going to be used in the study
2. Are you going to call patients who had surgery previously
3. Will you get clear outcome data on the retrospective numbers.
4. Primary outcome should be disease status at 3 months

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Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

5. Secondary outcome should be adherence to therapy
6. Calculate sample size based on healing rate
7. Information sheet very concise and should address the patient

Drs Markose Mathew and Suchita C were present during the presentation of the proposal and satisfactorily responded to the queries raised by the Members. After discussion, it was resolved to **ACCEPT the proposal after receiving the suggested modifications and answers to the queries.**

Note: 1. Kindly HIGHLIGHT the modifications in the revised proposal.
2. Keep a covering letter and point out the answer to the queries.
3. Reply to the queries should be submitted within 3 months duration the time of the thesis/ protocol presentation, if not the thesis/protocol has to be resubmitted to the IRB.
4. The checklist has to be sent along with the answers to queries.

Email the details to research@cmcvellore.ac.in and send a hard copy through internal dispatch to Dr. Biju George, Addl. Vice-Principal (Research), Principal's Office, CMC.

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board.

Dr. BIJU GEORGE
MBBS, MD., DM
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr. Suchita Chase, Department of Surgery - 4, CMC, Vellore.

IRB Min. No. 10951 dated 07.11.2017

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Ref: IRB Min. No. 10951 [OBSERVE] dated 07.11.2017

Dear Dr. Markose Mathew,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS, MD, DM,
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board
Christian Medical College, Vellore - 632 002

Cc: Dr. Suchita Chase, Dept. of Surgery - 4, CMC, Vellore

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Ref: IRB Min. No. 10951 [OBSERVE] dated 07.11.2017

Dear Dr. Markose Mathew,

The Institutional Review Board (**Blue**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "An observational study to assess the adherence to medical therapy, complications associated and outcomes in patients post-surgical debridement for atypical mycobacterial infection from January 2015 to March 2019" on November 07th 2017.

The Committee reviewed the following documents:

1. IRB application format
2. Patient information sheet and Consent form (English,Tamil, Hindi)
3. Proforma
4. Cvs of Drs. Markose Mathew, Suchita Chase, Priscilla Rupali, Beulah Roopavathana , Paul Trinity Stephen
5. No. of documents 1- 4

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Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on November 07th 2017 in the BRTC Conference Hall, Biostatistics Building, Christian Medical College, Vellore 632 004.

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC, Vellore	Internal, Clinician
Dr. B. J. Prashantham	MA(Counseling Psychology), MA (Theology), Dr. Min (Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. RatnaPrabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist
Rev. Joseph Devaraj	BSc, BD	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Dr. Sowmya Sathyendra	MBBS, MD (Gen. Medicine)	Professor, Medicine III, CMC, Vellore	Internal, Clinician
Dr. Visalakshi. J	MPH, PhD	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Sathish Kumar	MBBS, MD, DCH	Professor, Child Health, CMC, Vellore	Internal, Clinician
Dr. Jayaprakash Muliyil	BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, CMC, Vellore	External, Scientist &Epidemiologist
Dr. Asha Solomon	MSc Nursing	Associate Professor, Medical SurgicalNursing, CMC, Vellore	Internal, Nurse
Dr. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician

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Dr. Biju George, M.B.B.S., MD., DM.,
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Mrs. Pattabiraman	BSc. DSSA	Social Worker, Vellore	External, Lay Person
Dr. John Antony Jude Prakash	MBBS. MD	Professor, Clinical Microbiology, CMC, Vellore.	Internal, Clinician.
Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC, Vellore	Internal, Clinician
Dr. Mathew Joseph	MBBS. MCH	Professor, Neurosurgery, CMC, Vellore	Internal, Clinician
Dr. RekhaPai	BSc, MSc, PhD	Associate Professor, Pathology, CMC, Vellore	Internal, Basic Medical Scientist
Ms. Grace Rebekah	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mrs. Sheela Durai	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of Withdrawals for the study entitled: "An observational study to assess the adherence to medical therapy, complications associated and outcomes in patients post-surgical debridement for atypical mycobacterial infection from January 2015 to March 2019" on a monthly basis. Please sendcopies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:
A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an 1st Installment. The rest of the 50,000/- INR (Rupees Fifty thousand only) each will be released at the end of the first year as 2 nd Installment.

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
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LIST OF ABBREVIATIONS USED

NTM= Non tuberculous mycobacteria

M. sp= Mycobacterium species

MOTT= Mycobacteria other than tubercle

MAC= Mycobacterium avium complex

AIDS= Acquired immune deficiency syndrome

USA= United States of America

DNA= Deoxy ribonucleic acid

RNA= Ribonucleic acid

rRNA= ribosomal RNA

16S= 16S subunit

CD4= Cluster of differentiation 4

IU= International units

HIV= Human immunodeficiency virus

IFN= Interferon

IL= Interleukin

TNF= Tumour necrosis factor

CF= Cystic fibrosis

COPD= Chronic obstructive pulmonary disease

CO2= Carbon dioxide

SSTI= Skin and soft tissue infection

PCR=Polymerase chain reaction

MMAS= Morisky Medication Adherence Scale

TABLE OF CONTENTS

ABSTRACT

Background: Over the past several years the prevalence of nontuberculous mycobacterial infection of the skin has increased, which may be attributed to chronic diseases, poor immune status and also the increased awareness of the disease and its pathogenesis. Of the many species, the most common agent causing skin and soft tissue infections belong to the rapidly growing mycobacteria group. These organisms are detected by clinical presentation, site of infection, immune status, pathological biopsy and microbiological cultures. Limitations in diagnostic techniques, fastidious microorganisms and prolonged incubation periods have made the diagnosis and treatment challenging.

Aim: To study the wound status, adherence to medical therapy, adverse effects and outcomes associated with treatment in a patient post-surgical debridement for atypical mycobacterial infection of the skin

Materials and Methods: This was a bidirectional study and participants were patients who underwent surgical debridement for atypical mycobacterial skin infection. They were grouped as retrospective group- January 2015 to November 2017 and prospective group- December 2017 to March 2019. After initial biochemical and audiological assessment, they were started on medical therapy by the department of infectious diseases and followed up in the inpatient and outpatient department. The details of all the patients were analyzed and studied.

Result: A total of 96 patients were recruited (retrospective group-70 and prospective group-26). Most of the patients were young aged females (n=68) and 41 of these patients had undergone prior gynecological procedure. 64 patients had complete wound healing with no recurrence. Of the 32 patients with non-healed wound, 17 patients had recurrence of infection. Adherence to therapy was studied in prospective group and was medium-low in this group (n=15). 59 patients had multiple medical and surgical complications noted in the recruited patients.

Conclusions: NTM is not an uncommon infection and is more likely to occur after a surgical procedure, most commonly after laparoscopic surgery. Wound healing was noted after adequate surgical debridement and recurrence was low. However the risk of recurrence was more in patients whose wound did not adequately heal. The adherence to medications was mostly medium-low. There was no statistical significant association between the adherence to medical therapy and complications in the prospective group. There was statistically significant association between risk of recurrence of wound and non-healed wound.

INTRODUCTION

The atypical or non-tuberculous mycobacteria (NTM) include those organism species that are do not belong to the Mycobacterium tuberculosis complex. These bacteria garnered interest due to varied types of clinical presentation reported, facultative nature of pathogen and especially because of their association with surgical procedures and their tendency to disseminate in immune-compromised hosts.

NTM are widely distributed in the environment and they have high isolation rate worldwide. NTM diseases are seen world-wide and its incidence rates vary from 1.0 to 1.8 cases per 100,000 persons in industrialised nations. 90% of the cases involve the pulmonary system and the rest involve skin, soft tissue, lymph nodes and osteoarticular region. The most common organisms isolated are M. ulcerans, M. fortuitum, M. marinum, M. hemophilum, M.abscessus and M. chelonae. However prevalence of NTM is unknown in India as there is lack of awareness among clinicians, absence of systematic epidemiology coupled with lack of laboratory capacity to diagnose these infections. Among few reports available, NTM isolation rates are reported to range from 0.5% to 8.6% in India. Also data on adherence to medications and the outcomes associated have not been studied among the population.

Atypical mycobacterial infection of the skin usually occur after penetrating traumatic injury, surgery, intramuscular injections, cosmetic procedures,

environmental exposure of vulnerable wound to soil or contaminated water and medical devices occasionally contaminated with mycobacteria. Diagnosis is made by clinical presentation, culture and histopathological examination. The therapy recommended is a combination of surgical and medical management. Nonspecific, delayed clinical manifestations and inadequate laboratory services delay or even miss out on the diagnosis. This is complicated by prolonged antibiotic therapy on a culture negative wound, which may lead to various adverse effects affecting the patient and outcome of therapy which require to be identified and studied. Furthermore these factors may play a negative role in adherence to therapy in affected patients. Therefore it is imperative for an early diagnosis, combined with an early surgical approach, adequate antimicrobial agent and appropriate mode of delivery.

This study aims to explore the various outcomes in patients after surgical debridement of atypical mycobacterial infection of the skin and soft tissues who are started on medical therapy. Moreover this study will also help understand about the impact of various complications of the therapy on an individual's adherence to medications and it resultant effect on wound healing at the time of follow up. This may help in modifying the therapy for achieving better outcomes in these patients.

OBJECTIVES OF THE STUDY

The primary objective

To assess the wound healing at the first follow up visit to the department of general surgery.

The secondary objective

1. To assess the adherence to medical therapy and the adverse effects associated with medical therapy for patients treated with atypical mycobacterial infection.
2. To see the demographic profile of patients
3. To study the predisposing risk factors of infection in these patients.

REVIEW OF LITERATURE

HISTORICAL PERSPECTIVE AND ASSOCIATION WITH MYCOBACTERIA:

Robert Kochs discovery of mycobacterium tuberculosis was a milestone in microbiology. "Tubercle bacillus" translates to "Tuberkelbazillus" which included the causative agents of tuberculosis of mammals. New mycobacteria were being described within a few years. Initially the organisms were described as animal pathogens or environmental organisms with occasional diseases caused in humans. It soon became apparent that all bacilli were not all identical and that some similar infections were caused by mycobacterial species that existed as saprophytes in the surrounding environment. Later M bovis, M africanum and M microti were added into this genus.(1)

The atypical or nontuberculous mycobacteria (NTM) include those organism species that are do not belong to the Mycobacterium tuberculosis complex; hence they are also known as “nontuberculous mycobacteria” and “mycobacteria other than tuberculosis”. Infection by these organism became more evident once the prevalence of tuberculosis decreased. These organisms are also called “tuberculoid bacilli”, “MOTT” bacilli (mycobacteria other than tubercle) and “nyrocine mycobacteria” (2) and several other names.

NTM are emerging opportunistic pathogens that colonize household water systems, cause chronic lung infections and multiple other invasive infections in susceptible patients. Interest in the mycobacteria grew, due to the rising number of new species associated with the genus and the varied clinical presentation of the organism. Today

170 different species causing varying disease have been identified. Some of them have been implicated worldwide (M. avium complex, M. abscessus), while others (M. malmoense) are regionally important. NTM are geographically diverse and the spectrum of diseases caused include tuberculosis like pulmonary and extra-pulmonary disease, cervical lymphadenitis, visceral and disseminated disease. The clinical manifestations of this disease is varied and the number of organisms associated with this genus is increasing. Furthermore the associated complications and difficulty in treatment has spurred clinical interest. The disease manifestation being varied and atypical has resulted in diverse therapy for management.(3)

NON TUBERCULOUS BACTERIA BEFORE THE AIDS EPIDEMIC:

Prior to the advent of the AIDS epidemic, diseases caused by nontuberculous mycobacteria were pulmonary, cervical lymphadenitis, limited to skin, or they rarely presented as disseminated disease. Males in the sixth decade of life were frequently affected by pulmonary disease. Most of these patients had predisposing lung conditions (pneumoconiosis) or had occupational risk factors (farming or coal mining). It was also thought that M. tuberculosis infection conferred some degree of immunity to environmental non tuberculous mycobacteria. The major non tuberculous mycobacterial pathogens identified were M kansasii, M avium, and M intracellulare.(2)

NON TUBERCULOUS BACTERIA AFTER THE AIDS EPIDEMIC AND ITS RELEVANCE IN THE PRESENT SCENARIO:

The AIDS epidemic throughout the world has changed the picture of non-tuberculous mycobacterial infection. Non tuberculous mycobacteria infection was found in around 25 to 50% of those affected by AIDS in USA and Europe since 1982. Increase in prevalence of HIV has contributed to increase in NTM infections. However the increasing awareness of these organisms as human pathogens, improved and reliable methods of detection and culture, and increasing contact to environment rich in NTM have also contributed to the increased disease burden. The impact of NTM on AIDS and other immunodeficient patients has stimulated the initiation of studies of the epidemiology, ecology, genetics, molecular biology, and physiology of the organism.(2,4)

Evidence also shows that the prevalence of NTM is increasing over the past few years, though not exclusively in non HIV patients, with underlying lung disease. Various guidelines have been issued by various scientific bodies on the diagnosis and management of NTM infections in response to the increased identification of this organism. There is still difficulty in distinguishing patients with clinical disease related to NTM from those in whom the isolation of single clinical specimens raises a clinical suspicion of disease. Moreover various treatment regimens have been proposed by different scientific bodies. The success rates with these regimens are often poor, with failure to eradicate the organisms or recurrence of disease after cessation of therapy. NTM and the diseases they cause- Pulmonary and extra-pulmonary, remain a challenge for microbiologists and physicians. (5)

MICROBIOLOGY OF ATYPICAL MYCOBACTERIA

On the basis of microbiologic, clinical, and epidemiologic characteristics four groups of human pathogens can be delineated within the genus Mycobacterium. The Runyon Classification System recognizes these groups on the basis of growth rates, colony morphology and pigmentation.

Groups I, II and III are classified as slow growers, requiring a similar time to that required by M. tuberculosis to grow, while rapid growers like Group IV organisms grow well in routine bacteriologic media in less than seven days. The ability to produce yellow pigment further differentiates the slow growers.(5,6)

M. tuberculosis complex
- M. tuberculosis
- M. bovis
- M. africanum

M. leprae

Nontuberculous mycobacteria
- Slowly growing mycobacteria
  - M. kansasii (Photochromogens, Runyon group I)
  - M. marinum
  - M. gordonae (Scotochromogens, Runyon group II)
  - M. scrofulaceum
  - M. avium complex (Nonchromogens, Runyon group III)
    - M. avium
    - M. intracellulare
  - M. terrae complex
    - M. ulcerans
    - M. xenopi
- Rapidly growing mycobacteria (Runyon group IV)
  - M. fortuitum
  - M. chelonae
  - M. abscessus

This classification system is a tool for microbiologists, and allowed easier identification of individual NTM species by mycobacterial laboratories. Due to advances in mycobacteriology, including more rapid culturing techniques, DNA probes, and high-pressure liquid chromatography the Runyon Classification System has become less relevant in the recent years. In addition, this system may not contribute to the clinical diagnosis since organisms of the same class behaves in different manner.

More recently classification is based on clinical disease produced by the organism:

1. Pulmonary disease
2. Lymphadenitis
3. Cutaneous disease
4. Disseminated disease(5)

Currently, more than 170 NTM species have been catalogued.(3) However "variants" or "subspecies" of NTM with distinguishing cultural characteristics can be shown by simple tests. The distribution of these variants is related to ethnic groups and associated geographical locations, and they are of epidemiological importance which can have a bearing on diagnosis and treatment of the patient.(1)
The most common atypical mycobacteria isolated causing human disease has been listed below.(3,4,6)

Slowly growing mycobacteria

Mycobacterium avium complex
Mycobacterium avium
Mycobacterium intracellulare
Mycobacterium kansasii
Mycobacterium ulcerans
Mycobacterium malmoense
Mycobacterium lentiflavum
Mycobacterium scrofulaceum
Mycobacterium haemophilum
Mycobacterium szulgai
Mycobacterium genavense
Mycobacterium celatum

Rapidly growing mycobacteria

Mycobacterium abscessus
Mycobacterium chelonae
Mycobacterium fortuitum

The mycobacterial 16S rRNA gene is highly conserved, and that differences in the sequence of 1% or greater generally defined a new species. Recognition of a new NTM species is now relatively simple: to perform a 16S rRNA gene sequence analysis a new species and compare the within the databases. Numerous new species have been defined based on this method and it is likely that the number will continue to expand. (3,6)

NEWLY DISCOVERED OR EMERGING MYCOBACTERIA:

M . bohemicum	M. branderi	M. celatum
M. conspicuum	M. genavense	M. heckeshornense
M. heidelbergense	M. interjectum	M. intermedium
M. kubiacae	M. lentiflavum	M. triplex
M. tusciae	M. agri	M. alvei
M. bonickei	M. brumae	M. chitae
M. confluentis	M. hassiacum	M. immunogenum
M. magaritense	M. mucogenicum	M. novocastrense
M. porcinum	M. senegalense	M. septicum
M. goodie	M. peregrinum	M. wolinskyi

PATHOGENESIS OF ATYPICAL MYCOBCTERIAL INFECTION:

Three crucial observations have been made regarding the pathogenesis of atypical mycobacterial infection:

1. In many HIV infected patients, a CD4 T-lymphocyte count below 50/IU predisposed the individual to develop disseminated NTM infection. This suggested that T-cell products or activities is required for resistance to mycobacteria.

2. In HIV uninfected patient group, disseminated NTM infection were due to mutations in specific genetic sequences of interferon (IFN)-gamma and interleukin (IL)-12 synthesis and response pathways (IFN-gamma receptor 1 [IFN gamma R1], IFN-gamma receptor 2 [IFN gamma R2], IL-12 receptor -1 subunit [IL12R beta 1], the IL-12 subunit p40 [IL12p40], the signal transducer and activator of transcription 1 [STAT1], and the nuclear factor-K beta essential modulator [NEMO]).

3. It is also noted that there is an association between bronchiectasis, nodular pulmonary NTM infections and a particular body habitus, predominantly in postmenopausal women (e.g., pectus excavatum and scoliosis)

Host defence and immune system:

Macrophages phagocytose mycobacteria initially, which respond by production of IL-12, which in turn up regulates IFN gamma. Activated neutrophil and macrophages from IFN gamma kill pathogens, including mycobacteria. There exists a positive feedback loop between IFN gamma and IL-12, which is critical for the control of

mycobacteria, as well as many other intracellular infections. Disseminated NTM disease occurs as a definite manifestation of immunologic defect, either acquired, such as HIV and iatrogenic factors (steroids usage and immunosuppressive drug) or genetic-caused by defects in the above IFN gamma and IL 12 pathway genes.

TNF inhibition:

IFN gamma and IL 12 control mycobacteria phagocytosis largely through the up regulation of tumour necrosis factor (TNF)-alfa, predominantly made by monocytes and macrophages. TNF alfa blocking agents is associated with increased risk of disseminated disease, thereby establishing the critical role of TNF alfa in controlling intracellular infections.

Body Morphology:

Body morphology and association with NTM are most commonly associated with female sex. Similar clinical characteristics and body type like scoliosis, pectus excavatum, mitral valve prolapse, and joint hypermobility have been associated with patients with nodular NTM pulmonary infections with bronchiectasis. These phenotypic characteristics are important as they may represent markers for specific genotypes that affect body morphotype and NTM infection susceptibility. Alternatively, the morphotype as described itself may influence mycobacterial infection susceptibility, due to clinical features as poor tracheobronchial secretion drainage or ineffective mucociliary clearance.(3,7)

VIRULENCE ASSOCIATED WITH ATYPICAL MYCOBACTERIA

Biofilms are multicellular structure consisting and developed by free living bacteria. Biofilms help bacteria tolerate multiple stressors, including the host immune system and antibiotics. Nontuberculous mycobacteria (NTM) are emerging opportunistic pathogens that develop and utilize biofilms to adhere to environmental surroundings and avoid phagocytosis and destruction by host immune cells.

This adhesive biofilm matrix serves as a sturdy physical impediment against external stressors such as predation and desiccation, can interact with and segregate antimicrobial agents, and can short circuit phagocyte signalling. The three dimensional (3D) structure of biofilms creates a chemical gradient across a cellular population, resulting in a spectrum of physiologies and metabolisms. Along with genetic diversification and equivocal differences in gene expression they give rise to substantial cell to cell heterogeneity. A variety of models and experimental systems have demonstrated increased fitness of heterogeneous bacterial colonies as compared to homogenous bacterial colonies. Most antibiotics target rapidly dividing bacteria, so antibiotic tolerance is mostly conferred by slow growing and dormant cells in the bacteria.

Mycobacteria have evolved to enter and exit from the biofilm state in response to species and strain specific environmental signals. Cell-cell adhesion is a pivotal step in biofilm development in all bacteria, including nontuberculous mycobacteria.

Peculiarly, mycobacteria spontaneously aggregate under nearly all laboratory culture conditions, forming hydrophobic clumps in shaking cultures. This cumulative aggregation suggests either that non tuberculous mycobacteria express adhesive structures in response to very common signals in laboratory cultures or that they have adapted to always grow as aggregates in aqueous environments. The latter possibility is the dominant archetype, accentuated by the addition of detergents such as Tween 80 to mycobacterial cultures to prevent clumping. Therefore NTM are pathogens that utilize multiple forms of aggregation for survival and persistence, both in the environment and host organism.

From the baseline aggregated state, high iron in the environment can trigger the maturation of NTM biofilms. This biofilm formation is important for the NTM to survive standard water decontamination protocols and to persist in household water systems. NTM can infect healthy adults at risk, after repeated exposure and are especially dangerous to immunocompromised populations. Also patients with lung disorders such as cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) are at high risk of developing this infection.

Infections with NTM can be very difficult to treat. Mycobacterium abscessus in particular, require long courses of antibiotic cocktails that have limited efficacy and extensive adverse side effects. One of the reason could be that M. abscessus acquires mutations that reduce expression of surface glycopeptidolipids. This results in

development of rough colony morphology and forms cords, rope like structures in which the axis of each bacterial cell is parallel to the axis of the cord, in liquid culture. There is a role for Carbon and Nitrogen balance in dictating the transition between planktonic and aggregated states in NTM. Aggregation phenotype is mediated by surface adhesion. Mycobacteria produce a mycomembrane: a cell wall composed of peptidoglycan, lipoglycans, and proteins. The mycobacterial cell wall is unusually lipid rich which fits the longstanding observation that mycobacteria clump together into hydrophobic aggregates. Clumping is now recognized as a ubiquitous feature of mycobacteria infection.

Cell to cell adhesion is a requirement for biofilm formation and cording, therefore a more thorough understanding of the regulatory pathways responsible for mycobacterial aggregation holds promise for combating NTM infections in the future. The role of biofilm formation that renders the mycobacteria fractious to antibiotics and immune killing is a catalyst to develop novel anti biofilm strategies. There is a rising threat of NTM infections in the community. Hence a better understanding between increased aggregation and virulence lends motivation to probe the mechanisms of aggregation and dispersal in these enigmatic pathogens.(2,4,8)

GENETICS OF ATYPICAL MYCOBACTERIA:

Some progress has been made in describing the genetic basis of variation in the nontuberculous mycobacteria. Most of the information has been focused on members of the MAC and rapidly growing mycobacteria. The mycobacteria have a small number of rRNA genes, unlike many other species of bacteria which have multiple rRNA genes like E. coli *and* Streptomyces. Slowly growing species(according to the original Runyons classification) like M. tuberculosis, M. leprae, M. avium, M. paratuberculosis, M. intracellulare, M. simiae, and M. marinum only had a single copy of 16S, 23S, and 5S rRNA genes. In contrast to this, most rapidly growing species have two copies. There is a direct relationship between the number of ribosomes in cells and the rate of protein synthesis, therefore it is possible that the small number of rRNA genes limits the rate of protein synthesis and hence the rate of growth in the slowly growing mycobacteria. Moreover plasmids (extra chromosomal genetic elements) have also been discovered in members of the M. avium and M. fortuitum complexes. They encode of antibiotic resistance and virulence. Also transposable genetic elements have been found in these mycobacteria which confers resistance to sulphonamide and tetracycline group of drugs.

A number of newly developed methods greatly assist in identification of virulence genes of nontuberculous mycobacteria. They include methods for mRNA isolation and labelling with 5-fluorouracil.(2,8,9)

ECOLOGY AND PHYSIOLOGY OF ATYPICAL MYCOBACTERIA

The ecology and physiology of the nontuberculous mycobacteria is significantly different from those of M. tuberculosis. Many of the nontuberculous mycobacteria are free living saprophytes/commensals that have been detected in and isolated from a wide variety of environments including water, soil, dust, and aerosols. The exceptions include Mycobacterium ulcerans, Mycobacterium haemophilum and Mycobacterium genavense which have been isolated from humans. Nontuberculous mycobacteria have also been recovered from drinking-water distribution systems throughout the world. Thus the history of exposure involves interactions with natural and constructed sources, rather than infected patients which was common with the case for M. tuberculosis infection. Nontuberculous mycobacteria found in the environment or drinking water distribution systems are not noted as contaminants from another source, rather they are residents able to grow, persist, and survive in these environment. M. avium complex and M. scrofulaceum and M. fortuitum and M. chelonae grow in water. The mechanism of biofilm formation and quality of resistance to disinfection it imparts undoubtedly contributes to the ability of a number of organisms like Mycobacterium xenopi, M. avium, M. fortuitum, and M. chelonae to persist and thrive in drinking water systems.

Widespread presence of nontuberculous mycobacteria in natural and constructed environments warrants the study of their physiology. Nontuberculous mycobacteria grow over wide range of temperature, pH, salinity, and oxygen tension. Thus, nontuberculous mycobacteria can be isolated from multiple and diverse environmental samples. A well-known example of the relationship between mycobacterial

physiology and ecology is provided by the M. avium complex. They grow best between pH 5 and 5.5. Furthermore, M. avium complex strains grow equally well in waters with and without salt (i.e., up to 2%) and therefore they can be found commonly near brackish waters and swamps.

The widespread presence of nontuberculous mycobacteria in the environment could also be due to another characteristic. NTM are relatively resistant to heavy metals and oxyanions. In fact, some isolates of M. avium, M. intracellulare, or M. scrofulaceum are abnormally resistant to cadmium, mercury, silver, and tellurite. Therefore this demonstrates that mycobacterial physiology is a determinant of their ecology and geographic distribution. The persistence of M. avium complex organisms in hospital water systems and drinking water distribution systems is possibly because many systems use galvanized (zinc coated) pipes. NTM have also been found associated with sphagnum vegetation in large numbers. Also the high levels of humic and fulvic acids found in coastal swamps correlated with distribution of M. avium complex organism.

Physiological traits like hydrophobicity directly influence mode of transmission (e.g., aerosolization) and hence exposure of susceptible individuals. Hydrophobic mycobacteria are more readily aerosolized by natural processes. The distribution of nontuberculous mycobacteria in waters is influenced by hydrophobicity nature. Hydrophobic mycobacteria usually collect at air water interfaces and are found in large numbers at water surfaces. Hydrophobicity also partially responsible for the formation of biofilms.(2,3,7)

EPIDEMIOLOGY OF ATYPICAL MYCOBACTERIAL INFECTIONS

NTM are widely distributed in the environment and they have high isolation rate worldwide. Soil and water are the most common environment where these organisms are isolated, including natural and treated water sources. Organisms like M. kansasii, M. xenopi, and M. simiae are recovered exclusively from the municipal water sources and rarely from other environmental sources. NTM are geographically heterogeneous and cause a wide variety of diseases. Pulmonary NTM infections are commonly due to MAC and M. kansasii which cause a substantial burden of illness worldwide. (7) Isolation rates of NTM from environment is remarkably similar in diverse geographical areas. There is no evidence of animal to human or human to human transmission of NTM. Even in patients with cystic fibrosis, an apparently highly susceptible population, there has been no clear documentation of human to human transmission of NTM. It is suspected that human disease is acquired from environmental exposure, although the specific source of infection may not be clearly identified. (2)

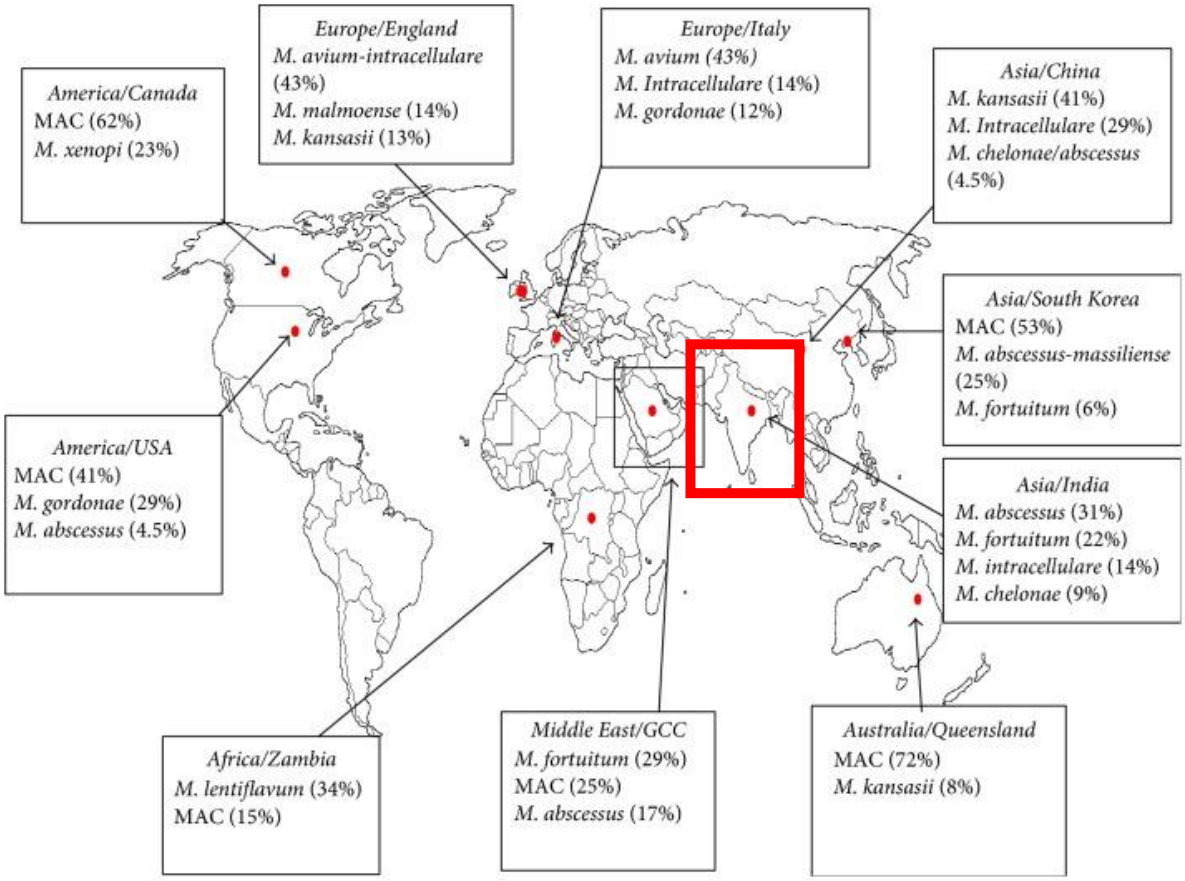
NTM diseases is seen world-wide and its incidence rates vary from 1.0 to 1.8 cases per 100,000 persons. These overall incidence rates are estimates based on numbers of NTM isolates reported.

MAC is the most common NTM species causing disease in most series, however many species have been implicated. These rates appear to be similar in most of the

developed countries, but there is limited surveillance information. NTM diseases is usually not reported since it is not communicable. Several reports have suggested that incidence of NTM diseases has increased over the past several decades, this observation has not been conclusively established due to the lack of comprehensive surveillance efforts. (3)

Pulmonary NTM disease in the United States is most commonly due to the M. avium complex (MAC), with M. kansasii in second place. In the United Kingdom, *M. kansasii* is the most common pathogen in NTM lung disease in England and Wales, while *M. malmoense* is the most common in Scotland. In south-east England, *M. xenopi* predominates. In Japan, the most common cause of NTM pulmonary disease is MAC, followed by *M. kansasii.* The pathogen most frequently responsible for NTM pulmonary disease in Korea is MAC.(5) Majority of atypical mycobacterial isolates in Scottish population was also MAC. (10) However the most common organism isolated from Hong Kong was Mycobacterium marinum. Other well-reported pathogens include MAI, *Mycobacterium ulcerans*, *M chelonae*, and *Mycobacterium fortuitum*. The route of infection is mainly via inoculation or trauma. *Mycobacterium chelonae* and *M fortuitum* are associated with injection and occur more often in immunocompromised individuals. These were similar findings also reported from Botswana(11,12) M. ulcerans causing buruli ulcer is mostly found in West and Sub-Saharan Africa where it affects children and adults in subsistence agricultural communities. However in countries with high burden of tuberculosis, including India, NTM diseases often go under recognized and misdiagnosed. Prevalence of NTM is

unknown in India as there is lack of awareness among clinicians coupled with lack of laboratory capacity to diagnose these infections. Among few reports available, NTM isolation rates are reported to range from 0.5% to 8.6% in India.



CLINICAL FEATURES OF ATYPICAL MYCOBACTERIAL INFECTION OF SKIN AND SOFT TISSUES

Atypical mycobacterial infection affecting skin are diverse in clinical presentation and geographical prevalence. These opportunistic infections are increasingly important, especially because of their association with surgical procedures and their tendency to disseminate in immunocompromised hosts. (13) Diagnosis is often delayed, as mycobacterial cultures are not routinely performed on skin biopsy specimens or surgical wound infections. Therefore a high index of suspicion is imperative for the diagnosis to be made.

The most common organisms causing skin and soft tissue infection are M. marinum, M. abscessus, M. chelonae, M. fortuitum, M. ulcerans and M. haemophilum. (3) These organisms enter skin after trauma, such as skin prick, motor vehicle accidents or surgery. Risk factors for NTM infection include immunocompromised status due to HIV infection, organ transplants and TNF-alfa inhibitors(14). Cutaneous NTM infections have also been reported in immunocompetent individuals after a variety of surgical, cosmetic or other procedures including punch biopsy(15), Mohs surgery(15), breast reconstruction(16), liposuction(17), filler injection(15), fractionated CO2 laser resurfacing(18,19), pedicures, acupuncture, body piercing, and tattooing(20). Aquatic environments and exposure to contaminated fresh or salt water, such as swimming pools or fish tanks can lead to M. marinum infection.(21) M. ulcerans infections occur commonly in tropical swamp dwelling areas due to water contamination.(2) The skin

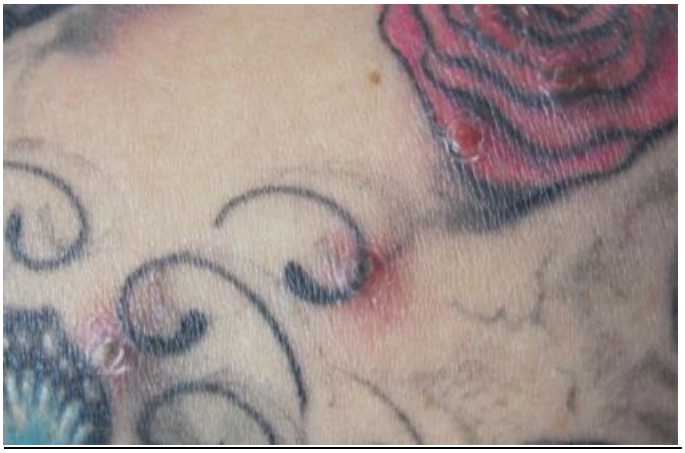
involvement of NTM can be classified into limited cutaneous disease and disseminated disease.

LIMITED CUTANEOUS DISEASE:

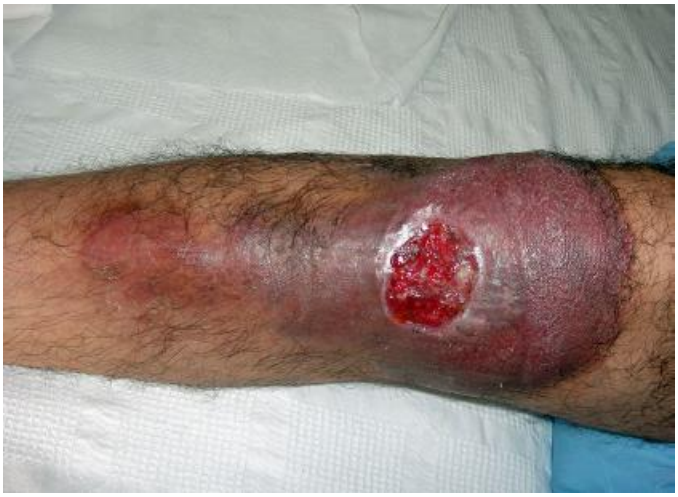
Papules, plaques, nodules, folliculitis, abscesses, cellulitis, ulcers, panniculitis, or draining sinus tracts is the most common presentation of SSTIs caused by NTM. These lesions may be solitary or multiple, and may present in a sporotrichoid pattern(22). NTM infections should ideally be suspected in any patient with lesions occurring at sites of prior trauma or procedures and negative routine bacteriological cultures who fail to improve with standard antibiotic therapy.

M. marinum infection usually begins as an indolent nodule around 2 to 3 weeks after inoculation, and can frequently develop into nodular lymphangitis. They usually present in a sporotrichoid pattern involving a hand or an arm. Lesions can present as an ulcer, abscesses, pustule or verrucous plaque. Deeper infections can lead to tenosynovitis and hence called “fish tank finger” (when involving the digits), bursitis, arthritis and osteitis. There may be history of fish handling or exposure to wet environment. These outbreaks have commonly been reported in association with fish markets and fish farms(23).

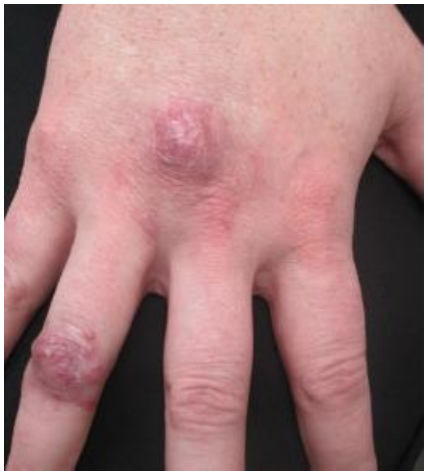
M. ulcerans causes a solitary, asymptomatic and firm nodule that presents after an incubation period of 3 to 4 months. Initially these lesions are typically less than 5 cm in diameter, but they can progress into necrotic ulcers with scalloped edges that expand in size. These ulcers are known as Buruli ulcers, named after the Buruli district in Uganda. Children are more commonly affected and the extremities are frequently involved, although the head, neck, trunk and genital regions can also be involved. Buruli ulcers may heal spontaneously, but can result in extensive scarring and deformities. If unhealed, they can progress to involve underlying tendons, joints and bones. Osteomyelitis has been reported in up to 14.5% of cases, and may lead to amputation(24). M. fortuitum, M. chelonae, and M. abscessus are the three most common RGM species that cause SSTIs, often following trauma or procedures(25). Skin lesions caused by this group is usually nonspecific, but a patient with M. fortuitum infection tend to be a younger and immunocompetent individual who present with a solitary lesion at the site of inoculation. Patients affected with M. chelonae or M. abscessus tend to be older, more likely to be immunosuppressed and they may have multiple skin lesions. M. kansasii and M. hemophilum are two other organism that can cause SSTI after surgical procedure(26).

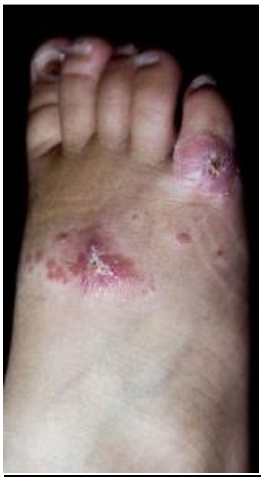


Erythematous nodules due to M chelonae infection



Buruli of ulcer of leg caused by M buruli



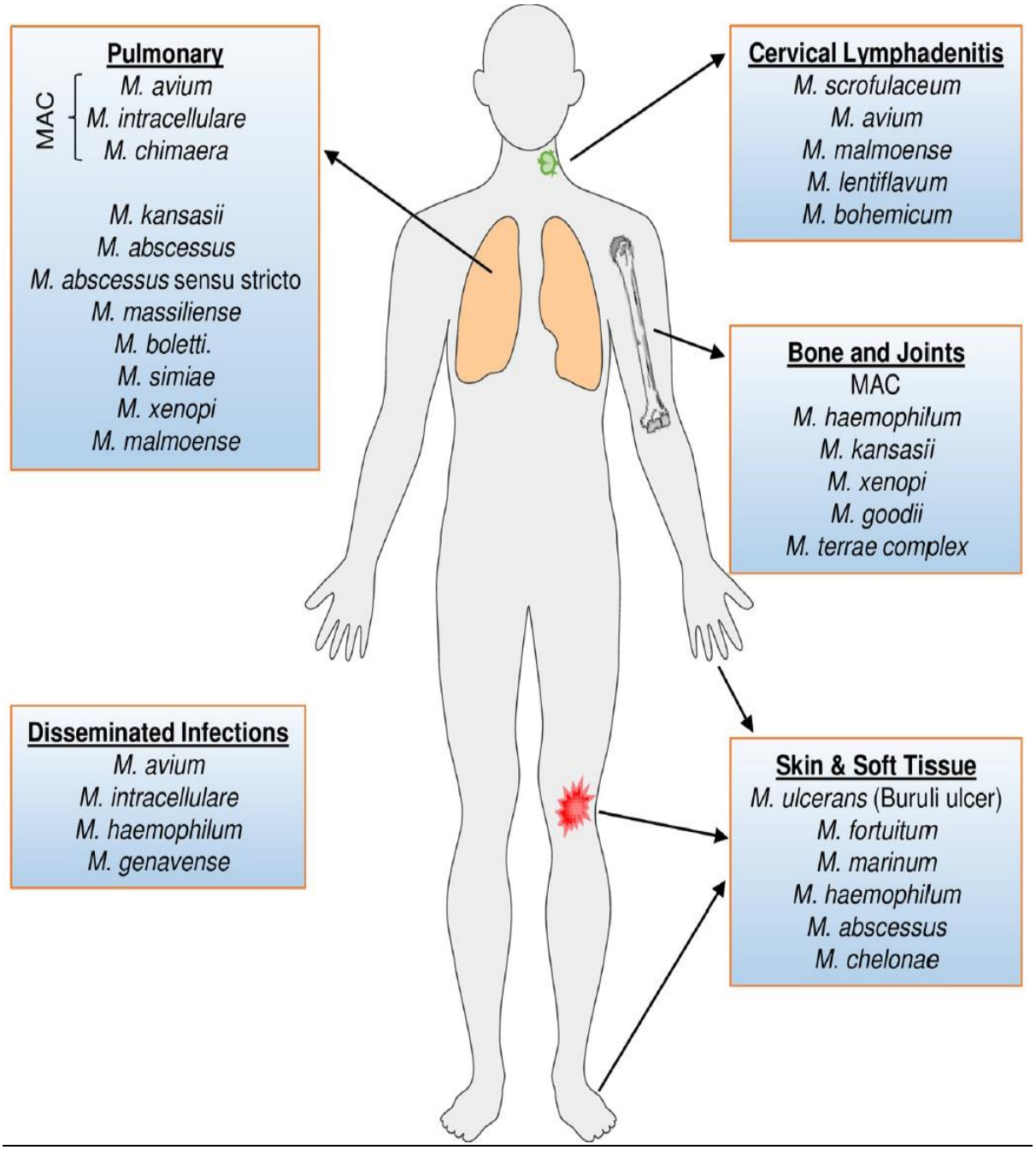


M marinum infection presenting as erythematous papules and nodules as sporotrichoid pattern on hand and foot

DISSEMINATED DISEASE:

The most frequent causative organisms in non-HIV–associated disseminated infections are the RGM species *M. fortuitum*, *M. chelonae*, and *M. abscessus*, but other species such as MAC, *M. kansasii*, *M. haemophilum*, and *M. scrofulaceum* have also been reported(27). Patients with disseminated NTM disease usually present with constitutional symptoms of fevers, night sweats and weight loss. Disseminated skin lesions can present as multiple subcutaneous nodules, pustules, draining abscesses, ulcerations, panniculitis or Sweets syndrome(28).

MOST COMMON BODY SITES AFFECTED BY NON TUBERCULOUS MYCOBACTERIA:



(7)

DIAGNOSIS OF ATYPICAL MYCOBACTERIAL INFECTION FOR SKIN AND SOFT TISSUE INFECTIONS

The increased incidence and prevalence of NTM infections have led to studies and development of methods for improved recovery, rapid detection, and rapid identification. Early control of NTM infections is dependent on methods for rapid identification and can reduce both morbidity and mortality through implementing the best course of drug therapy.

Diagnosis of infection by atypical mycobacterium differs depending on the site of infection. Individuals usually present with indolent or subacute courses and fever is the most common symptom. Suspicion of infection should be heightened in immunosuppressed individuals, or individuals with other comorbidities that damage host defences.(25)

Presumptive diagnosis is made primarily through signs and symptoms, physical examination findings, clinical history, geographic location, and if available, any preliminary evidence of the presence of AFB by pathology, smear or early culture reports. Probable or definitive diagnosis is made by adequate culture of any drainage or biopsy specimen.(26,27,29) The most commonly encountered organism are the rapidly growing organisms.(30)

For proper microbiological examination, optimal etiological source involves collecting high quality specimen. For atypical mycobacterial infection of the skin it entails adequate debridement, placing it in correct containers and rapidly transporting it to the laboratory.(23)

Identification of mycobacteria is made with several microbiological techniques that differ from methodologies used for standard bacteria. Despite several limitations, staining procedures are of critical importance. They take advantage of low permeability of lipid rich and mycolic acid containing cell wall for basic dyes. Permeability is enhanced by heat or prolonged exposure allowing colorization by basic dyes. The Ziehl-Neelson and Kinyoun stain are basic staining methods employed.(22,31,32) Flourescent microscopy reveals bright yellow rods against dark background, and this method is more sensitive than Ziehl-Neelson stain. Histopathological examination reveals features of granulomatous inflammation with either a foreign body or a tuberculous type of reaction in all the specimens. Moreover cultures may take several weeks to grow.(33–35)

PCR based molecular targets and commercially available molecular assays for *M tuberculosis* are used in the diagnosis of nontuberculous mycobacterial infection.(3,6,7) 16S rDNA sequencing is the gold standard for the molecular diagnosis of nontuberculous infection. Sometimes additional segments of 16S rDNA must be sequenced to differentiate these atypical mycobacteria.(2) Alternatively, other

genes, including hsp65, the gene coding for the 32-kDa protein, and the 16S-23S rRNA internal transcribed spacer, allow for the differentiation of all clinically important atypical mycobacteria.(21,36) Newer techniques like Line probe assays were developed for rapid and precise subspecies identification. The GenoType NTM-DR line-probe assay (Hain Lifescience, Nehren, Germany) is one such tool recently described.(8)

TREATMENT OF ATYPICAL MYCOBACTERIAL INFECTION OF SKIN AND SOFT TISSUES

NTM are uncommonly encountered clinical pathogens. Some species are much more likely to be isolated as a result of specimen contamination than as a result of disease. The clinician has not only to determine whether the organism is producing disease but also to distinguish the condition from tuberculosis and other diseases. This requires consideration of the clinical signs and symptoms, response to differential skin testing, histologic features and results of bacteriologic study. (24,36,37)

The main characteristic of almost all therapeutic protocols of NTM infections is the use of a combination of several drugs over long periods of time. The most important antibiotics all have the property of a high intracellular accumulation of drug. The use of a combination of drugs also allows a higher synergistic effect and minimizes the

appearance of antimicrobial resistances. Plasma concentrations of antibiotics can be affected by age, sex, HIV infection, dosage and by bilirubin and albumin levels, so an individualized evaluation of the patient is performed to prevent therapeutic failures due to low concentrations of antibiotics.(38)

Combination therapy using a macrolide (clarithromycin noted to have the best activity for M. abscessus or M. chelonae) or a quinolone (Specifically for M. fortuitum strains) and a parenteral antibiotic (amikacin, cefoxitin or imipenem) is recommended for severe infections. (2,3,6,7,15) However amikacin is the most preferred aprentral antibiotic as Cefoxitin and Imipenem have less predictable activity. For mild infections, monotherapy has been used with success for folliculitis or other skin and soft tissue infections. Monotherapy has been used for cutaneous disease without severe complications. Minocycline, doxycycline and clarithromycin are the antibiotics used. Foreign material such as prosthetic valves, pacemakers, orthopaedic prosthesis and catheters must be removed prior to therapy. The antibiotics used for RGM are different because these species are intrinsically resistant to first line antitubercular drugs. Biofilms are common among clinical isolates of RGM(39,40). Newer antibiotics that have shown in vitro activity against these mycobacteria include the new quinolones (moxifloxacin, levofloxacin), telithromycin, tigecycline, linezolid and other oxazolidinones.(41)

The duration of therapy varies depending on the nature of the infection and adherence to therapy. For severe diseases such as bone infections a minimum of 6 months

therapy has been recommended. Mild disease can be treated successfully for shorter periods. A combination of excisional surgery (or surgical debridement) and chemotherapy is usually performed for most patients. Surgical therapy includes complete debridement of skin and soft tissues associated with necrosis, but to leave adequate skin for closure if possible. Other therapy used are cryotherapy, X-ray therapy or electrodessication.(42)

Recent discoveries about the pathogenesis and resistance mechanisms of mycobacteria have also shed new light on the management of these infections. The discovery of biofilm development is important for the development of new therapeutic schemes in the following years because of their implications in conferring drug resistance. The number of drugs that can be used is still limited and new antibiotics must be tested against a high number of strains (usually by reference laboratories) to increase the possibilities for treatment.(43)

CHARACTERISTICS OF COMMONLY USED DRUGS FOR NTM OF SKIN AND SOFT TISSUES

DRUG	FIRST LINE THERAPY	ALTERNATIVE THERAPY	DOSAGE REGIMEN
Clarithromycin	MAC, M. abcessus, M. chelonae, M.fortuitum, M. haemophilum, M. marinum, M. ulcerans, M. xenopi	M. kansasii, M. malmoense, M. scrofulaceum	500mg 2 times/day
Azithromycin		Alternative to clarithromycin	500mg/day
Ciprofloxacin	MAC, M. fortuitum, M. haemophilum	M. chelonae, M. kansasii, M. malmoense, M. xenopi	500-750mg 2 times/day
Levofloxacin	MAC, M. fortuitum, M. haemophilum	M. chelonae, M. kansasii, M. malmoense, M. xenopi	500mg/day
Cefoxitin	M. abscessus, M. fortuitum	M. chelonae, M. hemophilum	2g IV every 4-6 hourly
Imipenem/Cilastatin	M. chelonae, M. fortuitum	M. abscessus	500mg IV every 6 hours
Doxycycline	M. fortuitum,	M. chelonae	100-200mg 2 times/day
Cotrimaxozole	M. fortuitum, M. marinum	M. chelonae, M. hemophilum, M. kansasii	80-1600mg 2times/day
Amikacin	M. abscessus	MAC, M. fortuitum, M. hemophilum, M . kansasii	6-7.5mg/kg IM or IV 1-2 times/day

(4)

COMPLICATIONS ASSOCIATED WITH TREATMENT REGIMEN

Macrolides:

Clarithromycin and Azithromycin had changed the treatment of atypical mycobacterial infection. They had allowed treatment regimens change to two or three drugs with superior outcomes. Gastrointestinal intolerance remains the most common adverse effect of macrolides. Clarithromycin is a potent inhibitor of CYP3A4 and other isoforms, affecting several drugs including warfarin, theophylline, anticonvulsants, benzodiazepines, protease inhibitors, estrogen and certain HMG coenzyme A reductase inhibitors.(39)

Flouroquinolones:

They have lower rates of GI intolerance and hypersensitivity as compared to macrolides. CNS effects such as headache, dizziness and agitation is observed in less than 10%. Tendon inflammation and tendon rupture have rarely been reported in adults. Other rare events include the potential for corrected QT interval prolongation and hepatotoxicity. Ciprofloxacin has variable CYP enzyme inhibition that affects drugs such as warfarin, theophylline, sulfonylureas and anticonvulsants. (30)

Aminoglycosides:

The primary toxicity of streptomycin is vestibular or auditory toxicity. Toxicity by aminoglycosides occur by degeneration of hair cells in the organ of Corti, predominantly in the basal turn which is required to sense high frequency sounds.

Therefore high frequency hearing loss is more common in patients developing ototoxicity related with aminoglycosides. Multiple factors have been associated with an increased risk of ototoxicity. Risk factors for ototoxicity and nephrotoxicity is age, male sex, low body weight, total dose of amikacin and duration of amikacin. These effects may result is symptoms such as dizziness, vertigo, ataxia, tinnitus and hearing impairment. Nephrotoxicity is also a potential concern. Therapy duration and total aminoglycoside exposure, supratherapeutic concentration are risk factors for toxicity. Clinicians should encourage patients to note any changes in hearing, tinnitus, dizziness or fullness in the ear. Therefore vestibular, baseline audiometric and renal function testing should be regularly repeated during course of therapy.
Aminoglycosides also tends to disrupts neuromuscular junction transmission through interference in Ach receptors. Patients at high risk are those who have myasthenia gravis and those receiving other drugs associated with neuromuscular blockade(muscle relaxants, anaesthesia or corticosteroid therapy) (41)

Beta-Lactams and Carbapenems:

Cefoxitin use may be associated with gastrointestinal intolerance, hypersensitivity reaction, interstitial nephritis, liver function enzyme elevation and blood dyscrasias. Imipenem-cilastatin is associated with neurological toxicity including seizures. (39)

A summary of complications and adverse events associated with medications have been summarised below:

DRUG	MAJOR SIDE EFFECTS
Macrolides(Clarithromycin, Azithromycin	Gastrointestinal disturbance(nausea, vomiting, diarrhoea, abdominal pain), Headache, Hypersensitivity (rash, pruritus, swelling)
Quinolones (ciprofloxacin, moxifloxacin, gatifloxacin)	Gastrointestinal disturbance (nausea, vomiting, diarrhoea), CNS disturbance (agitation confusion, insomnia, headache)
Aminoglycosides (streptomycin, amikacin)	Vestibular dysfunction and auditory toxicity, (vertigo, tinnitus, dizziness, hearing loss) Nephrotoxicity
Linezolid	Gastrointestinal disturbance (nausea, vomiting, diarrhoea), Haematological, (thrombocytopenia, anaemia, leukopenia), Hypersensitivity (rash, pruritus), Neuropathy
Cefoxitin	Hypersensitivity
Imipenem-cilastatin	Neurological toxicity
Doxycycline	GI sensitivity, phototoxicity
Cotrimaxozole	Bone marrow suppression, Hypersensitivity

SOCIAL, ECONOMIC AND PSYCHOLOGICAL CONSEQUENCES OF THE DISEASE IN THE POPULATION

Nontuberculous mycobacteria cause infections that can be clinically challenging at several levels. Proposed diagnostic criteria include clinical, pathological and microbiological criteria and the treatment is equally complex and taxing. It requires a prolonged multidrug regimen. Therapy by multiple drugs is frequently complicated by drug intolerances to first line agents, especially in the elderly, which can result in continuous use of second line antimicrobials to control the infection. The recent increase in prevalence of NTM in the population has made it important for clinicians and health care administrators. Successful clinical outcomes require that patients initiate treatment promptly and adhere to therapy regimen. Furthermore this disease can often lead to irreversible physical disabilities and can take a significant toll on affected patients and their households.(44)

Economic burden on the patient:

Despite the availability of healthcare access, patients face with major economic burden in the form of direct, indirect and intangible costs.

Direct costs: Patient and households' direct costs are expenditures incurred by the patient or household members in the course of treatment.

They could be divided as

(i) Irregular Medical Expenses and Hygiene Costs*:* These costs are related patients' personal hygiene when taking care of the wound (e.g. soap to wash and clothing dirtied due to the wound). Irregular expenses for extra medication and unofficial fees during treatment.

(ii) Feeding Costs: Food costs that was incurred by patients and caretaker.

(iii) Transportation Costs: It refer to the cost of transport for the patient, caretakers and other household members when travelling to and from the hospital.

(iv) Miscellaneous Costs: These include a variety of miscellaneous costs such as extra rent in the location of the hospital for caretakers, extra phone calls and debt to community workgroups due to illness.

Indirect costs: Refer to the value of lost productivity or earnings lost by the patient or household members in the course of treatment. Productivity lost could be calculated on individual's (patient and caretaker) earnings per calendar year. For children and youth, additional schooling can be taken into account.

Intangible costs: Certain consequences of NTM cannot be expressed or directly converted into monetary values. These include some of the long term consequences of the illness such as abandonment of schooling, disability and deformity, social exclusion, and psychosocial factors. These effects cannot tangibly be measured, but are nonetheless detrimental to the patients and their household. (45)

Social burden on the patient: Absence of the patient and their household members from the community results in increased vulnerability in times of crisis due to their

inability to maintain participation in the community. These communities require regular contribution. A common reflex strategy against the accumulation of the aforementioned unmanageable costs manifests in the breaking of ties with the individuals most taxing on the household economy- the patients. The importance of the above mentioned factors in relation to the economic and social impact of NTM disease is evident in the health seeking behaviour of the patient. The patients in underdeveloped countries prefer to seek traditional healing since it minimised or largely avoided such costs.(46)

Psychological burden on the patient:

Due to the nature of disease, prolonged treatment duration of the illness and associated economic and social costs to the patient can lead to adverse effect on psychology of the patient. (47) They can suffer from depression, anxiety about the illness, and in some cases adversely affect the treatment regimen altogether. Moreover some medications used in the treatment has the propensity to cause neurological toxicity which can further worsen the picture. Hypersensitivity reactions to the medications is not rare, and can cause some patients to altogether stop medical therapy. Furthermore difficulty in diagnosis, prolonged duration of treatment and persistent nature of the disease can dishearten a patient during the course of treatment, which can adversely affect the doctor patient relationship.

The rising prevalence of NTM infections has an increasingly large impact on population health and heath expenditures. However the cost of treating NTM disease

should never discourage therapy. The identification of new, less expensive alternative therapies may be most helpful for *NTM* infection and studies are underway to find more effective mode of therapies.

METHODOLOGY

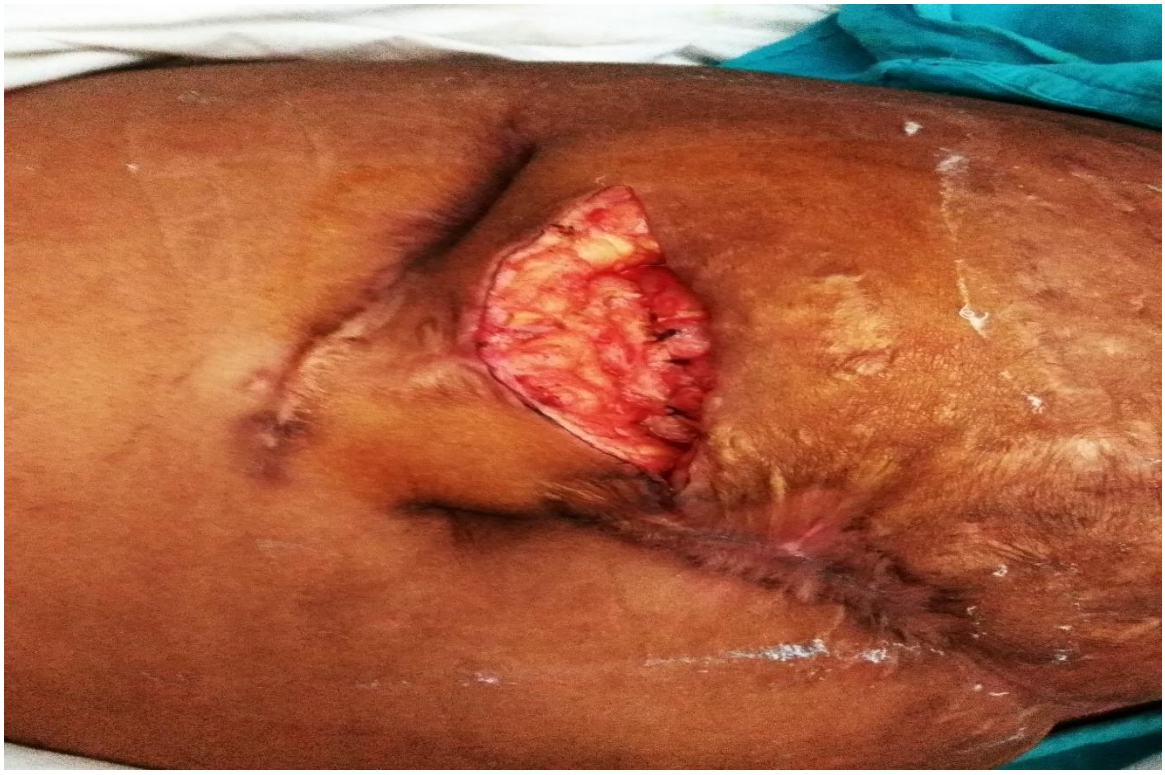
STUDY DESIGN

This study was designed as a bidirectional observational study, approved by the institutional review board. It was conducted over a period of 4 years from January 2015 to March 2019.

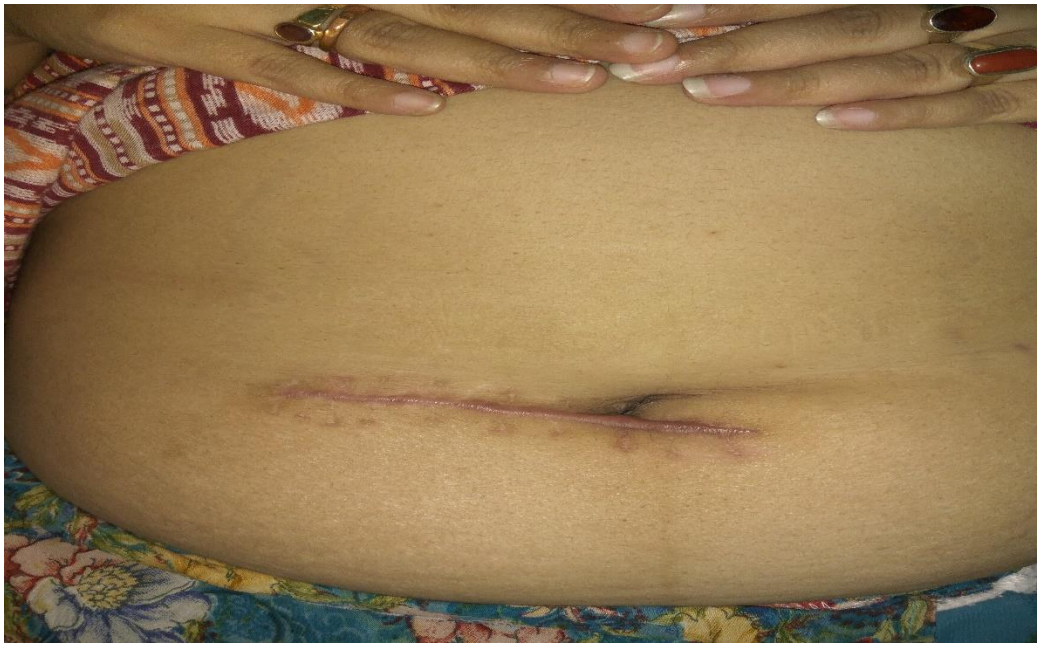
METHODOLOGY OF DATA COLLECTION

For prospective patients clinical history and examination was done at the time of recruitment in outpatient department or wards in the department of general surgery from December 2017 to January 2019. A proforma was filled by the primary investigator, and a separate patient proforma was given to the patient which was regularly entered by the individual. At first follow up visit after discharge, the data was collected in outpatient basis and various outcomes were studied. For retrospective patients, the study collected data of patients from the computer database only, who had been admitted under or attended the outpatient clinic of the department of General Surgery and Infectious Disease from period of January 2015 to November 2017. The retrospective patients were not called. An assessment of all the recruited patients was done and outcomes were analysed with the help of a statistician. For prospective patients, adherence to medical therapy was assessed based on follow up reports along with the other outcomes. For retrospective patients all outcomes except adherence to

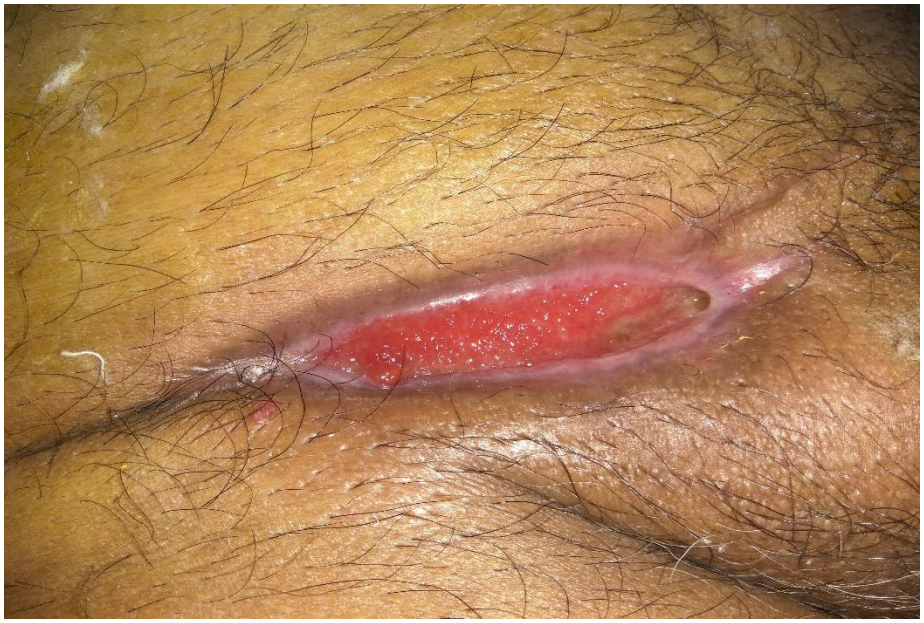
medical therapy was assessed. Hence a clear outcome data was expected in this study. Patients who have not followed up were considered as non-adherent to therapy.



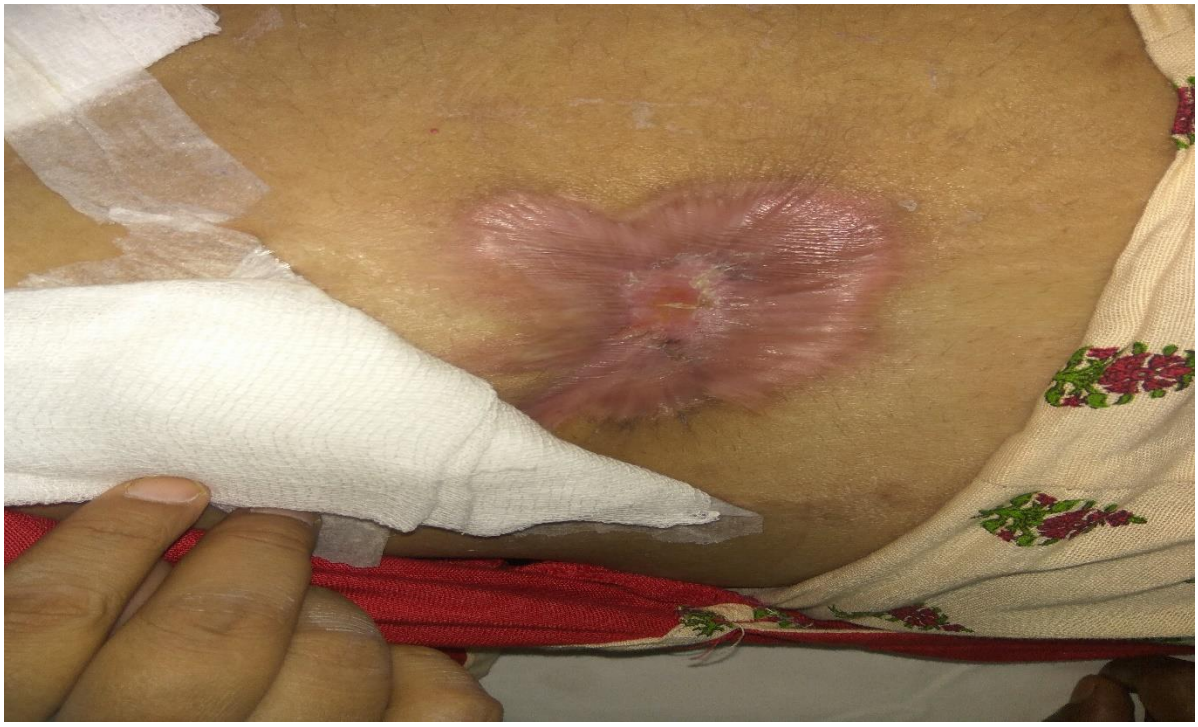
(Surgical site after debridement and prior to initiation of medical therapy)



(Surgical site at the first follow up visit-Healed wound)



(Surgical site at the first follow up visit- Unhealed wound)



(Surgical site- Recurrence of lesion)

Informed consent administration

All patients were given an information sheet with details about the study in English and in translated versions in Tamil, Hindi and Bengali (native language speakers). Informed consent was taken by the principal investigator from the patient after being given adequate opportunity to read the information sheet and ask questions.

Inclusion criteria

The inclusion criteria included patients with atypical mycobacterial skin infection who were initiated on therapy by Department of Infectious Disease after surgical debridement of skin lesion.

Exclusion criteria

1. Patients who were immunocompromised
2. Culture positive wound infection for organism other than nontuberculous mycobacteria.

MEASUREMENT OF DATA SOURCES

For the purpose of this study a patient proforma was designed for data collection of the adherence to medical therapy**.** Source of data for this study was from detailed case history, demographic information, lab investigations, treatment sheets and follow up reports from the intra-hospital clinical workstation, patient proforma provided to the

patient at the time of recruitment as well as periodic email and telephonic communication with the patient. These data sources was separately analysed and results was deducted from them.

The exposure for the patient will be medical therapy started by department of infectious disease after surgical debridement of the lesion. Confounding factors in this study will be infection with gram positive/gram negative bacteria. As concomitant infection can affect the clinical presentation, diagnosis and treatment, a pus culture/sensitivity report will be assessed prior to initiation of medical therapy.

Particular variables were described that had a bearing to the outcomes related to the study. They were utilised for subsequent follow up visits at the department of General Surgery. These variables helped to better categorise patients who were receiving medical therapy.

Comorbid illnesses: It was defined as the presence of a distinct clinical condition that has existed or may occur during the clinical course of a patient receiving therapy. Its presence may affect the clinical therapy and the often the outcome of these individuals.

Wound healing: It was defined as epithelialization of the raw surface at the first visit to the outpatient department following debridement of the skin lesion and subsequent medical therapy. It is a defining parameter for measuring success of therapy. Without re-epithelialization, a wound cannot be considered healed.

Recurrence of lesion: It was defined as occurrence of persistent discharging sinuses in spite of medical therapy, new papular or nodular lesions associated with previous wound and incidence of collection at the wound site noted clinically or radiologically.

Adherence to medications: It is defined as the degree to which the person's behaviour corresponds with the agreed recommendations from a health care provider. This will be a primary determinant for success of therapy. It signifies that the patient and physician collaborate to improve the patient's health by integrating the physician's opinion. A few factors affecting adherence was studied among these patients. This was assessed using Morisky Medication Adherence scale (MMAS 8 item).

BIAS IN THIS STUDY

There was no obvious bias to this study. Every consecutive patient was recruited into this study.

SAMPLE SIZE CALCULATION

A sample of 82 subjects was required to obtain a 95% confidence interval of ±10% around a wound heal prevalence estimate of 70% after the surgical debridement and medical therapy.

$$n = \frac{Z^2_{1-\alpha/2} p(1-p)}{d^2}$$

p= proportion of patients

d= population risk difference

Expected prevalence=70%

Precision=10%

z-value for 95% Confidence interval=1.96

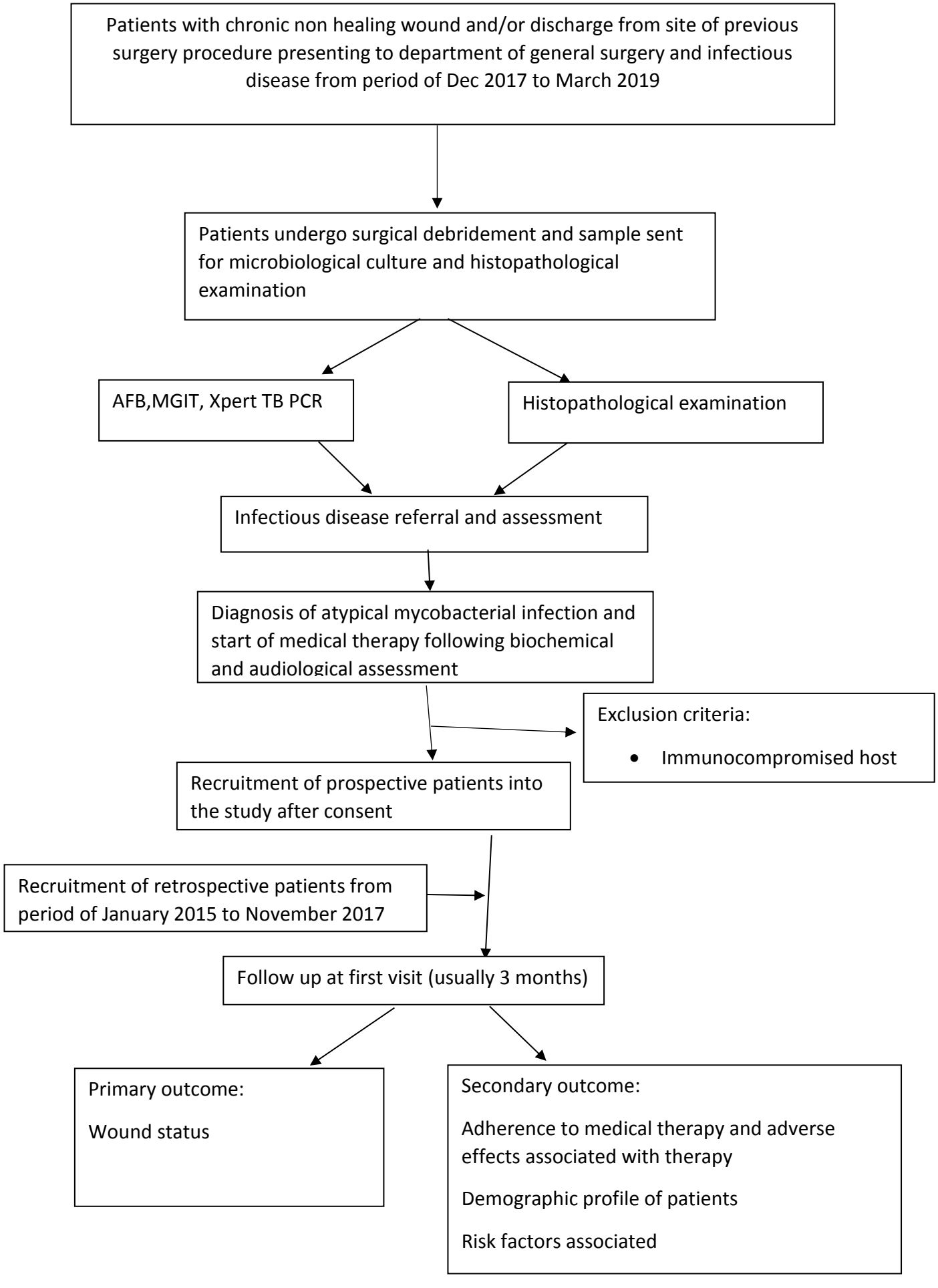
DATA ENTRY

Data collected from all cases and controls were entered using EpiData Manager and EpiData Entry Client(v4.2.0.0).

STATISTICAL METHODS USED IN THE STUDY

Categorical variables will be summarised using counts and percentages. Quantitative variables will be summarised using mean and standard deviation or median and IQR. Chi square test will be used to compare the proportions between the groups and two sample t tests will be used to compare means between the two groups. For all the analysis, 5% level of significance will be considered to be significant.

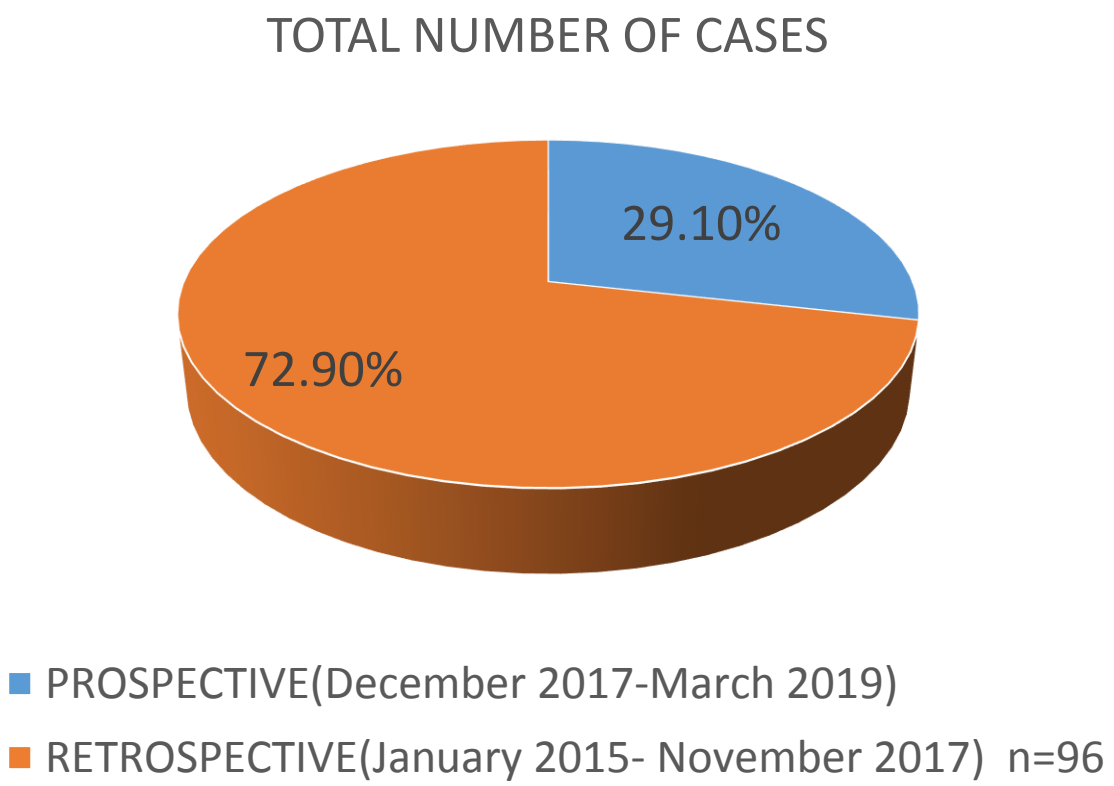
DIAGRAMATIC ALGORITHM OF THE STUDY



RESULTS

DISTRIBUTION OF CASES IN THE STUDY

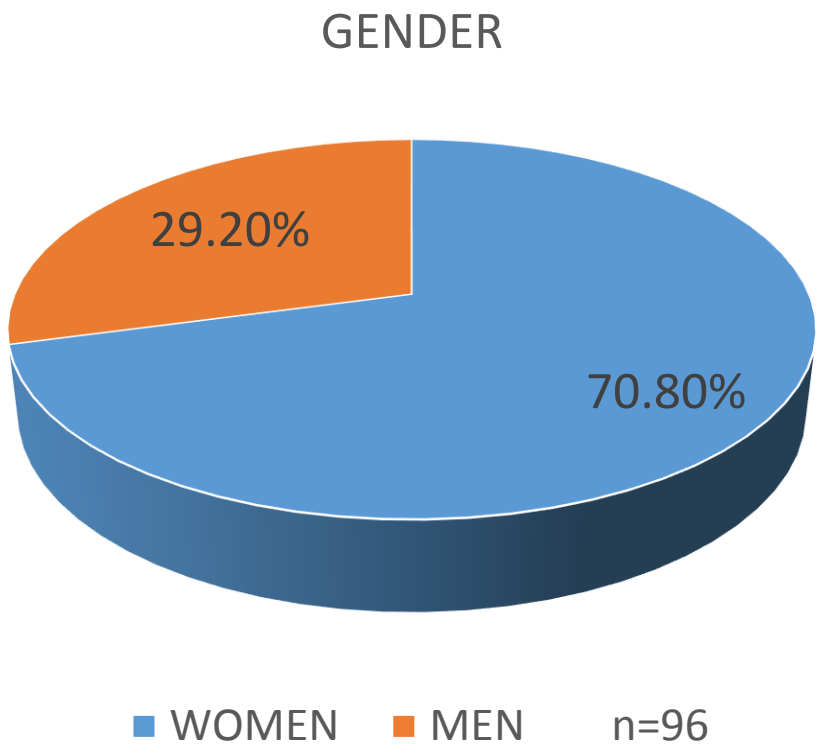
This study was undertaken at a tertiary care centre which caters to approximately 61000 out patients to the general surgery department every year. A total of 96 patients were enrolled during the said period out of which 70 were in the retrospective group and 26 were in the prospective group. These patients had surgical debridement for atypical mycobacterial infection of the skin and soft tissues following which they underwent medical therapy. The number of retrospective cases (72.9%) were noted to be more than the prospective cases (29.1%) because of the longer period chosen for the former group.

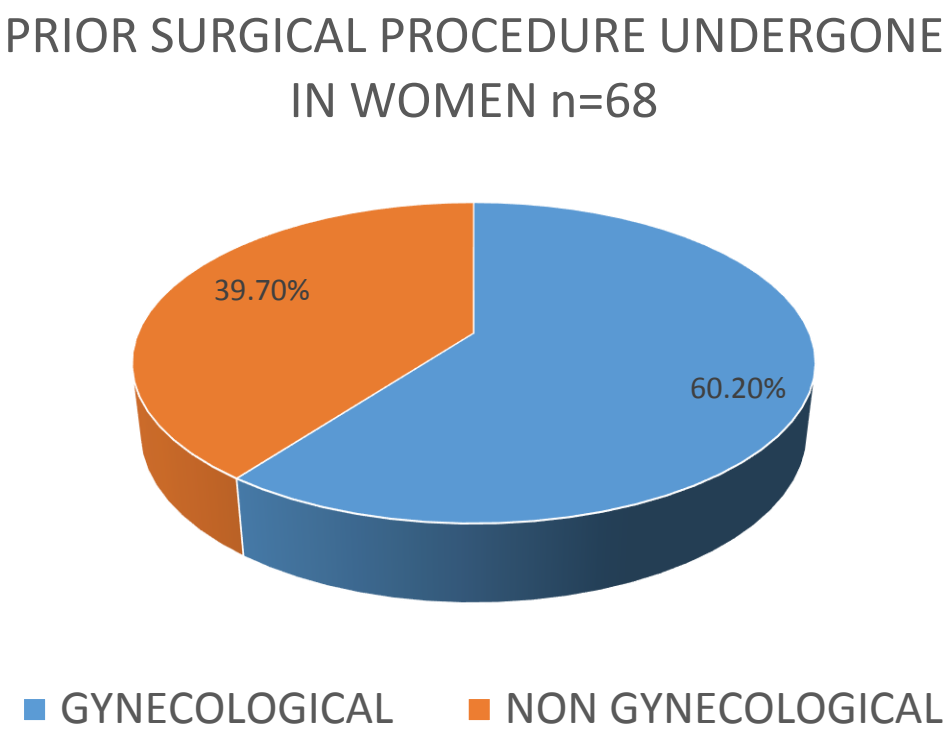


BASIC DEMOGRAPHIC DATA

GENDER

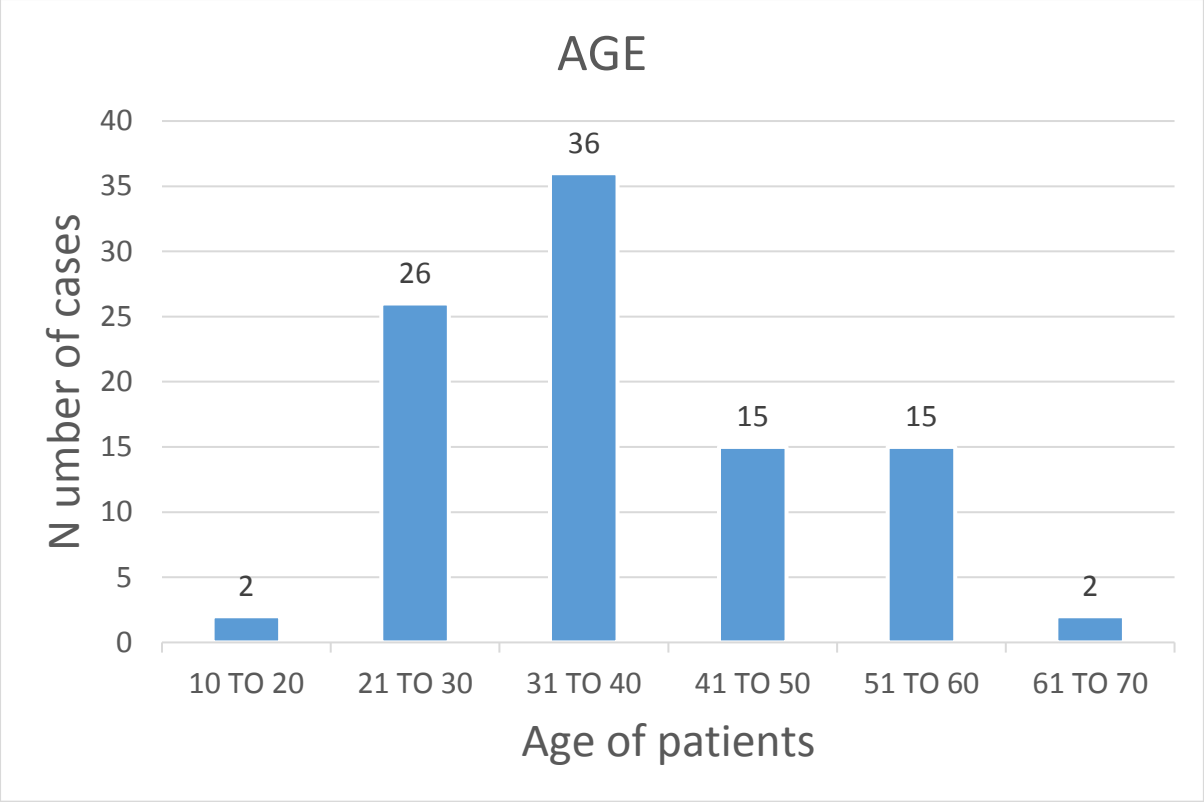
Of the 96 participants, 68 women and 28 men were recruited. Women were affected more commonly than men in this study. The female to male ratio was 2.42:1. The preponderance to women could be related to more number of gynecological procedures in this group.





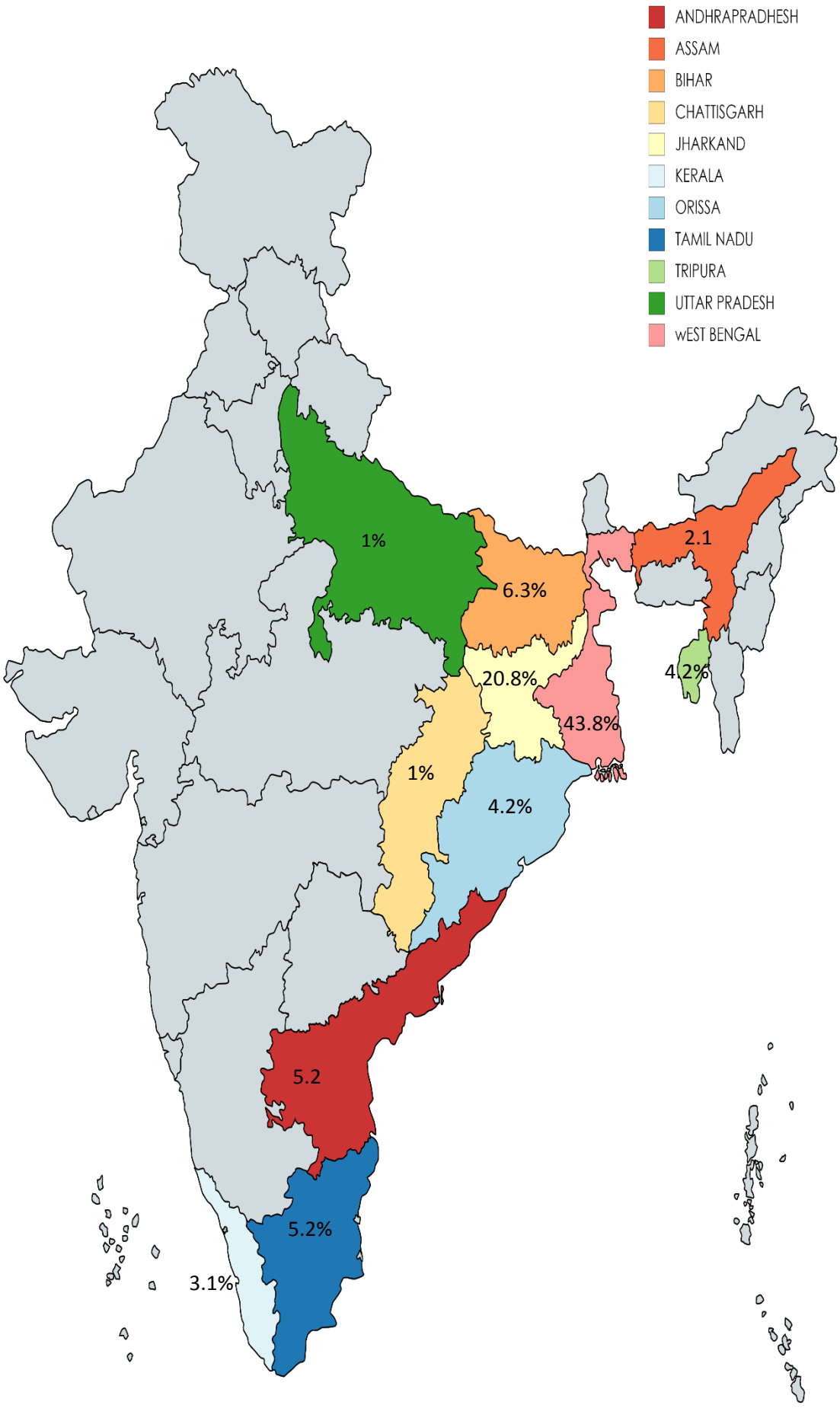
AGE

These patients were recruited by systematic sampling method. The cases recruited into the study were from the department of General Surgery and age ranged from 16 to 69. However the most common group of patients affected was of the age group from 20-40 years. The mean age of the patients were 37.073 years and the median age was 35 years. Most of the participants had undergone multiple abdominal surgeries in the past. The incidence of atypical mycobacteria infection is not uncommon in adolescent, middle and elderly groups as noted in the following results.



STATE OF ORIGIN

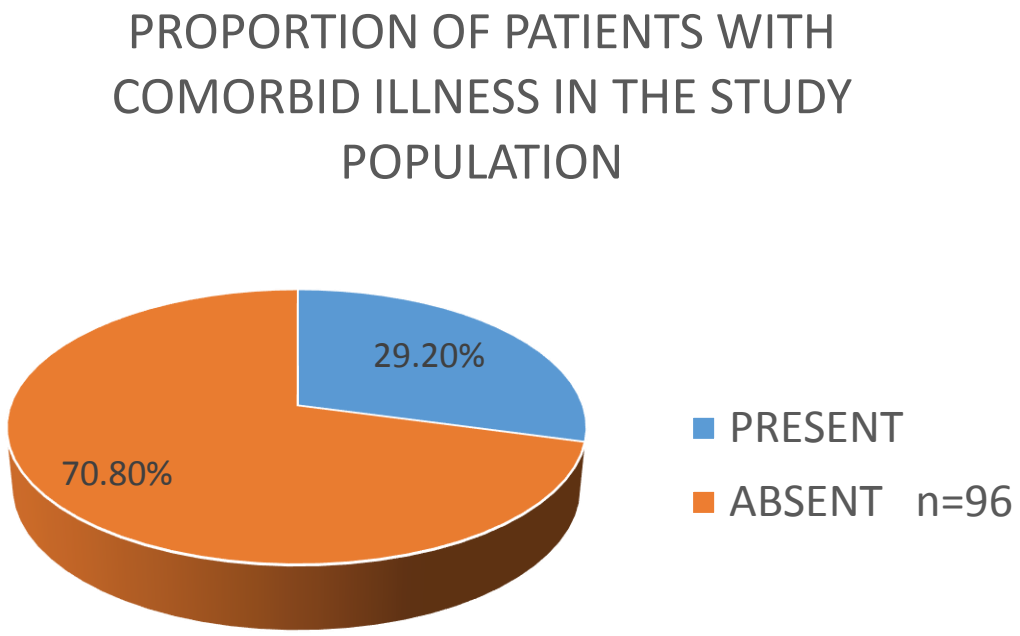
Majority of the patients were Indian except for 3 patients who hailed from Bangladesh (3.1%). In this distribution of cases, it is noted that most of them were not from native state. Rather a major proportion of the patients were from eastern (48%) and central (28.1%) part of the country. Also the proportion of patients from native state was noted to be only 5.2%

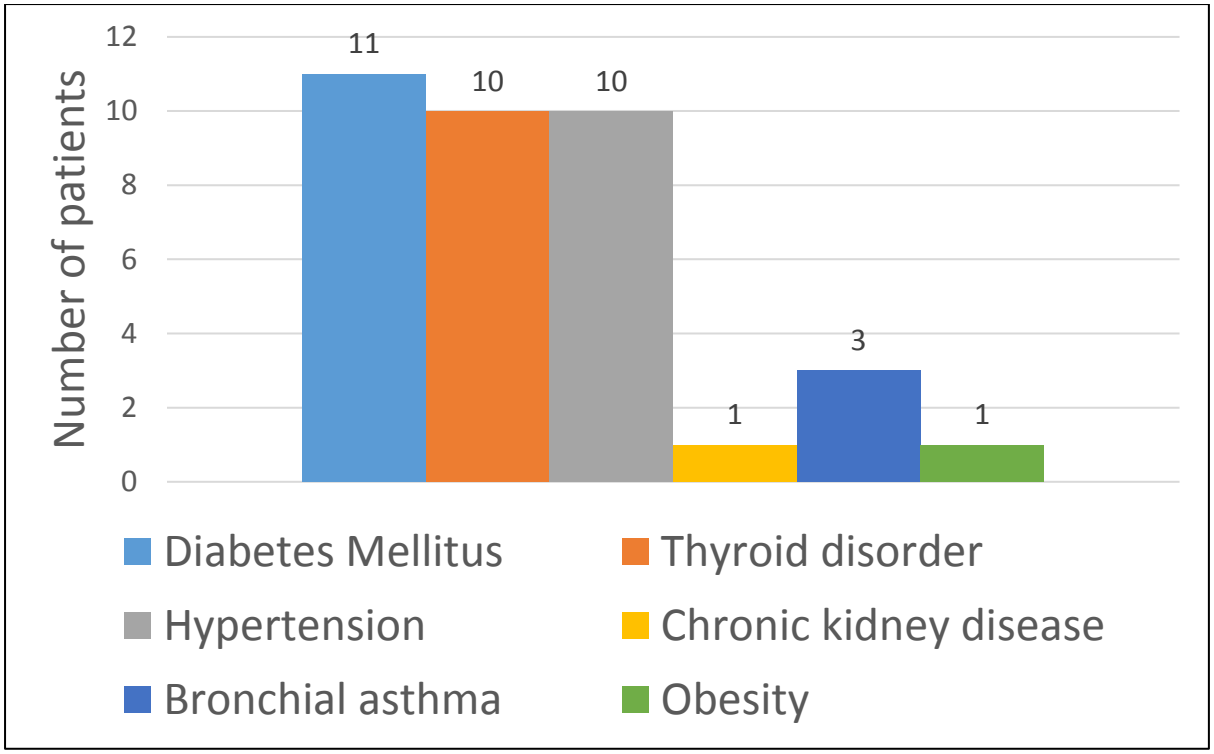


Created with mapchart.net ©

COMORBID ILLNESS

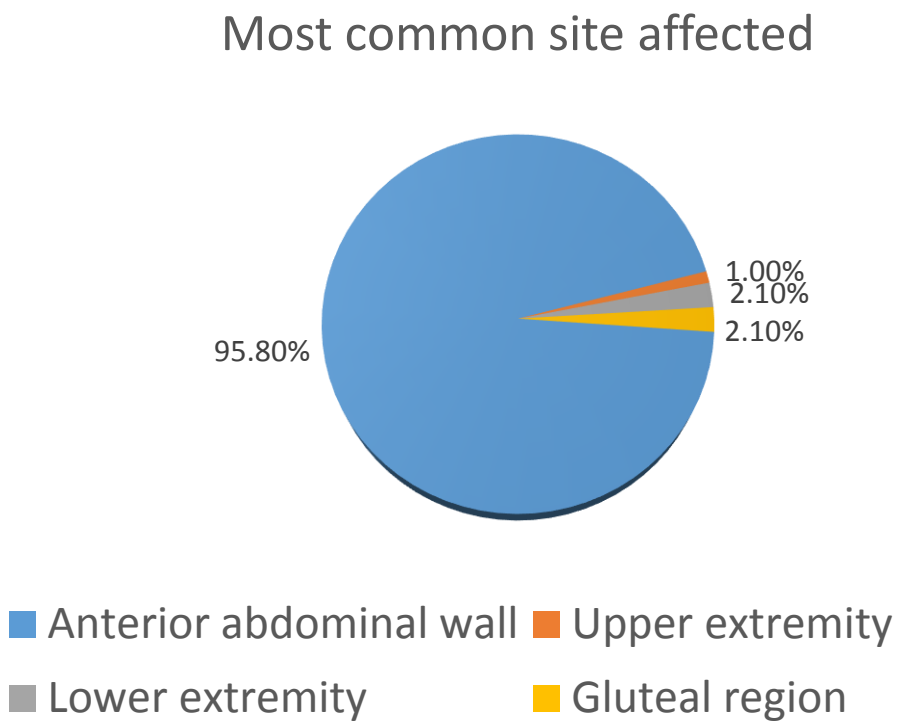
Participants with comorbid illnesses were optimised by the concerned medical specialties prior to starting medical therapy. Out of the total patients recruited, 28 patients had a single or multiple comorbid illnesses. Diabetes Mellitus, thyroid disorders, hypertension, chronic kidney disease, bronchial asthma and obesity were taken into account as they could affect treatment outcomes. It was noted that Diabetes Mellitus was the most common comorbid illness followed by hypertension and thyroid disorders.





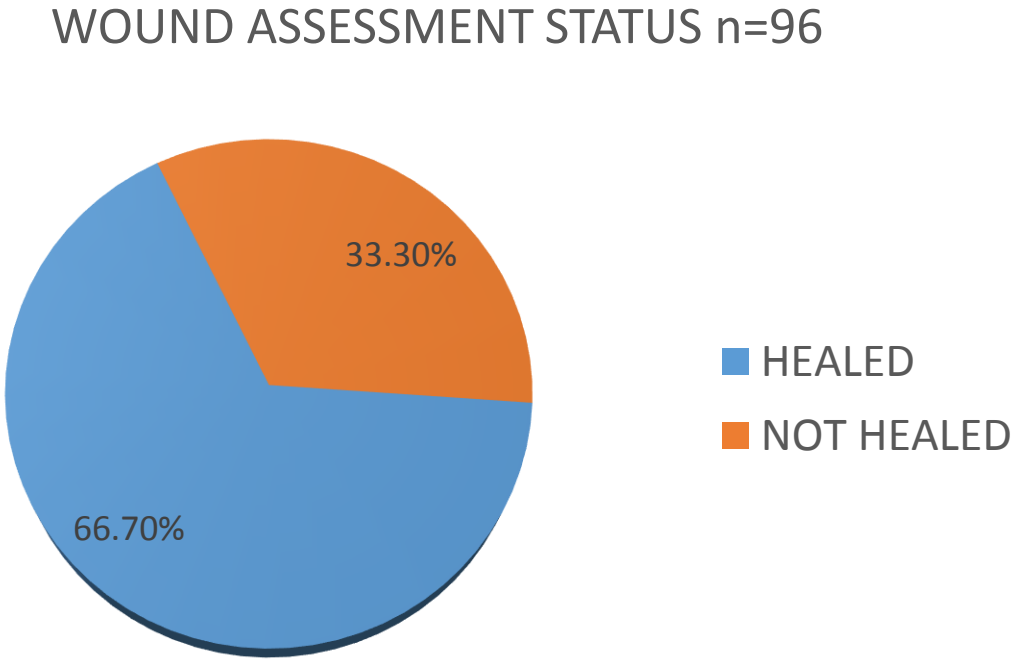
MOST COMMON SITE AFFECTED

The anterior abdominal wall was the most common site of post-operative infection (95.8%). This was in correlation with history of multiple surgery in the past. Also it was noted that more of cases occurred in the lower extremity as compared to the upper extremity (4.2% vs 1.0%). None of the patients in the study were noted to have infection involving the head, neck, chest or genitalia.



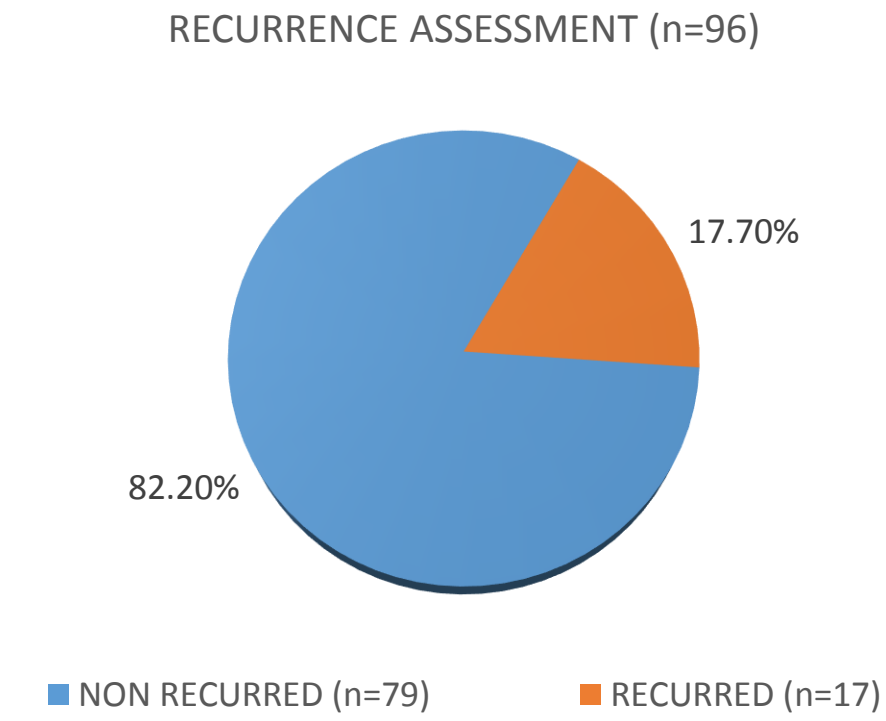
PRIMARY OUTCOME

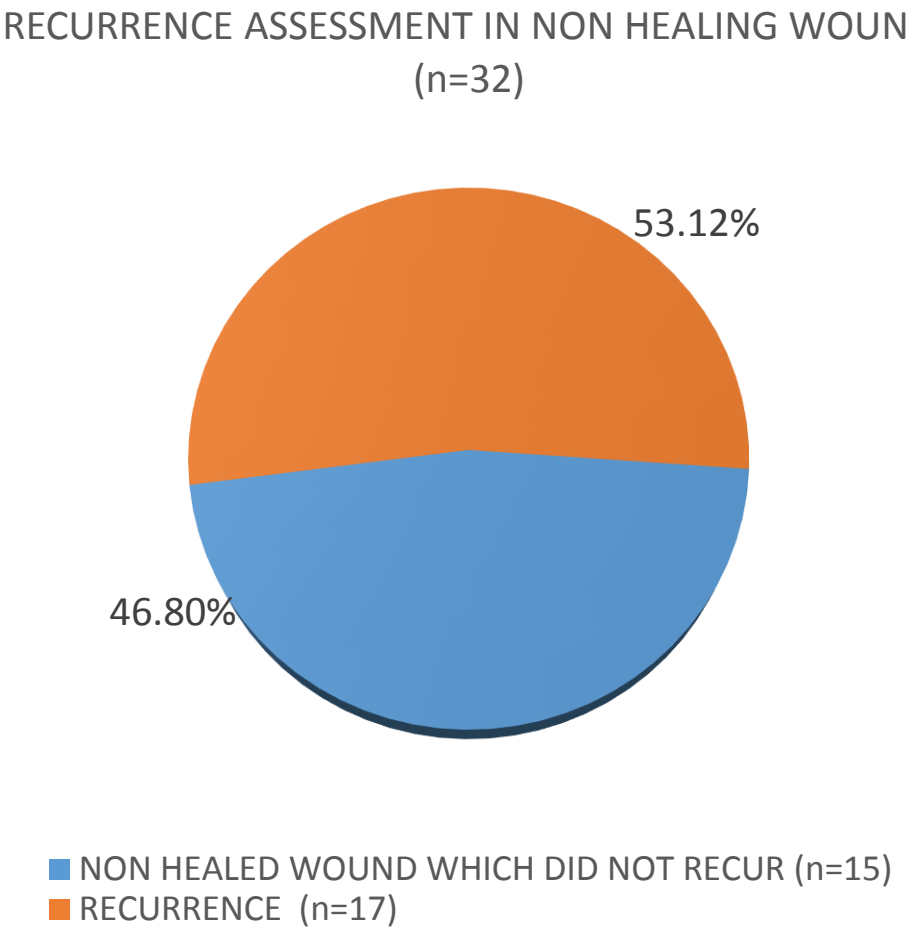
The primary outcome was wound healing as defined previously. 64 patients included in the study had adequate wound healing as noted at the first follow up visit (66.7%). 32 patients(33.3%) did not have adequate wound healing and these patients were assessed for recurrence of infection. Moreover it was noted that 100% of the patients had followed up at the outpatient department. The primary outcome was documented in the charts.



The recurrence of infection in patients was studied after adequate follow up of the patient. It was noted that 17 patients (i.e. 17.7%) had recurrence after surgical debridement out of the total number of 96 cases recruited.

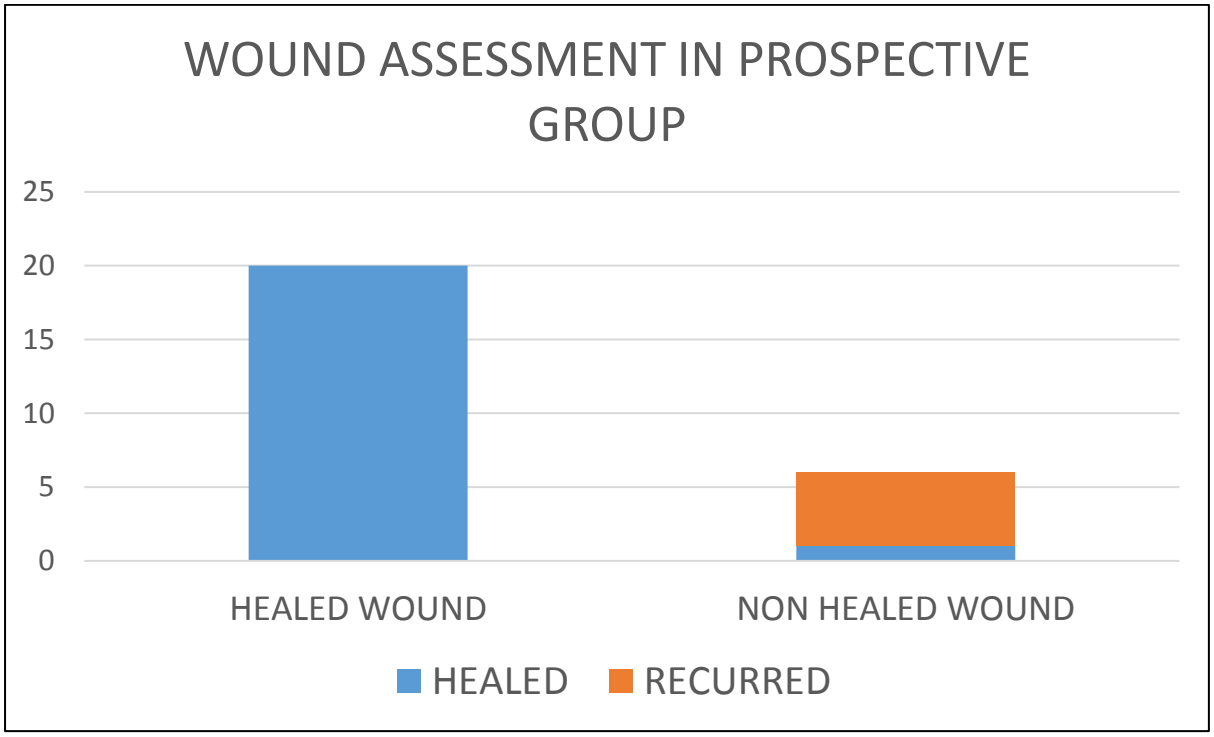
However the rate of recurrence was higher in the number of patients who did not have wound healing at the first follow up visit and they had to undergo additional surgical and/or medical therapy. The patients who had wound healing in the first follow up did not have recurrence of lesion in the subsequent follow up period. It was also noted that 100% of the patients who had recurrence underwent reoperation.


RECURRENCE ASSESSMENT (n=96)
17.70%
82.20%
NON RECURRED (n=79)
RECURRED (n=17)


RECURRENCE ASSESSMENT IN NON HEALING WOUND (n=32)
53.12%
46.80%
NON HEALED WOUND WHICH DID NOT RECUR (n=15)
RECURRENCE (n=17)

For the prospective group (n=26), it was noted that most of the patient's wound had healed (n=20) and only 6 patients had non-healed wound status. Of the patients whose wound had not healed, 5 had recurrence and had to be reexcised. Only one patient's wound had taken prolonged time to completely heal.

Of the patients whose wound had healed, none of the patient had recurrence.



SECONDARY OUTCOME:

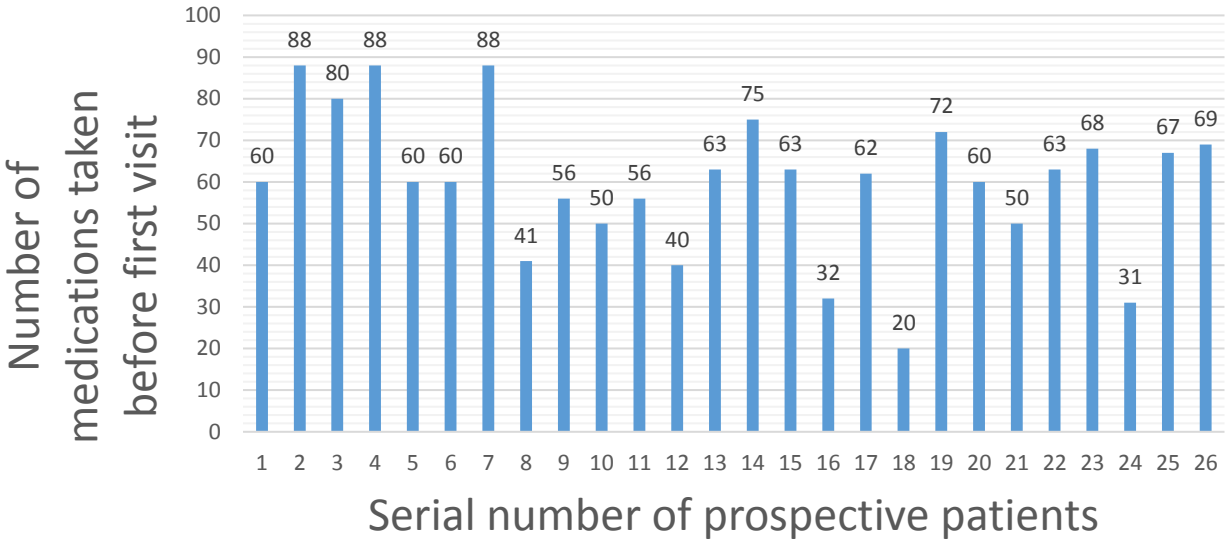
The adherence to medical therapy was assessed as the secondary outcome. The sample population was taken from the prospective group (n=26). The patient proforma for each individual was submitted and analysed. The patients were usually prescribed an injectable aminoglycoside and oral medications given in appropriate dosages and frequency.

Numeric variables like the number of medications taken by the patient before the first visit and the duration of therapy was documented. Finally the adherence to medication was calculated using Morisky Medication Adherence Scale (MMAS 8 item).

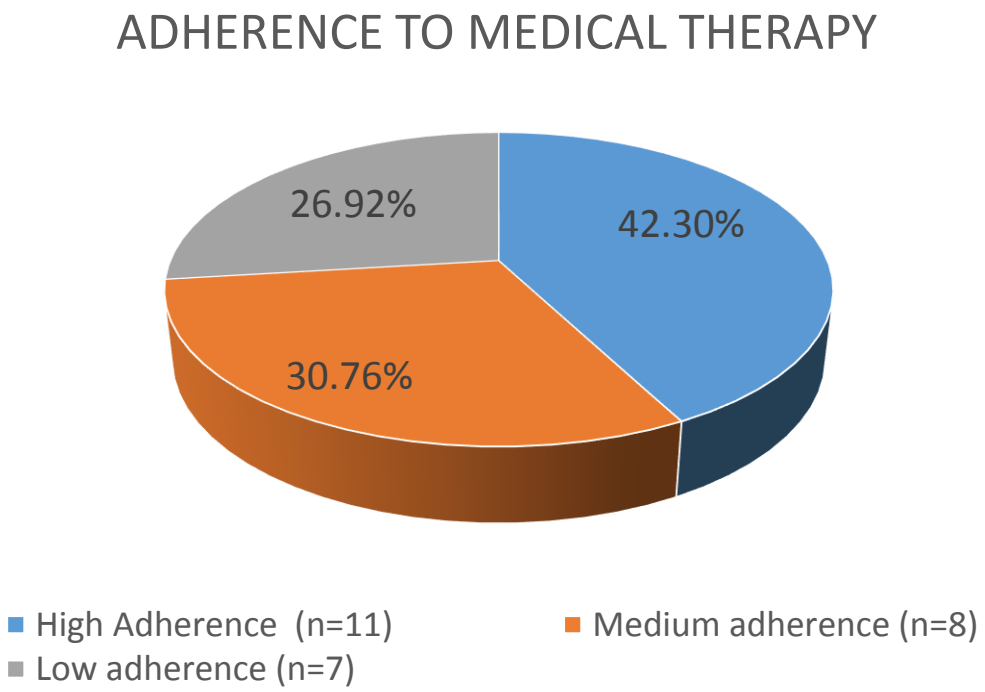
All the patient were successfully followed up at first visit and proforma was collected.

The mean number of days the medications taken by the patient before the first visit was 60.07 days.

The average duration for which the medications were prescribed was 8.808 weeks.

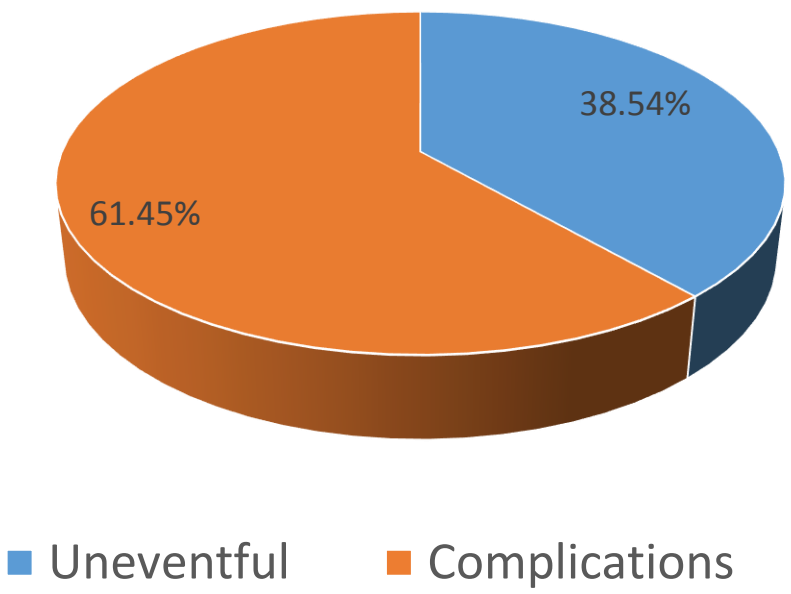


Adherence to medications was divided into High adherence, Medium adherence and Low adherence according to MMAS 8 item scale. Most of the patients had high adherence to therapy. However if medium and low adherence were collectively taken in the clinical context, the number would be greater.

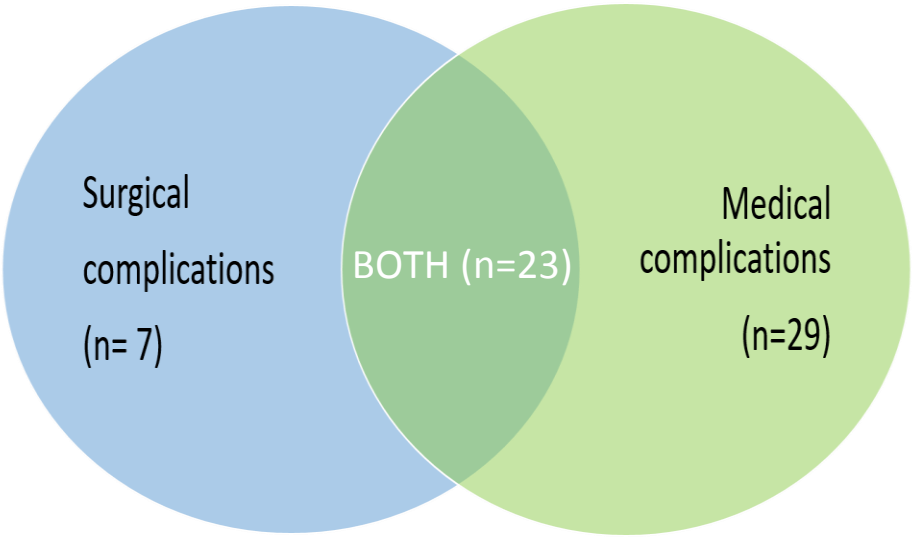


COMPLICATIONS ASSOCIATED WITH THERAPY

Multiple complications were noted with therapy, both surgical and medical (n=59). More than half of the patients were affected by these complications (61.45%). These complications were those described after undergoing surgical debridement for the lesion.

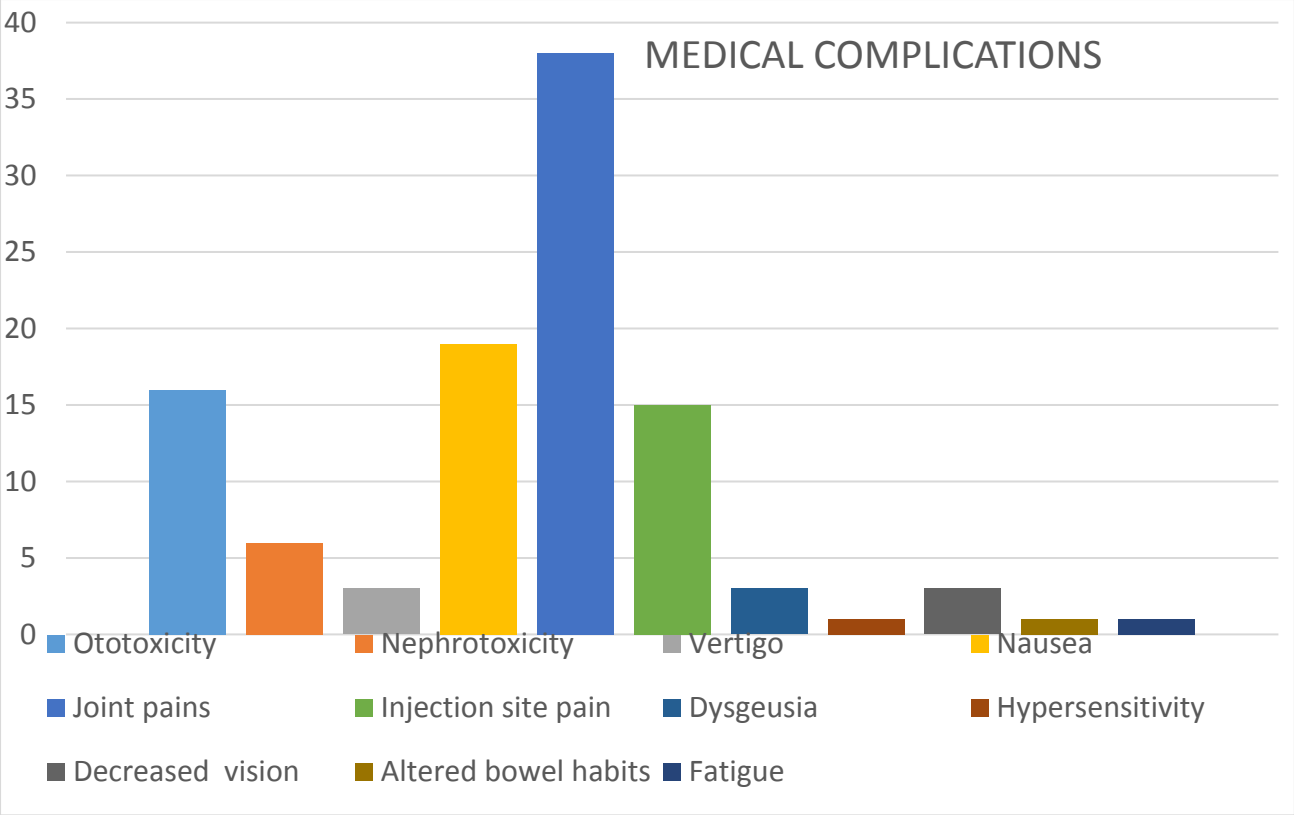


7 patients had only surgical complications, 29 patients had only medical complications and 23 patients had both surgical and medical complications. Therefore it was noted that the patients had more medical complications during therapy.



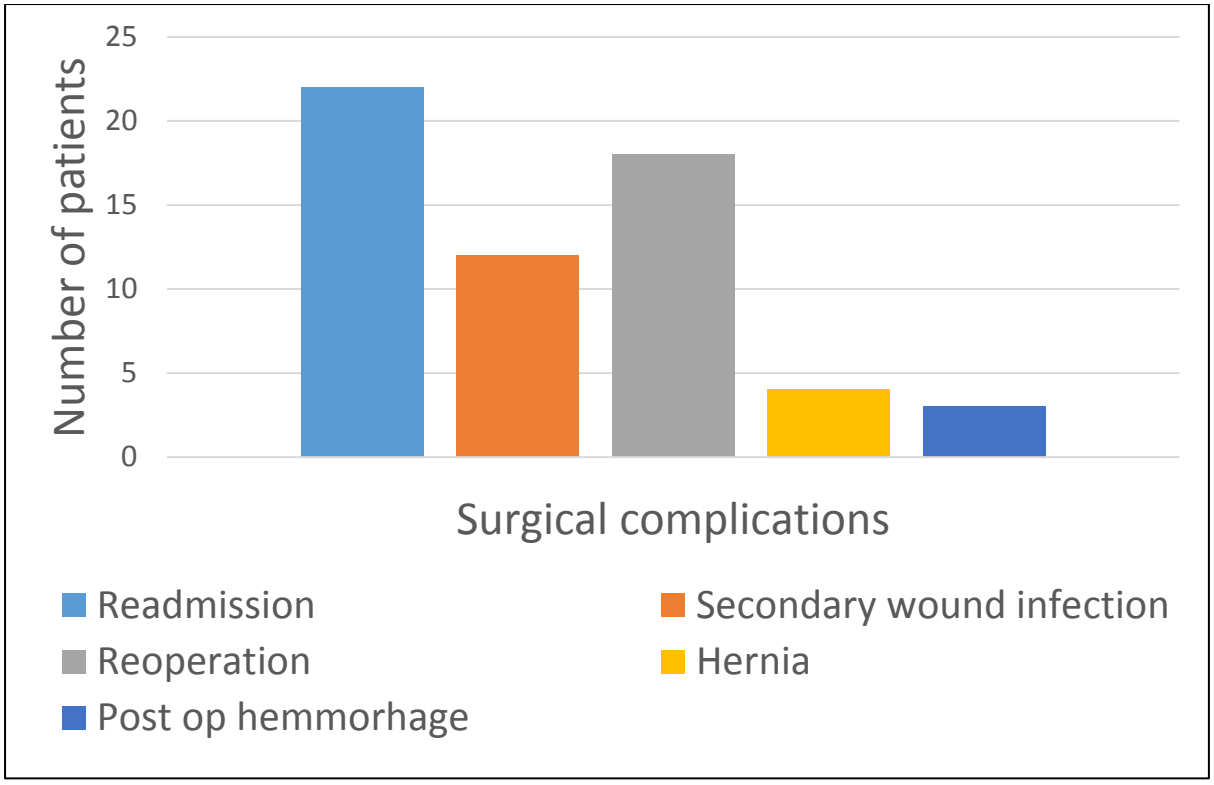
MEDICAL COMPLICATIONS ASSOCIATED WITH THERAPY

Majority of the patients had medical complications after starting medications. The most common medical complication was joint pains which occurred in very high frequency among this group (73.07%). The other complications noted were ototoxicity, nephrotoxicity, nausea, dysgeusia, decreased vision, vertigo, fatigue, altered bowel habits and hypersensitivity. Of these ototoxicity and nephrotoxicity has been specifically documented to occur after aminoglycoside therapy. These complications were adequately addressed by the concerned medical speciality(s).



SURGICAL COMPLICATIONS ASSOCIATED WITH THERAPY

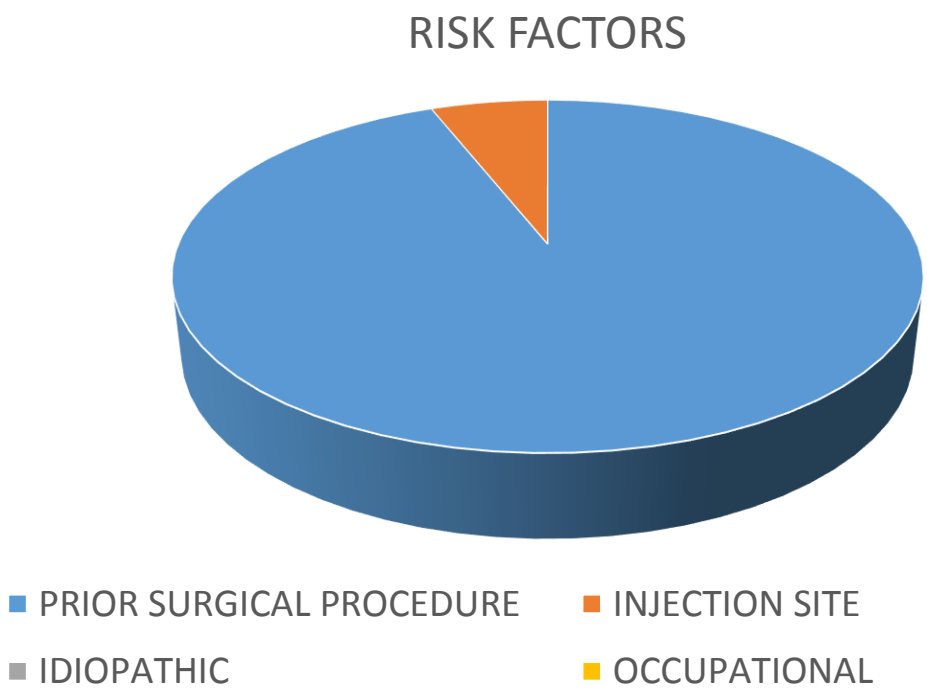
A total of 30 patients had surgical complications (57.69% of the total). Of these readmission was the commonest. 18 patients underwent reoperation. The other complications noted were secondary haemorrhage complicating course of therapy, post-operative haemorrhage from wound site and hernia.



RISK FACTORS ASSOCIATED

Multiple risk factors were proposed in the study, however most of the patients were only exposed to an invasive procedure- surgical procedure or injection prior to developing the infection. None of the patients had occupational exposure or idiopathic etiology as source of infection.

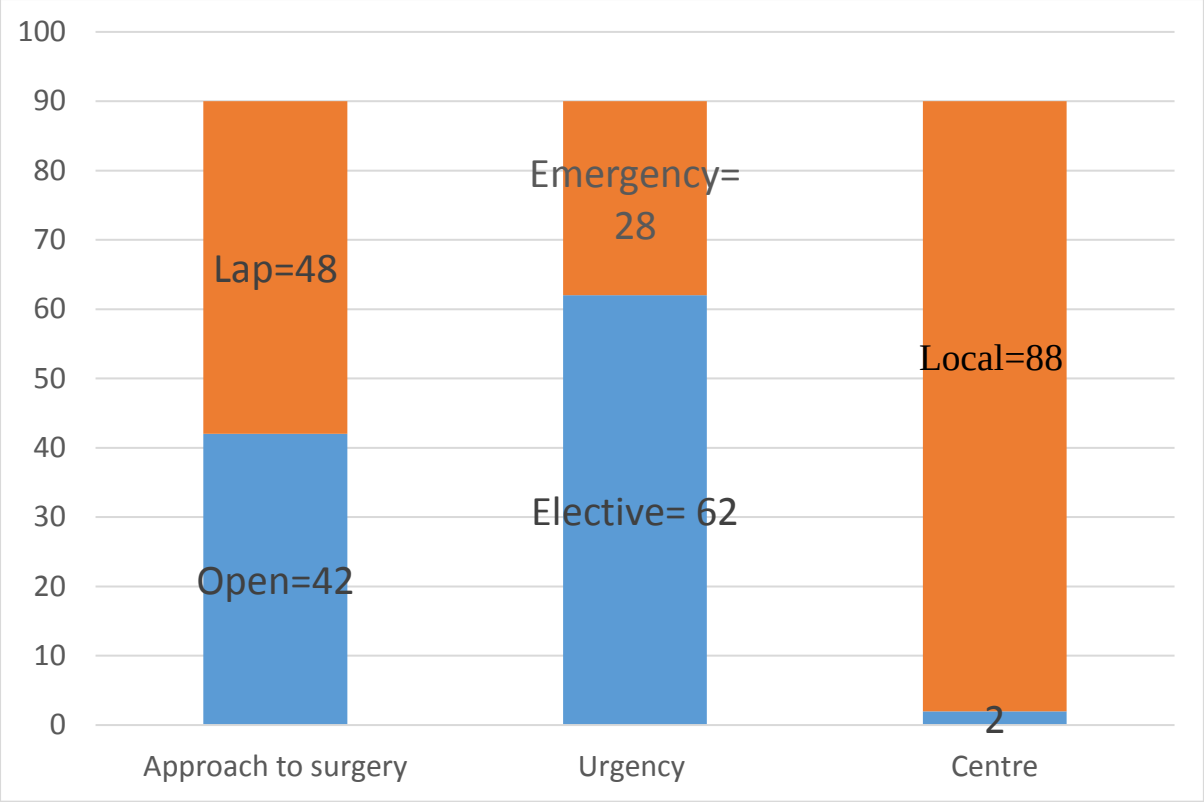
Most of the patients had undergone a prior surgery (single or multiple). The proportion of patients who underwent surgical procedure was 93.75% and those who had an injection were 6.25%.



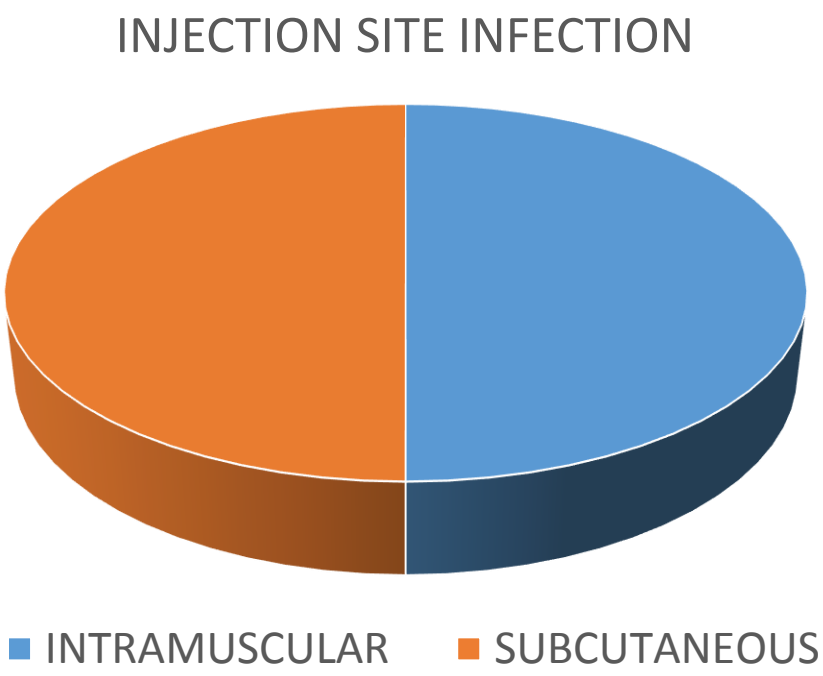
Of the patients who underwent prior surgical procedure, most of them had undergone a laparoscopic surgery (53.33%). Women were more implicated to develop this infection since most of the procedure were gynaecological. 46.67% of the patients underwent an open surgical procedure(s) for other reasons. A significant number of these patients were from western and central part of India.

Most of the procedure were done in the elective setting (68.88%). The rest were done in emergency or semi-elective setting.

Only 2 patients were noted to have a prior surgical procedure at the institution during the period of study (2.22%). The rest of patients recruited had a surgical intervention from an outside centre.



Of the patients who had infection at the prior injection site, there was an equal distribution between intramuscular and subcutaneous injection group (n=3). All of the patients in this groups had an exposure at their local centre. Most of these patients were from western part of India. There were no patients from the native state.



ANALYSIS

The association between two categorical variables i.e complications to medical therapy and adherence to medical therapy was studied in the prospective group. A fisher exact test was used to elicit any significant association between the two variables. The total number of patients with complications- surgical and medical were included in this group. Adherence to therapy was divided as high or medium/low adherence. Fisher's exact test was used and the resultant p value of 0.095 was obtained which was statistically not significant.

		ADHERENCE TO MEDICATIONS		TOTAL
		HIGH	MEDIUM/LOW	
COMPLICATIONS	NO	5 (45.5%)	2 (13.3%)	7 (26.9%)
	YES	6 (54.5%)	13 (86.7%)	19(73.1%)
TOTAL		11 (100%)	15 (100%)	26 (100%)

The association between adherence to medical therapy and primary outcome i.e wound healing was also studied. Fisher's exact test was used and the resultant p value was 1.000 which was statistically not significant.

		WOUND HEALING		TOTAL
		NOT HEALED	HEALED	
ADHERENCE	HIGH	3 (50.0%)	8 (40.0%)	11 (42.3%)
	MEDIUM/LOW	3 (50.0%)	12 (60.0%)	15 (57.7%)
TOTAL		6 (100%)	20 (100%)	26 (100%)

There was a significant association between recurrence of infection and wound healing in the prospective group. None of the patients whose wound healed had recurrence of infection, but most of the patients whose wound had not healed had recurrence of infection. Continuity correction was used which had shown significance of value (p=0.00)

		RECURRENCE OF INFECTION		TOTAL
		NO	YES	
DID THE WOUND HEAL?	NO	1 (4.8%)	5 (100%)	6(23.1%)
	YES	20 (95.2%)	0(0%)	20(76.9%)
TOTAL		21 (100%)	5 (100%)	26 (100%)

DISCUSSION

Atypical mycobacterial infection are caused by fastidious organisms that are difficult to isolate and becoming increasingly common in current clinical setting. NTM infections in immunocompetent hosts usually present in localised forms to the skin, in the form of SSTI's which was predominantly noted in this study. These infections did not cause any mortality but it had caused multiple morbidities in the recruited patients.

Women were more likely to be affected by this disease and it was more common in the younger age group in this study. This was similar to a few published literature where prevalence was found to be 1-1.6 times in elderly women than men(48). The reasons implicated are multifactorial like body habitus (pectus excavatum and scoliosis which indicate abnormality in fibrillin level that predispose to higher risk of infection due to modulated TGF-Beta expression), distribution of sex hormones (Luteinizing hormone (LH) and oestrogen that may contribute to altered leptin and adiponectin levels) and distribution of subcutaneous fat.

Geographical distribution showed predominance of cases from northern and eastern India which reflects the larger number of patients from that region visiting our centre every year. Recently environmental mycobacterium residing in soil and water have been increasingly isolated from these regions(49). Water plays the most important vector or medium for transmission for non-tuberculous mycobacteria, which correlates

with higher occurrence in areas having temperature 15-25 degree Celsius. But the rise in number and variety of NTMs in these regions should validate further research.

Most of the patients who presented to our centre underwent an operation at a semi-urban or rural centre. Many of them had had multiple surgeries in the past and were started on sub-therapeutic or non-therapeutic dosages of medications. Multiple surgeries in the past has been implicated as risk factor for infection(50).

Infection with atypical mycobacterial infections is mostly reported following laparoscopic procedures. In this study the majority of the infections were following laparoscopy surgery(51). The reason could be traced to improper mechanical cleaning of laparoscopic instruments and ports which leaves debris on these instruments. The contaminated instruments deposit the endospores on to the subcutaneous tissue during the surgical process. Due to development of biofilm, these organisms will exhibit antibiotic resistance and present as a persisting discharging sinus. The current practice in India is to immerse instruments in 2-2.5% glutaraldehyde solution for 20 minutes which achieves disinfection but not sterilization. Also boiled water is used to cleanse the instruments after immersion into glutaraldehyde solution, which can transmit the organisms. This is also highlighted in the fact that most of infections occurred in patients undergoing an elective operation, where sterilization of instruments can be done in controlled manner. Therefore the use of disposable laparoscopic instruments is the gold standard to prevent this infection. The instruments should be thoroughly

cleaned and rid of all organic soil before putting for disinfection. Possible alternative disinfectants include peracetic acid (0.2% to 0.35%), chlorine dioxide (700-1,100 ppm) and superoxidized water. Ethylene oxide gas sterilization is shown to be a better alternative and highly effective in reducing atypical mycobacterial infections following laparoscopy. If a liquid chemical sterilant is used, higher concentrations (3.4%) must be used and the exposure time should be increased to 8-12 hours to activate sporicidal activity. The water source used for cleaning should be from a clean source devoid of risk of contamination(52). The use of advanced sterilization systems such as STERRAD, which uses gas plasma technology to kill spores at low temperatures is strongly recommended for sterilization of insulated laparoscopic instruments.

6 patients had developed injection site abscess. 3 patients developed after intramuscular injections by health care personnel which shows the importance of penetrating trauma in cutaneous mycobacterial infections and significance of maintaining strict infection control precautions to prevent these iatrogenic infections.

The primary objective was to assess the wound healing rate of patients. It was noted that 2/3rd of the patients had wound healing with recommended therapy thus confirming that medical therapy following surgical debridement was an effective method to treat skin infection with atypical mycobacteria. But in patients who did not have wound healing at first follow up visit, it was noted that the post-operative healing was prolonged and they persisted to have superficial oozing wound that required regular dressings. More than half of these patients later developed recurrence of

infection. This shows that adequate early and aggressive debridement and medical therapy both were of utmost importance for clearance of organisms, to prevent recurrence of infection. Non-compliance to medical therapy was also a major cause of drug resistant strains, so establishment of directly observed therapy may be necessary.

Adherence to medical therapy was not high in this study group. More than half of the patients had a low or medium adherence. The main reasons for poor adherence found were forgetfulness to take medications, staying away from home (as in the case of patients from other state of origin) and complications of medical and surgical therapy. However statistically there was no significant association between adherence to medication and complications of medical therapy. Moreover there was also no statistical association noted between adherence to medication and wound healing rate in the prospective group. However it is important to note the small sample size used for this test.

Complications associated with medical therapy was both medical and surgical and required expert management. Nephrotoxicity, ototoxicity, vertigo and nausea are common side effects seen with aminoglycoside agents. Female sex and dose per kg body weight are important factors in this regard. This study had shown multiple patients complaining of injection site pain due to the mode of administration of aminoglycosides (i.e. intravenous or intramuscular). This could be a determinant for adherence which could affect outcome in these patients. Also multiple medications,

prolonged treatment and risk of recurrences can affect the adherence to medications. Moreover further research should progress to identify a more cost effective and practical alternative for local drug delivery.

The limitations in this study was mainly the low sample size that was used for prospective study. Also the period of follow up of adherence was limited and a greater amount of data could have been elucidated if the period of study was extended. For adherence to medications, there are multiple other factors which can affect outcomes like social, psychological and economic factors. Moreover there is a need for additional research in this field that will help to improve outcome in patients.

CONCLUSION

Most of the wounds healed after surgical debridement of skin infection and initiation of medical therapy at the first follow up visit. Surgical intervention was the most common risk factor associated with development of this infection. Adherence to medical therapy was medium- low in most of the patients. There was also no statistically significant association between the adherence to medical therapy and wound healing.

The complications associated with treatment for atypical mycobacterial infection of the skin and soft tissue include both medical and surgical, which needs to be addressed adequately for optimal outcome of the treatment regimen. However there was no statistically significant association between the complications associated with therapy and adherence to medical therapy. But in clinical context this is of importance as further study is required with larger numbers, to establish a correlation between the two.

It is noticed that complete surgical debridement would be the cornerstone for treatment as it eliminates the risk of recurrences. However this requires further study, preferably randomised controlled trials, to substantiate this.

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ANNEXURE-I

CHRISTIAN MEDICAL COLLEGE, VELLORE

Department of General Surgery

Patient Information Sheet

What is this study about?

Ans: This is a research to understand the population characteristics, risk factors, causes and results in individuals treated for atypical mycobacterial infection after surgery. The occurrence of this previously thought "uncommon" infection is progressively increasing, and despite an increase in knowledge of this infection, they are still a difficult condition to treat. The main aim of this study is to observe the various results after surgical treatment of atypical mycobacterial infection in a patient.

What is the duration of this study?

Ans: Duration of this study for a person with the disease will be of 3 months.

How is study going to be done? What should I do in this study? Is it necessary to be a part of this study?

Ans: You will be given this information sheet and explained about the study in detail by the primary investigator (Doctor). Consent will be sought from you at this time. Following consent and participation into the study, information like your physical profile, site of disease, medical therapy and any resultant complication and incidence of reappearance of lesion will be studied. You will also be provided a separate patient-proforma to fill during the course of therapy, which will not only help you to keep a track of daily medication taken, but also help the treating physician to monitor the adherence to medical therapy. On follow-up you are required to bring this patient-proforma and submit it to the treating physician. All the other requisite information will be obtained from the intra-hospital database and by communication with you through email and telephone.

Is there any adverse effects of this study? Is there any benefit of this study?

Ans: As this is an observational study, there will be no additional intervention to the existing therapy, and thereby no adverse effect or discomfort to you as a result of this study. The results of this study can help to determine the adherence to therapy, any reappearance of lesion with the current standard therapy, complications of medical therapy or surgical treatment and other related outcomes, and therefore help in the increase the knowledge and better the treatment to patients affected with the same disease. The patient-proforma sheet provided will help you to keep a record of

medications taken, record any missed medication and help the treating physician to tailor therapy when you come for follow up.

How will my confidentiality/privacy be maintained?

You will be allotted an enrolment number and this record will be maintained only by the primary investigator. All other information will remain confidential and will not be shared with anyone else. Therefore the primary investigator (Doctor) remains to have sole access to your medical records.

What are my responsibilities if I enroll in this study?

After enrolment into this study you are expected to responsibly come for followup and further treatment as advised. You will need to respond to questionnaire regarding the ongoing therapy and bring the patient-proforma provided.

Is it compulsory to participate in this study?

This is a research study which requires your voluntary participation, and you can withdraw from the study at any time. Refusal to participate will not involve any penalty or loss of benefits to which you are otherwise entitled to.

Whom can I contact if I have any queries?

Any queries related to the study will be clarified by the primary investigator(undersigned) by telephonic or email communication.

Primary Investigator

Dr.Markose Mathew
Post Graduate Registrar,
Department of General Surgery,
Christian Medical College, Vellore
Phone Number(M): 9815636239
Email- markose500@rediffmail.com

ANNEXURE-II

Informed Consent Form for Subjects

Study Title: An observational study to assess the adherence to medical therapy, complications associated and outcomes in patient post-surgical debridement for atypical mycobacterial infection from January 2015 to March 2019

Study Number: _____

Subject's Initials: _____

Subject's Name: _____

Date of Birth / Age: _____

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

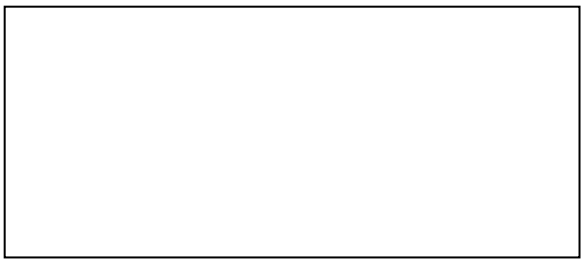
(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: _____ Signature:

Or 

Representative: _____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

ANNEXURE-III

Case No:

PATIENT PROFORMA SHEET

Kindly tick(√) the days when you have taken the medications, Kindly cross(X) the days when you have not taken the medications

Name of medication: Dosage: Date started:

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90

Reason(s) for not taking medication:

Name of medication: Dosage: Date started:

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90

Name of medication: Dosage: Date started:

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30
31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50
51	52	53	54	55	56	57	58	59	60
61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90

ANNEXURE-IV

CHRISTIAN MEDICAL COLLEGE,VELLORE

DEPARTMENT OF GENERAL SURGERY AND DEPARTMENT OF INFECTIOUS DISEASE

An observational study to assess the adherence to medical therapy, complications associated and outcomes in patient post-surgical debridement for atypical mycobacterial infection from January 2015 to March 2019

PROFORMA

Demographic profile:

Name: _____ Date of surgery: ☐☐☐☐☐☐

Age(years): ☐☐☐ Hospital No: ☐☐☐☐☐☐☐

Gender: ☐ M-1 ☐ F-2 Phone No: _____

Date of visit ☐☐-☐☐-☐☐

Comorbidities: Yes-1/No-0

DIABETES MELLITUS-2	
HYPOTHYROIDISM	
HYPERTENSION	
CHRONIC KIDNEY DISEASE	
OTHERS	

_____

Site of the lesion-. Yes-1/No-0

ANTERIOR ABDOMINAL WALL		
UPPER EXTREMITY		

LOWER EXTREMITY		
OTHER REGIONS		

Other regions: _____

Predisposing factor:

1.Surgical procedure- Yes-1/ No-0. If Yes-1 then:

OPEN SURGERY/LAPAROSCOPY/COSMETIC SURGERY	ELECTIVE SURGERY/EMERGENCY SURGERY	CMC/LOCAL CENTRE

2.Injection- Yes-1/ No-0. If Yes-1 then:

INTRAVENOUS	INTRAMUSCULAR	SUBCUTANEOUS

3.Occupation based- Yes-1/ No-0

4.Idiopathic- Yes-1/No-0

5. Others- _____

Adherence: Number of medications required to be taken(NUMERICAL)

Number of medications taken: (NUMERICAL)

Duration of medications taken (weeks): (NUMERICAL)

Were medications missed: Yes-1/ No-0

If medications were missed, was it due to medical therapy: Yes-1/ No-0

Patient proforma filled and submitted: Yes-1/ No-0

8 item morisky adherence scale score: High adherence-1

Medium adherence-2

Low adherence -3

Outcomes:

Whether wound healed at 3 months: Yes-1 /No-0

Recurrence: Yes-1/ No-0

Reoperation/re-debridement done or planned for the patient: Yes-1/No-0

Complications associated with therapy:

Surgical complication- Yes-1/ No-0

If Yes-1 then,

Wound Infection-

Bleeding from wound site-

Hernia-

Evisceration-

Others-

_____

Medical complication- Yes-1/ No-0

If Yes-1 then,

Ototoxicity-

Nephrotoxicity-

Vertigo -

Ataxia-

Nausea-

Others- _____

	YES	NO
1. Do you sometimes forget to take your medication?		
2. People sometimes miss taking their medications for reasons other than forgetting. Over the past 2 weeks, were there any days when you did not take your medication?		
3. Have you ever cut back or stopped taking your medication without telling your doctor because you felt worse when you took it?		
4. When you travel or leave home, do you sometimes forget to bring your medication?		
5. Did you take all your medication yesterday?		
6. When you feel like your symptoms are under control, do you sometimes stop taking your medication?		
7. Taking medication every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your treatment plan?		
8. How often do you have difficulty remembering to take all your medication? Never/Rarely…….. Once in a while…….. Sometimes…….. Usually…….. All the time……..		

Score= 0→ High adherence
1-2→Medium adherence
3-8→ Low adherence