Original Article

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Rapid Grower Non-Tuberculos Mycobacteria as Causative Agent for Surgical Site Infections: A Retrospective Study

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Abstract

Background: Rapidly growing mycobacteria (RGM) have become very important organisms responsible for surgical site infections (SSIs).

Aim: We retrospectively studied the occurrence of SSIs due to RGM seen in the western part of India, over a period of 3 years.

Methods: This retrospective study was done at six centers from January 2014 to December 2016, which included 73 patients aged 22–78 years, who had undergone open surgeries (coronary artery bypass grafting [CABG], caesarean section, hernioplasty, open cholecystectomy, breast surgery) and scope related surgeries (diagnostic laparoscopy, percutaneous nephrolithotomy, lap cholecystectomy), and had non-healing ulcers of >30 days. Gram stain and modified Ziehl–Neelsen staining methods were used for microbial examination. Culture media included sheep blood agar, chocolate agar, MacConkey agar and Sabourad dextrose agar. Isolates were identified using biochemical tests or molecular methods and the antimicrobial susceptibility pattern was studied by standard microbiologic procedures.

Findings: Mycobacterium fortuitum (42.5%), *Mycobacterium chelonae* (30.1%) and *Mycobacterium abscessus* (27.4%) were isolated by routine microbiological techniques. Amikacin, moxifloxacin and clarithromycin were given to all the patients. Overall, cure rate was achieved in 70 (95.9%) out of 73 patients; 3 were not cured - one with CABG having *M. fortuitum*, one with hernioplasty having *M. chelonae* and one lost to follow-up.

Conclusion: This study confirms the association of RGM with nonhealing SSIs. Treatment with combination of antibiotics such as clarithromycin, amikacin and moxifloxacin may be an ideal choice, and require at least six months of treatment for complete cure.

Keywords: Infection; Rapidly Growing Mycobacteria; RGM; Surgical Site Infection; SSI; Surgery.

Introduction

Surgical site infections (SSIs), a leading cause of healthcare-associated infections (HCAIs), are defined as invasive surgical procedure led infections in the wound. Numerous organisms are responsible for SSIs including the rapidly growing mycobacteria (RGM). Apart from the SSIs, RGM is responsible for pulmonary infections, lymphadenitis, disseminated infections, skin and soft tissue infections, musculoskeletal infections, prosthetic devise infections and catheter related infections [1]. Increasing reports in the literature, referral center experiences, and data from the Infectious Disease Society of America Emerging Infectious Disease Network suggest that RGM accounts for a large numbers of infections [2].

The RGM are ubiquitous environmental acid-fast bacilli, which after inoculums grow rapidly (within 7 days) in culture media [3]. M. fortuitum, M. chelonae and M. abscessus are important human pathogen RGMs. In the recent decades, the incidence of rapid growing non-tuberculous mycobacterium (NTM), especially with a trend of surgical procedures, has reportedly increased [4]. These atypical mycobacteria are resistant to sterilizers, standard disinfectants and antiseptics, and are widely distributed in the environment and

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may contaminate municipal water supply. These organisms can cause surgical wound infections and post-injection abscesses due to improperly sterilized instruments and endoscopes and usually result in localized infections; hence, systemic symptoms are often absent. Because the mycobacterial culture is not routinely done for surgical wound infections; the diagnosis is usually much delayed. These RGM organisms are most probably transmitted by aerosol, soil, dust, water, injection or by skin inoculation, but person to person spread is rare [5].

The RGM are hydrophobic organisms which are difficult to eradicate due to their resistance to disinfectants (alkaline glutaraldehydes, organomercurials and chlorine) owing to their ability to form biofilms for successful survival in the environment. The possible reason to acquire this infection could be due to brushing away of these organisms from the biofilm in a water pipe or device [6].

The frequency of non-tubercular mycobacteria (NTM) is increasing globally, however, the exact incidence is not known. Herein, we describe a retrospective data analysis of surgical site infections due to rapidly growing mycobacteria (RGM) seen in the western part of India, over a period of 3 years.

Materials and Methods

This study included 73 adult patients (Males: 34; Females: 39), 22 – 78 years of age, who had undergone two major types of surgeries – open surgeries and scope related surgeries, and presented with non-healing ulcers of >30 days. The study data was collected from six centers in Gujarat, India, from January 2014 to December 2016.

Open surgeries included caesarean sections, hernioplasty, coronary artery bypass graft (CABG) and open cholecystectomy. Laparoscopic surgeries were diagnostic laparoscopy, laparoscopic cholecystectomy and percutaneous nephrolithotomy. The key inclusion criteria were postoperative surgical site wound infections, signs of inflammation of the skin and abscesses or abscess drainage at the wound site with no complaints of fever, and antibiotic usage for pyogenic infections, but not responding. All acute postoperative wound infections of < 7 days duration from the time of surgery were excluded from this study. Patients with associated major diseases comorbidities (except hypertension and diabetes mellitus) or immunosuppression (e.g., HIV infection) were also excluded.

This study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and in accordance with the International Conference on Harmonization's Good Clinical Practice guidelines, applicable regulatory requirements, and in compliance with the protocol.

Microbiological Investigations

(1) Purulent material of 0.5-3.0 mL aspirate was collected with the help of a sterile syringe or

(2) two swabs from the wound site from the depth of sinus tract or

(3) excised tissue from sinus tract, were processed for the identification of the causative agents. Furthermore, in patients who had undergone herniotomy, the mesh was also sent for analysis. All the specimens were subjected to aerobic, fungal and mycobacterial culture and staining.

Specimens were inoculated in sheep blood agar, chocolate agar, MacConkey agar (without crystal violet and bile salts) and Sabourad dextrose agar. Postdigestion and decontamination by Petroff's method, specimens were inoculated in Lowenstein Jensen (L-J) medium and mycobacterial growth indicator tube (MGIT) medium. Specimen inoculated sheep blood agar, chocolate agar, MacConkey agar were incubated at 37oC±1 for 7 days, Sabourad dextrose agar were incubated at 25oC±1 and 37oC±1 for 14 days. L-J and MGIT were incubated at 37oC±1 for 30 days. Microscopy (gram stain and modified Ziehl Neelsen stain) was performed on direct specimens as well as from the culture growth. The isolated organisms were identified as per the standard bacteriological techniques. Acid fast stain positive colonies that were isolated from blood agar within 7 days of incubation were subjected to MPT-64 antigen testing. MPT-64 antigen negative cultures were further subjected to molecular typing (species identification) by Line Probe assay - GenoType Mycobacterium CM VER 2.0. [6]

Apart from the clinical specimens, water samples from different sources were collected from six hospitals (three had own bore water supply system and three had municipal water supply).

Outcome Measures

Evaluation criteria for cure were healing of the skin incision and disappearance of the sinus tract confirmed by ultrasound sonography.

Results

The baseline and demographic characteristics of the patients are provided in Table 1. A total of 73 patients (39 females and 34 males) with a median age of 47 years (range: 22-78 year) were identified with surgical site soft tissue infections due to RGM. Patients had undergone surgery in different surgical settings: scope related surgery (n=43), and open surgery (n=30). Overall, 72 patients completed the study and one patient was lost to follow-up.

Table 1: Baseline and Demographic Characteristics

Characteristic	Patients (N=73)
Gender, n (%)	
Male	39 (53.4%)
Female	34 (46.6%)
Age group (years), n (%)	
<20	0 (0%)
20-40	25 (34.2%)
41-60	31 (42.5%)
>60	17 (23.3%)
Scope related surgery, n (%)	**43 (58.9%)**
Diagnostic laparoscopy	21 (28.8%)
PCNL	16 (21.9%)
Lap cholecystectomy	06 (8.2%)
Open surgery, n (%)	**30 (41.1%)**
CABG	08 (11.0%)
Caesarean section	07 (9.6%)
Hernioplasty	07 (9.6%)
Open cholecystectomy	05 (6.8%)
Breast surgery	03 (4.1%)

CABG, coronary artery bypass grafting; PCNL, percutaneous nephrolithotomy.

Summary of 73 RGM cases

All the patients included in the study were initially treated by repeated incision and drainage of their lesions, and started on antibiotics such as amoxycillin+clavulanic acid, second- or third-generation cephalosporins (cefuroxime or cefixime), gentamicin, linezolid, or local application of mupirocin. Overall, cure rate was achieved in 70 (95.9%) out of 73 patients. The local healthcare record revealed that in majority (>90%) of the cases, pyogenic culture was sterile. Even after repeated incision and drainage and antibiotic treatment, wounds were not completely healed. The duration of non-healing wounds ranged from 50 to 120 days (Table 2).

Table 2: Days between surgery and sample collection in RGM positive cases

Days between surgery and sample collection	Patients, n (%) N=73
<50 Days	0 (0%)
50-60 Days	26 (35.6%)
60-70 Days	31 (42.5%)
70-80 Days	07 (9.6%)
80-120 Days	09 (12.3%)

RGM, rapidly growing mycobacteria

Discharging sinus with subcutaneous abscess (89.04%) with no systemic signs and symptoms of sepsis was the persistent feature of these chronic post-operative non healing wounds. Species identified were M. fortuitum, M. chelonae and M. abscessus. The frequency of RGM species is presented in Table 3.

Table 3: Frequency of RGM species

RGM species	Patients, n (%)
Isolated (N=73)	
Mycobacterium fortuitum	31 (42.5%)
Mycobacterium chelonae	22 (30.1%)
Mycobacterium abscessus	20 (27.4%)
Scope related surgeries (N=43)	
Mycobacterium fortuitum	16 (37.2%)
Mycobacterium chelonae	14 (32.6%)
Mycobacterium abscessus	13 (30.2%)
Open surgeries (N=30)	
Mycobacterium fortuitum	15 (50.0%)
Mycobacterium chelonae	08 (26.7%)
Mycobacterium abscessus	07 (23.3%)

Among the total study cases, 59 (80.8%) cultures were found to be sterile for routine pyogenic infections and 10 (13.7%) had isolated Staphylococcus epidermidis (likely to be contaminant), 2 (2.7%) Escherichia coli and 1 (1.4%) each of Morganella morganii and Proteus mirabilis (Table 4).

Table 4: Frequency of concomitant organisms isolated other than RGM

Concomitant other organism isolated	Patients, n (%)
No other bacterial pathogen isolated	59 (80.8%)
Staphylococcus epidermidis	10 (13.7%)
Escherichia coli	02 (2.7%)
Morganella morganii	01 (1.4%)
Proteus mirabilis	01 (1.4%)

RGM, rapidly growing mycobacetria

Overall, 32 (43.8%) cases were acid-fast bacilli (AFB) smear positive identified through the modified AFB stain from pus (Table 5).

Table 5: Direct modified AFB stain from pus

Parameter	Patients, n (%) N=73
Positive	32 (43.8%)
Negative	41 (56.2%)

Water samples were processed by membrane filtration technique and followed our NTM culture protocol with 2 samples positive among 6 specimens. Both isolates were M. fortuitum and the samples were from the hospitals with bore water supply systems.

Surgical excision and drainage of the abscess were performed in patients with sinus tract and abscess nodules. Wide surgical excisions were performed on patients having multiple sinus discharge. Foreign bodies (hernioplasty mesh) were removed.

All the patients were started with once daily injections of amikacin (10 mg/kg/day) for 3 months, once daily oral moxifloxacin 400 mg for a minimum of 6 months and thrice daily clarithromycin

250 mg for 6 months. In addition, imipenem injections (for 7days) and oral linezolid (for 2 weeks) were also administered in a few patients for the treatment of superadded infections.

All the patients responded well and were cured; skin incisions were healed and ultrasound sonography showed disappearance of the sinus tract, except three patients - one patient with coronary artery bypass graft (CABG) surgery having M. fortuitum, one patient with hernioplasty surgery having M. chelonae and one patient who was lost to follow-up.

Discussion

Mycobacterial species other than *M. Tuberculosis* complex and *M. leprae* are classified as NTM. The RGM are opportunistic pathogens, which are widely distributed in the environment. *M* chelonae, M abscessus and M fortuitum are classified as a rapid grower NTM according to the Runyon classification [7]. The NTM originated SSIs contribute to the high morbidity and mortality rates because of hospital acquired infections [8].

Globally, many outbreaks of SSIs due to RGM have been reported in countries like Dominican Republic [10], India [11] ,Karnataka, India [6], Sweden [12] USA [13-14], Korea [15], and Brazil [16]. There are also a large number of pulmonary RGM infections reported but our study included only SSIs due to RGM.

There are many causative agents for postoperative SSIs. The most common pyogeinc organisms are *Staphylococcus aureus*, *Enterobacteriaciae*, and *Enterococcus* species [18,19]. Initially, clinicians were treating the SSIs as pyogenic infections; however, repeated pyogenic cultures showed no growth. In our study too, 59 (80.8%) of 73 cases showed no growth, and 10 (13.7%) were reported to have *Staphylococcus epidermidis,* which may be considered as a skin contaminant. The suspected RGM caused postoperative wound infection can generally be identified in some weeks to some months. Despite repeated sterile pyogenic cultures and routine antibiotics, non-healing wounds with pus discharge and sinus tract formation are the characteristics of RGM infection and should be the reason for suspicions of RGM infection [6]. Similarly, in our case series, the incubation period ranged from 50 days to 120 days as seen in the similar study by Kavita et al [6]. On the contrary, infections due to other pyogenic bacteria have a shorter incubation period as compared to RGM which have a longer incubation period ranging from several days to several months. After antimicrobial administration against pyogenic bacteria and sterile routine cultures from the infected sites, there were no signs of clinical response, which is indicative of RGM infection.

The *M. tuberculosis* and NTM patients require an entirely different management, and prompt isolation, detection, and differentiation are necessary for suitable management procedures. Positive microscopy alone cannot differentiate *M. tuberculosis* complex from NTM infection, and this may cause diagnostic and clinical dilemmas; hence the correct identification of NTM is very important for optimal management [20-21]. In our study 32 (43.8%) cases were AFB smear positive and required differentiation between NTM and *M. tuberculosis*. Identification of RGM up to species level using biochemical and molecular methods is required as different therapeutic as well as prevention strategies have to be adopted [22]. Though, there are many outbreaks and sporadic cases of postsurgical RGM infection that have been reported, the exact species specific prevalence cannot be measured. In our study, the overall prevalence of *Mycobacterium fortuitum* (42.5%) was the highest followed by *Mycobacterium chelonae* (30.1%) and *Mycobacterium abscessus* (27.4%). Similarly, in patients with scope

related surgeries, the isolation of *Mycobacterium fortuitum* (37.2%) was the highest followed by *Mycobacterium chelonae* (32.6%) and *Mycobacterium abscessus* (30.2%) and in post open surgeries the highest was *Mycobacterium fortuitum* (50.0%) followed by *Mycobacterium chelonae* (26.7%) and *Mycobacterium abscessus* (23.3%).

The RGM has a widespread distribution in the environment; detectable in soil and water, including both natural and treated water sources [23]. In our study, 2 water samples were positive among 6 specimens taken from different sources in the hospitals where majority of the surgeries were done. Both isolates were *Mycobacterium fortuitum.* Breeched sterile technique and nonsterile water exposure are the leading causes of acquired infections by these organisms in healthcare settings [24]. Mycobacteria are relatively resistant to disinfectants and may be able to grow in a wide range of temperatures (especially high temperatures). Furthermore, resistance of these mycobacteria to quaternary ammonium compounds, phenolics, iodophors, and glutaraldehyde, which are commonly used disinfecting agents, is also established [24]. The lack of adherence to the specified cleaning and maintenance requirement also contribute to increased NTM growth [24]. Most of the health care associated mycobacterial outbreaks and pseudo-outbreaks have involved RGM, especially *M. fortuitum* and *M. abscessus [24]*. Health care associated pseudo-outbreaks have most commonly been associated with scopes including the use of contaminated topical anesthesia, contaminated and/or malfunctioning individual scopes, contaminated terminal rinse water (tap water), and contaminated automated endoscope washers that used a terminal tap water rinse cycle [24]. There are also possibilities of the inoculation of these environmental bacteria into the open or sutured wound after discharge from the hospital.

According to an official ATS (American Thoracic Society)/IDSA (Infectious Disease Society of America) Statement for diagnosis, treatment, and prevention of Nontuberculous Mycobacterial Diseases based on the various studies, *M fortuitum* isolates are susceptible to amikacin (100%), ciprofloxacin and ofloxacin (100%), sulfonamides (100%), cefoxitin (50%), imipenem (100%), clarithromycin (80%), and doxycycline (50%) [24].

A ≥ 4 month of therapy with a minimum of two agents with *in vitro* activity against the clinical isolate is recommended to achieve high cure rates against serious skin, bone, and soft tissue infections. *M. abscessus* isolates are uniformly resistant to the standard antituberculous agents, whereas these are 100% susceptible to clarithromycin, 90% to amikacin, and 70% to cefoxitin, with low or intermediate MICs to linezolid for some isolates. Macrolides are the only oral agents that have *in vitro* activity against *M. abscessus.*

Clarithromycin at a dose 1,000 mg/day or azithromycin 250 mg/day in combination with parenteral medications (amikacin, cefoxitin, or imipenem) should be administered for serious skin, soft tissue, and bone infections caused by *M. abscessus* [25-26] .

M. chelonae isolates are susceptible or intermediate in susceptibility to tobramycin (l00%), clarithromycin (l00%), linezolid (90%), imipenem (60%), amikacin (50%), clofazimine, doxycycline (25%), and ciprofloxacin (20%) [27]. In our study, to reduce the bacterial load, all the patients initially underwent surgical procedures to drain existing abscesses and also to resect any immature nodules. In addition, mesh was also removed in the herniotomy patients. A cure rate of 95.9% (70 out of 73 patients) was achieved. Though cefoxitin is recommended for *M fortuitum* and *M. abscessus,* it is not available in India and hence not used.

Conclusion

Nonhealing wounds with pus discharge and sinus tract formation in spite of the repeated sterile pyogenic culture and antibiotic use are indicative of RGM infections. Strict aseptic precautions and sterilization protocols for medical equipments, particularly laparoscopes and fiberoptic scopes and instruments as per manufacturer's instruction should be followed in all set ups. Tap water should not be used for automated endoscopic washing machines or for manual cleaning. Post disinfection, the instruments should have a terminal alcohol rinse or sterile saline rinse. Open wounds should not be washed or contaminated with tap water. RGM infection should not be treated with monotheraphy. A combination of clarithromycin, amikacin and moxifloxacin may be an ideal choice and should be administered based either on in vitro susceptibility or epidemiological data. To have complete cure and prevent recurrence, treatment should be continued for at least six months.

Limitations

Antimicrobial susceptibility testing were not performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

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