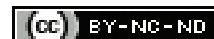


Nosocomial Outbreak of Port-site Infection due to Atypical Mycobacteria following Laparoscopy: Suggested Infection Control Strategies

MUKESH SHARMA¹, DIVYA GAUTAM², LEIMAPOKAM SUMITRA DEVI³, MOUMITA SARDAR⁴

ABSTRACT

Introduction: Atypical mycobacteria can survive in conditions that make them hard to eradicate, despite using the standard decontamination procedures and protocols. Thus, errors in sterilisation techniques for laparoscopic instruments can be responsible for outbreaks caused by such bacteria and make it a problem mainly affecting developing countries including India.

Aim: To investigate the outbreak of postlaparoscopic wound infection caused by atypical mycobacteria.

Materials and Methods: An institution based cross-sectional study was conducted over a two month, period from January to February 2020. A total of 14 patients presented with postlaparoscopic surgical site wound infections were evaluated with Ziehl-Neelsen (ZN) staining and pus culture on Lowenstein Jensen (LJ) medium and subsequently treated with appropriate antibiotics. For further

investigation of the outbreak, environmental samples were collected and isolation rates (percentage) of atypical mycobacteria from these samples were analysed.

Results: All the patients included in the study were diagnosed with postlaparoscopic surgical site wound infections caused by atypical mycobacteria. Infection control investigation of the Operation Theatres (OTs) revealed multiple sources of atypical mycobacterial contamination viz., laparoscopic surgical instruments, used disinfectant (gluteraldehyde disinfectant solution) and tap aerators.

Conclusion: Negative routine bacterial culture report of samples collected from port-sites should be further investigated for other aetiology e.g., atypical mycobacteria which do not grow on routine bacterial culture. Since, high indices of suspicion followed by timely and efficient management of patients with postlaparoscopic surgical site infection are of critical importance.

Keywords: Laparoscopic wound infections, Non tuberculous mycobacteria, Used disinfectant

INTRODUCTION

Atypical mycobacteria or Non-Tuberculous Mycobacteria (NTM) species can be commonly seen in samples of soil and water in geographic locations [1-3]. Their prevalence is unknown in India due to limited data available in conjunction with a lack of laboratory capacity to diagnose these infections. The overall isolation rate of atypical mycobacteria in India has been reported to range from 0.5- 8.6% [4].

Due to their ability to form biofilms, atypical mycobacteria are able to survive in conditions that make them hard to eradicate, despite using the standard decontamination procedures and protocols [1]. Usually atypical mycobacteria are less virulent in humans compared to *Mycobacterium tuberculosis* and therefore in a healthy host they tend not to cause disease [5]. It is usually in cases where host defenses are compromised, and these organisms manifest clinically.

Nosocomial infection outbreaks are generally caused by the rapid grower species and are almost always reported in context of contaminated instruments and procedural equipment [6]. Rapidly growing *Mycobacterium* indicates that the species is able to grow within seven days from the time of inoculation in culture medium [7,8].

Since, atypical mycobacteria are able to colonise tap water, they can easily contaminate solutions including disinfectants. These infections have thus been a source of significant morbidity for patients recovering from laparoscopic surgeries [5]. Errors in sterilisation techniques for laparoscopic instruments are mostly responsible for such outbreaks. This becomes a problem affecting mainly developing countries like India where single use instruments are not as widely available as in the West [9]. Earlier reports from India have also suggested prolene material (used in sutures) as a possible cause of infection [10,11]. Since, skin and skin structure infections caused by atypical mycobacteria are variable in clinical presentation [12], the initial diagnosis is a clinical one dependent on history, physical examination and high level of suspicion based on the prevalence of atypical mycobacteria in the geographical location.

Early identification and diagnosis of such cases are critical to the successful outcome as these bacteria do not respond to the conventional anti-mycobacterial treatment and second line chemotherapy is the principle management option [5]. Strict adherence to the recommended sterilisation protocol is a must for prevention of postlaparoscopic port-site infections. The present study location is an upcoming tertiary care hospital, thus identification of such infections is necessary in order to evaluate the sterilisation protocol being followed in the hospital. Therefore, the present study was undertaken to investigate outbreak of postlaparoscopic wound infection caused by atypical mycobacteria.

MATERIALS AND METHODS

The present institution based cross-sectional study was carried out over a period of two months between January to February 2020 at an upcoming tertiary care Medical College Hospital in rural belt of Haryana, Northern India. A total of 14 patients with postlaparoscopic port-site infection were included in the study. Informed consent was obtained from all the study participants. This study was approved by Institutional Ethical Committee (FMHS/IEC/F/012/01/20/33).

All patients with postlaparoscopic wound infection, presenting three to four weeks following surgery, over a period of two months (January 2020 to February 2020) were included in the study.

Inclusion criteria: The patients presented with non healing persistent discharging sinuses at port-sites, with wound suppuration and limited erythema, pain and fever. At the time of discharge from the hospital, none of them had showed any signs of surgical wound infections or complained of febrile illness were included in this study.

Exclusion criteria: Patient with wound infection presenting after non laparoscopic surgery were excluded in this study.

Study Procedure

Specimen collection and processing: Pus was collected from the site of wound infection using standard protocol. Margins of the wound were avoided to decrease risk of cross contamination of the sample. All pus samples were evaluated with ZN staining and culture on LJ medium [13].

Environmental sampling and processing: To further investigate the source of the outbreak, samples were also collected from surgical instruments, used disinfectant solution and from bottom of the disinfectant tray, mouth of the tap aerators and supplying water tank reservoir and were analysed.

From laparoscopic instruments: Sterile swabs premoistened with sterile saline immediately before use was used to collect sample within the outer surface of re-usable laparoscopic surgical instruments.

For used disinfectant solution: Two sterile swabs were used for collection of samples viz., used disinfectant solution and from bottom of the disinfectant tray from all the major OTs to check the effectiveness of the disinfectants and presence of biofilms.

From tap aerators: The inner side of tap aerator mouth were swabbed using sterile swabs premoistened with sterile saline immediately before use to detect presence of residual biofilms.

From water tank reservoir: Approximately, 200 mL of water samples from all the water tank reservoirs were collected in sterile glass stoppered bottles and immediately transported to the laboratory.

All the environmental swab samples and the residue obtained after filtration of the water samples from reservoir tanks were subjected to ZN staining and conventional culture on LJ medium.

STATISTICAL ANALYSIS

Descriptive analysis was done and data was calculated in percentages.

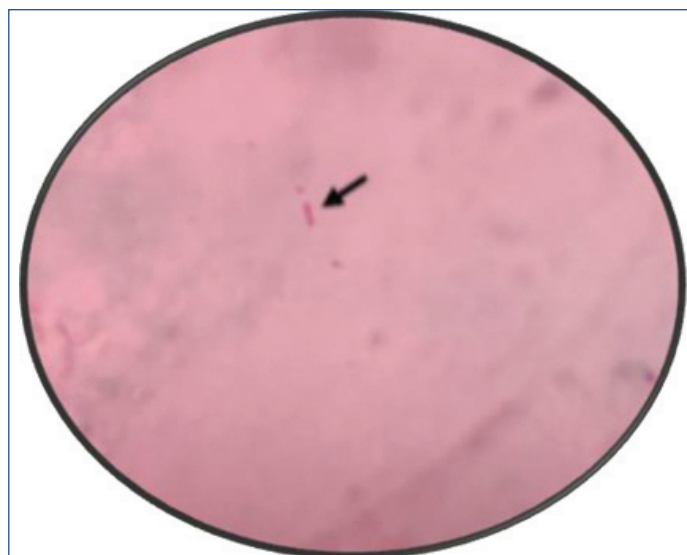
RESULTS

A total of 14 patients (eight males and six females, of median age 45 years) with laparoscopic port hole infection who presented three to four weeks postsurgery, were positive for Acid Fast Bacilli (AFB) on ZN staining and conventional culture of pus on LJ media revealed growth of atypical mycobacteria (rapid growers) within seven days of incubation [Table/Fig-1,2]. All the patients were treated with clarithromycin (500 mg), linezolid (600 mg) and ciprofloxacin (500 mg) twice daily for three months along with open drainage of nodules and dressings.

Sr. No.	Age (years)/Sex	Type of laparoscopic surgery	Number of ports	
			Total	Infected
1	28/Male	Cholecystectomy	4	1
2	42/Male	Appendectomy	3	1
3	51/Male	Cholecystectomy	4	1
4	60/Male	Cholecystectomy	4	2
5	68/Male	Cholecystectomy	4	1
6	45/Male	Appendectomy	3	1
7	60/Male	Cholecystectomy	4	2
8	25/Male	Cholecystectomy	4	1
9	47/Female	Cholecystectomy	4	1
10	62/Female	Appendectomy	3	1
11	60/Female	Cholecystectomy	4	1
12	35/Female	Cholecystectomy	4	1
13	29/Female	Cholecystectomy	4	1
14	42/Female	Cholecystectomy	4	2

[Table/Fig-1]: Demographic and clinical data of the study participants.

Atypical mycobacteria were first suspected in the microbiology laboratory from a single pus sample taken from the surgical site infection of a postlaparoscopic surgery patient in the month of



[Table/Fig-2]: Acid fast bacilli seen in Ziehl-Neelsen stained slide prepared from pus sample (under 100X).

January 2020. Gram staining of the sample did not reveal any microorganism and bacteriological culture on routine media was also sterile. This raised the suspicion and therefore ZN staining was performed which revealed AFB. The results of ZN staining were immediately reported to the concerned surgeon. Subsequently, culture on LJ medium revealed atypical mycobacteria (rapid grower) on the fourth day of incubation. A combination of ciprofloxacin and amikacin were given to the patient for 28 days and the patient responded appropriately to the treatment. Within a week, another postlaparoscopic surgery patient presented to the Outpatient Department with similar clinical presentation and laboratory findings. Thus, an OT investigation request was received and set up by the Hospital Infection Control (HIC) team. Environmental samples were collected from surgical instruments, used disinfectant solution and from bottom of the disinfectant trays as well as from mouth of the tap aerators. Water tanks supplying corresponding OTs were identified and samples collected.

Out of two laparoscopic surgical instruments swabs collected, one was positive for atypical mycobacteria. A total of 14 swabs, seven each from the used disinfectant solutions and from bottom of the disinfectant tray were collected and analysed, positivity for atypical mycobacteria was found to be similar for both the sample types 3 (42.9%) out of seven. Out of three tap aerator swabs collected during the outbreak investigation, two samples were positive for atypical mycobacteria. However, none of the samples from water tank were positive for atypical mycobacteria [Table/Fig-3].

Environmental source		Total number of samples, n	Positive for atypical mycobacteria, n (%)
Laparoscopic surgical instruments		02	01 (50)
Disinfectants	Used solution	07	03 (42.9)
	Tray	07	03 (42.9)
Tap aerators		03	02 (66.7)
Water tank reservoir		02	00

[Table/Fig-3]: Isolation of atypical mycobacteria from environmental samples.

DISCUSSION

Infections due to atypical mycobacteria in surgical patients have been reported from a wide variety of settings like injection site abscesses, cellulitis following rhinoplasty, after liposuction and augmentation mammoplasty, outbreaks of sternal wound infections, endocarditis after cardiac surgery, vein graft harvest site infections, keratitis after laser in situ keratomileusis and use of contaminated endoscopes [14-18]. Port-site infections with NTM, are being increasingly recognised as a significant source of morbidity in postoperative laparoscopic

cases [14,16,18]. The source of infection, in most cases, has been recognised as direct or indirect contamination of the port-site due to contaminated water.

The NTMs have a predilection for skin and soft tissues resulting in patients presenting with port-site infections three to four weeks postsurgery, and usually with five clinical stages [19].

Stage 1: A small tender nodule near the port-site.

Stage 2: Increase in the size and tenderness with inflammation of nodule, followed by pus discharge.

Stage 3: Reduced pain with continuously discharging sinus and necrosis of the overlying skin.

Stage 4: Chronic sinus with white or serous discharge.

Stage 5: Hyperpigmentation with necrosed skin and nodules appear at the other site.

Thus, when postlaparoscopic patients presented with non healing discharging sinuses at port-sites, which were sterile on routine gram staining and conventional bacteriological culture, suspicions were raised and the samples were processed for ZN staining and cultured on LJ media, which revealed the growth of atypical mycobacteria. The microbiology laboratory received samples of the pus from patients with similar clinical picture as the previous cases and proceeded to test for atypical mycobacteria due to a high level of suspicion. Present study revealed 14 such cases of port-site infections over a period of two months caused by atypical mycobacteria (rapid growers). Vijayraghavan R et al., reported a series of 145 port-site infections following laparoscopy, due to atypical mycobacteria, source being contaminated rinse water used for cleaning. The concerned doctor was immediately alerted, and the patients responded to a combination of clarithromycin and amikacin for 28 days [18].

The HIC team immediately took notice and acted on it, trying to locate the cause for the outbreak. They carried out an investigation in the major OTs, collecting samples from surgical instruments, used disinfectant solution and their trays, as well as from tap aerators. During the first OT investigation, swab testing revealed the glutaraldehyde solution, used for disinfection of surgical instruments, positive for atypical mycobacteria. Furthermore, tap aerator swabs were positive which triggered investigation of the hospital OT water source. Water tanks were identified and processed; however, they did not show presence of atypical mycobacteria. A second investigation was carried out in the minor OT, focused on the glutaraldehyde solutions and the trays used for disinfecting the scopes, which were also found to be positive for atypical mycobacteria. The investigation was launched due to high level of suspicion arising from a single case that originated from the general surgery department and a proactive HIC team.

The NTMs can colonise in tap water, natural water, sewage, and soil, thereby easily infecting solutions and disinfectants used in hospitals [20]. Duarte RS et al., in their study observed various factors to be responsible for postsurgical NTM infections: spread in aquatic environments for a long time, inadequate mechanical cleaning of surgical instruments, or dissemination inside commercially available non activated glutaraldehyde solutions [21].

Multiple approaches have been suggested as a part of an improved infection control strategy in light of these infections. Standard infection control policies advocates, all instruments should be cleaned and disinfected, potentially using ultrasonic technology [22], only after they have been dismantled so that organic material can be removed and patient to patient transmission of infection can be prevented. Moreover, reusable laparoscopic instruments sometimes have an outer sleeve where biofilms could easily form, if they are soaked in disinfectant fluids for prolonged periods, which will permit the survival of opportunistic pathogens [18]. Thus, such instruments must be dismantled and thoroughly brushed. According to Spaulding's classification, scopes that normally enter sterile tissues needs to be sterilised before each

use; if not feasible, must receive high level disinfection [23]. Rinsing of items should be with sterile water to prevent contamination with atypical mycobacteria in hospital water supply.

Current infection control guidelines recommend a minimum exposure time of 8-12 hours to achieve the desired level of sporicidal activity and the use of higher concentrations (3.4%) of glutaraldehyde disinfectants for scopes [20]. Despite clear guidelines, however, the practice in many locations in India, including the current setting, is to immerse instruments in 2-2.5% glutaraldehyde solution for 20 minutes which achieves disinfection but not sterilisation [24]. Spores often survive, gets deposited in the subcutaneous tissue during laparoscopic procedures, which later germinates, resulting in port-site infections after an incubation period of three to four weeks.

Lorena NSO et al., reported *Mycobacterium massiliense* to be resistant to higher concentration of glutaraldehyde (GTA, 7%), thus suggesting glutaraldehyde might not be effective for rapidly growing mycobacteria. Orthophthaldehyde (OPA; 0.55%) with a contact time of 12 minutes, which destroys all bacteria, fungi, and mycobacteria, and peracetic acid may be used for high-level disinfection with good efficacy [25]. Hydrogen peroxide (gas plasma and vaporised form) are also effective against NTM [26]. For heat sensitive instruments, Ethylene oxide (ETO) is also a good alternative. Authors suggest using higher concentrations of glutaraldehyde as per the guidelines and follow the correct exposure time to achieve desirable results [26]. Thus, HIC plays an important role in formulating institutional policies for sterilisation and disinfection protocols to be followed and ensuring strict adherence to them.

Furthermore, proper disposal of glutaraldehyde based disinfectants should be followed. These chemicals can be used for maximum of 100 cycles or a period of 14 days (2.5% glutaraldehyde) or 28 days (3.4% glutaraldehyde) [20]. In present study, HIC team noticed that no record of cycles count was being kept in the hospital and thus the chemicals did not have the right potency to achieve the desired level of sterilisation. Moreover, inadequate cleansing of disinfectant trays may be responsible for organisms surviving within biofilms which in turn, contaminated the instruments. Authors would like to highlight the importance of internal audit and record keeping and the responsibility to log the use of the solution so that it can be disposed of in a timely manner.

Authors also suggest replacing glutaraldehyde solution disinfection procedures of laparoscopic equipment with ETO gas sterilisation, as this has been shown to be highly effective in reducing atypical mycobacterial infections following laparoscopy, in various studies [18]. Keeping the laparoscopic instruments in a formalin chamber for 24 hours is another suggested alternative to glutaraldehyde solution however this method also requires strict protocol for cleaning of the instruments prior to placement in the chamber [20].

The practice of rinsing the instruments with boiled tap water to rinse off the glutaraldehyde may have caused the reintroduction of mycobacterial spores on the instruments as the tap aerators were contaminated [26]. A way to tackle this issue would be to use sterile water for rinsing so that recontamination is prevented. Furthermore, sites like tap aerators should be regularly disinfected to avoid colonisation. As revealed in present report, the water source was also found to be colonised with atypical mycobacteria. Regular cleaning of these areas is also suggested with monthly chlorination and annual cleansing of the tank. Finally, the use of disposable laparoscopic instruments, as is done in Western countries, is strongly advocated [9].

The treatment of atypical mycobacterial wound infections usually requires a multidisciplinary approach. There is no concrete agreement on regimen and duration of treatment. However, multiple sources in the literature state that a combination of antimicrobials has shown the greatest benefit [7,20]. The development of resistance during therapy is a recognised problem when mycobacterial infections are

treated with only a single active drug [24]. The literature supports antibiotics being given for a minimum period of three months or for a period of three to six weeks after the wound heals completely in order to prevent recurrence [27], however, this was not done in present study setting. Although, in some cases, response can be rapid after just one dose of therapy [28], it is important to stay vigilant as these infections are treatable and may have devastating outcomes if left untreated and may require surgical wound debridement [10]. There is currently a lack of data supporting the use of antibiotic prophylaxis for the prevention of port hole infections. Where recommendations are available, the need for the same is optional for laparoscopies of the upper gastrointestinal and biliary surgeries [29].

Limitation(s)

Due to non availability of facilities for further identification of the atypical mycobacteria/rapidly growing mycobacterial isolates, identification up to species level was not done.

CONCLUSION(S)

In this way, it can be seen that skillful work, with a high level of suspicion for atypical mycobacteria, can lead to efficient infection control strategies in order to improve and optimise patient care. These infections require to be diagnosed specifically also because they need to be treated with drugs other than the routine anti-tuberculous drugs. With the help of this report, authors want to make clinicians aware that atypical mycobacteria should be put in mind before starting treatment and that all acid fast bacteria positive smear should be further processed by culture in appropriate media. Proper sterilisation of instruments and adherence to strict infection control protocol is essential to prevent the occurrence of postlaparoscopic wound infections with atypical mycobacteria.

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