Abstract

**Background:** Port-site infection (PSI) is a prevailing, chronic, nagging, treatment refractory complication of laparoscopic surgery (LS). It neutralizes the advantages of minimally invasive surgery and increases morbidity, treatment cost of patient, leading to loss of confidence on operating surgeon. PSIs are preventable with appropriate preoperative, intraoperative, and postoperative measures. Atypical mycobacterium is most commonly associated with nonhealing postlaparoscopic wound infections, causing outbreaks or sporadic cases worldwide. **Purpose:** We retrospectively studied the occurrence of nontuberculous mycobacterium (NTM) from PSIs following LS that did not respond to antibiotics used for pyogenic infections and having sterile routine aerobic cultures and their antimicrobial susceptibility pattern to guide proper management. **Methods:** The study was done in a tertiary care hospital of Eastern India over a 1-year period which included PSI cases with delayed onset not responding to antibiotics, following different types of LS. Pus/discharge from 32 patients was collected and examined for isolation and identification of the causative agents. Gram stain and Ziehl–Neelsen staining methods were used for direct examination. Culture media included blood agar, Robertson’s cooked meat broth, MacConkey agar, and Lowenstein–Jensen medium. Isolates from the cases were identified using biochemical tests or molecular methods and studied the antimicrobial susceptibility pattern by the standard microbiologic procedures. **Results:** Mycobacterium abscessus (13) and Mycobacterium fortuitum (2) were isolated from 15 serosanguinous drainage obtained from 32 cases by routine microbiological techniques. All isolates analyzed for antimicrobial susceptibility pattern were highly sensitive to clarithromycin (93.3%), amikacin (93.3%), and imipenem (80%) but were variable to ciprofloxacin, ofloxacin, and linezolid. **Conclusions:** Our present study shows frequent association of NTM with laparoscopic port-site nonhealing chronic infection or wound dehiscence. Although direct microscopy can give us a clue to diagnosis, culture isolation is required for speciation and antimicrobial susceptibility testing, which helps formulate therapeutic regimen.

**Keywords:** Laparoscopic surgery, nontuberculous mycobacterium, port-site infection, rapid growing mycobacterium

Introduction

Minimal access surgery also commonly termed laparoscopic surgery (LS) is a weapon of modern surgery limiting invasiveness to reduce morbidity such as postoperative pain, quicker return to normal activity, and less postoperative complications. However, LS has a whole lot of unique complications. Besides, major complications such as bowel or vascular injury, port-site infections (PSIs), port-site herniation, pyoderma gangrenosum, and metastasis at the port site following laparoscopic oncosurgery are indolent but growing problem nowadays. Rate of PSI varied from 3.3% to 8% depending on area of reporting and type of surgery. PSI is a type of surgical-site infection (SSI) confined to skin and soft tissue or rarely muscles around the ports through which surgeons gain access into the abdomen and present within a month of the operative procedure. Most LS belongs to Classes 1 (clean) and 2 (clean-contaminated) wounds as per the CDC criteria for SSI 2015. It soon erodes the advantages of LS, with the patient becoming worried with the indolent and nagging infection and losing confidence on the operating surgeon. The cosmetic purpose of LS unsightly wound, and the quality of life of patients is seriously affected.
Methods

This study was carried out in the Surgery Department of a large teaching hospital of Kolkata from January 2015 to March 2016.

Study population

Patients those suspected to have developed PSI following different LS with the evidence of delayed wound healing, breakdown of wounds after initial healing, redness or discharge from any wound, nodules in or around the vicinity of the wounds, and nonresponsive to empiric antibiotic therapy were included in the study. No emergency surgical cases were included. Detailed pre- and post-operative history and physical examination were done on predesigned pro forma.

Sample processing

Patients were sent to Microbiology Department where any available discharge or fine needle aspirates or scraping from the wounds or nodules was subjected to Gram stain and Ziehl–Neelsen (ZN) stain. Then, all the samples were cultured on blood agar, MacConkey agar, Robertson’s cooked meat broth, Wilkins Chalgren anaerobic agar (HiMedia®, India) supplemented with 5% sheep blood, and two sets of Lowenstein–Jensen (LJ) medium at 37°C. Any positive nontuberculous mycobacterium (NTM) culture was confirmed by repeating process with the second sample. Screening was also done from water source, staining solutions, oil for microscopy used in laboratory to exclude contamination of saprophytic NTM from environmental sources.

Identification of isolates

Any growth on LJ medium was examined by ZN stain; growth rate and pigmentation were noted. Aerobic bacterial isolates were identified by routine laboratory methods, and mycobacterial isolates were tested with TB Ag MPT64 Rapid test (SD Bioline). All the NTM isolates were identified and specified by line probe assay using GenoType Mycobacterium CM/AS (Hain Lifescience GmbH, Nehren, Germany).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for the rapidly growing NTM was carried out on Mueller-Hinton agar by the disc diffusion Kirby-Bauer method to the following antibiotic discs (HiMedia®, India): Clarithromycin, erythromycin (15 μg), amikacin (30 μg), gentamicin (10 μg), imipenem, linezolid, cefoxitin, tetracycline (30 μg), vancomycin (30 μg), ofloxacin (5 μg), ciprofloxacin (5 μg), co-trimoxazole (25 μg), and polymyxin B 300 (300 μg).

Results

In our study, 32 patients with chronic PSIs were included. The majority of patients were female (20 females, 12 males), with a median age of 38.5 years (range 8–67 years). All patients had undergone different types of LS, and laparoscopic cholecystectomy was the most commonly performed procedure.[Table 1] which developed PSI. No specific comorbidities or causes of immunosuppression (e.g., HIV infection, diabetes) were identified among the patients. Most of the patients complained of serosanguinous discharges from the nonhealing wound dehiscence at port sites on several occasions. The wounds were healed initially after surgery over 1–2 weeks. Then, induration appeared at port sites followed by swelling which subsequently ruptured to form sinus. Wound was nonresponsive to 1-week antibiotic therapy (either amoxicillin-clavulanic acid combination or ofloxacin). No patient was reported to have any pain, fever, or systemic complications. The average interval between surgical procedure and onset of discharge from port site was 28–64 days.

On examination, 18 (56.3%) cases had serous discharge from 1 to 2 mm wound gapping, 29 (90.6%) had multiple ports involvement, and all were superficial PSIs. Epigastric and umbilical ports were commonly affected. One patient who underwent laparoscopic hernioplasty also developed incisional hernia and abscess.

Of the 32 pus/discharge specimens examined, 20 were positive for acid-fast bacilli (AFB) by ZN staining and 15 revealed growth of NTM (% isolation rate) which also were AFB positive by ZN staining. All the isolates were rapid growers, of which 13 were of Mycobacterium abscessus and two belonged to Mycobacterium fortuitum. Three of the cases had mixed infection of Staphylococcus aureus and Pseudomonas spp. with NTM [Table 2].

Antibiotic susceptibility testing was done for all the rapid growing mycobacterium isolates, and they showed highest susceptibility

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**Table 1: Summary of 32 port-site infection cases included in the study**

| Variables                      | Value     |
|--------------------------------|-----------|
| Age, years (median [range])    | 38.5 (8-67) |
| Sex (n)                        |           |
| Male                           | 12        |
| Female                         | 20        |
| Types of laparoscopic surgery (n) |         |
| Laparoscopic cholecystectomy    | 19        |
| Laparoscopic hernioplasty       | 6         |
| Laparoscopic orchiopey          | 2         |
| Laparoscopic appendectomy       | 1         |
| Laparoscopic-assisted hemicolecctomy | 1      |
| Laparoscopic common bile duct repair | 1   |
| Diagnostic laparoscopy          | 1         |
| Laparoscopic hydatid cystectomy | 1         |
| Incubation period from surgery to symptom onset, days (median [range]) | 36 (28-64) |
| Number of ports affected (n)    |           |
| Single port-site infection      | 4         |
| ≥2 port sites infection         | 28        |
| Types of port affected (n)      |           |
| Supraumbilical port             | 32        |
| Subxiphoid port                 | 28        |
| Lateral port                    | 16        |
| Medial port                     | 3         |
to both clarithromycin and amikacin (93.3%) Table 3. Only one isolate of *M. abscessus* was resistant to these two drugs. Susceptibility to imipenem, gentamicin, ofloxacin, and linezolid in the case of *M. abscessus* was 77%, 61.5%, 41.5%, and 30.8%, respectively. All 13 isolates of *M. abscessus* were resistant to tetracycline, cefoxitin, and polymyxin B. Of the two isolates of *M. fortuitum*, one showed sensitivity to all antimicrobials tested, except vancomycin. The other isolate of *M. fortuitum* was resistant to cefoxitin, tetracycline, erythromycin, ciprofloxacin, and vancomycin.

**DISCUSSION**

Infections at the port sites of LS broadly classified into two varieties based on the timing of presentation. The “early type” occurs immediately within a week of surgery, by Gram-positive or Gram-negative bacteria which are contracted from endogenous source, the skin or bowel, and can be treated with commonly used topical or oral antibiotics.[10] Early type of PSI was most commonly caused by *S. aureus* although few studies found *Bacteroides* or *Pseudomonas* as the common offending organism following LS.[5,6] Second variety, the “delayed type,” has 3–4 weeks incubation period, is caused by NTM.[2] Although there are many reports of PSI due to NTM from different parts of the body, no structured study on clinico-microbiological profile of chronic type PSI is present.[2,7,8]

In our study, 59.4% of the infections associate with laparoscopic cholecystectomy, whereas 18.7% after laparoscopic hernioplasty. Similar association was shown by previous reports and might be due to the higher rate of this LS.[1] Umbilical port site was the most common site of PSI, followed by epigastric port site. In the literature, there is great emphasis on the increased frequency of umbilical site PSIs and the role of umbilical flora in the development of PSIs.[9]

In contrast to previous reports, our results show lesser sensitivity of culture than ZN-stained direct smear for detection of mycobacteria.[10,11] Inappropriate sampling technique such as swab sample which inhibits transfer of highly hydrophobic mycobacterium to solid media or inadequate pretreatment before sample collection could explain the mismatch.

Over thirty species of rapidly growing mycobacteria (RGM) have been identified, but most common are *M. abscessus*, *Mycobacterium chelonae*, and *M. fortuitum* group, which are important causes of skin and soft tissue infections, especially following penetrating trauma or surgery with possible soil or water contamination.[2,10] In our study, RGM constituted 15/15 (100%) of all NTM isolates, *M. abscessus* being the predominant (87%) isolate. Postoperative wound infections caused by RGM generally appear some weeks to some months following the procedure; similarly, in our study, median incubation period was 36 days. Initial healing with delayed onset, absence of thick pus, or abscess formation with no fever, no clinical improvement with short-term antimicrobial therapy established pathogenic potency of RGM isolates. Few aerobic isolates might cause superficial colonization or secondary infection.

Susceptibility pattern of RGM varied widely with different species, with isolates from different geographical areas. The Infectious Diseases Society of America and American Thoracic Society recommended routine susceptibility testing of clinically significant RGM isolates, and susceptibilities should be reported and used as a clinical guide for treatment.[12] Although broth microdilution is the method recommended by the CLSI for susceptibility testing of NTM, disc diffusion method provides a good screening technique for RGM.[13,14]

Clarithromycin and amikacin were found to be active against nearly all RGM isolates. 92.3% *M. abscessus* and 100% *M. fortuitum* were sensitive to both the drugs. Previous studies also demonstrated similar data.[10,14,15] Imipenem exhibited good activity against both the species as documented earlier.[15] Among the fluoroquinolones, 46% of *M. abscessus* and 100% of *M. fortuitum* were susceptible to ofloxacin.
followed by ciprofloxacin (30.8% and 50%). In contrast to previous findings, our results showed decreased susceptibility of *M. abscessus* to fluoroquinolones but active against *M. fortuitum.*[14,15] Although the study suggested excellent potential of linezolid against RGM, high MIC<sub>90</sub> had been reported among common RGM species.[15,16] Our results supported this finding.

In the absence of species identification and susceptibility results, for empirical therapy of RGM depending on site of infections, multidrug regimens containing macrolide and aminoglycoside are usually recommended.[12] Different combinations for variable duration, even intralesional injection, had been tried by previous workers.[7,10] Localized, nagging, chronic, difficult-to-treat PSI due to NTM may benefit from surgical curettage usually with adjunctive antibiotic coverage. It has been recommended that to prevent recurrence, antibiotic treatment should be given for a minimum of at least 3 months or at least 3–6 weeks after the wound heals. We wish to emphasize that reporting of RGM from clinical settings along with their sensitivity patterns is an absolute need of the hour.[17]

Prevention of healthcare-related NTM infections requires that surgical wounds, injection sites, and intravenous catheters are not be exposed to tap water or tap water-derived fluids. Endoscopes cleaned in tap water and clinical specimens contaminated with tap water are also not acceptable.

**Conclusions**

Awareness about the rising incidence of RGM infections in postlaparoscopic port sites should be generated as it has immense role in the choice of therapeutic regimen. Unnecessary use of broad-spectrum antibiotics or antitubercular drugs could be minimized for the treatment of delayed PSI. Culture and species identification followed by susceptibility testing of RGM is necessary to select an optimal antimicrobial therapy.

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**Conflicts of interest**

There are no conflicts of interest.

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