

An outbreak of atypical mycobacterial port site infections in a newly established medical college & hospital of West-Bengal: A preventable nuisance

Dr. Nandita Pal¹, *Dr. Sanjit Kumar Patra², Sikha Gupta³

¹ Assistant Professor, Department of Microbiology, CMSDH, Kamarhati, Kolkata

² Associate Professor, Department of Microbiology, CMSDH, Kamarhati, Kolkata

³ 2nd Professional MBBS Student, CMSDH, Kamarhati, Kolkata

Corresponding Author: * Dr. Sanjit Kumar Patra

Abstract

Introduction: Laparoscopic surgery (LS) has multiple advantages like less pain, rapid healing and early ambulation. Yet, port site infection is an emerging nuisance. Early onset port site infections (PSIs) are usually caused by non-fastidious microbes and are easily preventable or treatable with conventional antimicrobial therapy following a culture and sensitivity testing. However, the late-onset PSIs are mostly caused by the *Mycobacterium* spp. and if not managed properly, tend to become chronic non healing lesions leading to neutralize the benefits of LS.

Objectives: To determine the microbial aetiology of late onset PSIs at our institution, to assess the intramural infection rate of such PSIs and to observe the sterilization protocols followed for LS in General Surgery operation theatre (GSOT) & suggest amendments (if required) to combat these port site infections.

Material & Methods: The study was conducted on 192 post LS patients (undergoing laparoscopic surgery for various indications) from January 2016 to June 2017 at the GSOT of our hospital. All these patients were observed for PSIs during post-operative follow-up for six months. Simultaneously, various ongoing sterilization and disinfection activities in the GSOT were scrutinized. Aspirated pus or swab taken from the port site wounds were processed for microscopy, culture (aerobic, anaerobic, mycobacterial and fungal cultures) and Cartridge Based Nucleic Acid Amplification Test (CB-NAAT) for *Mycobacterium tuberculosis*. Cultured bacterial colonies from Lowenstein Jensen (LJ) media were subjected to Ziehl-Neelsen (ZN) and auramine staining followed by light and fluorescent microscopy respectively for presence of acid fast bacilli (AFB) and tested with immunochromatographic lateral flow rapid assay for detecting MPT64 TB antigen.

Results: All detected PSIs were caused by rapid growing atypical mycobacteria. Intramural infection rate of mycobacterial PSI was 14.53% in 2016. No such occurrence was noted in December 2016 and in the 1st half of 2017. Amendments included in sterilization and disinfection protocols in mid-November 2016 played the key role in remission and further prevention of the concerned nosocomial outbreak of atypical mycobacterial late onset PSIs in our institution.

Conclusion: Nosocomial menace like late onset PSI is controllable by inculcating optimum protocols for proper sterilization of laparoscopic instruments.

Keywords: laparoscopic surgery, port site infection, atypical mycobacteria, sterilization

Date of Submission: 21-11-2017

Date of acceptance: 02-12-2017

I. Introduction

Laparoscopic surgery is a variant of minimal invasive surgery (MIS). Benefits of LS are early postoperative recovery, less pain and improved aesthesis.^{[1],[2]} Port site infections (PSIs), at times tending to chronicity, are emerging as one of the annoying complication of MIS and eventually neutralizing these benefits.^[3]

PSIs are of two broad varieties based on the timing of their presentation:

- Early onset PSI: Presenting within a week of the surgical procedure. Gram positive or Gram negative bacteria are the usual offending organisms which are contracted endogenously or exogenously on the surgical site.^[4]
- Delayed or late onset PSI: Presents late (usually three to four weeks after surgical procedure) and is mainly caused by atypical mycobacteria.^{[1],[5]} Despite advances in sterilization techniques and surgical techniques, late onset PSIs are emerging with ascending trend and may even cause sporadic outbreaks.^{[1],[6]}

Our medical college was established in 2011 following up-gradation of a state general hospital in North 24 pargana district of West Bengal. General Surgery department started performing few selective LS in 2015. Then, the GSOT was shifted in new premises in January 2016 and LS started in full swing from April 2016. We detected frequent late onset PSIs in the 2nd quarter of 2016. In this context we designed the present observational study to determine the microbial aetiology and the intramural incidence of such PSIs and simultaneously we also scrutinized the sterilization protocols followed for LS in GSOT to suggest amendments required for combating these port site infections.

II. Materials and Methods

The observational study was conducted maintaining all ethical guidelines. Our study included 192 patients who underwent laparoscopic surgeries for various indications in General Surgery Department of our institute over a period of 18 months from January 2016 to June 2017. Sterilization protocols followed for LS in GSOT were observed throughout the study period. Certain changes were implemented within the ongoing sterilization and disinfection protocols being followed in GSOT from mid-November 2016. Post-LS patients were followed-up for late onset PSIs during their follow-up visits for next six months. Known immunosuppressed individuals and patients having malignancy taking radiotherapy and/or chemotherapy were excluded from the study. Also, cases with history of LS being converted to open procedures and cases presenting with early onset PSI within a week of LS, were excluded from the study.

Late onset PSI was diagnosed based on the following criteria:

1. Evidence of delayed port site wound healing characterised by breakdown of wounds after initial healing, redness or discharge from any wound, nodules in or around the vicinity of the wounds.
2. Patients presenting with one or more non healing/refractory PSI after taking antibiotics (used singly or in combination or in series) like beta-lactam antibiotics, tetracyclines, lincomycins, oxazolidinones and cotrimoxazole.

Information regarding age, sex, surgery-infection interval, associated co-morbidities (diabetes mellitus, immunosuppression, malignancy, radiotherapy and antibiotic use) was noted. FNAC was done from the infected sites in few randomly selected patients in the Department of Pathology. Samples (aspirated pus, wound swab) obtained from the site of infection were processed as follows for identification of the microbial agent causing the infections:

1. Staining & microscopy: Gram stain, ZN stain, fluorescent staining (Auramine stain) and KOH mount
2. Collected samples were cultured in 10% Sheep Blood agar, MacConky agar, Lowenstein Jensen media, Sabourauds Dextrose agar, and anaerobic gas jar with gas pack system following proper incubation temperature and duration.

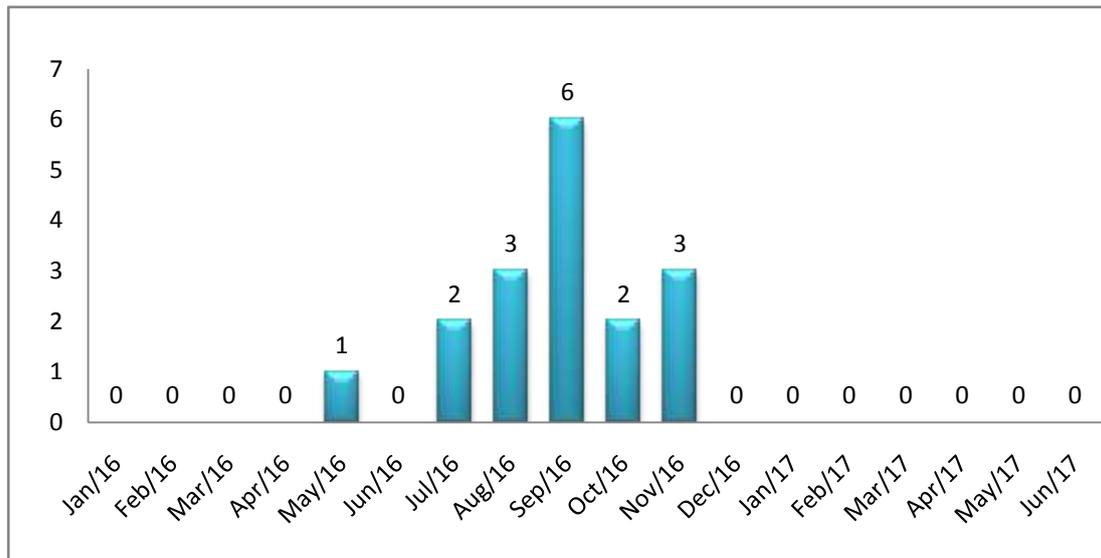
Samples were sent for CB-NAAT (with assistance of RNTCP programme) to exclude *M. tuberculosis* infection. Identification of the colonies grown on LJ media was done by immune-chromatographic test using MPT64TB Ag Kit for detection of *M. tuberculosis*.

III. Results

Total number of 192 laparoscopic surgeries (mainly laparoscopic cholecystectomy) was performed in GSOT of our institution during the study period. During the study, 17(11 females and 6 males) patients developed late onset PSI in 2016. Gram stain of the collected samples showed plenty of pus cells but no microorganism was detected. Microscopy of the ZN stained smears and showed plenty of acid fast bacilli (AFB) and fair number of pus cells. Fluorescent microscopy of the smears also showed same results. FNAC report showed presence of epithelioid granulomas, Langhans giant cells, plenty of acute inflammatory cells and lymphocytes in all examined cases. Bacterial, fungal and anaerobic cultures showed no growth, but growth (white to cream coloured, circular, entire, smooth emulsifiable colonies) was detected within one week of incubation in LJ media and ZN stain & fluorescent stain from these colonies showed plenty of AFB. On examining the colonies with immune-chromatographic test using MPT64TB Ag Kit for detection of *M.tuberculosis*, non reactive result was obtained. Also, all the samples processed for CB-NAAT showed negative result, hereby, excluding presence of *Mycobacterium tuberculosis* and hence confirming the rapid growing atypical mycobacterial port site infection.

Monthly distribution of number of PSIs detected over the 18 month study period has been shown in Figure:1. The intramural infection rate of PSI in 2016 was 14.53% (17/117). However, no such infection was detected in December 2016 and in the 1st half of 2017(January to June). This indicates a nosocomial outbreak of late onset PSIs in the 2nd quarter 2016 followed by its remission by the year end.

Figure-1: Monthly distribution of number of PSIs detected during the 18 month study period



Sterilization and disinfection techniques followed till mid-November 2016 and the changes implemented henceforth are summarised below:

Instruments/ Accessories	Protocols followed till mid of November 2016	Protocols followed after mid of November 2016
Number of set of instruments used	All patients scheduled for laparoscopic procedures on the same day (2-3 LS/day approx.) were operated upon with single set of instruments following chemical disinfection of port, trocar and hand instruments.	<ol style="list-style-type: none"> 1. Three sets of sterile instruments available for use. 2. In inevitable circumstances if the number of scheduled LS exceeded number of instrument sets, then port, trocar & hand instruments were chemically disinfected with 2% Glutaraldehyde solution for 1 hr.
Port and trocar	<ol style="list-style-type: none"> 1. Washed with sterile water and immersed in 2% Glutaraldehyde solution for 20-30 min, then, washed with sterile water and dried before commencing the 1st scheduled LS and in between the subsequent LS 2. Washed and chemically disinfected as before and placed in formalin chamber [Picture:1] till the next day of surgery. 	<ol style="list-style-type: none"> 1. Washed with chemo-sterilizant (15% Cetrimide W/V + Chlorhexidine Gluconate solution I.P. 7.5% V/V) and then, washed with sterile water and dried after single use and placed in formalin chamber. 2. Standard autoclaving (121°C, 15lb/sq.inch, 15 min) was done on previous day of operation [Picture:2].
Hand instruments	<ol style="list-style-type: none"> 1. At the end of all LS on the same day, instruments are washed with chemo-sterilizant (cetrimide-chlorhexidine solution), washed with sterile water and dried 2. Placed in formalin chamber till the next operation scheduled date. 3. Immersed in 2% Glutaraldehyde solution for 20-30 min, then, washed with sterile water and dried before commencing the 1st scheduled LS and in between the subsequent LS. 	<ol style="list-style-type: none"> 1. Washed with chemo-sterilizant and followed by washing with sterile water and drying after single use 2. Placed in formalin chamber till the day before next operation scheduled date. 3. Immersed in 2% Glutaraldehyde solution overnight (12-18 hrs) before operation 4. Washed with sterile water, dried and used
Laparoscope	Used multiple times on same day and then placed in formalin chamber till the next operation date.	Used once in a day and placed in formalin chamber till the next operation date.
Tray for activated Glutaraldehyde solution	<ol style="list-style-type: none"> 1. Old plastic tray [Picture:3] 2. Routine change of Activated 2% Glutaraldehyde solution was not properly supervised 	<ol style="list-style-type: none"> 1. New stainless-steel tray. [Picture:4] 2. Activated 2% Glutaraldehyde solution routinely changed every fortnight



Picture 1: Formalin chamber



Picture 2: Autoclaved Port and Trocar



Picture 3: Old Plastic Tray for Chemical Sterilization



Picture 4: New Stainless-Steel Tray for Chemical Sterilization

IV. Discussion

Post LS 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.^[7] Most LS belongs to Classes 1 (clean) and 2 (clean-contaminated) wounds as per the CDC criteria for SSI 2015.^{[1],[2]} We found that 100% (17/17) late onset PSIs were caused by rapid growing atypical mycobacterial (RGAM), similar to the findings of Ghosh R *et al.*^[7] Sasmal PK *et al* in a review article, has mentioned RGAM like *M.chelonae*, *M.fortuitum*, *M.flavescens*, *M.massiliense*, *M.abscessus* etc, as the main cause of late onset PSIs.^[1]

Past studies have reported variable late onset PSI rates ranging from 2-8%.^{[1],[8]} Compared to these reports, our rate was far higher. However, Vijayaraghavan R *et al* has reported an outbreak of 35 such infections among 156 post LS patients over 6 months which amounts to the infection rate of 22.4%.^[6] Such variation in PSI rates among studies may be attributed to differences in environment, sterilization techniques and population under study which differ from one health set-up to another.

Atypical mycobacterium colonize tap water, natural waters, and soil and thus can easily contaminate solutions and disinfectants used in hospital settings.^[9] Mir *et al* mentioned in his report that the cause of PSI could be due to reusable trocars.^[8] Vijayaraghavan R *et al* isolated *M. chelonae* from post LS port site wounds during a PSI outbreak in his institution as well as from the hospital water supply used for washing/rinsing of laparoscopic instruments.^[6] He also recovered the same mycobacteria from scrapings obtained from the bottom of glutaraldehyde trays and proposed that the concerned NTM formed biofilm within the outer sleeves of the reusable laparoscopic instruments. According to Krishnappa R *et al*, the source of infection is often the boiled tap water used for cleansing of the instruments after immersion in glutaraldehyde. Improper mechanical cleaning of the instruments and ports leaves debris on these instruments.^[10] Usually the current practice in India is to immerse instruments in 2% glutaraldehyde solution for 20 minutes which achieves disinfection but not sterilization.^[11] Svetlikova Z *et al* showed that atypical mycobacteria were showing increased resistance to these chemicals due to defects in porin expression in the bacterial cell walls.^[12]

In our OT set up, we observed that sterile water was being used for rinsing instruments throughout the study period. Here, the infections totally disappeared after opting for the following enlisted amendments:

1. Increase in number of instrument sets omitted chances of breach in sterilization amidst subsequent LS on the same day.
2. Autoclaving of port and trocar achieved the recommended sterilization assurance level.
3. Increasing exposure time of instruments to glutaraldehyde (from 30 minutes previously to overnight exposure at present) assured high level chemical sterilization.
4. Replacing plastic disinfectant tray with autoclavable new stainless steel disinfectant tray remitted chances of bacterial biofilm formation.

Autoclavable laparoscopic instruments have come up as an excellent preventive remedy against mycobacterial PSIs.^[1] Other applicable option may be the use of 0.55% ortho-phthalaldehyde (OPA) for sterilization of heat sensitive laparoscopic instruments. OPA has many advantages over the glutaraldehyde such as; it does not require activation, low vapour property, better odour, more stable during storage, increased mycobactericidal activity.^{[13],[14]} Also, ethylene oxide sterilisation or plasma sterilization are certain low temperature physical methods of sterilization which may be used as alternatives to chemical sterilization of heat sensitive lumen containing instruments in resourceful sterilization units.^{[15],[16]}

V. Conclusion

PSI is one of the morbid nosocomial complications of laparoscopic surgery. This menace is controllable by inculcating optimum protocols for proper sterilization of laparoscopic instruments.

Limitation of the study: We were unable to identify the isolated atypical mycobacterium to species level and also couldn't perform drug sensitivity testing of the mycobacterial isolates in our resource poor set-up. This may be feasible in further research with assistance from state level reference laboratory under RNTCP where line probe assay facility is available.

VI. Acknowledgements

We express sincere gratitude to the Principal, Medical Superintendent cum Vice Principal and surgical OT sisters Mrs Nivedita Sarkar and Mrs Srabani Roychoudhury of our institution for their active support and co-operation throughout the study. We specially extend our thanks to all the staff of the department of Microbiology and all participants included in this study.

Bibliography

- [1]. Sasmal PK, Mishra TS, Rath S, Meher S, Mohapatra D. Port site infection in laparoscopic surgery: a review of its management. *WJCC* 2015;3(10):864.
- [2]. Pal N, Guhathakurta R. Surgical site infection in surgery ward at a tertiary care hospital: the infection rate and the bacteriological profile. *IOSR Journal of Pharmacy* 2012;2(5):1-5.
- [3]. Sharma AK, Sharma R, Sharma S. Port Site Infection in Laparoscopic Surgeries. *Indian Medical Gazette* 2013;224-8.
- [4]. Falkinham JO. Epidemiology of infection by nontuberculous mycobacteria. *Clin Microbiol Rev* 1996; 9:177-215
- [5]. Ramesh H, Prakash K, Lekha V, Jacob G, Venugopal A, Venugopal B. Port-site tuberculosis after laparoscopy: report of eight cases. *Surg Endosc* 2003;17: 930-932
- [6]. Vijayaraghavan R, Chandrashekar R, Sujatha Y, Belagavi CS. Hospital outbreak of atypical mycobacterial infection of port sites after laparoscopic surgery. *Journal of Hospital Infection* 2006;64(4):344-7.
- [7]. Ghosh R, Das S, De A, Kela H, Saha ML, Maiti PK. Port-site infections by nontuberculous mycobacterium: A retrospective clinico-microbiological study. *Int J Mycobacteriol* 2017;6:34-7
- [8]. Mir MA, Malik UY, Wani H, Bali BS. Prevalence, pattern, sensitivity and resistance to antibiotics of different bacteria isolated from port site infection in low risk patients after elective laparoscopic cholecystectomy for symptomatic cholelithiasis at tertiary care hospital of Kashmir. *Int Wound J* 2013;10:110-13.
- [9]. Moghim S, Sarikhani E, Nasr Esfahani B, Faghri J. Identification of Nontuberculous Mycobacteria Species Isolated from Water Samples Using Phenotypic and Molecular Methods and Determination of their Antibiotic Resistance Patterns by E-Test Method, in Isfahan, Iran. *Iranian journal of basic medical sciences* 2012;5(5):1076-82.
- [10]. Krishnappa R, Samarasam I. Int Atypical mycobacterial infection in post laparoscopy surgical wounds: our observations and review of literature *Surg J* 2017 Sep;4(9):2943-2946
- [11]. Chaudhuri S, Sarkar D, Mukerji R. Diagnosis and Management of Atypical Mycobacterial Infection after Laparoscopic Surgery. *The Indian Journal of Surgery* 2010;72(6):438-42.
- [12]. Svetilkora Z, Skovierova H, Niederweis M, Gaillard JL, McDonnell G, Jackson M – role of porins in the susceptibility of mycobacterium smegmatis and mycobacterium chelonae to aldehyde based disinfectant and drugs. *Antimicrobial agents Chemotherapy* 2009;53(9):4015-18.
- [13]. "Infection Control in the Physician's Office" . College of Physicians and Surgeons of Ontario. 2004.
- [14]. Tsuda M, Hata M, Nishida RIE, Oikawa S. Chemically amplified resists proton-catalyzed degradation mechanism of poly(phthalaldehyde). *Journal of Photopolymer Science and Technology* 1993;6 (4): 491.
- [15]. Schneider PM. Low-temperature sterilization alternatives in the 1990s. *Tappi J* 1994;77:115-9.
- [16]. Jacobs PT, Smith D. The new Sterrad 100S sterilization system: Features and advantages. *Zentr. Steril* 1998;6:86-94.

,*Dr. Sanjit Kumar Patra. "An Outbreak of Atypical Mycobacterial Port Site Infections in A Newly Established Medical College & Hospital of West-Bengal: A Preventable Nuisance." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)* , vol. 16, no. 11, 2017, pp. 89–93.