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Molecular Detection of NontuberculousMycobacteria (Mycobacterium Chelonae, MycobacteriumAbscessus Group and Mycobacterium Fortuitum Complex) Among Patient with Post-Operative Wound Infection in Khartoum State - Sudan

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ABSTRACT

Background

Nearly 100 mycobacterium belong to rapidly growing mycobacterium (RGM), the most common pathogens being *M. chelonae* ,*M. abscessus* groups and *M. fortitum* complex,, *M. abscessus* is more resistant to antibiotic and disinfection. Nontuberculosis mycobacteria(NTM) have been isolated from various sites viz., cutaneous and soft tissue infection, after skin injury following inoculation and after surgical procedures and also, known to cause systemic infection in AIDS and immunocompromised patients. Surgical site infection due to atypical mycobacterium usually has along incubation period.

Material and Method

Swaps from infected surgical wound were collected randomly from 40 patients, the bacterial DNA were extracted from the samples by using the CTAB method. Different primers were designed to detect *M. abscessus*, *M. chelonae*, and *M. fortitum* complex. DNA amplification was performed under different optimization PCR condition for different nontuberculosis mycobacteria species. The PCR products were separated by electrophoresis using 2% agarose and the products were visualized in gel documentation system. A 100pb ladder was used for comparison of product length.

Result

The prevalence of NTM infection among patients with post-operative wounds was 17.5% for *M. abscessus*, 15% for *M. fortitum* and 0% for *M. chelonae*. Infection was higher in males (17.5%) in comparison to females (7.5%). NTM organism were detected in all delayed healing periods from 7 days to two years.

Conclusion

Our results showed that the *M. abscessus* and *M. fortitum* infection were found among patient with post operative wounds in Sudan. The infection are higher in males than females. Further investigation is needed to identify strains of NTM found in hospitals for proper control and to study whether this microorganism can cause nosocomial infection.

Keywords:NTM,CTAB, DNA, RGM, MFC, MC–MAG, PCR

I. INTRODUCTION

The genus Mycobacterium is composed of *Mycobacterium leprae*, *Mycobacterium tuberculosis* complex and nontuberculous Mycobacteria (1).According to the Runyon classification system, Rapid growing mycobacteria (RGM) represent the Nontuberculous mycobacteria group (2). The marked increase in the past years of infections caused by Nontuberculous mycobacteria, such as those caused by RGM, in resource-rich countries is getting the attention of the scientific community (4). Nearly 100 Mycobacterium species belong to the RGM. However, the most common RGM pathogens belong to the *Mycobacteriumchelonae*,*Mycobacterium abscessus* group ((MC–MAG)) and *Mycobacterium fortuitum* complex (1,15). MC – MAG consists of the following species' *M. chelonae, M. abscessus* group (*M. abscessus* subspecies *abscessus, M. abscessus* subspecies *bolletii* and *M. abscessus* subspecies *massieliense*), *M. immunogens*, and *M. salmoniphilum* (6,8). Even though *M. chelonae* and *M. abscessus* are the MC-MAG species most frequently isolated in infection. The M. abscessus group is more resistant to antibiotics and disinfectants (9). For this reason, the guidelines for diagnosis and treatment of nontuberculous mycobacteria recommend the rapid identification of MC–MAG to the species level(1). MFC comprises 12 species, only *M. fortuitium, M. peregrinum*, *M. alvei*, *M. farcinogenes*, *M. senegalense* and *M. porcinum* are considered as potential pathogens (16). Nontuberculosis mycobacterium are usually saprophyte found in water and sewage water but can be opportunistic pathogen transmitted by aerosols, dust, water, ingestion or by skin inoculation whereas its person to person transmission is rare (1). NTM have been isolated from various sites viz., cutaneous and soft tissue infection, after skin injury following inoculation or minor trauma and after surgical procedure including plastic surgery procedures like rhinoplasty and median sternotomy (6, 11). They are also known to cause systemic infection in a patients with AIDS and in other immunocompromised individual while in immunocompetent hosts the infections are of localized nature such as cutaneous and soft tissue infection (2,13).Typically surgical site infections due to atypical mycobacteria have long incubation periods, This investigation was undertaken to report the occurrence of nontuberculous mycobacteria, associated with non-healing post-operative wound infection that did not respond to antibiotics used for pyogenic

infection and to address the need for simple and rapid assay with broader identification scope, such as PCR techniques. Such techniques are rapid, sensitive, minimize waiting for culture result and represent reliable alternatives for the diagnosis of mycobacterial infection (19).

II. METHODS AND MATERIAL

Data collection

Demographic data of patients were collected using structured questionnaire, which included the following particulars: name, gender, a sign of a wound, antibiotic usage, and types of surgical procedure..

Study area

The study was conducted in Khartoum Hospitals (Omdurman hospitals, Military forces hospital, Chinese hospitals, and Libya specialist hospital) During the period April to December 2017

Sample collection

The present study included 40 patients of different ages and gender who had undergone surgery for various ailments. These patients developed wound infection at the surgical site (10 days to 3wk or more) that were not responding to antibiotics treatment. The skin was sterilized using 70% ethanol then the pus/discharge was collected by sterile Dacron swabs without touching the margin of the wound. After collection, the swabs were inserted in 0.085% normal saline or peptone water to avoid dryness of specimen and to maintain microorganism's viability. If there were any delay in processing specimen in the lab the specimens were preserved at 4 °C for 24h-48h.

DNA Extraction

Bacterial DNA extracted from post-operative wound samples using CTAB method. One and half ml of the bacterial suspension was centifuged at 12000 rpm/2min ,the supernatant was then discarded the pellet was re-suspended in 567µl TE buffer, plus 30µl of 10% SDS and 3µl of 20 mg/ml proteinase K , mixed thoroughly and incubated for 1hr at 37°C. A100µl of 5M NaCl was added and mixed thoroughly. Then 80µl of CTAB/ NaCl was added ,well mixed and incubated for 10min at 65 °C. then extracted with an equal volume of 25:24:1phenol/chloroform /isoamyl alcohol and spun in a microcentrifuge for 5min. The aqueous phase was transferred to a fresh tube, and 100 % ethanol was added,thenincubated overnight After incubation the supernatant was discarded, and the precipitated nucleic acid was washed with 70% ethanol to remove residual CTAB and re-spun for 5 min at room temp t. The supernatant was removed and the pellet was left to dry. Lastly, the pellet was dissolved in 50µl TE buffer or sterile distilled water, stored at -20°C till used.

Demographic data of patients were collected usi

Polymerase chain reaction (PCR)

Different sets of primers designed to target different genes (16S rRNA, SOD, rpoB) for detecting nontuberculous mycobacteria (*M. chelonae, M. fortitum complex, M. abscessus*). 16S rRNA gene of *M. Chelonae was* amplified with nucleotides sequence 16S –chelonae –F (5' ACCACACACTTCATGGTGAGTGG 3') and 16S –chelonae –R (5' GCCCGTATCGCCCGCACGCKCAC 3') for *M. fortitum* complex we used primers that target SOD gene oligo sequence *M. fortitum* –F (5'CCAAGCTCGATGAGGCGCGG- 3,) and *M. fortitum*- R (5' CCGATCGCCCAGGCTGTCGT 3'). Rpo B gene for detection the *M. abscessus*

subspecies *abscessus* was amplified by using oligonucleotide sequence MYCO F (5' GGCAAGGTCACCCCGAAGGG'3) and MYCO R (5' AGCGGCTGCTGGGTGATCATC '3). The amplification was performed in a thermo cycler machine. Briefly 13 µl of sterile DW was added to each ready master mix tube containing (dNTPs, MgCl, KCL,TrisHCl,Taq DNA polymerase), 1 µl of each forward and reverse primer and 5 µl aliquot of each DNA sample were added. PCR condition for 16S rRNA region of *M. chelonae* was optimized as follows : first cycle of denaturation 9 at 5°C for 5 min ,followed by 35 cycles consisting of denaturation at 94°C for 15 sec, annealing at 64°C for 15 sec, extension at 72°C for 30 sec and at final extension cycle at 72°C for 10 min. For SOD region of *M. fortitum* complex the cycles were optimized as follows : first cycle of denaturation at 95°C for 5 min ,then 35 cycle of, 94°C for 15 sec , 61°C for 15 sec and 72°C for 30sec, with final extension step at 72°C for 10min. PCR conditions for rpoB gene of *M. abscessus* subspecies *abscessus* was : first cycle at 95 °C for 2min followed by 35 cycles of 94°C for 30sec, 61°C for 30sec and 72°C for 2 min, with a final extension step at 72°C for 5 min. The PCR products were separated by electrophoresis using 2% agarose gel in 1X TBE buffer(Tris-borate EDTA) at 100 volts, and 60 ampere current for 30 min. The gel were stained with ethidium bromide (0.5micro gram/ml) and the products were visualized in gel documentation system for comparison of product length (*M. chelonae* 441bp, *M. fortitum* 275 bp, and *M. abscessus* 723bp), Two ul of 100bp ladder was used as molecular size marker.

Ethical clearance

The study was reviewed and approved by the Faculty of Medical Laboratory Science Post Graduate Collage Al-neelain University, Khartoum Sudan.

III. RESULTS AND DISCUSSION

Table 1. Show gender and positive in each Mycobacterium species

Gender	No. of Samples (%)	No. of positive (%)	No *M.abscessus* (%)	No. *M.fortitum* (%)	No. *M.chelonae* (%)	No of Mixed (*M.abscessus*& *M.fortitum*) (%)
Male	21 (52.5%)	7 (17.5%)	4 (10%)	5 (12.5%)	0(%)	2 (5%)
Female	19 (47.5%)	3 (7.5%)	3(7.5%)	1(2.5%)	0(%)	1(2.5%)
Total	40(100%)	10 (25%)	7 (17.5%)	6(15%)	0(%)	3(7.5%)

*%calculated from total No. of samples (n=40)

Table 2. Show period of wound healing and patient included

period ofhealing	No of patient	No of positive (%)	No of negative (%)
2 -6 days	5	0 (0)	5 (100)
7 – 21 days	10	3 (30)	7 (70)
21 – 60 days	17	5 (29.4)	12(70.6)

3 -6month	6	1 (16.6)	5 (82.4)
6 month – 2 years	2	1 (50)	1 (50)
Total	**40**	**10 (25%)**	**30 (75%)**

Figure 1: show ladder 100pb, controlnegativeand sample positive of M. abscessus.

Of the 40 patients included in the study 21(52.5%) were males and 19(47.5%) females, the surgical infection with NTM were higher in males (17.5%) in comparisons to females (7.5%). Four male patients were positive for *M. Abscessus* (10%) females while three female patient were positives (7.5%) *M. fortitum* infection was positive in 5 male patients (12.5%) and in only one female (2.5%). M. chelonae infection were not detected in any of the patients. Overall the prevalence of infection of the post-operative wound in Khartoum State was as follows *M. Abscessus (*17.5%) *M. fortitum* (15%%) and M. Chelonae(0%). Three (30%), 5(29.4), 1(16.6%) and 1 (50%) of the patients with delayed healing periods of 7-12 days, 21-60 days, 3 months 6 month to two years respectively were positive for NTM infection (**Table 2**).The total percentage of positive in all delayed healing wound patients groups was 10 (25%) .

In our study most of the patient had presented with discharge after 7 days to 2 years post-operative. On the contrary infections due to other pyogenic bacteria have a shorter incubation period as compared to RGM which have long incubation period ranging from several days to several months (9). Water, soil, animals and marine life have been mentioned as a source of *M. fortitum*. *M. chelonae* complex (4). Typically, wound infection due to atypical mycobacteria don't occur as an immediate post-operative complication (22. 23). There is apparent immediate post-operative healing and gradually over a variable period of times, the scar breaks down to persistent nonhealing superficial wound with discharging sinuses but systemic manifestation like fever and chill are rare (11). Such wound does not respond to the antibiotic used for acute infection and persists for prolonged period of time. But even when culture are detected positive and the presence of mycobacteria were confirmed by Z.N staining it's not yet possible to confirm it as MTC or NTM (18). Polymerase chain reaction has been used to aid in diagnosing these condition (26). In our study we identified *M. abscessus* in 7 cases and *M. fortitum* in 6 cases in Khartoum state, Variety of reasons including contamination of aqueous solution or surgical equipments used have been reported as a cause of wound infection (18,19). Our PCR assay indicated the presence of *M. abscessus* and *M. fortitumn* as the cause of wound infection. *M. abscessus* is known to be a major cause of skin infection, it causes nosocomial infection, post-injection abscess and wound infection

following surgeries (2, 8, 10). Cutaneous infection with rapidly growing mycobacteria can manifest in a variety of ways including ulceration, abscess, draining sinuses or nodules (2, 8). The median 7 weeks delays from surgery **(Table 2)** to the onset of symptoms seen in our cases is similar to the reports in a previous outbreak of post-surgical *M. abscessus* infection (17, 22, 24, 33,34).

IV. CONCLUSION

Our result showed that *M. abscessus* and *M. fortitum* infections were found among patients with post operative wounds in Sudan. The infection are higher in males than in females. The misdiagnosis of NTM is a common occurrence and can easily be overlooked and not be cared for. Further investigations areneeded to identify strains found in hospitals for better control and to study if these microorganism can cause nosocomial infection.

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