International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 9, Issue 10, 2017, pp.-959-962.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

INCIDENCE OF *M. mucogenicum* INFECTION IN TERTIARY CARE HOSPITAL INDIA: RECENT INCREASE IN NUMBER OF NTM CASES

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Received: August 16, 2017; Revised: September 26, 2017; Accepted: September 27, 2017; Published: October 28, 2017

Abstract- Nontuberculous mycobacteria (NTM) are emerging pathogens that affect both immune-compromised and immune-competent patients. Recently *Mycobacterium mucogenicum* (*M. mucogenicum*) has been identified as significant cause of post surgical wound infection, soft tissue infection, catheter related sepsis, peritonitis, following peritoneal dialysis, bacterimia in patients undergoing haemodialysis, and central venous line associated sepsis, meningitis, pneumonia and lymphadenitis. **Aims and objectives**: The present study was designed for retrospective analysis to identify incidence of *M. mucogenicum* from various clinical samples and to do comparative analysis with reference to clinical syndrome, predisposing factors and its demographic information. **Materials and Methods**: Various clinical samples were received from suspected NTM infection with symptomatic and compatible radiographic findings. Isolation, identification of NTM and *M. mucogenicum* were done by standard conventional methods and liquid culture in automated MB BacT culture system. Molecular genotyping were done by Line probe assay (LiPA) for identification of NTM and *M. mucogenicum*. **Results:** A total of 13 strains of *M. mucogenicum* were identified out of 30 NTM strains. Of the total *M. mucogenicum*; 11 [85%] of the strains were isolated from extra pulmonary origin and 2 [15%] strains were from lung infections; of which 1 was of the paediatric patient having tuberculous lymphadenitis and 1 case was from geriatric age group having tuberculous appendicitis only one case was immune-compromised while remaining 12 cases were immune-competent. Isolation of *M. mucogenicum* from case of endometrium and sub ovarian cyst were rare findings from present study. **Conclusion:** Attention should be given to *M. mucogenicum* isolates as a possible etiology of infection. Clinicians should be alert to those unique aspects of NTM disease concerning diagnosis with advanced molecular methods and successful treatment with limited options.

Keywords- NTM, *M. mucogenicum*, LiPA.

Citation Vyawahare Chanda R., et al., (2017) Incidence of *M. mucogenicum* Infection in Tertiary Care Hospital India: Recent Increase in Number of NTM Cases. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 9, Issue 10, pp.-959-962.

Introduction

Nontuberculous mycobacteria (NTM) are emerging pathogens that affect both immune-compromised and immune-competent patients. The incidence and prevalence of NTM lung disease are increasing worldwide and rapidly becoming a major public health problem.The American Thoracic society (ATS) and Infectious Diseases Society of American (IDSA) published clinical guidelines for NTM in 2007.[1-3] Because of the difficulty in distinguishing between NTM isolation and diseases, clinical and microbiological criteria are needed for the diagnosis of NTM lung disease. Currently recommended treatment regimens, drug resistance patterns, and treatment outcomes differ according to the NTM species, and management is a lengthy complicated process with limited therapeutic options. [4,5] In NTM *Mycobacterium mucogenicum (M.mucogenicum)* has emerged as a frequent cause of infection in healthy as well as in immune-compromised patients. *M.mucogenicum* was frequently isolated from tap water, drinking water, aquatic environments like cooling towers, pools and shower water. *M. mucogenicum* was first recognised as a human pathogen in late 1970s, when it was isolated from several patients who were undergoing chronic peritoneal dialysis. [6-8] *M.mucogenicum* has been identified as water contaminant in hospital settings and could be the source of nosocomial infections such as post surgical wound infection, soft tissue infection, catheter related sepsis, peritonitis, following peritoneal dialysis, bacteraemia in patients undergoing haemodialysis, and central venous line associated sepsis, meningitis, pneumonia and lymphadenitis. *M. mucogenicum* was also reported as principal cause of infection involved in bacteraemia patients after bone marrow transplantation at a hospital in Minnesota. [9-11] However, some cases have occurred in immune-competent patients. Increasing incidence of rapidly growing Mycobacteria (RGM) infections in various clinical infections and rising incidence of *M. mucogenicum* infections in hospital settings drew attention to the prevalence of *M. mucogenicum* in our set-up.Since treatments and outcomes differ depending on the NTM species, its identification is clinically important. Traditional biochemical tests or high performance liquid chromatography for NTM identification have been replaced by molecular methods such as line probe hybridization, polymerase chain reaction (PCR), restriction fragment length polymorphism analysis, real-time PCR, and DNA sequencing. Some commercial kits are available, including the AccuProbe system (Hologic Inc.), INNO-LiPA Mycobacteria system (Fujirebio Europe, Ghent, Belgium), and GenoType Mycobacterium system (Hain Lifescience, Nehren, Germany) [4,12-13] The present study was designed for retrospective analysis to identify *M. mucogenicum* from various clinical samples and to do comparative analysis with reference to clinical syndrome, predisposing factors and its demographic information.

Material and Methods

Study was conducted in tertiary care hospital From January 2013 to December 2013 in department of Microbiology. Various clinical samples were received from

suspected NTM infection with symptomatic and suggestive radiographic findings. Related sociodemographic details and clinical findings of the patient were also documented.

Clinical samples: Various pulmonary and extra-pulmonry samples like sputum, bronchoalveolar levage (BAL), plural fluid, ascetic fluid, pus, FNAC aspirate, Lymph Node tissue, endometrial tissue, appendix tissue, CSF etc. received in the Department of Microbiology.

Ethical approval was waived for this retrospective laboratory database study

Microbiological investigation:

All the samples except samples from the sterile body sites were decontaminated by using N-acetyl L-cysteine and sodium hydroxide (NALC-NaOH) method[14]and followed as per the routine protocol for mycobacterial identification which includes: Direct microscopic examination of samples by Ziehl-Neelsen (Z-N) staining technique, culture on solid Lowenstein Jensen (L.J.) [10]media as well as liquid culture in automated MB BacT culture (Biomeuriex USA), Line probe assay (LiPA): DNA extraction and PCR- based DNA amplification and reverse hybridization. Those samples showing negative results for mycobacterium tuberculosis complex by LiPA were considered as non tuberculous mycobacteria (NTM) and then subjected for further speciation using Genotype mycobacterium CM and AS kit. [15-18]

Direct Microscopic examination of samples by Z-N staining technique:

All the samples were examined microscopically after Z-N staining for the presence of acid fast bacilli in the direct samples.

Culture on solid Lowenstein Jensen media as well as liquid culture in automated MB BacT culture (Biomeuriex USA): The MB/BacT system consists of a bottle containing 10 ml of modified Middlebrook 7H9 broth enriched with casein, bovine serum albumin, and catalase. Before inoculating specimen, bottles were supplemented with 0.5 ml of MB/BacT MAS supplement (amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, and vancomycin) which was reconstituted with 10 ml of MB reconstituting fluid according to the manufacturer's instructions. Bottles were placed inside the BacT Alert 3D instrument (Bio Merieux Durham, USA) and incubated at 37°C for 6 weeks. Any bottle which displayed as positive was taken out of the instrument.

Similarly all the samples were also inoculated on LJ medium and incubated at 37°C for 8 weeks. Bottles were examined for growth every week and L.J. bottles failing to show any growth after 8 weeks were discarded as negative.

Microscopy: Any growths obtained in the bottle were stained by Z-N staining for detection of acid fast bacilli (AFB).

Line probe assay (LiPA): DNA extraction and PCR- based DNA amplification and reverse hybridization were performed with a TwinCubator (Hain Lifescience GmbH, Nehren, Germany).(9) Results were read by lining strips code provided with the kit. In order for results to be valid, CC (Conjugate control) and AC (Amplification control) bands appeared for every sample.

GenoType® Mycobacterium CM and **GenoType® Mycobacterium AS:**

All isolates suggestive of NTM were then subjected to two commercial kits for further speciation, Genotype mycobacterium CM for detection of common NTMs. Isolates not identified by this were further tested with the Genotype AS assay for additional species of NTM. This test is based on the DNA-STRIP® technology and permits the identification of various Mycobacterial species such as *M. simiae, M. mucogenicum, M. goodie, M. celatum, M. smegmatis, M. genavense, M. lentiflavum, M. heckshornense, M. szulgai, M. phlei, M. haemophilum, M. kansasii, M. ulcerans, M. gastri, M. asiaticum and M. shimoidei*

DNA extraction: DNA extraction was performed by sonication. Identification of Mycobacterium tuberculosis complex (MTBC) and NTM species were carried out by using specific sets of primers designed to amplify a species specific 23S rRNA gene sequence of *Mycobacterium* species.

Amplification: Amplification mixture was prepared & amplification carried out in thermal cycler which involved 01 cycles of denaturation solution (DEN) at 95°C for 15 min, annealing of primers at 95°C for 30 s, 2 min at 58°C for 10 cycles, then 20 cycles at 95°C for 25 s, 53°C for 40 s and 70°C for 40 s and final primer extension at 70°C, 8 min for 01 cycle.

Hybridization: Hybridization was done on TwinCubator. 20 µl of amplified product was mixed with 20 µl of DEN (blue) added and incubated for 5 min at room temperature. Then 1 ml of pre-warmed hybridization buffer (HYB, Green) was added, followed by gentle shaking. Now strip was placed in a manner to make sure complete flooding of solution over strips. Then tray was placed in TwinCubator and was incubated for 30 min at 45°C, followed by complete aspiration of HYB. Washing was done by 1 ml of stringent (STR) wash solution followed by ringer solution wash to each strips and incubated. Then 1 ml of diluted conjugate was added to each strips and incubated for 30 min on TwinCubator. Strips were washed again with 1 ml of ringer solution for 1 min, after that 1 ml of diluted substrate were added to each strips and incubated for 3-20 min in the absence of light without shaking. Rinsing was done twice with distilled water to stop the reaction. Strips were removed and dried between two layers of absorbent paper.

Interpretation: Evaluation and interpretation of results were done based on the presence and absence of different bands and compared with reference band as provided in the kit.

Result:

A total of 13 strains of *M. mucogenicum* were identified out of 30 NTM species from various samples received in the department of Microbiology from the suspected cases of NTM infection. The absence of any other pathogenic agent supports the potential clinical significance of *M. mucogenicum*. Of the total 13 *M. mucogenicum* strains 10(76.92%) strains were showed acid-fast bacilli in direct Z-N staining [Fig-1], 11 were also grown on Mac-Conkey's agar and were stained with Gram's staining showed gram positive bacilli and were catalase test positive. All these strains were grown on L-J medium in 3-7 days of incubation at 37°C [Fig-2]. Growths of all 13 strains were also detected in liquid culture in automated MB BacT 2-5 days.[Fig-3]

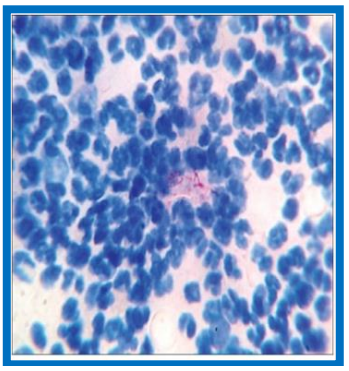

Fig-1 Acid-fast Bacilli from clinical specimen

Fig-2 Growth on L.J medium

Fig-3 Growth in liquid medium: BacT/ALERT

Table-1 *Details of the isolated M.mucogenicum strains*.

Sr.no	Age (yrs)	Sex	Diagnosis	Sample
1	45	M	Lung infection	Bronchoalveolarlevage (BAL)
2	52	M	Tubercular empyema	Pus
3	43	F	Right axillary swelling	Lymph Node tissue
4	28	F	Breast cancer	FNAC fluid
5	51	M	Pleural effusion	Pleural fluid
6	28	M	Osteomyelitis (Left femur)	Drain tip
7	6	M	TB lymphadenitis	Lymph Node tissue
8	28	M	Miliary Tuberculosis	BAL
9	65	F	Tuberculous appendicitis	Excised appendix tissue
10	48	M	Pleural effusion	Pleural fluid
11	30	F	Tuberculous appendicitis	Excised appendix tissue
12	20	F	Endometritis	Endometrial tissue
13	30	F	Right subovarian cyst	Endometrial tissue

Of the 13 samples 7(53.84%) samples were from male and 6(46.15%) samples were from female. 11 [85%] of the samples were from extra pulmonary origin and 2 [15%] ceases were from lung infections [Table-1]

Among the 13 cases only 1 was of the paediatric patient having tuberculous lymphadenitis and 1 case was from geriatric age group having tuberculous appendicitis, rest all were from young adults.

MB BacT liquid cultures were positive in all the 13 cases.

Following the Genotype assay for mycobacterium speciation, all the 13 strains were identified only as Mycobacterium spp. by using CM assay, when further followed with AS assay they identified as *M.mucogenicum*. [Fig-4]

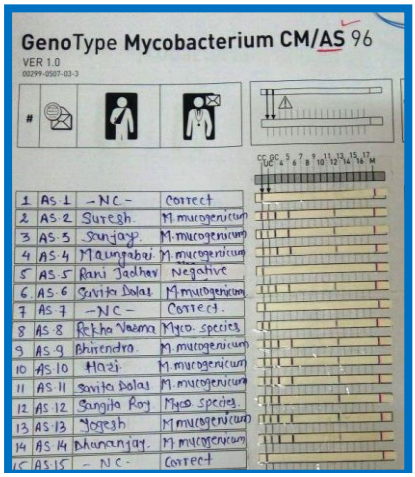

Fig-4 Genotype Mycobacterium CM/AS

Discussion

For the diagnosis of NTM disease, patients suspected to have NTM infection are required to meet all clinical and microbiologic criteria as per the American Thoracic Society (ATS) 2007[3]. Conventional biochemical tests used to identify different mycobacterial species are complex and time consuming. The development of molecular methods allows the characterization of new species and NTM identification at a subspecies level. Even after the identification of NTM species from clinical specimens, clinicians should consider the clinical significance of such findings. Besides the limited options, treatment is lengthy and varies by species, and therefore a challenge. Treatment may be complicated by potential toxicity with discouraging outcomes.

In the study mentioned period we isolated 30 NTM species; on further evaluation with Gernotype Mycobacterium CM/AS assay for speciation, 13 were *M. mucogenicum*. We could also able to isolate *M.fortuitum* in 7 samples. However, 10 isolates could not be speciated by either kit

M. mucogenicum is considered as clinically significant species of NTM. It was described in 1982 as *M. chelonae* like organism and in 1995 was delineated as a unique species *M. mucogenicum*; because of mucoid appearance of colonies.[7]

In the present study we have isolated 13 *M. mucogenicum* from various clinical samples were received from suspected NTM infection with symptomatic and suggestive radiographic findings.

Of the total 13 NTM isolates; 85% clinical specimens were from extra pulmonary site while 2 [15%] clinical samples were from lung disease and both are young immune-competent male patients.

With emergence of case reports and series from diverse countries and regions, it has become clear that the distribution of NTM species that are isolated from clinical samples differs greatly by region.

Catheter related infections are the most clinically significant cases of *M. mucogenicum* infection. From the present study we have isolated only one *M. mucogenicum* in drain tip of osteomyelitis infection and patient was immune-competent. Han XY *et al.,* (2007)[1] reported 52% *M. mucogenicum* of total NTM from blood stream and catheter related infections while in contrast Gaviria *et al.,* (2000)[19] reported 9% *M. mucogenicum* from catheter related infections which shows that frequency of isolation of clinically significant *M. mucogenicum* is increasing.

Skin and soft tissue *M. mucogenicum* infections by Shehan JM *et al.,* (2008)[20], reported skin infection associated with etanercept and Gomez-Moyano *et al.,* (2009) [21] reported furuncle-like lesions in immuno-competent patient. In the present study we have isolated *M. mucogenicum* from two endometrial tissues specimen from endometritis and right sub ovarian cyst of sexually active females and both are immuno-competent. So far there are no documented reports of isolation of *M. mucogenicum* from endometrial tissue. *M. mucogenicum* was also isolated from fine needle aspiration cytology (FNAC) fluid of breast cancer patient. There are not well documented reports of *M. mucogenicum* infection in breast cancer patients.

In present study, *M. mucogenicum* were isolated from 2 cases of disseminated infection *i e.* Tuberculous lymphanditis of six year old male patient and right axillary swelling of 43 year old female patient. Chetchotisakd P *et al*., (2000) reported *M. abscessus* infection in 16 Thai patients manifested as lymphadenopathy and multiple-organ involvement. Toidi A et al., (2006)[22] reported two fatal cases of *M. mucogenicum* central Nervous System Infection in immuno-competent patients from France.

Our study did not perform the drug susceptibility of these isolate, howeverall *M.mucogenicum* infections showed successful treatment outcome when treated by amikacin, cefoxitin, claritromycin, imipenem, trimethoprim-sulfamethoxazole, amoxicillin, amoxicillin-clavulanate, erythromycin, azithromycin, ofloxacin, gatifloxacin, levofloxacin and linezolid according to type of infection. Han *et al*.,[1] reported 100% of *M. mucogenicum* isolates were susceptible to amikacin, cefoxitin, claritromycin, imipenem and trimethoprim-sulfamethoxazole Despite availability of this in vitro data, the management of *M.mucogenicum* infection is largely based on clinical experience.

Conclusion

Attention should be given to *M. mucogenicum* isolates as a possible etiology of infection. As it is estimated that the most common origin of this bacterium is the water supply from hospital settings, consequently standard regular monitoring of hospital water and updating the infection control measures are obligatory preventive measures for patient care. High index of suspicion can detect NTM which would provide correct treatment modalities.Clinicians should be alert to those unique aspects of NTM disease concerning diagnosis with advanced molecular methods and successful treatment with limited options.

Acknowledgement / Funding: Author are thankful to Department of Microbiology, Dr. D Y Patil Medical College, Pune

Author Contributions: All author equally contributed

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Han X.Y., Dé I. and Jacobson K.L. (2007) *Am J ClinPathol.,* 128, 612-21.
[2] Marshall C., Samuel J., Galloway A. and Pedler S. (2008) *J ClinPathol.,* 61, 140-41.
[3] Griffith D.E., Aksamit T., Brown-Elliott B.A., Catanzaro A., Daley C., Gordin F., et al. (2007) *Am J Respir. Crit. Care Med.,* 175, 367-416.
[4] Ryu Y.J., Koh W.J. and Daley C.L. (2016) *Tuberc Respir Dis.,* 79, 74-84.
[5] Pagnier I., Merchat M., Raoult D., La Scola B. (2009) *Emerg Infect Dis.,* 15, 121–22.
[6] Covert T.C., Rodgers M.R., Reyes A.L. and Stelma G.N. Jr. (1999) *Appl Environ Microbiol.,* 65, 2492–96
[7] Springer B., Bottger E.C., et al. (1995) *Int J. Syst. Bacteriol.,* 45, 262-7.
[8] Hawkins C., Qi C., Warren J and Stosor V. (2008) *Diag. Microbiol Infect Dis.,* 61, 187-191.
[9] Hayes P. S., McGiboney D. L., Band J. D. and Feeley J. C. (1982) *Appl. Environ. Microbiol.,* 43,722–24.
[10] Garcia-Agudo L. and Garcia-Martos P. (2011) *A. Méndez-Vilas (Ed.)*: 363: 377.
[11] De Groote M. A. and Huitt G. (2006) *Clinical Infectious Diseases,* 42, 1756-63.
[12] Amresh Kumar Singh, Anand Kumar Maurya et al. (2013) *J Lab Physicians.,* 5(2), 83–89.
[13] Gandham N.R., Sardar M., Jadhav S.V., Vyawahare C.R. and Misra R.N.(2014) *Journal of Clinical and diagnostic research*, 8(5), PD 01-PD 03.
[14] Pfyffer G.E. (2007) Mycobacterium: general charectristics, laboratory detection and staining procedures. In P.R Murray, E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed). Mannual of clinical microbiology, 9th edition. Vol 1. ASM press, Washington DC, P 543-573.
[15] Sharma M., Misra R.N., Gandham N.R., Jadhav S.V., Angadi K.M. and Wilson V. (2012) *Medical J D Y Patil Univ.,* 5(2). 97-100.
[16] Vyawahare C.R., Misra R.N., Gandham N.R., Angadi K.M., Ghatole M. and Kotadia S.N. (2012) *BMC infect Dis.,* 12(Suppl), P 17.
[17] Jadhav S.V, Misra R.N., Gandham N., Angadi K., Vyawahare C. and Gupta N. (2015) *International J Microbiology Research,* 7(3), 636-40.
[18] Jadhav S.V., Vyawahare C.R., Chaudhari N., Gupta N.S., Gandham N.R. and Misra R.N. (2013) *Journal of Clin and Diagnostic Research,* 7(9), 1996-98.
[19] Gaviria J.M., Garcia P.J., Garrido S.M., Corey L. and Boeckh M. (2000) *Biol Blood Marrow Transplant,* 6, 361-69.
[20] Shehan J.M. and Sarma D.P. (2008) *Dermatology Online Journal*, 14(1), 5.
[21] Gomez-Moyano E., Del Boz Gonzalez J., Bermudez- Ruiz P. and Sanz-*Trelles A. Med Clin (Barc),* 132, 370.
[22] Adékambi T. (2009) *ClinMicrobiol Infect.,* 15, 911-18.