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Molecular diagnostic methods in detection and identification of non-tuberculous mycobacteria in post-operative wound infections: A systematic review

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Abstract: *Background:* Post-operative wound contamination with non-tuberculous mycobacterial (NTM) organisms is not rare, however, it poses difficulty in identification and isolation using routine standard laboratory tests. *Objective:* In past decades, numerous molecular techniques were introduced to solve this issue however, the results were heterogeneous. There is a need for identification of rapid, sensitive, and effective molecular methods for NTM detection. *Methods:* Published articles relevant to research questions were searched in the database (PubMed, Scopus, Web of Science). Additionally, studies were searched through snowballing. The articles were included as per eligibility for data extraction. The included articles were assessed on quality reporting and results were presented using the descriptive analysis.*Results:*27 studies articles (131 patients) out of 3439 searches were included. 12 and 15 were case series and case reports respectively. Cases were reported mostly from India, Brazil, South Korea, and the United States of America. 16srRNA gene sequencing and reverse hybridization techniques were the common techniques used. Most frequently isolated NTM reported in POWI was M. abscessus followed by M. chelonae. Overall quality of case reports and case series were high to moderate, however, in general evidence level from case report and case series is considered a slow. *Conclusion:* The 16sr RNA gene sequencing and reverse hybridization techniques were the most common, faster, sensitive and specific diagnostic method sutilized in hospitals to detect NTM. However, most of this evidence is presented from the case reports and case series, prospective randomized clinical trials with a quality methodology should be conducted to ascertain the real effect estimate.
Keywords: Post-operative wound infection (POWI),Non-tuberculous mycobacteria (NTM), Molecular method

Introduction

Post-surgical wound infection (POWI) is a common but preventable complication. With over 23% cases of surgical site infections (SSI) reported each year that can lead to an increase in antibiotic resistance in bacteria as well as delayed recovery [1]. *Staphylococcus aureus*, Coagulase- negative *staphylococci (CoNS)*, *Enterococcusspp.* and *Escherichia coli* are the most common organisms responsible for post-SSI [2]. In recent years, opportunistic infection of post-operative surgical wound by Non-tuberculousmycobacteria (NTM) has emerged as one of the main cause of complications and morbidity [3].

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Although surgical safety checklist (SSC) by WHO has decreased the incidence of POWI significantly, it still remains a major concern in developing countries [4-5]. Clinicians and surgeons face a major challenge in identifying NTM in non-healing surgical wounds. This is attributed to difficulty in identification of NTM with conventional detection methods [6].

Non-detection or delayed identification of NTM, has a prolonged impact on the social and economic well-being of patients. Another cause of delayed diagnosis is time taken for culturing and isolation of NTM from the sample such as pus. Not only rapid but also accurate identification of NTM is essential to control and treat the POWI. The current laboratory protocol follows the gold standard of culture and isolation of NTM, however, it is time consuming and less capable to identify the species. This obstacle can be overcome using the newer advanced techniques however, the cost, technical expertise limits their use in limited resource hospitals.

Recent introduction to strip-based PCR and gene based analysis techniques with some cost-effectiveness has provided some sort of promise to stakeholders. However, there are numerous techniques with multiple modifications causing the dilemma overuse of one technique over another. There is a need for identification of the most feasible and relevant detection method that can be employed in minimal resources, especially in developing countries. Considering it, we conducted systematic review to gather evidence on diagnostic methods for detection /isolation of NTM species in POWI.

Material and Methods

This review was undertaken as part of an Indian Council of medical research funded extramural project (Project Id No- 2019-1394), Institutional Ethics Committee Approval was taken for the project (PCMS/OD/2019/470-1).

There view was registered on review registry.com (Unique identification Number- review registry 987, dated on September 042020). This systematic review is reported in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Supplementary file1: PRISMA checklist) [7].

Eligibility criteria: Studies were selected based on PICOS (population exposure – comparator – outcomes – study design) framework.

- *Population:* human population without age, gender, or demographics restrictions
- *Exposure:* POWI caused by NTM
- *Outcomes:* Identification/detection/confirmation of POWI caused by NTM using either conventional or molecular technique.
- *Study design:* Retrospective cohort study, case-control study.

Inclusion and Exclusion criteria: The published cases must have had documentation of: (i) method(s) of diagnosis, (ii) species of NTM. The reports mentioned in conference abstracts, editorials, poorly described cases, and narrative reviews were excluded.

Search strategy: Initial search with predefined keywords was performed and later detailed search strategy was developed. We searched Medline/PubMed Scopus, Google Scholar, EMBASE, Cochrane database for eligible case reports. Articles published between Jan 2010 to Dec 2020 were included for assessment in the present systematic review (most of the Technologies were developed in last 10years). No language restrictions were used in search strategy. Bibliographical references of potentially related articles were checked and searched for additional articles.

Study Selection: Two reviewers independently screened the retrieved titles and abstracts of articles for relevance after duplicates were removed. Those found eligible as per inclusion and exclusion criteria were selected for full-text retrieval. Two reviewers performed full-text screening (Ashok Mhaske and Shubhangi M).The articles were excluded based on agreement between two reviewers. Any differences were resolved through consensus and discussion with a third reviewer (Monal Yuwanati). Final eligible articles were selected for data extraction.

Data collection and extraction: Data were extracted in standardized Microsoft excel format. All authors reviewed, verified the

data. Inconsistencies were rectified through discussion and revisiting the full text of cases reports needed. Extracted data includes following details:

- Details of study (author(s), year, country)
- Particulars of patient (clinical manifestation, surgical procedure, location of wound, age, gender etc.)
- Laboratory methods of Diagnosis, type of NTM species.

Quality Assessment tools: The Joanna Briggs Institute Critical Appraisal checklist for case reports and series was used to assess the quality of reported cases [8]. To summarize the overall quality of case reports and series, these were grouped into the following categories

1. High quality / Low risk of bias (case series/reports that met at least 75% of the quality criteria)
2. Moderate quality/ risk of bias (case series/reports that met between 50% and 74%of the quality criteria)
3. Low quality/High risk of bias (case series/reports that met less than 49% of the quality criteria)

Summary measures and statistical analysis: Publication and patient characteristics were estimated and summarized descriptively. Case reports were grouped based on type of method used for identification, type of NTM species. Variables are reported as frequency and percentage, proportions, means and standard deviations or medians and inter-quartile range as appropriate.

Results

Search result and Study characteristics: The PRISMA flowchart (Figure 1) summarizes the results of a literature search and process of study inclusion and exclusion. Total 27 publications out of 3439 articles were identified through online and snowballing literature search [9-35]. Table 1 contains the characteristics of included studies. 27 articles (15 case reports and 12 were cases series) reporting 131 cases were included in systematic review after removing the duplicates and non-eligible studies. Most of the cases were reported from India (52/131), followed by Brazil (41), South Korea (26) and the United States of America (12/131).

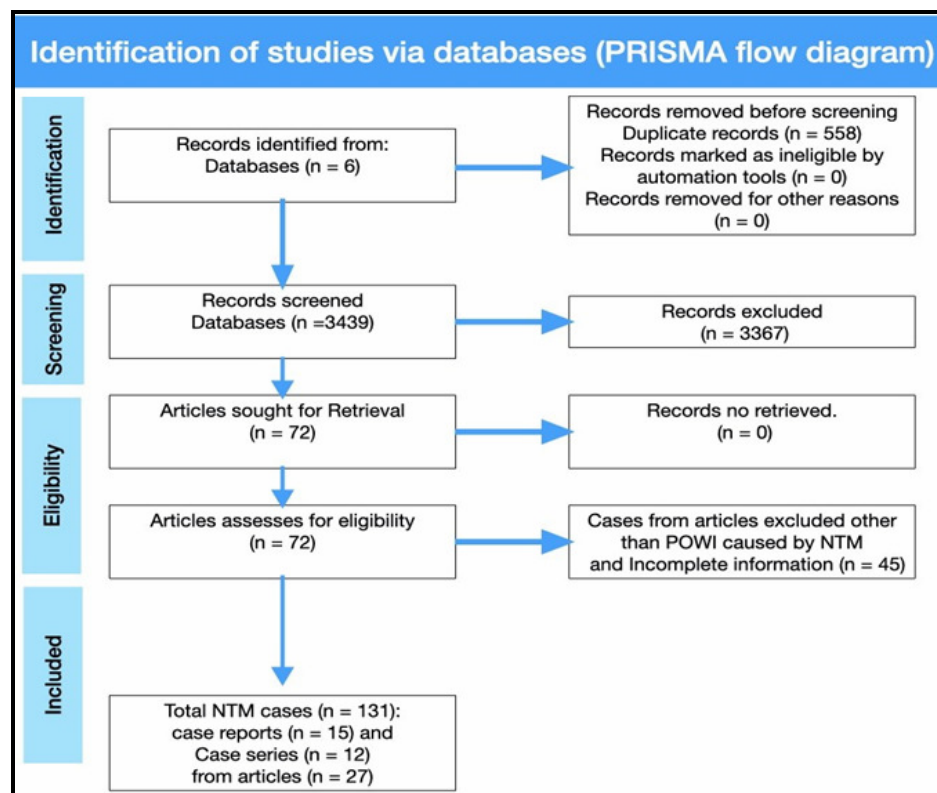**Fig-1:** PRISMA checklist of selected studies

Table-1: Characteristics of investigations included in the systematic review							
Country	Author	Year	No. of cases	Age	Sex	NTM species	Diagnostic method
India	Pandey et al. [9]	2019	6	38	F	*M. abscessus*	16s rRNA gene sequencing
				42	F	*M. senegalenese*	16s rRNA gene sequencing
				44	M	*M. abscessus*	16s rRNA gene sequencing
				53	F	*M.abscessus*	16s rRNA gene sequencing
				48	M	*M.aviumcomplex*	16s rRNA gene sequencing
				16	M	*M.abscessus*	16s rRNA gene sequencing
	Jagadeesan et al. [10]	2018	3	36	F	*M.chelonae*	Multiplex PCR (*Mycobacterium* Chip, X Cyt on Diagnostics)
				72	F	*M.chelonae*	Multiplex PCR (*Mycobacterium* Chip, X Cyt on Diagnostics)
				68	M	*M.chelonae*	Multiplex PCR (*Mycobacterium* Chip, X Cyt on Diagnostics)
	Chogtu et al. [11]	2017	1	51	M	*M.fortuitum*	Geno Type *Mycobacterium* CM kit
	Ghosh R et al. [12]	2017	13			*M.abscessus*	Line probe assay using Geno Type *Mycobacterium* CM/AS
			2			*M.fortuitum*	Line probe assay using Geno Type *Mycobacterium* CM/AS
	Sasikumar et al. [13]	2017	1	30	F	*M.abscessus*	Line probe assay
	Misra et al. [14]	2017	1	49	F	*M.abscessus*	Line probe assay
	Vijayalaxmi et al. [15]	2016	18			*M.chelonae*	PCR
	Achra et al. [16]	2016	1	38	F	*M.fortuitum*	16S rRNA gene-PCR and DNA sequencing
	Madhusudhan et al. [17]	2016	1	28	F	*M.fortuitum*	Line probe assay
	Singh et al. [18]	2015	1	46	F	*M.szulgai and M.Intermedium*	Geno Type *Mycobacterium* CM/AS assay
	Haider et al. [19]	2013	1	40	F	*M.abscessus*	GenoType *Mycobacterium* CM/AS assay

Country	Author	Year	No. of cases	Age	Sex	NTM species	Diagnostic method
	Sharma et al. [20]	2012	1	62	F	*M.fortuitum*	SOD gene PCR and sequencing
	Sarma et al. [21]	2011	1	75	M	*M.abscessus*	Genotype *Mycobacterium* CM kit
	Gandhi et al. [22]	2010	1	45	F	*M.abscessus*	Reverse line blot hybridisation assay
United States	Romero et al. [23]	2017	1	64	F	*M.xenopi*	Partialsequencingofthe16SrRNAgene
			1	47	F	*M.chelonae*	Partial sequencing of the 16S rRNA gene followed by MALDI-TOF
			1	56	F	*M.fortuitum*	MALDI-TOFMS
	Unai et al [24]	2013	1	60	M	*M.chelonae*	PCR
	Callen EC et al [25]	2011	2	21	F	*M.fortuitum*	High-Performance Liquid Chromatography (HPLC)-Mycolicacid analysis
				35	F	*M.fortuitum*	High-Performance Liquid Chromatography (HPLC)-Mycolicaci danalysis
	Hammond SE et al. [26]	2017	1	40	F	*M. chelonae*	PCR-rpoB gene sequencing
	Ariza-Heredia EJ et al. [27]	2011	5	84	M	*M.wolinskyi*	16S-rRNA gene sequencing
				28	F	*M.wolinskyi*	
				73	M	*M.wolinskyi*	
				16	M	*M.wolinskyi*	
				78	M	*M.wolinskyi*	16S-rRNAgenesequencing
Brazil	Monego et al. [28]	2011	36			*M.massiliense*	PCR and sequencing of rpoB gene
			1			*M.abscessus*	PCR and sequencing of rpoB gene
			1			*M.chelonae*	PCR and sequencing of rpoB gene
			1			*M.fortuitum*	PCR and sequencing of rpoB gene
	Coelho et al. [29]	2010	1			*M.massiliense*	hsp 65 gene PCR
	Lima et al. [30]	2013	1			*M.wolinskyi*	rpoB gene analysis

Country	Author	Year	No. of cases	Age	Sex	NTM species	Diagnostic method
Korea	Choi et al [31]	2018	5			NA	PCR
						M.abscessussubsp bolletii	PCR
						MOTT	NA
						M.abscessus	NA
						NA	NA
	Kim et al. [32]	2014	12			*M.abscessus*	NA
						M.abscessus	Advan Sure TB/NTM real-time PCR
						NA	Advan Sure TB/NTM real-time PCR
						M.chelonae	Advan Sure TB/NTM real-time PCR
						NA	NA
						NA	NA
						NA	Advan Sure TB/NTM real-time PCR
						NA	Advan Sure TB/NTM real-time PCR
						M.fortuitum	Advan Sure TB/NTM real-time PCR
						NA	Advan Sure TB/NTM real-time PCR
						M.fortuitum	Advan Sure TB/NTM real-time PCR
	Lee et al. [33]	2014	3			*M.conceptionense*	PCR
						M.conceptionense	PCR
						M.conceptionense	PCR
	Lim et al. [34]	2012	5			*M.abscessus*	PCR-REBA
						M.conceptionense	PCR-REBA, gene sequencing of 16 S rRNA and rpoB
						M.fortuitum	PCR-REBA
						M.fortuitum	PCR-REBA
						M.abscessus	PCR-REBA
	Shin H et al [35]	2020	1			*M.septicum*	PCR

NTM in post-operative wound infection: M. massiliense (37/131), followed by *M. abscessus* (29/131), *M. chelonae* (26/131), *M. fortuitum* (14/131) were commonly isolated NTM species from the POWI (Table 2). Among the 52 cases reported from India, *M. abscessus* (22/52) followed by *M. chelonae* (21/52), *M. fortuitum* (6/52), *M. senegalense*, *M. szulgai* and *M. intermedium*, *M. avium* complex (1/52 each), were identified as causative NTM for POWI. In the 41 cases from Brazil, *M. massiliense* (37) was the most identified NTM

species with reported cases. This was an uncommon strain not found in other countries. Brazil had an outbreak of *M. massiliense* infection following video-assisted surgeries in a hospital. Other species detected in Brazil were *M. fortuitum*, *M. chelonae*, *M. abscessus*, *M. wolinskyi*. In South Korea, multiple species were found to be equally prevalent such as *M. abscessus* (5/26), *M. fortuitum* (4/26), *M.*

conceptionense (4/26) and other identified species (8/26). The lesser common species detected in South Korea were *M. chelonae*, *M. bolletii*, M. s*epticum* and unidentified NTM species with one case each detected. In the United States of America, *M. wolinskyi* (5/12), *M. fortuitum* (3/12), *M. chelonae* (3/12) and *M. xenopi*(1/12)were identified.

Table-2: Country Wise distribution of NTM identified in Post operative wound infection																					
Country	Total	M.fortuitum	M.chelonae	M.kansasii	M.abscessus	M.Abscessussp.bolletii	M.massiliense	M.maltophilia	M.conceptionense	M.wolinskyi	M.senegalense	M.Septicum	M.flavescens	M.szulgaiandM.intermedium	M.xenopi	M.magerienseandfortuitum	M.neoarumandM.massiliense	M.aviumcomplex	M.abscessuscomplex	MOTT	Others
India	52	6	21		22						1			1				1			
Brazil	41	1	1		1		37			1											
Korea	26	4	1		5	1		1	4			1								1	8
United States	12	3	3							5						1					
Total	131	14	26	0	28	1	37	1	4	6	1	1	0	1	1	0	0	1	0	1	8

Molecular Detection Methods: The data was tabulated for each species identification by different molecular diagnostic methods (Table1). It was observed that country wide there was a predilection for using molecular diagnostic methods of choice for the same species in different geographical locations.

Most commonly used methods were *16sr RNA* gene sequencing (7.6%) followed by Advan Sure TB/NTM real-time PCR (6.8%), PCR (5%), *rpo B* gene analysis (4.5%), Line probe assay using Geno Type *Mycobacterium* CM/AS(3%), Line probe assay (2.2%), PCR-reverse blot hybridization assay (PCR-REBA) (2.2%), Multiplex PCR(*Mycobacterium* Chip, X Cyton Diagnostics) (2.2%), Geno Type *Mycobacterium* CM kit (1.5%), and hsp 65 gene PCR (0.76%), SOD gene PCR and sequencing (0.76%), High-Performance Liquid Chromatography (HPLC) - Mycolic acid analysis (0.76%), and Reverse line

blot hybridization assay (0.76%) methods for 131 cases.

In India, most widely used molecular methods were *16s rRNA* gene sequencing, Multiplex PCR (*Mycobacterium* Chip, X Cyton Diagnostics), Line probe assay using Geno Type *Mycobacterium* CM/AS, *SOD* gene PCR and sequencing and Reverse line blot hybridization assay (Table1).

In Brazil PCR and sequencing of *rpo B* gene and *hsp 65* gene PCR were most commonly used methods. In South Korea, Advan Sure TB/NTM real-time PCR and PCR-REBA methods were used preferably. In the United States of America, Partial sequencing of the *16S rRNA* gene followed by MALDI-TOF was used. High-Performance Liquid Chromatography (HPLC)-Mycolic acid analysis was also reported.

Quality of Included Case Reports assessing Risk of Bias: The quality assessment of included case reports are presented in Table 3. Although all 12 case series were of low risk bias (case series/ reports that met at least 75% of the quality criteria), only three studies clearly mentioned consecutive patient selection processes. Out of15 case reports, 80% met with quality criteria and were having low risk of bias.

Table-3: JBI Critical Appraisal Checklist for 15 Case reports															
JBI Check list questions	Chogtuetal(2017) [11]	Sasikumaretal (2017) [13]	Misraetal(2017) [14]	Achraetal(2016) [16]	Madhusudhanet al(2016) [17]	Singh etal.2015 [18]	Haideretal(2013) [19]	Sharma et al.2012) [20]	Sarmaetal (2011) [21]	Gandhi etal(2010) [22]	Unaiet al(2013) [24]	HammondSEetal (2017) [26]	Coelhoetal(2010) [29]	Limaetal (2013) [30]	ShinH etal (2020) [35]
Were the patient's demographic characteristics clearly described?	Yes	Yes	Yes	No	Yes	unclear	Yes	Yes	Yes	Yes	Yes	Yes	unclear	Yes	Yes
Was the patient's history clearly described and presented as a time line?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	yes	Yes	Yes
Was the current clinical condition of the patient on presentation clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	unclear	Yes	Yes
Were diagnostic tests or assessment methods and the results clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	yes	Yes	Yes
Was the intervention or treatment procedure(s) clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	yes	Yes	Yes
Was the post-intervention clinical condition clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were adverse events(harms) or Unanticipated events identified and described?	Yes	Yes	no	Unclear	Yes	no	Yes	Yes	Yes	Yes	Yes	Yes	yes	Yes	Yes
Does the case report provide take away lessons?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	yes	Yes	Yes

Discussion

The present systematic review of published cases of POWI of NTM conducted to provide an insight on epidemiology, clinical manifestations, diagnostic methods and bacterial species involved in POWI. *16s rRNA* gene sequencing was the most common molecular method to detect the causative microorganism. However, in most of the cases this was not the first choice of investigation since most hospitals and laboratories rely on standard laboratory investigation to detect the causative organism.

Further more, the requirement of costly equipment and high-level technical expertise are a hindrance to requesting these molecular investigations. In the last few years, there has been advancement in technology, which makes these technologies affordable, as well

as an increase in their sensitivity and specificity. In addition, hospitals and patients are keen on rapid diagnosis to reduce economical as well as health burden. Further, due to variability, longer turnaround time for NTM identification, investigators focus has shifted to molecular methods in the last few decades [36-38].

Rapid detection and speciation is possible and advantageous to clinicians for prompt management of infection. There are several other molecular technologies especially polymerase chain based techniques, currently identification and speciation of microorganism including nucleic acid hybridization probes, line probe hybridization assays, matrix-assisted laser desorption /ionization time-of-flight mass spectroscopy (MALDI-TOFMS), and DNA sequencing. These techniques can give results within 5 to 12 hours' time frame although they require qualified experts. Gen-Xpert, a nucleic acid base molecular technique has 71 % sensitivity, 98.6% specificity, as compared to ZN stain [39].

Geno type *Mycobacterium* Assay is another, strip based assay which can identify 156 mycobacterial strains including the NTM [40]. Nevertheless, some of the molecular techniques commonly used cannot differentiate between all the NTM organisms and complexes among organisms of *Mycobacterium* [41]. rpoB partial gene sequencing, 16S-rRNA, hsp65 gene technique can assist in discriminating the species. However, these techniques are costly and available only in selected levels.

There are different NTM species involved in postoperative infection and varies from site to site, has different clinical courses and antibiotic sensitivity. Hence, it is empirical to not only diagnose it rapidly but also find the exact species involved in infections. The post-operative wound infection mostly presents itself as a non-healing wound with sero-sanguinous or purulent discharge. Incase of *M. abscessus* which is the most common NTM species observed and detected in post-operative, wound *i*nfections, it usually presents itself as nodules, erythematous papules/ pustules, and papular eruptions or abscesses. Second most frequently observed NTM species *M. chelonae* shows odorless and watery discharge rather than purulent. Further,

these two species may present first symptoms at different timings after the initial surgery. Post-operative wound infection with *M. chelonae* may show first symptoms after 11months.

However, long-term studies are required to confirm it and multiple factors will have influence on manifestations. Nevertheless, it is important to identify species at earliest considering the variation in antimicrobial susceptibility of these two variants. Post-operative wound infections (POWIs) usually reported 3 to 7 weeks' post-surgical procedure, during hospitalization or home care with wide spectrum of clinical presentation [42-43]. According to the World health organization (WHO) and Centre of Disease Control (CDC), USA, POWI occurs within 30 days after the operative procedure [44].

Purulent discharge, redness, erythema, fever, in addition to swelling, nodules, or draining sinus are the typical signs and symptoms. However, many other pathogenic infections can also present with similar features. Although the clinical presentation of POWI is not pathognomonic of NTM infection, a negative or non-response to routine antimicrobial and negative /sterile culture, one should suspect NTM infection [43]. There are various risk factors associated with POWI [44-48].

Most of these risk factors are existing underlying medical conditions or disease. The significant portion of cases in this review had underlying conditions and comorbidity at the time of post-operative infection. This underlying conditions/ comorbidities indicate the ubiquitous opportunistic characteristic nature of this low virulence microbe atypical mycobacteria or NTM, which can complicate the successful surgical procedures in patients.

Diabetes mellitus and hypertension were the most common comorbidity associated with NTM infection in all cases irrespective of geographic location. Furthermore, there is a strong relationship with NTM in chronic obstructive pulmonary disease, chronic respiratory failure, bronchiectasis, HIV,

interstitial lung disease, rheumatoid arthritis, and hematopoietic malignancies [49]. These co-morbidities in at the time of NTM infections can cause death [50].

Hence, the prompt identification of the pathogen is paramount for the clinician to quickly implement the treatment protocol. However, the accurate identification of pathogensis dependent on sample (specimen), collection, handling and processing of samples [51]. Although laboratories follow standard guidelines and protocols, isolating NTM pathogens is difficult in many countries in a short period. In addition, contamination of samples excessive time and the availability of advanced infrastructure such as molecular methods can influence it [52].

The Gram stain is not a routine requested diagnostic test for identification of causative microorganism [36]. This could be attributed to the fact that POWI with NTM is not usually suspected [52-53]. Although gram stains can assist in initial diagnosis of NTM infection, however, they occasionally show positivity and are visible on smears. In addition, it requires rich experience and takes time to screen a large field in the smear. Currently, there are no cohort studies substantiating the Gram stains role in identification of NTM. This may be perhaps due to less research in this aspect as well as non-sensitivity of NTM to Gramstain [54].

In contrast, Ziehl-Neelsen stain is a universally accepted microbiological stain for AFB, but it has lower sensitivity and specificity especially if it is carried out on tissue samples. NTM are difficult to grow in tissue as compared to culture media. It is advisable to culture the tissue to obtain the microbial colonies. This review found that pus was collected as a sample for isolation and identification of organisms. Fluorescent stains were also utilized in identification of NTM in smear which has better sensitivity as compared to AFB stain but only on smears prepared from culture or directly from pus or fluid samples. Auramine-O-Rhodamine (AO) is a simple and faster technique although less specific than AFB stain [55]. Only a few case reports employed AO for fast identification of NTM in either tissue or pus samples.

Culturing growth of NTM is a gold standard method for identification of NTM organisms even in the majority of case reports, the identification as well as confirmation was performed on the culture method. Both solid and liquid media were used, and recently automated culture systems were recommended for heavy patient sample load setup. Traditionally LJ medium shows good growth but it is consistent for all mycobacterial species including NTM, and it usually takes more time for growth. LJ media was used in 64% of case reports for diagnosis of NTM infection.

Surprisingly only one case report used 7H11 liquid media for NTM growth. The reason behind this observation could not be ascertained although it may be laboratory preference or protocol in NTM specimen processing. Most of the cases reported in literature fail to identify the causative microorganism resulting in the increased morbidity, unwarranted medication, additional surgical procedures, and hospitalization. Rapid and accurate identification using the molecular technique can avoid these problems. In addition, it can help in resolution of postoperative infection with help of antibiotic susceptibility testing of identified NTM.

Limitations: The existing review provides detailed information about most common molecular diagnostic methods and NTM species in POWI. A comprehensive search was carried out for journal databases using a systematic and logical search method to identify and include cases in this review. Notwithstanding having applied strict exclusion and inclusion requirements, it is not possible to rule out the risk of losing any essential cases or facts, if certain particular patient details were not accessible in these published articles. Since many journals have stopped publishing case reports or have policies to publish only rare and novel case presentations, chances of publication bias may limit review to only rare and atypical findings excluding common or frequent observation [56].

Practice implications: The findings of this study have helped to assess common molecular methods that can rapidly detect the NTM in non-healing POWI that are not responsive to routine antimicrobial therapy. Well-established PCR based molecular techniques must be utilized for identification of less common or new pathogenic species.

Early and quick identification will enable the clinician to initiate drug regime at earliest on a case-by-case basis and prevent NTM related mortality and morbidity. Potential work should concentrate on optimizing and integrating molecular techniques as well as assessing the importance of utilizing molecular techniques into the diagnostic protocol routinely.

Conclusion

The 16sr RNA gene sequencing and reverse hybridization techniques were the most common, faster, sensitive and specific diagnostic method sutilized in hospitals to detect NTM. However, most of this evidence is presented from the case reports and case series, prospective randomized clinical trials with a quality methodology should be conducted to ascertain the real effect estimate.

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