Nontuberculous Mycobacteria: An Underestimated Cause of Bioprosthetic Valve Infective Endocarditis

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Background. Atypical mycobacteria, or nontuberculous mycobacteria (NTM), have been barely reported as infective endocarditis (IE) agents.

Methods. From January 2010 to December 2013, cardiac valve samples sent to our laboratory as cases of blood culture-negative suspected IE were analyzed by 16S rDNA polymerase chain reaction (PCR). When positive for NTM, *hsp* PCR allowed species identification. Demographic, clinical, echocardiographic, histopathological, and Ziehl-Neelsen staining data were then collected.

Results. Over the study period, 6 of 370 cardiac valves (belonging to 5 patients in 3 hospitals) were positive for *Mycobacterium chelonae* (n = 5) and *Mycobacterium lentiflavum* (n = 1) exclusively on bioprosthetic material. The 5 patients presented to the hospital for heart failure without fever 7.1–18.9 months (median 13.1 months) after biological prosthetic valve implantation. Echocardiography revealed paravalvular regurgitation due to prosthesis dehiscence in all patients. Histopathological examination of the explanted material revealed inflammatory infiltrates in all specimens, 3 of which were associated with giant cells. Gram staining and conventional cultures remained negative, whereas Ziehl-Neelsen staining showed acid-fast bacilli in all patients. Allergic etiology was ruled out by antiporcine immunoglobulin E dosages. These 5 cases occurred exclusively on porcine bioprosthetic material, revealing a statistically significant association between bioprosthetic valves and NTM IE ($P < .001$).

Conclusions. The body of evidence confirmed the diagnosis of prosthetic IE. The statistically significant association between bioprosthetic valves and NTM IE encourages systematic Ziehl-Neelsen staining of explanted bioprosthetic valves in case of early bioprosthesis dysfunction, even without an obvious sign of IE. In addition, we strongly question the cardiac bioprosthesis conditioning process after animal sacrifice.

Keywords. bioprosthetic valve; infective endocarditis; nontuberculous mycobacteria.

Infective endocarditis (IE) is a rare but serious disease with a high mortality rate, even in Western countries [1]. Appropriate antibiotic treatment is the cornerstone of therapeutic care and relies on identification of the causative microorganism. At present, approximately 5% of IE cases remain non-microbiologically documented, mainly due to prior antibiotic administration or to fastidious organisms [2, 3]. It is thus essential to improve the diagnosis and management of such blood culture-negative IE. Of the approximately 174 *Mycobacterium* species that have been described and classified into 2 major groups, or "rapidly growing" and "slowly growing" nontuberculous mycobacteria (NTM), also known as atypical mycobacteria [4], approximately 80 species have been described in human disease. These

species have been mainly reported in lung or skin and soft-tissue infections and in immunocompetent patients or patients with impaired immunity [5]. These organisms are also involved in waterborne nosocomial outbreaks [6]. Very few have been reported as agents of IE, which are mostly *Mycobacterium fortuitum*, *Mycobacterium abscessus*, and *Mycobacterium chelonae* [7–10]. Since the first reports in the late 1970s [10–12], approximately 40 papers have been published on this topic. Our laboratory performs bacteriological analysis of cardiac valves, vegetations, and prostheses for a cardiac teaching hospital (Hôpital Louis Pradel) and surrounding private heart surgery clinics (Clinique du Tonkin and Infirmerie Protestante) in Lyon, France. In case of IE suspicion, 16S rDNA polymerase chain reaction (PCR) is performed when cardiac samples and blood cultures remain negative on conventional media. Between January 2010 and December 2013, 5 cases of bioprosthetic valve failure were found to be NTM IE. The strong association of NTM with bioprosthetic valve IE that we put in evidence alerted us and urged us to report those cases so that the medical community can be warned and public health authorities can get involved.

METHODS

Study Population and Study Design
Polymerase chain reaction data for cardiac samples analyzed from January 1, 2010 to December 31, 2013 were retrospectively collected. Demographic data on the related patients, the valve type, and its anatomic localization were compiled. Samples with 16S rDNA PCR positive for NTM underwent *Mycobacterium*-specific *hsp* PCR for species identification. The clinical records of the patients were collected, including their medical history, time of disease occurrence, and clinical symptoms at presentation, and echocardiographic signs, the types of lesions observed during surgery, antibiotic treatment, and outcomes were obtained from their medical files. Histopathological observations were collected. Ziehl-Neelsen staining and a specific culture on Löwenstein-Jensen medium (Bio-Rad, Marnes-la-Coquette, France), Coletsos (Bio-Rad, Marnes-la-Coquette, France), and Supplemented Mycobacteria Growth Indicator Tubes (MGIT, Becton Dickinson, Le Pont-de-Claix, France) were performed.

Molecular Analysis
DNA was extracted from cardiac sample fragments after mechanical cell disruption (MagNA Lyser, Roche Diagnostics, Meylan, France), proteinase K enzymatic digestion, and DNA isolation using a MagNA Pure Compact Nucleic Acid Isolation Kit according to the manufacturer's recommendations (Roche Diagnostics, Meylan, France). Next, 16S rDNA was amplified using the primers 13BS (GCCCGGGAACGTATTCAC) and 91E (TCAAAKGAATTGACGGGGGC), as previously described [13]. Propidium MonoAzide (Interchim, Montlucon, France) was added to the 16S rDNA PCR mix (2.5 µM final) to remove unspecific DNA background, as described by Hein et al [14]. The mycobacterial *hsp65* (*gro*-EL2)-specific sequence was amplified by nested PCR using the primers hsp1 (ACCAACGATGGTGTG TCCATC) and hsp2 (CTTGTCGAACCGCATACCC), and the internal primers hsp3 (CCAAGGAGATCGAGCTGG) and hsp4 (TCGGTGAGCTCSAGCTG) were adapted from Telenti et al [15]. After double-stranded sequencing of the 16S rDNA and *hsp* amplicons (Biofidal, Vaulx-en-Velin, France) with the primers 13BS and 91E or hsp3 and hsp4, respectively, bacterial identification was performed by analyzing the obtained sequences using the leBIBI gene database, a software environment for sequence-based phylogenetic identification of prokaryotes (https://umr5558-bibiserv.univ-lyon1.fr/lebibi/lebibi.cgi) [16]. In brief, the method involves a conventional phylogenetic approach combining a supervised consensus extraction from the chromatograph, the construction of an ad hoc reference data set, alignment, selection of the best evolution model, and phylogenetic reconstruction by using a maximum likelihood method [17].

Antiporcine Epithelium Immunoglobulin E Dosages
Antiporcine epithelium immunoglobulin (IgE) was quantified in sera by fluoro-enzyme immunoassay (ImmunoCAP 250, Thermo Fischer, Illkrich, France) according to the manufacturer's recommendations.

Ethics Committee Approval
According to French law and the Helsinki declaration, this retrospective observational study did not require local institutional review board approval.

RESULTS

From January 2010 to December 2013, 284 patients undergoing surgery for possible or definite IE episodes [18] had their 370 cardiac valves analyzed by 16S rDNA PCR because of a lack of documentation by conventional bacteriology (Figure 1). These 370 samples belonging to 284 patients (288 IE episodes) involved 221 native valves (181 IE episodes), 70 mechanical prostheses (51 IE episodes), and 79 biological prostheses (56 IE episodes) and were further examined.

Of the 284 patients, 200 (70.4%) were men. The patients' median age was 62 years old (range, 7 months to 86 years old). Of the 370 samples, 120 (related to 110 IE episodes) yielded a positive 16S rDNA PCR. Streptococci accounted for 52% of cases (n = 57) (52% of specimens, n = 63), among which oral streptococci were predominant (18 cases, 20 specimens), followed by *Streptococcus gallolyticus* (12 cases, 12 specimens) and enterococci (8 cases, 9 specimens) (Table 1). In addition, 16S rDNA PCR was positive for staphylococci in 16% of cases (n = 17) (15% of specimens, n = 18), most of which involved non-*aureus* staphylococci (11 cases, 12 specimens). Cases of Whipple disease (n = 2), Q fever (n = 1), *Streptobacillus*
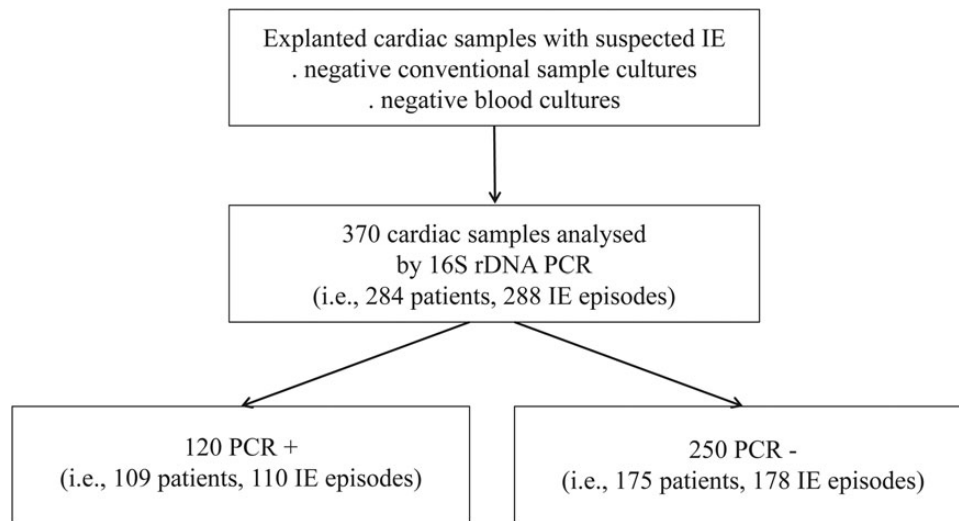
Figure 1. Flow chart of sample inclusion.

moniliformis (n = 1), and HACEK group pathogens (ie, *Haemophilus* species, *Aggregatibacter actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae*) (n = 2) were also diagnosed. Over the study period, 16S rDNA PCR was positive for *Mycobacterium* species in 5 samples, all bioprostheses, from 5 patients (Tables 1 and 2). *Mycobacterium*-specific *hsp65* PCR was performed on these 5 samples and confirmed the presence of *M chelonae* (patient nos. 1, 2, 3, and 5) and *Mycobacterium lentiflavum* (patient no. 4) (Supplementary Figure 1 and 2). Patient no. 1 also had his bioprosthetic mitral valve explanted at the same time, which remained 16S rDNA PCR negative but was positive for *M chelonae* by *Mycobacterium*-specific *hsp* PCR (Supplementary Figure 1). The clinical, microbiological, and histopathological data of the 5 patients were reviewed.

The 5 patients had undergone aortic valve bioprosthesis implantation in the past months or years for severe calcific aortic stenosis. One patient (patient no. 1) had undergone associated mitral valve bioprosthesis implantation because of mitral regurgitation. The 5 patients were admitted to the hospital 7.1–18.9 months after surgery (median 13.1 months) for an impaired general condition and moderate to severe heart failure (Table 2). None of them had fever or reported having fever episodes. Transthoracic and/or transesophageal echocardiography revealed paravalvular regurgitation due to prosthesis dehiscence in all patients, plus perforation of the aortic bioprosthesis in one (patient no. 1). A typical vegetation was detected on echocardiography in only 1 patient. A periannular abscess was found in 2 others, and the last 2 patients did not show additional echocardiographic abnormalities beyond a valvular leak (Table 2). These echocardiographic findings in the 5 patients were confirmed during surgery. Macroscopic examination of the explanted material by the pathologist-microbiologist team

revealed multifocal ulcerated lesions of the tissue in all 6 samples (Figure 2A). Gram staining and conventional cultures of the cardiac material remained negative in all cases, as did the routine blood cultures that were concomitantly sampled. Mycobacteria-specific culture was positive for only 1 of 6 samples (patient no. 4) on Löwenstein-Jensen medium with *M abscessus*.

Infective endocarditis was histologically proven for all patients: inflammatory infiltrates composed of neutrophils, mononuclear cells, and fibrin layers associated with several giant cells were observed in 3 of 6 specimens (patient nos. 1, 2, and 5) (Figure 2B–E). In patient nos. 3 and 4, histology revealed neutrophil infiltrates in conjunctive tissue, with few macrophages and without giant cells. Examination by polarized light microscopy revealed the absence of a foreign body. Ziehl-Neelsen staining of the histological sections revealed the presence of numerous acid-fast bacilli in all patients (Figure 2F). No eosinophil infiltrates were observed. In 3 of the 5 patients, sera were available, and antiporcine epithelium IgE dosages were performed. Patient no. 3 showed no sensitization to the antigen, and patient nos. 4 and 5 had subnormal antibody titers.

Because the NTM IE cases exclusively occurred on bioprosthetic material, the hypothesis of a significant association was posed. Indeed, we found that NTM IE was statistically significantly associated with biological prosthetic valves compared with native valves ($P < .001$) and all other valve types ($P < .001$) (Fischer's exact test) (Table 3). Because antibiotic susceptibility testing was not available, treatment consisted in empirical combined therapy with cefoxitin or imipenem and clarithromycin followed by a fluoroquinolone regimen for a minimum of 6 weeks; 2 patients were still under treatment at the time of manuscript submission. One patient died from a postoperative hemorrhage. The other 4 patients had a favorable clinical evolution.
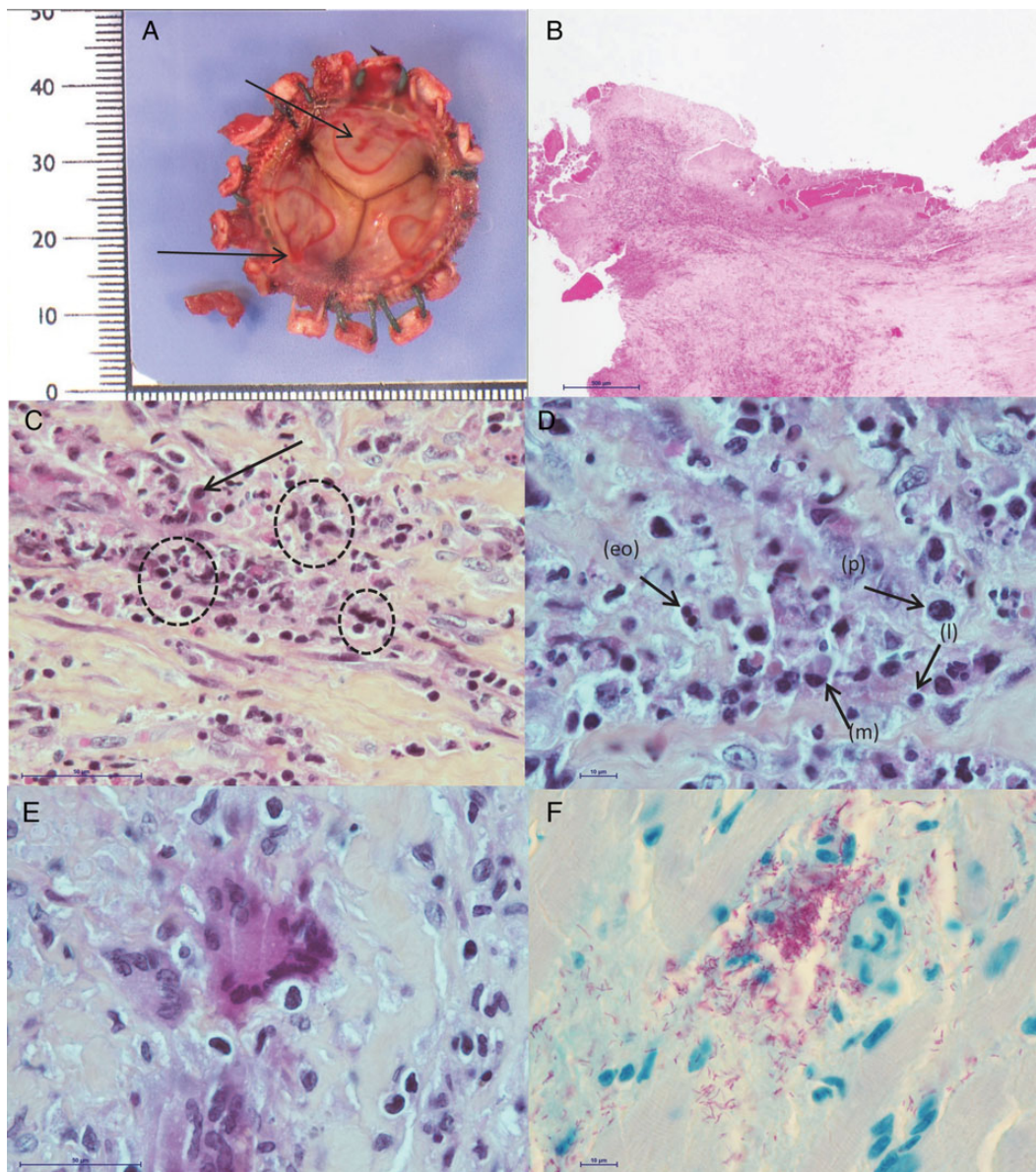
Figure 2. Histological examinations and Ziehl-Neelsen staining of explanted bioprostheses. A, Macroscopic aspect. Endothelial ulcerations (black arrows) with fibrin replacement. B, Vegetation with leukocytes and fibrin matrix at the surface of the prosthesis (hematoxylin-eosin staining) (scale bar = 500 µm) (low magnification). C, Enlargement of (B): focus on the inflammatory infiltrate with macrophages (black arrow) and foci of necrotic neutrophils (dotted circles) (scale bar = 50 µm). D, Enlargement of (C): focus on plasma cells (p), lymphocytes (l), macrophages (m), and rare eosinophils (eo) (scale bar = 10 µm). E, Enlargement of (C): focus on few scattered multinucleated giant cells (scale bar = 50 µm). F, Ziehl-Neelsen staining revealing numerous acid-fast bacilli (in pink) (scale bar = 10 µm).

Overall, the presence of significant histopathological patterns associated with positive Ziehl-Neelsen staining, positive PCR results, and relevant clinical symptoms allowed the diagnosis of NTM prosthetic valve IE.

DISCUSSION

From January 2010 to December 2013, 6 bioprostheses of 370 cardiac samples analyzed by PCR for possible or definite blood culture-negative IE were positive for NTM. The combination of clinical and molecular evidence and the results of Ziehl-Neelsen staining and histopathological analysis ascertained a diagnosis of bioprosthetic valve IE. All of the patients had undergone valvular replacement 13.1 months earlier (median) with a biological prosthesis, revealing a significant association between such prosthesis and NTM IE ($P < .001$). We think that systematic Ziehl-Neelsen staining of explanted bioprosthetic valves, in case of early bioprosthesis dysfunction, could help NTM

Table 1. The 16S rDNA Polymerase Chain Reaction (PCR) Results of Culture-Negative Valves in Infective Endocarditis

Identified Microorganism	No. of Samples (Episodes) n = 120 (110)	% of Episodes
Staphylococci	18 (17)	16
Non-*aureus* staphylococci	12 (11)	10
Staphylococcus aureus	6 (6)	5
Streptococci	63 (57)	52
Oral streptococci	20 (18)	17
Streptococcus gallolyticus	12 (12)	11
Enterococci	9 (8)	7
Group B streptococci	4 (4)	4
Streptococcus pneumoniae	3 (2)[a]	
Other[b]	15 (13)	12
Enterobacteriaceae	9 (7)	6
HACEK group[c]	3 (2)	2
Propionibacterium acnes	6 (6)	5
Mycobacteria	5 (5)	5
Mycobacterium chelonae[d]	4 (4)[a]	
Mycobacterium lentiflavum[d]	1 (1)[a]	
Coxiella burnetii	1 (1)[a]	
Tropheryma whipplei	2 (2)[a]	
Actinomyces oris	1 (1)[a]	
Streptobacillus moniliformis	1 (1)[a]	
Other[e]	11 (11)	10

[a] Percentages were not reported because of very small numbers.

[b] Including *Streptococcus lutetiensis* (n = 1), *Streptococcus gordonii* (n = 4), *Streptococcus* species (n = 4), *Gemella haemolysans* (n = 2), *Gemella morbillorum* (n = 2), *Gemella bergeri* (n = 1), and *Gemella* species (n = 1).

[c] Including *Aggregatibacter actinomycetemcomitans* (n = 1) and *Cardiobacterium hominis* (n = 2).

[d] Obtained by *Mycobacterium*-specific *hsp* PCR.

[e] *Acinetobacter* species (n = 1), *Bacillus* species (n = 1), *Brevibacterium* species (n = 1), *Burkholderia* species (n = 1), *Corynebacterium* species (n = 1), *Friedmanniella capsulata* (n = 1), *Hypho-microbium denitrificans* (n = 1), *Micrococcus* species (n = 1), *Neisseria flava* (n = 1), *Paracoccus* species (n = 1), or contaminated DNA sequence (n = 1).

IE diagnosis and that the bioprosthesis disinfection process after animal explantation should be explored as a route of contamination.

Histopathological analysis revealed the association of neutrophil infiltrates with either giant cells or macrophages. The diagnosis of foreign body granuloma was excluded by examination by polarized light microscopy. The presence of giant cells and macrophages associated with acute inflammation is very unusual and should alert one to mycobacterial infection. Such association was previously noted in a case of *Mycobacterium chimaera* IE with multiple septic emboli [19]. In the current study, specific mycobacterial culture was positive only in 1 patient (patient no. 4), which could have been related to the detrimental effect of peroperative antibiotic prophylaxis or the presence of metabolic variants associated with viable but noncultivable (VBNC) mycobacteria, as previously observed in latent infections [20].

In our study, for the first time, we showed a significant association between NTM IE and biological prosthetic valves ($P < .001$). This association is all the more significant because bioprostheses accounted for only 20% of the 370 total analyzed samples and because none of the 7084 other 16S rDNA PCRs performed in the same time period on diverse sample types was positive for NTM. The 5 cases occurred on porcine bioprostheses only, but no significant association with porcine or bovine origin could be inferred because of missing information for 19 bioprosthetic samples. Only possibly minimal sensitization to porcine antigens was suggested in 2 of 3 patients in our study, excluding allergy as a differential diagnosis [21, 22]. In addition, the time of appearance of symptoms (median 13.1 months, range 7.1–18.9 months) is inconsistent with both mechanical dysfunction, which usually occurs after 10–15 years [23], and with postoperative IE due to common pathogens such as *Staphylococcus aureus* and non-*aureus* staphylococci, which develop earlier and in a more aggressive way. The timeline here suggests a very indolent infectious process that could thus be misdiagnosed as noninfectious, leading to an underestimation of NTM prosthetic IE.

The aforementioned body of evidence confirmed the diagnosis of NTM prosthetic valve IE, which is of great importance because NTM infections are known to be difficult to treat, requiring long-lasting multitherapy in most cases [24]. Moreover, no recommendation concerning NTM IE treatment is included in current guidelines, most likely due to the extremely low incidence of this entity. Addition of a second antimicrobial agent, tailored on NTM species, beyond macrolides is recommended [24, 25]. Tobramycin is the recommended aminoglycoside for the treatment of *M chelonae* infections [26]. Of importance, *M abscessus* is known to be resistant to tobramycin and is generally susceptible to cefoxitin, unlike *M chelonae* [27]. Thus, imipenem is preferred to cefoxitin for *M chelonae* infections [26]. Oxazolidinone and glycylcyclines could be valuable and have already been tested as rescue therapy. However, we still lack data for a possible use as first-line therapy [28]. Less is known about the treatment of *M lentiflavum*-disseminated infection, and some reports state variable susceptibilities for ethambutol and rifampicin. Treatment duration is irregularly mentioned in reported cases but, when specified, extends from 10 to 17 weeks [29, 30]. There are no data on the optimal duration of antibiotic therapy for NTM IE, but treatment lasting several months could be considered, as recommended for disseminated infections [24]. However, the use of antibiotic therapy needs to be balanced because we cannot exclude the fact that a proportion of NTM IE, misdiagnosed as mechanical dehiscence, can be cured solely by surgical replacement. Regarding the potential contamination sources, preoperative contamination of implanted valves or peroperative surgical inoculation

Table 2. Clinical and Laboratory Data of the 5 NTM IE Cases[a]

Patient	Cardiology Center	Age (y)/ Sex	Valve Localization	Valve Type	Interval Between Valve Implant and First Suspect Symptoms (Months)	Clinical Symptoms	TTE/TEE Vegetation/ Abscess	TTE/TEE Cardiac Dysfunction
1.	CT	78/M	Aortic	Biological (porcine Labcor)	17.1	Acute valve failure	Vegetation	Severe paravalvular leak. Prosthetic valve perforation
			Mitral	Biological (porcine Medtronic)	17.1	Acute valve failure	Vegetation	Severe paravalvular leak
2.	IP	76/M	Aortic	Biological (porcine Labcor)	7.1	Acute pulmonary edema due to valve failure	Perivalvular abscess	Severe paravalvular leak
3.	CT	81/M	Aortic	Biological (porcine Labcor)	18.9	Acute valve failure	Periannular abscess	Severe paravalvular leak
4.	HLP	76/M	Aortic	Biological (porcine Vaskutek)	13.1	Acute valve failure	No	Severe paravalvular leak
5.	HLP	73/M	Aortic	Biological (porcine Vaskutek)	8.9	Progressive aortic and mitral failure	No	Moderate to progressive severe paravalvular leak

have been proposed [7, 9, 31, 32]. The latter hypothesis is very unlikely because the 6 valves involved in our study were implanted at 3 different hospitals and by different surgical teams. In addition, in case of peroperative contamination, mycobacteria would theoretically affect all patients undergoing valve replacement, and the global incidence of NTM IE would thus be much higher. Our series shows a strong and significant association of NTM IE with bioprosthetic valves only, suggesting contamination of these devices before their implantation, as already proposed by Strabelli et al [9] and pointed out by the Centers for Disease Control and Prevention several decades ago [32]. The valves analyzed in our study belonged to different manufacturer's brands, originating from different pig-breeding farms, as in previously published studies [33, 34]. Regardless of the brand and the breeding farm, the decontamination process of bioprosthetic valves after explantation from animals uses chemicals such as glutaraldehyde [32]. However, mycobacteria, which are highly prevalent in the environment, are known to be resistant to those disinfectants (including 8% aqueous formaldehyde, 2% alkaline glutaraldehyde, and free chlorine), and the disinfection step has recently been suspected in

NTM infections [35, 36]. Thus, one can speculate that in very rare instances, deficient decontamination following explantation from animals could lead to bacterial survival and proliferation—although in a very slow process associated with VBNC conditions [20]—eventually causing IE. Such environmental contamination at the time of sacrifice is supported by case no. 4, in which the valve contained both *M lentiflavum* (PCR) and *M abscessus* (culture). In this last case, we postulate an initial environmental contamination with both species, the dominant *M lentiflavum* being detected by PCR and the subdominant *M abscessus* being detected by culture.

At present, precision and clarification about the bioprosthetic valve manufacturing process are needed. Thus, it is of great concern to mobilize public health authorities. In addition, NTM bioprosthetic IE may be considerably underdiagnosed because it mimics mechanical prosthesis dehiscence and thus is not considered for histological and bacteriological analyses. Finally, we could not rule out the possibility that infection of the bioprosthesis occurred postoperatively; to confirm such a hypothesis, the specificity of superinfection for bioprosthetic valve remains to be elucidated, along with the identification of a route of entry.

Table 2 continued.

Histopathology	Ziehl-Neelsen Staining	Mycobacterial Culture	16S rDNA PCR	*hsp65* PCR	Treatment	Outcome
Necrosis, fibrin with neutrophils infiltrates. Few giant cells	Negative	Negative	Positive: *Mycobacterium* spp	Positive: *M chelonae*	Aortic valvular replacement (mechanical prosthesis)	Death. (Postoperative hemorrhage)
Necrosis, fibrin with neutrophils infiltrates. Few giant cells and rare eosinophils	Rare acid-fast bacilli	Negative	Negative	Positive: *M chelonae*	Mitral valvular replacement (mechanical prosthesis)	
Necrosis, fibrin with neutrophils infiltrates. Few giant cells and rare eosinophils	Numerous acid-fast bacilli	Negative	Positive: *Mycobacterium* spp	Positive: *M chelonae*	Aortic valvular replacement (mechanical prosthesis). Cefoxitin (8 wk) + clarithromycin (6 wk) + moxifloxacin (6 wk)	Recovery (44-mo follow-up)
Neutrophils infiltrates in conjunctive tissue. Few macrophages and rare eosinophils	Numerous acid-fast bacilli	Negative	Positive: *Mycobacterium* spp	Positive: *M chelonae*	Aortic valvular replacement (bioprosthesis). Ceftriaxon + vancomycin (4 wk)	Recovery (32-mo follow-up)
Neutrophils infiltrates in conjunctive tissue. Few macrophages	Numerous acid-fast bacilli	Positive: *M abscessus*	Positive: *Mycobacterium* spp	Positive: *M lentiflavum*	Aortic valvular replacement (bioprosthesis). Imipenem + tobramycin, ofloxacin + clarithromycin (4 wk). Switch to moxifloxacin + azithromycin per os	Recovery. Ongoing treatment
Necrosis, fibrin with neutrophils infiltrates. Few giant cells	Numerous acid-fast bacilli	Negative	Positive: *Mycobacterium* spp	Positive: *M chelonae*	Imipenem + ofloxacin + azithromycin (3 mo) then switch to azithromycin per os	Recovery. Ongoing treatment

Abbreviations: CT, Clinique du Tonkin; HLP, Hôpital Louis Pradel; IE, infective endocarditis; IP, Infirmerie Prostestante; rNTM, nontuberculous mycobacteria; EE, CliniquHLPHpital lymerase haireaction; TEE, transesophageal echocardiography; TTE, transthoracic echocardiography.

[a] Follow-up duration was defined from surgical treatment to the end of the study.

CONCLUSIONS

In this study, we report 5 cases of NTM bioprosthetic IE diagnosed from 2010 to 2013 in our laboratory. Regardless of the mechanism of contamination, the strong association between NTM and bioprosthetic material demands specific attention to a possible diagnosis of mycobacterial IE in the presence of prosthesis dysfunction with or without obvious signs of IE. In this context, careful histological and microbial examination of the explanted material, including at least a systematic Ziehl-Neelsen staining, followed by a molecular technique whenever possible, should be considered.

Table 3. PCR-Analyzed IE Episodes

	IE Episodes	
	NTM-Positive	NTM-Negative[a]
Native valve	0	181
Bioprosthetic valve	5	51
Mechanical valve	0	51

Abbreviations: IE, infective endocarditis; NTM, nontuberculous mycobacteria; PCR, polymerase chain reaction.

[a] Includes 16S rDNA negative PCR or positive for another bacterium. *P* values: $P < .001$ between bioprosthetic and native valves, $P < .001$ between bioprosthetic valves and both native and mechanical valves. Statistical association was estimated using Fisher's exact test.

Supplementary Material

Supplementary material is available online at *Open Forum Infectious Diseases* (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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