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Emergence of *Mycobacterium gordonae* in heater cooler units: a five-year prospective surveillance on devices frequently subjected to chloramine-T booster disinfection

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Keywords: heater-cooler; non-tuberculous mycobacteria; disinfection; resistance; *Mycobacterium gordonae*.

Summary

Background: Worldwide, the detection of *Mycobacterium chimaera* in LivaNova heater-cooler units (HCUs) has led to their replacement with other HCUs, although non-tuberculous mycobacteria (NTM) have been reported also for HCUs produced by other manufacturers. In almost all hospitals of our region, LivaNova HCUs have been replaced with Maquets HCU40, regularly disinfected with chloramine-T.

Aim: To report the results of the surveillance over a 63-month operation period of the Maquet devices, and to provide a trend in NTM positivity over time.

Methods: Twenty-nine Maquet devices (HCU40 and HU35) were monitored by two culture methods and PMA-PCR method. The trend in NTM positivity rate was evaluated through the Locally Estimated Scatterplot Smoothing regression and then modelled over time through segmented logistic regression.

Findings: The data acquired during the study period demonstrate a remarkable increase in the positivity rate, especially after their third year (maximum slope change at 1280 days).

Non-tuberculous mycobacteria were isolated in 150 water samples (37.2%); 100% and 62% of HCU40 and HU35 devices respectively were colonized with non-tuberculous mycobacteria. The most frequently detected species were *Mycobacterium gordonae* (73%) followed by *Mycobacterium chelonae* (41%) and *Mycobacterium paragordonae* (11%).

Conclusion: Preventive strategies by disinfection with chloramine-T did not effectively reduce non-tuberculous mycobacteria colonization of Maquet devices. Although, to date, no cases of post-operative invasive infections linked to Maquet devices have been reported, our microbiological results emphasize the need for 1) designing changes to increase safety of devices and 2) researching and developing new disinfection protocols including alternative molecules.

Introduction

Heater-cooler units (HCUs) are devices used during open-chest heart surgery to regulate the temperature of patients' blood and cardioplegia circuits in extracorporeal circulation. These machines consist of a tank, which provides a temperature-controlled fluid (usually water) to an external heat exchanger or warming/cooling blanket through a closed circuit. Contaminated fluid could enter other parts of the device, with potential transmission of bacteria through the air (aerosolized) through the exhaust vent of the device, or other unsealed openings, into the environment and to the patient. One of these devices, the Stockert 3T, manufactured by LivaNova PLC (Sorin Group Deutschland GmbH, Norderstedt, Germany), was shown to increase the risk of patient infection due to colonization by *Mycobacterium chimaera* [1,2].

The first cases occurred in 2011 and were discovered in 2013 [3]; since then, more than 140 severe infections by *M. chimaera* have been reported worldwide in patients who had undergone cardiothoracic surgery with extracorporeal circulation [4]. As previously acknowledged, cases of NTM infection in patients have been reported to the US Food and Drug Administration (FDA) after undergoing cardiac surgery with extracorporeal circulation using contaminated LivaNova HCUs (Stockert 3T), as well as contaminated devices from Cincinnati Sub-Zero 333W, Hemotherm and Terumo [5,6].

In 2016, the Sorin Group released a safety warning regarding both 1T and 3T devices, recommending microbiological monitoring by measuring total viable counts and checking the possible presence of *Pseudomonas aeruginosa*, coliform bacteria, and non-tuberculous mycobacteria (NTM). In 2016, **Maquet Getinge Group (Rastatt, Germany)** also issued a new instruction manual that advised monitoring atypical mycobacteria in water circuits [7]. The water system of the HCU40 is separated and sealed from the technical cooling airflow through the device. The only spatial connection of the tank water surface to the environment is the filling lid [8].

Subsequently, since June 2017, a microbiological surveillance programme was started for NTM on HCUs used in the main cardiac surgery ward in Piedmont. In 2019, following the guidelines

of the Italian Ministry of Health [9,10], all cardiac surgeries in Piedmont such as other surgeries in Italian regions, began microbiological surveillance on devices and clinical surveillance on patients.

Previously, we reported the results of a microbiological surveillance from June 2017 to October 2019 on Stockert 3T (LivaNova) and Maquet HCU40 (Getinge) devices, which showed a NTM prevalence of 65.5% in a total of 308 HCU water samples analysed. Stockert 3T was the most frequently colonized device with NTM (88.2%), with a frequency of positive samples of 59.5%. Maquet HCU40 devices were less frequently colonized by NTM (33.3%), with a frequency of positive water samples of 13.6%. Moreover, **the same study showed that, particularly for** NTM, disinfection procedures were poorly effective in reducing the total viable counts [11].

The present study is intended to report data collected on the regular microbiological surveillance examinations of Maquet devices used in cardiothoracic surgery theatres in Piedmont region over a 63-month period. Moreover, this study aims at assessing the trend in positivity rates and counts of NTM detected in the monitored devices over time.

Methods

Settings

From February 2019 to April 2024, water samples from HCU40 and HU35 devices manufactured by Maquet Getinge Group (Rastatt, Germany) were collected from four cardiac surgery facilities, located in Piedmont (Italy), currently under active surveillance and microbiological investigation.

Disinfection procedure

All the cardiac surgery facilities enrolled in this study adopted the disinfection protocol suggested by the manufacturer. According to Getinge, suggested disinfection protocols include a weekly treatment with 2% chloramine-T for routine disinfection for 150–200 min or treatment with 5% chloramine-T solution for 24 h if atypical mycobacteria are found in the water system. The manufacturer recommended a terminal water filter with a pore size of 0.2 μm was used to fill the Maquet HCU40 and HU35 water tank.

Sampling

Samples from HCU and HU35 devices were collected during regional microbiological surveillance program. 2.5 litre of circulating water from each HCU (from the patient circuit and the cardioplegia circuit) and 1.5 litre from each water tank of thermoregulatory devices used for HU35 treatment, were collected in sterile plastic bottles containing sodium thiosulphate (10% w/v).

Microbiological analysis

From February 2019 to June 2020 the microbiological surveillance was conducted by molecular method [12] and cultural method suggested by European Centre for Disease Prevention and Control (ECDC) [13]. Later, the surveillance continued, also adding an internal culture method consolidated in our laboratory. In previous studies [14,15] we reported the usefulness of Elite agar that has been shown to have high performance for the recovery of NTM from environmental samples.

Propidium monoazide polymerase chain reaction method (PMA-PCR)

One litre (HCU40) or 0.5 litre (HU35) of each water sample was concentrated by filtration through a 0.45 µm polycarbonate filter (Millipore, Billerica, MA, USA). The filter was then treated with PMA and DNA was extracted as described previously [8,9]. The extracted genomic DNA was analysed using a qualitative multiplex PCR assay (Anyplex™ plus MTB/NTM MDR-TB Real-Time Detection, V2.0; Seegene Technologies, Inc., Seoul, South Korea). Interpretation of the results was performed according to cycle threshold (CT≤ 42) values outlined in a previous study from our group [12].

Culture method according ECD guidelines (reference method)

One litre (HCU40) or 0.5 litre (HU35) of each water sample were concentrated by filtration through a 0.45 µm polycarbonate filter (Millipore, Billerica, MA, USA). The filter membrane was aseptically washed to detach the bacteria and then the eluted specimens were decontaminated with BBL MycoPrep Kit (Becton Dickinson Diagnostic Systems, Franklin Lakes, NJ, USA) and inoculated on Lowenstein Jensen solid medium and BBL MGIT liquid medium (Becton Dickinson). Incubation at 37°C was continued for 42 days for the MGIT 960 system and 60 days at 35 ± 2°C for

Lowenstein Jensen [12,13]. No enumeration of NTM from water samples was undertaken, this method just confirmed presence or absence of NTM.

Culture method on NTM Elite agar

NTM isolation was performed by filtration of 100 mL and 10 ml of each water sample through mixed cellulose esters filter. The filters were directly added to a Petri dish containing NTM Elite agar **an industrial version** (bioMérieux, Marcy-l'Étoile, France) of the RGM medium [16] (0.47% Middlebrook 7H9 base, 0.5% glycerol, 1.3% agar, 0.4% yeast extract, 0.2% glucose, 0.5% bovine serum albumin, 0.0056% oleic acid, and 0.0494% of a mix of selective antibiotics, *i.e.* colistin methanesulfonate, fosfomycin, glucose-6-phosphate, amphotericin and C-390) and incubated at 30° C in a sealed plastic bag to prevent dehydration. Starting from the seventh day, cultures were examined weekly for seven weeks because mycobacteria from environmental samples needed a longer time to grow. Using this protocol based on direct plating of membrane filters on culture medium, we were able to count NTM on filters and obtain quantitative results for each water sample analysed [14,15].

All colonies isolated from MGIT, Lowenstein Jensen and Elite agar, were confirmed as AFB by Kinyoun stain and then subcultured on Middlebrook 7H11 medium and identified by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF). Spectra were acquired by Bruker Microflex LT (Bruker Daltonics, Bremen, Germany) MALDI-TOF MS and analyzed by MBT Mycobacteria RUO Library v.4.0 (Bruker Daltonics, Bremen, Germany). The results from NTM Elite agar analyses were reported as CFU/100 mL.

Statistical analysis

The presence of a positivity in any of the cultures or molecular tests over time was initially assessed via visual inspection of a scatterplot, where the trend in the positivity rate was evaluated through the Locally Estimated Scatterplot Smoothing (LOESS) regression. Following the progressively increasing trend observed, with greater slopes over time, the “critical point” with the

highest instant increase in the slope was identified by finding the maximum value of the second derivative of the LOESS fit.

Moreover, given the non-linear nature of the increase, as the LOESS fit predicted positivity rates with confidence intervals not exceeding 30% in the first years of operation of the devices, the first time point with a predicted positivity rate $\geq 30\%$ was identified and considered as a “threshold point”. Segmented logistic regression was then used to model the trend in positivity rate over time, by adjusting for confounding factors such as device type (HCU vs HU35) and hospital, and by allowing for the presence of an intercept and/or slope change between before and after the “threshold point”.

The presence of a trend in the quantitative recovery of NTM colonies over time was also inspected: to this purpose, a Tobit model was built by right-censoring NTM counts at 1000 CFU/100 ml (full scale of the detection capacity), and non-linear effects were sought using restricted cubic splines. The R statistical program (version 4.3.1)[17] and its “rms” (version 6.7-1) [18] and “vglm” (version 1.1.9) [19] libraries were used for all the plotting and computation.

Results

The study period comprised 63 months of surveillance (February 2019 - April 2024) and a total of 403 water samples from 13 HCU40 and from 16 HU35 devices (Maquet, Getinge Group, Rastatt, Germany) were collected from four cardiac surgery theatres., all operating in hub hospitals (>450 beds) of the same region, located within a 100-km (70-mile) distance from one another.

Overall, mycobacteria were detected in 150/403 samples, with a positivity rate of 37.2%. NTM were detected in all the thirteen HCU40s monitored and in 10 out of the sixteen HU35s (Table I). In 26.6% of the samples all the three tests were carried out (PCR, ECDC cultures, Elite agar cultures), while only two tests (i.e., PCR and ECDC culture or PCR and Elite culture) and 1 test (ECDC culture) were carried out in 60.5% and 12.9% of samples respectively.

The detection rate by test was similar by PCR and Elite agar culture (Table II). Of note, 25 of 26 samples positive with ECDC cultures were also positive with Elite agar cultures.

The most common NTM isolated by culture was *Mycobacterium gordonae* (73%), followed by *Mycobacterium chelonae* (41%), and *Mycobacterium paragordonae* (11%). *M. gordonae* was isolated from twelve HCU40 and from three HU35 devices, alone or in association with *M. paragordonae* or *M. chelonae*; furthermore, the latter was isolated only using Elite agar.

The NTM detection rate rose over the years of surveillance, even though the increase was not linear over time. Taking into account the positivity at any of the tests, the likelihood of a positive sample was almost stable (around 15-30%) for around 3 years (Figure 1): the 30% “threshold” point for the positivity rate was reached at 1150 days (≈ 3.2 years). The period following the “threshold” point was characterised by a remarkable increase in the positivity rate, with a “critical point” (i.e., with maximum slope change) at 1280 days (≈ 3.5 years, see Supplementary Figure S1 for the plot of the second derivative of the trend).

The segmented logistic regression model (considering the trend only) showed a statistically significant increase over time after the “threshold point” ($p < 0.001$ for the slope change), but not before ($p = 0.369$ for the trend in the first period). Table III presents the results of the model in the form of average predicted positivity rates at different time points from the first month to the end of the surveillance period, when estimated positivity rates even above 90% were eventually attained, while the detailed model coefficients are reported in the Supplementary Material (Supplementary Table S1).

An additional multivariate model was built to consider the possible impact of confounders on trend computations: of note, a potentially significant confounding effect was observed from the hospital in which devices were sampled (apparently with a slightly higher likelihood of a positive sample in one of the hospitals, $p = 0.013$), but not from the device type ($p = 0.323$), even though the above-described trend was confirmed also in the adjusted model (Supplementary Table S2).

Furthermore, in addition to the increase of the positivity rate, an upward trend could be also found in the quantitative recovery of NTM colonies, with a significant, non-linear increase in the

counts ($\beta = 1.755, p = 0.040$), which was particularly noticeable in the last year of surveillance (Figure 2). A visual example of an increase in the NTM counts over time in samples from the same device is shown in Figure 3.

Discussion

Worldwide, the detection of *M. chimaera* in LivaNova HCUs has led to their replacement with HCUs by other companies. Afterwards the FDA warnings, in Duke University Medical Center (North Carolina, US) in June 2018, a multidisciplinary team recommended replacing the Stockert 3T LivaNova 3T HCUs with CardioQuip MCH(i)-1000 HCUs (CardioQuip, College Station, TX) and using strongest disinfectant (bleach) according to the manufacturer's instructions, as well as an increased frequency for deep cleaning (monthly instead of quarterly). Despite adherence to the enhanced protocols, a cluster of three NTM patient infections was reported in 2020 [20] with patients developing sternal surgical site infections by *Mycobacterium abscessus* after cardiothoracic surgery.

To date there have been some reports of Maquet devices (HCU 20, HCU40 and HU35 devices) contamination with *M. chimaera* worldwide, but there are no reports confirming NTM infection in patients treated with HCU40, HCU30, or HU35 [1,21–23]

International [24] and national [9,10] reports of devices contamination associated with human infections led to increased sensitivity and adherence to maintenance, disinfection and microbiological monitoring protocols by healthcare workers of cardiac surgeries. Even in our region, after the first Italian case of infection by *M. chimaera* [9], disinfection and maintenance activities have been intensified and LivaNova HCUs have been replaced with Maquet HCU40 in almost all hospitals.

This study analyses data from the surveillance of Maquet devices operating for more than 5 years in four hospitals of the Piedmont Region. Our laboratory was involved in multi-year microbiological surveillance and the data acquired demonstrate that, despite rigorous compliance to the manufacturers' instructions (use of filtered water to fill the water tanks, performing cleaning and disinfection more frequently than required, and following the recommended maintenance schedule),

a significant increase of positive devices and positive samples with *M. gordonae* was observed during the surveillance period.

The results obtained from the surveillance, and from the subsequent models, provide ground for speculating that reiterated and intensive (5% chloramine -T) booster disinfections of the tanks of the HCU40 and HU35 included in this study may have caused NTM resistance and adaptation phenomena, similarly to what happens in other water pipelines.

Our result agrees with other studies [23,25]. NTM resistance to disinfection treatments had emerged already in the first years of our surveillance on HCU40 Maquet [11]: the data acquired in the last 5 years have strengthened the previous evidence, with an increase from a positive sample rate of 13.6% in 2019 to 80% in the last year. Notably, all samples with >1000 CFU/100ml of NTM (16/89) were collected between 2023 and early 2024.

The occurrence of resistance to disinfectants in the microbial population of water is widely documented for drinking water distribution systems (DWDSs): *Mycobacterium* spp. is widely detected in chloraminated DWDSs [26,27] due to its high content of complex lipids and mycolates: mycolic acids are major lipid components of the mycobacterial cell envelope and guarantee the survival of mycobacteria. Thanks to mycolic acids, mycobacterial cells are extremely hydrophobic, which eases their binding to various surfaces, facilitates biofilm formation [28], and contributes to their resistance to phagocytosis, disinfectants, and antibiotics [29–31].

According to Zang *et al.* [32], booster disinfection promotes the growth of such genera and increases the relative abundance of antibiotic resistance genes (ARGs). In addition, mycobacteria are more resistant thanks to the properties of the highly hydrophobic membrane, the ability of growing inside amoebae and the ability of producing biofilms themselves which represent a shield against chlorine and chloramine. As a result, mycobacteria organized in biofilms are difficult to eradicate with common decontamination practices and are relatively resistant to standard disinfectants such as chlorine.

Our analysis found that *M. gordonae* is the most resistant species to chloramine-T and it has been detected in 92% of HCUs, either alone or in association with *M. paragordonae* or *M. chelonae*, perhaps due to its ability to form biofilm. According to a recent study [33], *M. gordonae*, after 72 hours on stainless steel, has been reported to produce a significantly larger volume of biofilm than *M. avium* and *Mycobacterium intracellulare*, which confirmed previous observations of a significant presence of *M. gordonae* in the biofilm on metal surfaces of water supply systems [30].

M. gordonae, a slow-growing pigmented mycobacteria, is a ubiquitous environmental mycobacteria commonly found in water and soil [34] and has been long considered an opportunistic pathogen, causing infections only in immunocompromised hosts[35–37]. Currently, the literature regarding disease caused by *M. gordonae* is extremely limited: in the past, pseudo-outbreaks in hospitals due to water supply contamination were reported [38,39], and a recent study reported that *M. gordonae* could be pathogenic and causing infection not only in the immunocompromised host, but also in the otherwise healthy population [40]. We searched and reviewed the literature on *M. gordonae* infections and identified a case report on the infection of a prosthetic aortic valve in a 53-year-old patient in February 1974, who showed a disease characterized by myalgia, arthralgia and fever symptoms that caused several hospitalizations five months after the surgical operation. One year later, *M. gordonae* was cultured from the abscess of aortic valve and from the bone marrow, urine and sputum. The risk factors associated with the infection were the cardiac surgery and a tooth extraction two weeks before the surgery without antibiotic prophylaxis. It should be emphasized that, in those years, cardiac surgery needing extracorporeal circulation was performed with water heat exchangers, conceptually similar to those used today and probably even less safe [41].

It must be noted that this is the first report in which *M. gordonae* has been isolated from a large number of Maquet devices in a multicentre surveillance. Laboratory analyses have been conducted using more diagnostic methods: among these, the Elite agar culture, besides granting a high sensitivity, has allowed obtaining quantitative results, which have been useful to better estimate the phenomenon of NTM resistance to monochloramine.

Of course, other factors may play a role in the likelihood to find NTM-positive samples in the devices, for example the local hospital water supply. Indeed, the adjusted model seems to hint at a possible difference between hospitals, with a slightly higher likelihood of a positive sample in one theatre compared to the others. However, the effect size and direction of such differences must be interpreted very cautiously, as they emerged from a model where this variable was simply conceived as a confounder [42]. More confidently, the adjusted model provides ground for claiming that the non-linear trend observed in the univariate regression, with a definite increase in NTM counts and positivity rates (particularly after the third year of operation), is still evident after considering the concomitant effect of other possible hospital-related variables.

Among the limitations of this study, it must be certainly acknowledged that positivity rates, especially in the last years of surveillance, might be even underestimated: in fact, deep disinfection protocols used to decontaminate devices with NTM-positive samples are unlikely to eradicate the pathogens from the machines, hence some negative samples obtained in such machines immediately after the disinfection may actually hide the presence of very low counts of NTM, rather than represent true negatives [43]. Moreover, this surveillance includes centres within the same region (Piedmont); therefore, conducting similar analyses in other settings might provide useful insights about possible geographical differences.

Conclusions

In conclusion, strong and continued booster disinfections (5% chloramine-T) of our HCU tanks could have favoured NTM resistance and adaptation similarly to what is observed in DWDSs. Given the paucity of the evidence, sometimes consisting of very old studies only, some questions remain open: for instance, the clinical relevance of HCU contamination with NTM other than *M. chimaera* is still poorly defined. It is therefore unknown whether, with the increase in HCU contamination by NTM such as *M. gordonae*, more cases of invasive *M. gordonae* infections are to be expected following cardiac surgery. No cases of post-operative invasive infection caused by *M.*

gordonae have been reported recently, thus more evidence is needed to correctly estimate the latency period of infection.

Eventually, further investigations are warranted to explore whether the origin of contamination of Maquet devices by *M. gordonae* could have occurred at the manufacturing site, as it had happened in the case of LivaNova devices, or at the local hospital. Should this be confirmed, in the time needed to develop devices less prone to NTM colonization, HCU manufacturers should research improved disinfection protocols, based on alternative molecules still effective on the colonizing NTM.

Conflict of interest

None declared.

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Supplementary data

Supplementary data to this article can be found online at.....

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Table I. Summary of NTM contamination by hospital and device type

Hospital A	Number of samples	Positive samples (%)	Number of devices	Positive device (%)
HCU40	142	54 (38.0)	5	5 (100.0)
HU35	70	10 (14.3)	12	6 (50.0)
Total	212	64 (30.2)	17	11 (64.7)
Hospital B				
HCU40	48	36 (75.0)	4	4 (100.0)
HU35	18	6 (33.3)	2	2 (100.0)
Total	66	42 (63.6)	6	6 (100.0)
Hospital C				
HCU40	27	8(29.6)	2	2 (100.0)
e	0	0 (0.0)	0	0 (0.0)
Total	27	8 (29.6)	2	2 (100.0)
Hospital D				
HCU40	54	17(31.5)	2	2 (100.0)
HU35	44	19 (43.2)	2	2 (100.0)
Total	98	36 (36.7)	4	4 (100.0)
Total	403	150 (37.2)	29	23 (79.3)

Table II. NTM detection rate by test type

Type of test	Number of samples	Positive samples (%)
PCR	330	103 (33.9)
ECDC culture	305	26* (8.5)
Elite agar culture	226	89 (39.4)

*Of the 26 samples with positive ECDC culture results, 18 (69.2%) were positive at the BBL MGIT and 24 (92.3%) on the Lowenstein

Table III. Positivity rates at different time points from the first month to the end of the surveillance period, as predicted by the segmented regression model.

Model prediction: first period (before day 1150)		Model prediction: second period (after day 1150)	
Day	Predicted positivity rate [95% CI]	Day	Predicted positivity rate [95% CI]
30	0.137 [0.078 - 0.228]	1200	0.225 [0.107 - 0.414]
60	0.138 [0.081 - 0.226]	1230	0.253 [0.128 - 0.437]
90	0.140 [0.084 - 0.224]	1260	0.282 [0.153 - 0.461]
120	0.142 [0.087 - 0.222]	1290	0.314 [0.181 - 0.485]
150	0.143 [0.090 - 0.221]	1320	0.347 [0.213 - 0.510]
180	0.145 [0.093 - 0.219]	1350	0.382 [0.249 - 0.535]
210	0.147 [0.096 - 0.218]	1380	0.418 [0.288 - 0.560]
240	0.149 [0.099 - 0.217]	1410	0.455 [0.330 - 0.586]
270	0.150 [0.103 - 0.215]	1440	0.492 [0.374 - 0.612]
300	0.152 [0.106 - 0.215]	1470	0.530 [0.419 - 0.638]
330	0.154 [0.109 - 0.214]	1500	0.567 [0.464 - 0.665]
360	0.156 [0.112 - 0.214]	1530	0.604 [0.507 - 0.693]
390	0.158 [0.115 - 0.214]	1560	0.639 [0.548 - 0.721]
420	0.160 [0.117 - 0.214]	1590	0.673 [0.587 - 0.749]
450	0.162 [0.120 - 0.215]	1620	0.705 [0.621 - 0.778]
480	0.164 [0.122 - 0.216]	1650	0.736 [0.653 - 0.805]
510	0.166 [0.124 - 0.218]	1680	0.764 [0.681 - 0.831]
540	0.168 [0.126 - 0.220]	1710	0.790 [0.706 - 0.855]
570	0.170 [0.127 - 0.222]	1740	0.814 [0.729 - 0.877]
600	0.172 [0.128 - 0.225]	1770	0.836 [0.750 - 0.896]
630	0.174 [0.129 - 0.229]	1800	0.855 [0.769 - 0.913]
660	0.176 [0.130 - 0.233]	1830	0.873 [0.787 - 0.928]
690	0.178 [0.130 - 0.238]	1860	0.889 [0.803 - 0.940]
720	0.180 [0.130 - 0.243]	1890	0.903 [0.818 - 0.951]
750	0.182 [0.130 - 0.248]		
780	0.184 [0.130 - 0.254]		
810	0.186 [0.129 - 0.261]		
840	0.188 [0.128 - 0.267]		
870	0.190 [0.128 - 0.274]		
900	0.193 [0.127 - 0.282]		
930	0.195 [0.126 - 0.289]		
960	0.197 [0.125 - 0.297]		
990	0.199 [0.123 - 0.305]		
1020	0.202 [0.122 - 0.314]		
1050	0.204 [0.121 - 0.322]		
1080	0.206 [0.120 - 0.331]		
1110	0.208 [0.118 - 0.340]		

Figure 1.

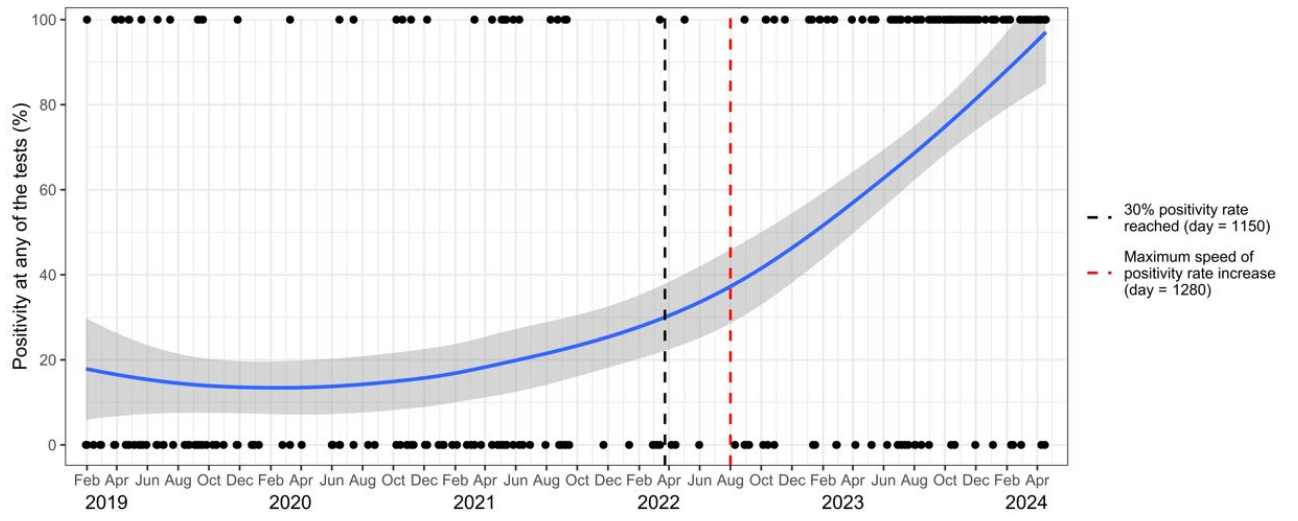
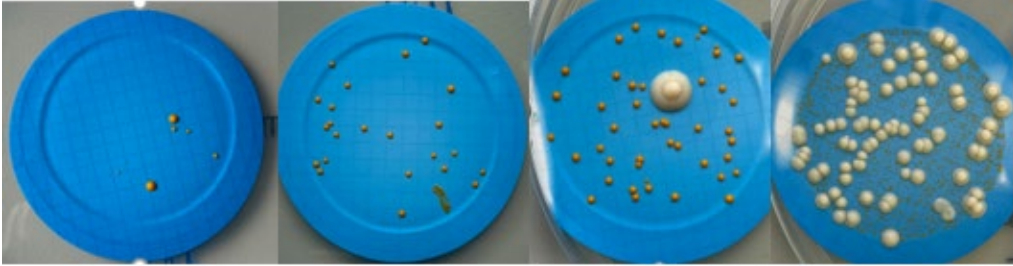


Figure 3.



June 2021

November 2022

February 2023

April 2024

Figure 1. Trends in the rate of NTM positivity to any of the tests (culture, molecular) in samples over time. Each dot on the top or bottom line represents, respectively, a positive or negative sample. The blue line depicts the trend in positivity rate over time, as computed by the LOESS estimator, along with its shaded 95% confidence interval. The black dashed line (time = 1150 days) represents the first time point with a positivity rate $\geq 30\%$ (“threshold point”), while the red dashed line (time = 1280 days) represents the point with greatest increase in the trend slope (“critical point”).

Figure 2. Trends in NTM counts yielded on the filter in samples over time. The y-axis is shown on a logarithmic scale, and zero counts indicate negative samples. The blue line depicts the trend in NTM counts over time, as computed by the LOESS estimator, along with its shaded 95% confidence interval. Red dots indicate observations greater than the full-scale value of 1000 CFU/100 ml.

Figure 3. Example of an increase in NTM count observed during three years of surveillance on one HCU40 Maquet. NTM counts yielded on the filter plated on Elite agar in four samples obtained from the same device over time. Culture after 3 weeks of incubation at 30°C with *M. gordonae* (orange colonies) and *M. chelonae* (white colonies).