An Unusual Antibiotic Susceptibility Pattern in a Mycobacterium Cosmeticum Strain Isolated from the Chesapeake Bay

Vajini Atukorale, Nicholas Boire, Kim Dionne, Stefan Riedel, Nicole Parrish
Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, USA

Abstract

Objective/Background: Mycobacterium cosmeticum, first described in 2004, was recovered from a patient undergoing a cosmetic procedure. Subsequently, this species was associated with an outbreak in a nail salon. In all cases, the isolates were susceptible to all antibiotics tested. Recently, however, we recovered a strain of M. cosmeticum from the Chesapeake Bay, resistant to 11 of 14 antimicrobials. The objective of this work was to present our findings on the resistance and susceptibility of this isolate to various antibiotics. Materials and Methods: Surface water samples were collected from 10 sites in the Chesapeake Bay and upper tributaries to assess microbial diversity and antibiotic resistance. Site selection was based on proximity to agricultural runoff, industrial contaminants, and sewage effluents. Samples were processed and recovered organisms were identified and subjected to antimicrobial-susceptibility testing. Results: One nontuberculous species, identified as M. cosmeticum, was recovered from Sandy Point State Park. Resistance was detected to several antibiotics: doxycycline (16 µg/mL), ligeycleline (≥4 µg/mL), clarithromycin (8 µg/mL), trimethoprim/sulfamethoxazole (≥8/152 µg/mL), imipenem (32 µg/mL), cefoxitin (32 µg/mL), ethionamide (≥20 µg/mL), and streptomycin (16 µg/mL). Of the 14 antibiotics tested, only the fluoroquinolones, linezolid, and amikacin demonstrated potent activity with susceptible minimum inhibitory concentrations. Conclusion: The antimicrobial resistance identified in this M. cosmeticum isolates from the Chesapeake Bay raises some important concerns: (a) why is the susceptibility pattern in this isolate so different from the previously published reports, (b) how did resistance emerge in this isolate, (c) is there a source of environmental exposure to antibiotics, (d) is it a human isolate transferred to the watershed, or (e) is it the result of lateral gene transfer with other resistant organisms in the Bay?

Keywords: Antibiotic resistance, Chesapeake Bay, Mycobacterium cosmeticum

INTRODUCTION

Microbial resistance is a growing public health concern in human and veterinary medicine. Antibiotics are often over-prescribed and disposal of antimicrobial waste is not routinely monitored, both of which may contribute to the development of resistance. The emergence of resistance in the mycobacteria is not completely understood. While it is known that some species demonstrate intrinsic resistance to specific drugs, it is thought that acquired resistance occurs due to exposure to a particular antibiotic, resulting in a chromosomal mutation, or accumulation of multiple mutations.[1-4] For the nontuberculous mycobacteria (NTM), which are typically found in soil, water, and even on plants, antibiotic exposure could occur in the environment due to antimicrobial runoff in water and sewage effluents or during treatment of a patient for a specific mycobacterial infection. At this time, horizontal gene transfer is not thought to play a role in the development of mycobacterial resistance.

Recently, at our institution, a number of NTM isolates were recovered from patients with infections acquired due to direct exposure with the waters of the Chesapeake Bay.[5] The species recovered, Mycobacterium marinum, typically susceptible to most antibiotics including rifampin and doxycycline,
demonstrated high-level resistance to both drugs. Because none of the patients from which the isolates were recovered had been treated with any of the drugs in question, no explanation was possible as to how resistance emerged in these strains. This prompted our group to undertake a pilot survey of specially selected sites on the Chesapeake Bay and upper watershed in an effort to determine the type and scope of antimicrobial resistance present in these waters.

In the summer of 2012, we recovered a strain of *Mycobacterium cosmeticum*, a rapid-growing (RG) mycobacterial species, which was first described in 2004.[6-8] Initially isolated from a patient undergoing a cosmetic procedure, *M. cosmeticum* has subsequently been associated with an outbreak in a nail salon.[6-8] The exact environmental niche for *M. cosmeticum* has not been definitively identified. However, it is thought to exist in a water-based habitat and the *M. cosmeticum* strains recovered initially were susceptible to most antibiotics tested.

In this study, we describe the isolation of a strain of *M. cosmeticum* from water samples obtained from the Chesapeake Bay resistant to a number of antibiotics, including doxycycline. Recovery of this drug-resistant isolate raises important questions regarding antibiotic use and disposal not only in human medicine but also in agriculture.

**Materials and Methods**

A pilot survey of the Chesapeake Bay and upper tributaries was conducted in July 2012 to assess bacterial diversity and antimicrobial resistance. Ten locations were selected based on sites previously sampled in 1976 to be used as a comparative reference and those with current proximity to industrial agriculture, human habitation, and sewage runoff.[9] Two sampling sites per location were chosen and global positioning system (GPS) coordinates were recorded for each site [Table 1]. For Chesapeake Bay sites, sampling was done proximal to the shore (within several yards) as well as further out at a range from 137.16 m (150 yards) to 274.32 m (300 yards). Sampling for the upper watershed sites, consisting of rivers and creeks, was done close to the bank, where little current was visible, and further out, where the flow was most rapid. Personnel collecting and processing surface water samples wore gloves to avoid contamination of samples by normal skin flora. Aliquots (500 mL) were collected in sterile, glass, screw-capped bottles that were not opened until they were submerged in the water. All samples were chilled on ice and subsequently transported back to the laboratory for processing. Water samples were poured through glass filtration units containing preresterilized 0.2-µm bacterial recovery filters (Millipore, Billerica, MA, USA). For recovery of mycobacteria, filters were subsequently placed in Middlebrook 7H9 (M7H9) broth containing a commercially available mixture of antibiotics (PANTA: nalidixic acid, polymyxin B, amphotericin B, trimethoprim/sulfamethoxazole, and azlocillin; Becton Dickinson, Sparks, MD, USA). Samples were incubated at 30°C and 37°C for up to 6 weeks to permit recovery of mycobacteria. All broth tubes were visually examined daily for detection of growth. Positive broth cultures were plated on Middlebrook 7H10 (M7H10) agar to obtain isolated colonies. Ziehl–Neelsen staining was used to identify acid-fast bacilli, which were subsequently identified to the species level using 16S ribosomal DNA (rDNA) sequencing.

Following species-level identification, antimicrobial-susceptibility testing (AST) was performed using a commercially available microbroth dilution assay (Sensititre, Thermo Scientific, TREG Diagnostic Systems, Cleveland, OH, USA), and the minimum inhibitory concentration (MIC) was determined for each antimicrobial agent. The following antimicrobial agents were tested: imipenem, cefoxitin, amikacin, tobramycin, doxycycline, ciprofloxacin, clarithromycin, linezolid, trimethoprim/sulfamethoxazole, tigecycline, rifabutin, rifampin, ethambutol, and ethionamide. In brief, isolate suspensions were prepared using fresh growth samples from M7H10 agar, which was added to a test tube containing 10–20 sterile beads submerged in 5 mL of Müller–Hinton (MH) broth (TREG Diagnostic Systems). Suspensions were thoroughly vortexed and subsequently allowed to settle for 5–10 min. The supernatant was then adjusted to a 0.5 McFarland standard and further diluted (50 µL in 11-mL MH broth) to obtain a target inoculum of 5 × 10^6 colony-forming unit (CFU)/mL. Table 1: Sampling sites and coordinates for surface water samples collected for this study

| Sampling site                  | GPS coordinates |
|-------------------------------|-----------------|
| Chesapeake bay                |                 |
| Northpoint State Park         | 39.208-76.423   |
| Inner Harbor, Baltimore City  | 39.285-76.610   |
| Eastern Neck Wildlife Refuge  | 39.046-76.234   |
| Sandy Point State Park        | 39.016-76.395   |
| Gunpowder Falls State Park    | 39.344-76.356   |
| Potomac river                 | 39.361-76.340   |
| Shenandoah river              | 39.443-77.382   |
| Shenandoah river, point of rocks | 39.396-77.563 |
| Shenandoah river, point of rocks | 39.436-77.800 |
| Shenandoah river, point of rocks | 39.273-77.786 |
| Shenandoah river, point of rocks | 39.274-77.539 |
| Shenandoah river, point of rocks | 39.274-77.539 |
| Shenandoah river, point of rocks | 39.274-77.539 |

[6-8] All sites are located in Maryland with the exception of the Shenandoah river site, which is located in West Virginia. Two sampling sites were selected per location for water collection on the Chesapeake Bay as indicated by the pairs of GPS coordinates. The first site in each pair was proximal to the shore; the second site was offshore, a minimum of 137.16 m (150 yards) to 274.32 m (300 yards). This was done because the Chesapeake Bay sites represented large open bodies of water, whereas the upper watershed sites were more discreet. Only one GPS coordinate was obtained for upper watershed sites as the locations were close in proximity and did not differ significantly. GPS: Global positioning system

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which was confirmed by determination of viable counts. For all RG-NTM species, including *M. cosmeticum*, microtiter plates were inoculated with 100 µL of suspension per well, covered with an adhesive film, and incubated at 30°C. All AST plates were visually interpreted using a mirror box on day 3 or 5 according to the manufacturer’s guidelines (TREK Diagnostic Systems). MIC determination and interpretation were performed using the Clinical and Laboratory Standards Institute (CLSI)-recommended guidelines and criteria for RG-NTM. In case of antibiotics for which no interpretive guidelines currently exist, if the MIC value was equal to or over the top of the test range, the isolate was considered resistant.

## Results

During this pilot survey of the Chesapeake Bay and upper tributaries, only one RG-NTM was recovered. This isolate was recovered from a water sample at GPS coordinates 39.016-76.395 (Sandy Point State Park, Maryland, USA). At the time of sampling, public access to the area was restricted because of a high number of jellyfish that had been observed in the waters. Microbial colonies grew within 5 days on M7H9 broth and in 2 days on M7H10 agar. Colonies were glossy and tan-colored. Ziehl–Neelsen staining revealed long, thin, acid-fast rods. The isolate was identified by 16S rDNA sequencing as *M. cosmeticum*.

Susceptibility testing results were performed in triplicate by three different medical technologists [Table 2]. At the time of testing, a quality control strain of *Mycobacterium peregrinum* (ATCC 700686) was also tested according to CLSI guidelines and results were within expected limits. The *M. cosmeticum* isolate recovered from the waters off Sandy Point State Park was only susceptible to amikacin, linezolid, and ciprofloxacin. Resistance was found to imipenem, tobramycin, doxycycline, clarithromycin, and trimethoprim/sulfamethoxazole. The isolate demonstrated intermediate resistance to ceftazidime with an MIC of 32 µg/mL [Table 2]. These MICs were then compared with those reported for the original type strains, first described in 2004, which were isolated from a female patient in Venezuela and a hair salon in Ohio. As shown in Table 2, the original *M. cosmeticum* strains were susceptible to all but one of the antibiotics tested including imipenem, cefoxitin, amikacin, doxycycline, clarithromycin, and ciprofloxacin. For the *M. cosmeticum*-type strains, tobramycin was the only drug for which resistance was detected with an MIC of 4 µg/mL (intermediate resistance); the Sandy Point isolate had an MIC to tobramycin of 8 µg/mL (resistance). Additional antimicrobial agents selected for AST against the isolate included tigecycline, rifabutin, rifampin, ethionamide, and streptomycin. Tigecycline, rifampin, rifabutin, and ethionamide had MICs greater than the highest concentration present in the microtiter plate [Table 2] and thus were considered resistant for the purposes of this study. For streptomycin, the MIC was 16 µg/mL, a concentration considered resistant for other mycobacterial species including *Mycobacterium tuberculosis*.

Interestingly, no mycobacteria were recovered from any of the other geographic sites that were sampled in this study.

## Discussion

To date, *M. cosmeticum* isolates obtained from various clinical and other sources were reported to be susceptible to most antimicrobial agents with little to no evidence of resistance.

### Table 2: Minimum inhibitory concentration comparisons for various antibiotics between prior published strains and the Sandy Point State Park Strain

| Antibiotic                  | *M. cosmeticum* (published data) | *M. cosmeticum* (Sandy Point isolate) |
|-----------------------------|----------------------------------|---------------------------------------|
|                             | MIC (µg/mL)                      | Interpretation (S, I, or R)           | MIC (µg/mL)                      | Interpretation (S, I, or R) |
| Imipenem                    | ≤1                                | S                                     | 32                              | R                              |
| Cefoxitin                   | 8                                 | S                                     | 32                              | I                              |
| Amikacin                    | 4                                 | S                                     | 2                               | S                              |
| Tobramycin                  | 4                                 | I                                     | 8                               | R                              |
| Doxycycline                 | ≤0.25                             | S                                     | 16                              | R                              |
| Ciprofloxacin               | 0.25                              | S                                     | 0.5                             | S                              |
| Clarithromycin              | 0.25                              | S                                     | 8                               | R                              |
| Linezolid                   | NT                                | NT                                    | 4                               | S                              |
| Trimethoprim/sulfamethoxazole | NT                                 | NT                                    | ≥8/152                          | R                              |
| Tigecycline<sup>a</sup>     | NT                                | No interpretation                     | ≥4                              | No Interpretation              |
| Rifampin<sup>a</sup>        | NT                                |                                       | >8                              |                                 |
| Rifabutin<sup>a</sup>       | NT                                |                                       | >8                              |                                 |
| Ethionamide<sup>a</sup>     | NT                                |                                       | ≥20                             |                                 |
| Streptomycin<sup>a</sup>    | NT                                |                                       | 16                              |                                 |

*For some antibiotics, no interpretive guidelines currently exist as indicated in the table; however, all MICs for these drugs were at the top of the test range established for other mycobacterial species. Current Clinical and Laboratory Standards Institute guidelines for susceptibility testing of rapid-growing mycobacteria were used for the following interpretations where available: S: Susceptible, I: Intermediate, and R: Resistant. MICs: Minimum inhibitory concentrations, NT: Not tested.*
In this work, we report antimicrobial-susceptibility data for an *M. cosmeticum* isolate recovered from the waters off Sandy Point State Park (Chesapeake Bay, Maryland, USA), with resistance to most of the antimicrobial agents commonly used for AST for such isolates, including imipenem, cefozitin, doxycycline, tobramycin, trimethoprim/sulfamethoxazole, and tigecycline. Such resistance in *M. cosmeticum* is highly unusual based on published data,[6-8] however, unexplained antimicrobial resistance is not without precedent in bacterial isolates recovered from the Chesapeake Bay. Previously, we published a case of an unexplained antibiotic resistance that was observed in another mycobacterial species, *M. marinum*, isolated from a patient who suffered a fish hook injury while engaging in recreational activities on the eastern shore of the Chesapeake Bay.[5] As with the *M. cosmeticum* recovered in this study, this particular *M. marinum* strain demonstrated resistance to doxycycline and rifampin, an unusual finding for this species.[5]

Like *M. cosmeticum*, *M. marinum* is generally considered to be susceptible to most antimicrobial agents. For that reason, AST is not routinely recommended and/or performed for this species. In addition, intrinsic or acquired resistance, especially to rifampin, has not been reported for *M. marinum* and the patient in this case had never been exposed to rifampin or any other rifamycins as part of a treatment regimen.[5] Thus, selective pressure in the conventional sense (i.e., exposure of a bacterium *in vivo* to a drug) is unlikely to have been the cause of resistance. Instead, it was suggested that this particular *M. marinum* strain may have acquired resistance through antibiotic exposure in the natural environment.[5]

In this report of *M. cosmeticum*, it is not known if the resistance was due to exposure of the organism to antibiotics in the natural environment. Previous *M. cosmeticum* strains, all of which had been recovered from infected patients, were susceptible to most antimicrobial agents tested.[6-8] Presumably, all of these cases were due to environmental exposure, primarily through water containing these organisms (e.g., medical devices, foot baths).[6-8] Although it was previously proposed that *M. cosmeticum* may exist in environmental reservoirs, no definitive evidence to support this concept has been found.[6-8] To the best of our knowledge, this is the first report of *M. cosmeticum* being isolated directly from a natural environment. Most NTM isolates are ubiquitous in the environment and inhabit a variety of ecological niches, including soil and water (marshland, streams, rivers, and estuaries). It is entirely possible that this body of water and associated drainage area represent typical habitat for the *M. cosmeticum* species. Sandy Point State Park, Annapolis, Maryland, is located on a peninsula on the northwestern shore of the Chesapeake Bay, in proximity to the Chesapeake Bay Bridge. This particular body of water is openly connected to the Chesapeake Bay; the beach area along the shore is used for recreational human activities, especially in the summer months. Thus, it is also possible that this *M. cosmeticum* strain may be a human isolate transferred to the watershed.

Determination of the origin of the *M. cosmeticum* strain recovered in this study and the specific molecular determinants of resistance were beyond the scope of this project. Nonetheless, as with the *M. marinum* strain described in a prior study,[5] the observed antibiotic resistance raises some interesting questions. Antimicrobial resistance in mycobacteria is thought to be primarily due to chromosomal mutations, which arise from exposure of the organism to a particular drug. This exposure can occur *in vivo* during treatment or alternatively in the environment should a particular antibiotic be present in sufficient concentration. The *M. cosmeticum* strain recovered in this study was not only resistant to some standard antimicrobial agents used for treatment of NTM infections (clarithromycin, tobramycin, doxycycline, and tigecycline) but also resistant to other agents used for the treatment of tuberculosis (rifampin and ethionamide). In the latter case, it is possible that resistance to tuberculosis-specific drugs may be inherent in this species as has been observed with other NTM, especially the RG-NTM such as *Mycobacterium abscessus, Mycobacterium fortuitum*, and *Mycobacterium chelonae.[11,12] Intrinsic resistance may also play a role with respect to doxycycline. It is well documented that tetracycline (e.g., doxycycline and minocycline) resistance has limited the use of this class of drugs for the treatment of many NTM infections. However, given that the clinical strains of this species recovered to date were susceptible to this drug, this seems less likely.

Perhaps, most puzzling of the susceptibility results obtained in this study was the resistance of this *M. cosmeticum* strain to tigecycline (MIC ≥4 μg/mL). Tigecycline, a glycylcycline, is not as heavily used therapeutically, and thus, resistance of NTM to tigecycline has not been as widespread when compared with other antibiotics. Glycylcyclines are second-generation tetracyclines that are considered superior to tetracyclines because they are generally unaffected by common tetracycline-resistance mechanisms such as efflux pumps and ribosomal protection.[13,14] To date, tigecycline has shown strong activity against most RG-NTM species tested, including tetracycline-resistant strain.[15,16] Because *M. cosmeticum* is an RG-NTM, the relatively high MIC to tigecycline was unexpected and at this time unexplained. Evidence is emerging supporting horizontal gene transfer between mycobacterial species as well as between mycobacteria and other bacteria occupying the same microenvironment.[17] Further investigations are needed to confirm this possibility with respect to the *M. cosmeticum* recovered from the waters off Sandy Point State Park.

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**Conflicts of interest**
There are no conflicts of interest.

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