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Analysis of a Panel of Rapidly Growing Mycobacteria for Resistance to Aldehyde-based Disinfectants

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Summary

After several accounts across the globe of mycobacterial outbreaks associated with medical procedures and aldehyde disinfectants resistance, we undertook an analysis of mycobacteria isolated from patients seen in a hospital in the United States between 1994 and 2008 to determine prevalence of resistance to aldehyde-based disinfectants. Out of the 117 clinical isolates screened, six isolates belonging to the emerging *Mycobacterium abscessus* group were found to display significant levels of resistance to glutaraldehyde and *ortho*-phthalaldehyde.

Infections with rapidly growing, non-tuberculous, mycobacteria (RGM) of the *Mycobacterium abscessus* group are increasing in prevalence throughout the world.¹ A recent outbreak of widespread *M. abscessus* subsp *massiliense* infections (more than 2,000 possible cases) following video-assisted surgery in Brazil caused by a single highly virulent clone displaying high-level resistance to glutaraldehyde (GTA) highlights the pathogenic potential of these organisms and the important role that disinfectant failure may play in their spread.²⁻³ Although by far the largest RGM-associated outbreak ever reported, small outbreaks (9 to 26 cases) of nosocomial infections and pseudo-infections caused by mycobacteria resistant to aldehyde-based disinfectants have also been reported in the United States, and the recovery of RGM capable of surviving more than one to 24 hour exposure to 2% GTA or 2 to 10% formaldehyde from bronchoscope/endoscope-reprocessing machines and processed dialyzers has been reported globally.⁴⁻⁵ GTA and another aldehyde-based

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disinfectant, *ortho*-phthalaldehyde (OPA), remain the most widely used chemical disinfectants in hospitals worldwide for the high-level disinfection of semi-critical, temperature-sensitive medical devices. Our previous work has identified reduced porin expression as a possible mechanism through which RGM isolates may develop high-level resistance to aldehydes.⁶ Given the known impact of porins on both the virulence and resistance of RGM to a variety of antibiotics and biocides⁶, this finding raises concerns that aldehyde-resistant RGM strains may become a threat to infection control while causing infections that are more difficult to treat. Since RGM clinical isolates are typically not screened for disinfectant resistance unless associated to an outbreak, current data on the prevalence and trends of disinfectant resistance is lacking. The present study was therefore undertaken with the goals of determining the prevalence of GTA and OPA resistance among a panel of randomly selected RGM clinical isolates from the United States and assessing whether GTA or OPA resistance correlated with increased resistance to antibiotics.

One hundred seventeen sequential RGM isolates isolated between 1994 and 2008 from patients seen at a single university teaching hospital (University of Colorado Hospital, Denver) were sub-cultured and their speciation confirmed by standard molecular biology techniques. The panel included isolates of *M. abscessus* subsp *abscessus* (n=44), *M. fortuitum* (n=35), *M. chelonae* (n=12), *M. peregrinum* (n=4), *M. mucogenicum* (n=4), *M. abscessus* subsp *massiliense* (n=3), *M. abscessus* subsp *bolletii* (n=4), *M. phocaicum* (n=3), *M. porcinum* (n=4), *M. farcinogenes* (n=1), *M. goodii* (n=1), *M. immunogenum* (n=1), and *M. canariensis* (n=1). These isolates were primarily from pulmonary sources but also from skin and soft tissue, sinus, eye, ear and disseminated infections. 64% of patients were from Colorado and others originated from all regions of the United States including Southeastern (32%), Northeastern (16%), West (30%), Midwest (15%) and Hawaii (7%).

GTA was used in the University of Colorado hospital for many years until a switch to OPA was made in 2006. An initial screen for GTA- or OPA-resistant isolates was performed at 25°C in small volume suspension tests in freshly prepared GTA or OPA solutions as described in Fig. 1. The concentrations of GTA and OPA, temperature and incubation times were chosen to meet the processing and minimum effective concentration (MEC) requirements for high-level disinfection recommended by the manufacturer (i.e., minimum of 20 min at 25°C for 2.1% GTA/Cidex Plus®; minimum of 5 min at 25°C for 0.3% OPA/Cidex® OPA). Out of the 117 RGM isolates tested in this primary screen, 13 demonstrated greater than 6 log reduction in CFU counts after the indicated times of exposure to OPA or GTA but yielded a few viable CFUs. They belonged to the *M. chelonae* (n = 3), *M. abscessus* (n = 4), *M. bolletii* (n = 2) and *M. fortuitum* (n = 4) groups. Three to five subsequent repeat testing on these isolates yielded in most cases no survivors. The presence of a few viable CFUs in some tests may be explained either by bacterial clumping⁷ or to the existence in some culture batches of a sub-population of, e.g. stationary phase, phenotypically tolerant bacteria. Alternatively, the propensity of these 13 isolates to yield survivors may reflect reduced susceptibility to the disinfectants. To assess the latter hypothesis, the 13 strains were further compared to their reference ATCC strain as well as to other isolates selected at random from our panel for their ability to survive exposure to more diluted solutions of OPA (0.04 to 0.2%) and GTA (0.2 to 0.5%). As shown in Fig. 1, all six

M. abscessus subsp *abscessus* and *M. abscessus* subsp *bolletii* isolates (# 34, 43, 53, 78, 91 and 97) consistently showed a significantly higher resistance to 0.5% GTA or 0.2% OPA than the control strains. The relatively high level of resistance to OPA of the *M. abscessus* subsp *abscessus* isolates # 91 and 34 in particular is remarkable as Cidex® OPA was only diluted 1.5-fold from the recommended MEC and killing was incomplete even after 15 min. The *M. chelonae* isolates # 3, 6 and 9 and the *M. fortuitum* isolates # 7, 41, 42 and 96, in contrast, only yielded survivors at the longest times of exposure when the disinfectant solutions were diluted to 0.2% GTA and 0.04% OPA (7.5 to 10-time dilutions of the recommended MEC) and their susceptibility to the disinfectants was in fact comparable to that of other isolates selected at random from the collection (Fig. 1).

While MIC results for the entire collection demonstrated the expected antimicrobial drug susceptibility by species, the MICs measured for isolates # 34, 43, 53, 78, 91 and 97 were comparable to those of other *M. abscessus* group isolates (data not shown). Thus, no correlation was found between disinfectant susceptibilities and the MIC of the drug classes tested therein.

In conclusion, the recurrent finding of *M. abscessus* isolates displaying significant levels of resistance to aldehyde-based disinfectants (OPA in particular) in the present study and earlier ones should increase awareness of the existence of such organisms, particularly in clinical settings where the use of disinfectants may promote their emergence, selection and spread in the environment. These organisms are indeed prone to surviving within the tubing of reprocessed heat-sensitive medical devices (e.g., endoscope channels, hemodialysis tubing), washer disinfectors, or any other kind of settings favoring the development of biofilms⁸⁻⁹.

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References

1. Tettelin H, Davidson RM, Agrawal S, Aitken ML, Shallom S, Hasan NA, et al. High-level Relatedness among *Mycobacterium abscessus* subsp. *massiliense* Strains from Widely Separated Outbreaks. *Emerg. Infect. Dis.* 2014; 20:364–71. [PubMed: 24565502]
2. Leao SC, Viana-Niero C, Matsumoto CK, Lima KVB, Lopes ML, Palaci M, et al. Epidemic of surgical-site infections by a single clone of rapidly growing mycobacteria in Brazil. *Future Microbiol.* 2010; 5:971–980. [PubMed: 20521940]
3. Shang S, Gibbs S, Henao-Tamayo M, Shanley CA, McDonnell G, Duarte RS, et al. Increased virulence of an epidemic strain of *Mycobacterium massiliense* in mice. *PLoS ONE.* 2011; 6:e24726. [PubMed: 21931831]
4. Bolan G, Reingold AL, Carson LA, Silcox VA, Woodley CL, Hayes PS, et al. Infections with *Mycobacterium chelonae* in patients receiving dialysis and using processed hemodialyzers. *J. Infect. Dis.* 1985; 152:1013–9. [PubMed: 4045242]
5. Center for Disease Control. Nosocomial infection and pseudoinfection from contaminated endoscopes and bronchoscopes - Wisconsin and Missouri. Vol. 40. *MMWR*; 1991. p. 675-8.

6. Svetlíková Z, Škovierová H, Niederweis M, Gaillard J-L, McDonnell G, Jackson M. The role of porins in the susceptibility of *Mycobacterium smegmatis* and *Mycobacterium chelonae* to aldehyde-based disinfectants and drugs. *Antimicrob. Agents Chemother.* 2009; 53:4015–8. [PubMed: 19581465]
7. Nomura K, Ogawa M, Miyamoto H, Muratani T, Taniguchi H. Antibiotic susceptibility of glutaraldehyde-tolerant *Mycobacterium chelonae* from bronchoscope washing machines. *Am. J. Infect. Control.* 2004; 32:185–8. [PubMed: 15175610]
8. Bisset L, Cossart YE, Selby W, West R, Catterson D, O'Hara K, et al. A prospective study of the efficacy of routine decontamination for gastrointestinal endoscopes and the risk factors for failure. *Am. J. Infect. Control.* 2006; 34:274–80. [PubMed: 16765205]
9. Gilbert P, McBain AJ. Biofilms: their impact on health and their recalcitrance toward biocides. *Am. J. Infect. Control.* 2001; 29:252–5. [PubMed: 11486267]
10. Griffiths PA, Babb JR, Bradley CR, Fraise AP. Glutaraldehyde-resistant *Mycobacterium chelonae* from endoscope washer disinfectors. *J. Appl. Microbiol.* 1997; 1997; 82:519–28. [PubMed: 9190297]

Figure 1. GTA and OPA susceptibility of *M. abscessus* subsp *abscessus*, *M. abscessus* subsp *bolletii*, *M. chelonae* and *M. fortuitum* isolates

GTA and OPA susceptibility tests were performed in small volume (1 ml) suspension tests containing ~ 10^8 to 10^{10} CFUs in freshly prepared alkaline 2.1% GTA solution under the formulated form of Cidex Plus® (Johnson & Johnson) or 0.3% OPA solution under the form of Cidex® OPA (Johnson & Johnson) essentially as described.¹⁰ Results are expressed as Log₁₀ CFU counts upon exposure of the test organisms to the indicated concentrations of disinfectant for 0 to 30 min. The MEC of 2.1% GTA and 0.3% OPA of the concentrated stock solutions were verified prior to each test using test strips specific to each disinfectant (3M Comply™ ColdSterilog™; Cidex OPA Solution Test Strips). Disinfectant neutralization was validated for each disinfectant tested and tests were performed in tri- to pentaplicates on independent culture batches. MAB, *M. abscessus* subsp *abscessus*; MBO, *M. abscessus* subsp *bolletii*; MCH, *M. chelonae*; MFO, *M. fortuitum*. The ATCC reference strains used in these studies (black squares) include *M. abscessus* subsp *abscessus* ATCC 19977, *M. abscessus* subsp *bolletii* ATCC 14472, *M. fortuitum* ATCC 6841 and *M. chelonae* ATCC 35752. The control clinical isolates selected at random from the collection (dashed

lines) include the *M. abscessus* subsp *abscessus* isolates # 24, 30, 52, 63 and 73, the *M. fortuitum* isolate # 59 and the *M. chelonae* isolates # 13, 28 and 49.