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SUSCEPTIBILITY

# *In Vitro* Evaluation of Povidone-Iodine and Chlorhexidine against Outbreak and Nonoutbreak Strains of *Mycobacterium abscessus* Using Standard Quantitative Suspension and Carrier Testing

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**ABSTRACT** Povidone-iodine (PI) and chlorhexidine (CHX) are widely used antiseptics active against conventional *Staphylococcus aureus*, *Enterobacteriaceae*, *Candida* species, and viruses, but their efficacy against *Mycobacterium abscessus* remains unproven. We determined the *in vitro* potency of alcoholic PI and CHX against *M. abscessus* subsp. *abscessus* (ATCC 19977), *M. abscessus* subsp. *bolletii* (BCRC 16915), and our outbreak strain of *M. abscessus* subsp. *massiliense* (TPE 101) in reference to *Staphylococcus aureus* (ATCC 29213) by standard quantitative suspension and carrier methods (EN 14563). By suspension, all mycobacterial strains compared to *S. aureus* were significantly more resistant to CHX, but not PI. By carrier, the mean logarithmic reductions (LR) achieved by PI under clean (dirty) conditions were 6.575 (2.482), 5.540 (2.298), 4.595 (1.967), and 1.173 (0.889), while those achieved by CHX under clean (dirty) conditions were 3.164 (5.445), 5.307 (2.564), 3.844 (2.232), and 0.863 (0.389) for *S. aureus*, *M. abscessus* subsp. *bolletii*, *M. abscessus* subsp. *abscessus*, and *M. abscessus* subsp. *massiliense*, respectively. *M. abscessus* subsp. *massiliense* (outbreak strain) was significantly more resistant than the other tested strains to PI and CHX. By both methods, the mean LR achieved by PI was higher than for CHX for all mycobacterial strains, but under dirty conditions, neither antiseptic was effectively mycobactericidal (LR $<$ 5). These preliminary findings caution against the universal replacement of PI with CHX as the first-line skin antiseptic, since all *M. abscessus* isolates were resistant to CHX. More studies are needed to establish the best practice for skin antisepsis if mycobacterial infections are also to be prevented.

**KEYWORDS** *Mycobacterium abscessus*, *Mycobacterium abscessus* subsp. *massiliense*, antiseptic resistance, chlorhexidine, disinfectants, *in vitro*, outbreak, povidone-iodine

The expansion of body-modifying procedures and vulnerable populations has led to a global rise in outbreaks of nontuberculous mycobacterial (NTM) infections following cosmetic and medical procedures (1). The most common NTM causing such infections belongs to the *Mycobacterium abscessus* complex, whose members are notoriously difficult to treat, since they are inherently resistant to multiple antimicrobials (2–4). The *M. abscessus* complex comprises 3 subspecies of rapidly growing mycobacteria: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii* (5, 6).

Previously, in Taiwan, we found the emergence of a dominant clone of *M. abscessus* subsp. *massiliense* (TPE 101, MLST sequence type 48 [ST48], clonal complex 3) causing a prolonged outbreak of skin and soft tissue infections, with a peak attack rate in 2012

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(7, 8). Outside Taiwan, an epidemic of surgical site infections in Brazil between 2004 and 2011 by a related clone (BRA100, ST23, clonal complex 3) coinciding with pulmonary infections among cystic fibrosis cohorts in the United Kingdom and United States has led to the identification of a globally successful clone of *M. abscessus* subsp. *massiliense*, perhaps with enhanced virulence and transmissibility (9–13).

Although the significance and specific virulence traits of these *M. abscessus* subsp. *massiliense* outbreak strains are only beginning to be unraveled, one untested hypothesis for their association with invasive procedures is their relative resistance to routine skin antisepsis (14). Povidone-iodine (PI) and chlorhexidine (CHX) are widely used antiseptics active against conventional *Staphylococcus aureus*, enteric bacteria, *Candida* species, and viruses, but their efficacy against individual strains of *M. abscessus*, particularly outbreak strains of *M. abscessus* subsp. *massiliense*, remains unproven (15, 16). Since various landmark studies in the last decade showing superiority of preoperative cleansing of the patient's skin with CHX over PI for preventing vascular catheter infections and surgical site infections after clean-contaminated surgery, there has been a shift toward recommending CHX over PI as the first-line skin antisepsis for a wide variety of procedures (17–19). We hypothesized that this secular trend has had an impact on the increasing frequency of rapidly growing NTM, particularly *M. abscessus* subsp. *abscessus*, isolated from catheter and surgical sites (20–24).

In this study, we determined the *in vitro* potencies of commonly applied formulations of alcoholic PI and CHX against our outbreak strain of *M. abscessus* subsp. *massiliense* (TPE 101) in reference to *S. aureus* (ATCC 29213), *M. abscessus* subsp. *abscessus* (ATCC 19977), and *M. abscessus* subsp. *bolletii* (BCRC 16915) by standard quantitative suspension and carrier (EN 14563-2008) methods.

## RESULTS

**Suspension method.** The mean log reductions (LRs) following suspension with PI and CHX under the various test conditions (exposure times and dilutions) for the three mycobacterial isolates and reference *S. aureus* strain (ATCC 29213) are shown in Table 1. There was a significant difference in the behaviors of the two antiseptics against the 4 tested strains ($P < 0.05$) (Table 1). PI consistently produced higher mean LRs than did CHX for all strains by the suspension method. However, there were significant interspecies and subspecies differences in the magnitude of the effect.

The effects of PI and CHX for *S. aureus* described below are consistent with previous studies (15). PI achieved an average LR of greater than 5 for most dilutions at 30 s for *S. aureus*. In contrast, CHX showed only a minimal killing effect, failing to attain an LR of greater than 5 under any of the test conditions for *S. aureus*. The effects of dilution and exposure time did not fit the decay model for PI, whereas for CHX, the predicted effect was obtained. This is well illustrated in Table 1, which shows a regular decay of activity of CHX throughout the dilution range ($P < 0.036$) and increased activity of CHX with increased contact time ($P < 0.028$), which is slowly bactericidal. In contrast, PI is rapidly lethal, acting mainly during the first 30 s and showing little enhanced killing when the time of exposure was increased.

For the clinically prevalent *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* outbreak strains, the LRs for CHX were less than 1, while those for PI were less than 5 for all test conditions. No enhanced activity for CHX was observed with increased contact time. The potency of PI against the less frequently clinically implicated *M. abscessus* subsp. *bolletii* approached that of *S. aureus*, with LRs of greater than 5 for most dilutions and maximal killing at 30 s. However, no mycobactericidal effect of CHX could be demonstrated against *M. abscessus* subsp. *bolletii*, with LRs of less than 1 for all test conditions. Taken together, *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* demonstrated moderate *in vitro* resistance against PI and high resistance against CHX in comparison to *S. aureus*, while *M. abscessus* subsp. *bolletii* appeared to be as susceptible to PI as *S. aureus* and as resistant to CHX as the other two mycobacterial strains. The comparative activities of PI (4,000 mg/liter) and CHX (800

**TABLE 1** Mean LRs achieved by alcoholic PI and CHX for all tested *M. abscessus* strains and a reference *S. aureus* strain at each exposure time and dilution by quantitative suspension test

| Exposure time (s)[a] | Mean LR[b] at dilution: 1:25 | 1:50 | 1:100 | 1:200 | 1:400 | 1:800 | $P^{1c,e}$ | $P^{2d,e}$ |
|---|---|---|---|---|---|---|---|---|
| *S. aureus* (ATCC 29213) | | | | | | | | |
| PI | | | | | | | | |
| 30 | 5.08 | 5.08 | 3.88 | 5.08 | 5.08 | 4.54 | 0.170 | Ref |
| 60 | 4.16 | 5.09 | 5.09 | 4.05 | 5.09 | 5.09 | 0.623 | |
| 90 | 4.32 | 4.71 | 5.15 | 4.68 | 5.15 | 5.15 | 0.667 | Ref |
| $P^3$ | 0.665 | 0.536 | 0.102 | 0.572 | 0.592 | 0.414 | | |
| CHX | | | | | | | | |
| 30 | 2.37 | 2.21 | 1.88 | 1.69 | 0.49 | 0.04 | **0.036** | Ref |
| 60 | 2.86 | 3.31 | 2.63 | 2.71 | 1.79 | 0.34 | **0.019** | |
| 90 | 3.93 | 2.90 | 3.35 | 4.22 | 2.25 | 1.06 | 0.141 | Ref |
| $P^{4e}$ | 0.382 | 0.443 | 0.277 | 0.208 | **0.028** | **0.023** | | |
| *M. abscessus* subsp. *bolletii* (BCRC 16915) | | | | | | | | |
| PI | | | | | | | | |
| 30 | 5.43 | 5.43 | 5.43 | 5.43 | 5.43 | 3.89 | **0.047** | 1.000 |
| 60 | 5.37 | 5.37 | 4.78 | 4.46 | 5.37 | 5.37 | 0.553 | |
| 90 | 5.35 | 5.35 | 5.35 | 5.35 | 4.82 | 5.35 | 0.480 | 1.000 |
| $P^3$ | 0.277 | 0.277 | 0.423 | 0.388 | 0.377 | 0.129 | | |
| CHX | | | | | | | | |
| 30 | −0.04 | −0.03 | −0.01 | −0.05 | 0.05 | 0.01 | 0.637 | **0.011** |
| 60 | −0.10 | −0.12 | −0.09 | −0.07 | −0.04 | −0.02 | 0.208 | |
| 90 | −0.13 | −0.18 | −0.10 | −0.07 | −0.02 | −0.07 | 0.085 | **0.007** |
| $P^4$ | 0.099 | 0.066 | 0.204 | 0.860 | 0.184 | 0.459 | | |
| *M. abscessus* subsp. *abscessus* (ATCC 19977) | | | | | | | | |
| PI | | | | | | | | |
| 30 | 4.08 | 3.62 | 3.35 | 3.64 | 2.71 | 2.22 | 0.635 | 1.000 |
| 60 | 2.97 | 4.74 | 4.10 | 4.06 | 3.05 | 3.35 | 0.705 | |
| 90 | 2.95 | 4.15 | 3.35 | 4.11 | 4.12 | 3.34 | 0.904 | 0.495 |
| $P^3$ | 0.451 | 0.616 | 0.825 | 0.934 | 0.563 | 0.666 | | |
| CHX | | | | | | | | |
| 30 | −0.06 | −0.10 | −0.17 | −0.04 | −0.02 | −0.01 | 0.058 | **0.010** |
| 60 | −0.23 | −0.13 | −0.10 | −0.15 | −0.10 | −0.11 | 0.448 | |
| 90 | −0.14 | −0.07 | −0.12 | −0.12 | −0.05 | −0.07 | 0.846 | **0.008** |
| $P^4$ | 0.075 | 0.670 | 0.497 | 0.458 | 0.674 | 0.151 | | |
| *M. abscessus* subsp. *massiliense* (TPE 101) | | | | | | | | |
| PI | | | | | | | | |
| 30 | 4.08 | 4.08 | 3.64 | 4.08 | 3.27 | 3.32 | 0.776 | 1.000 |
| 60 | 3.60 | 4.04 | 3.17 | 3.44 | 4.04 | 2.15 | 0.199 | |
| 90 | 4.07 | 4.07 | 4.07 | 3.15 | 2.78 | 2.74 | 0.300 | 0.177 |
| $P^3$ | 0.385 | 0.911 | 0.351 | 0.617 | 0.446 | 0.641 | | |
| CHX | | | | | | | | |
| 30 | −0.04 | −0.00 | −0.02 | −0.12 | −0.02 | −0.04 | 0.935 | **0.011** |
| 60 | −0.04 | −0.02 | −0.03 | 0.00 | −0.06 | −0.06 | 0.986 | |
| 90 | −0.04 | −0.06 | 0.01 | 0.02 | −0.03 | −0.03 | 0.821 | **0.010** |
| $P^4$ | 0.999 | 0.792 | 0.893 | 0.138 | 0.795 | 0.981 | | |

[a] $P^3$, *P* values for whether the differences with increasing exposure times are statistically significant for povidone-iodine; $P^4$, *P* values for whether the differences with increasing exposure times are statistically significant for chlorhexidine.

[b] The experiments were conducted three times, and an average (mean) LR was obtained for each test condition. A mean LR of greater than 5 was considered biocidal.

[c] $P^1$, *P* values for whether the differences across the range of dilutions within species are statistically significant.

[d] $P^2$, *P* values for the interspecies comparison (whether the activities of PI or CHX is significantly different between mycobacterial strain and the reference *S. aureus* strain) at a dilution of 1:25 with exposure times of 30 s and at a dilution of 1:800 with exposure times of 90 s. Ref, reference.

[e] *P* values of $<0.05$ are in boldface.

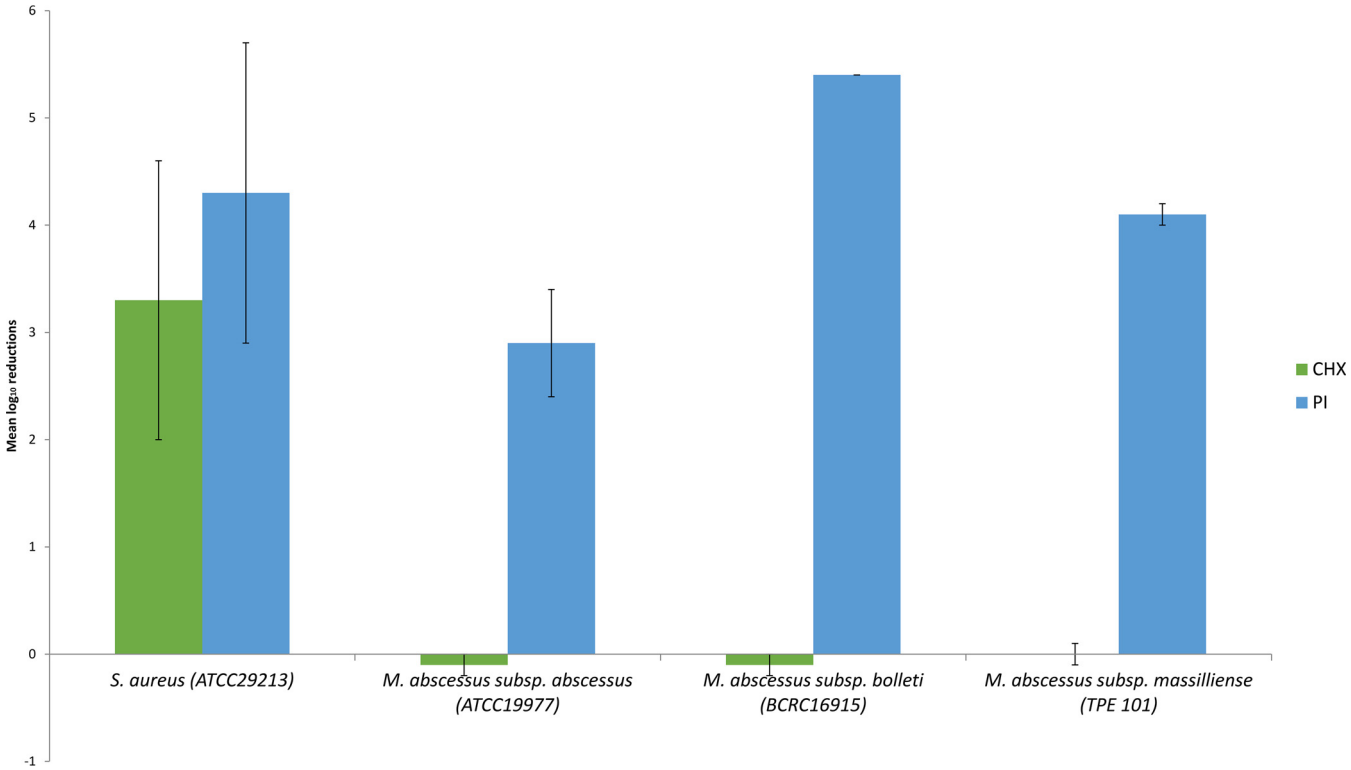


**FIG 1** Potency of alcoholic PI and CHX by quantitative suspension testing against different strains of *M. abscessus* expressed as the mean logarithmic reductions of CFU at the maximal concentrations of 4,000 mg/liter (PI) and 800 mg/liter (CHX) and at the maximal exposure time of 90 s. The error bars indicate SD.

mg/liter) at the maximal concentration (1:25 dilution) and contact time (90 s) against all strains tested are shown in Fig. 1.

**Quantitative carrier method.** The mean (standard deviation [SD]) LRs achieved by PI under clean versus dirty conditions were 6.575 (0.255) versus 2.482 (0.851), 5.540 (1.123) versus 2.298 (1.471), 4.595 (1.431) versus 1.967 (1.665), and 1.173 (0.658) versus 0.889 (0.501), while those achieved by CHX under clean versus dirty conditions were 3.164 (0.581) versus 5.445 (2.159), 5.307 (0.643) versus 2.564 (1.556), 3.844 (1.519) versus 2.232 (1.602), and 0.863 (0.433) versus 0.389 (0.306) for *S. aureus*, *M. abscessus* subsp. *bolletii*, *M. abscessus* subsp. *abscessus*, and *M. abscessus* subsp. *massiliense*, respectively (Table 2). *M. abscessus* subsp. *massiliense* (outbreak strain) was significantly more resistant than the other tested strains to PI and CHX ($P < 0.05$) (Fig. 2). While the mean LR achieved by PI was higher than for CHX for all mycobacterial strains, this was not statistically significant. For *S. aureus*, the efficacy of PI was significantly ($P = 0.019$) reduced under dirty conditions but that of CHX activity was not affected ($P = 0.293$) (16). Neither antiseptic was effectively mycobactericidal under dirty conditions (LR $< 5$). The least killing activity (LR $< 2$) was observed for the outbreak *M. abscessus* subsp. *massiliense* strain regardless of antiseptic or the presence or absence of organic debris.

## DISCUSSION

This *in vitro* study using two established methodologies to assess the susceptibility of *M. abscessus* subsp. *massiliense* (TPE101; outbreak strain), *M. abscessus* subsp. *abscessus* (ATCC 19977; nonoutbreak strain), *M. abscessus* subsp. *bolletii* (nonoutbreak strain), and *S. aureus* (reference strain) to commercial formulations of chlorhexidine and povidone-iodine suggests that clinically prevalent *M. abscessus* strains are highly resistant to the most commonly used commercial formulation of 2% chlorhexidine-alcohol and only partially susceptible to 10% povidone-iodine-alcohol. Since the skin is the major source of pathogens following an invasive procedure and a potential reservoir for mycobacteria contaminating hospital water or products applied to the skin prior to the

**TABLE 2** Mean LRs achieved by alcoholic PI and CHX for all tested *M. abscessus* strains and a reference *S. aureus* strain by quantitative carrier test (EN 14563-2008)

| Condition[a] | Mean LR[b] (SD) | | | | $P^{1c,e}$ | $P^{2d,e}$ |
|---|---|---|---|---|---|---|
| | ***S. aureus* (ATCC 29213)** | ***M. abscessus* subsp. *bolletii* (BCRC 16915)** | ***M. abscessus* subsp. *abscessus* (ATCC 19977)** | ***M. abscessus* subsp. *massiliense* (TPE 101)** | | |
| PI | | | | | | |
| Clean | 6.58 (0.25) | 5.54 (1.12) | 4.60 (1.43) | 1.17 (0.66) | 1.000 (1.000) | |
| Dirty | 2.48 (0.85) | 3.00 (1.47) | 1.97 (1.66) | 0.89 (0.50) | 0.226 (1.000) | |
| $P^{3e}$ | **0.019** | 0.228 | 0.435 | 1.000 | **0.001** (0.883) | |
| CHX | | | | | | |
| Clean | 3.16 (0.58) | 5.31 (0.64) | 3.84 (1.52) | 0.86 (0.43) | | 0.117 (0.324) |
| Dirty | 5.45 (2.16) | 2.56 (1.57) | 2.33 (1.60) | 0.39 (0.31) | | 1.000 (0.224) |
| $P^4$ | 0.293 | 0.168 | 1.000 | 1.000 | | **0.084** (0.025) |
| $P^{5e}$ | **0.050** (0.100) | 1.000 (1.000) | 1.000 (1.000) | 1.000 (1.000) | | |

[a] $P^3$ is the *P* value for comparisons of clean versus dirty conditions for PI within species; $P^4$ is the *P* value for comparisons of clean versus dirty conditions for CHX within species; $P^5$ is the *P* value for comparisons of PI versus CHX under clean (dirty) conditions within species.
[b] The experiments were conducted three times, and an average (mean) LR was obtained for each test condition. A mean LR of greater than 5 was considered biocidal.
[c] $P^1$ is the *P* value for interspecies comparisons under clean (dirty) conditions for each mycobacterial species in reference to *S. aureus* in the following order: *M. abscessus* subsp. *bolletii*, *M. abscessus* subsp. *abscessus*, and *M. abscessus* subsp. *massiliense* for PI.
[d] $P^2$ is the *P* value for interspecies comparisons under clean (dirty) conditions for each mycobacterial species in reference to *S. aureus* in the following order: *M. abscessus* subsp. *bolletii*, *M. abscessus* subsp. *abscessus*, and *M. abscessus* subsp. *massiliense* for CHX.
[e] *P* values of $<0.05$ are in boldface.

invasive procedure, it is conceivable that inadequate mycobactericidal activities of the currently used antiseptics may lead to an increase in postprocedural mycobacterial infections (1, 4, 7, 21).

The inactivity of CHX in suspension against these *M. abscessus* strains is particularly worrisome, as there is a potential for the antiseptic itself, commonly stored in multiuse

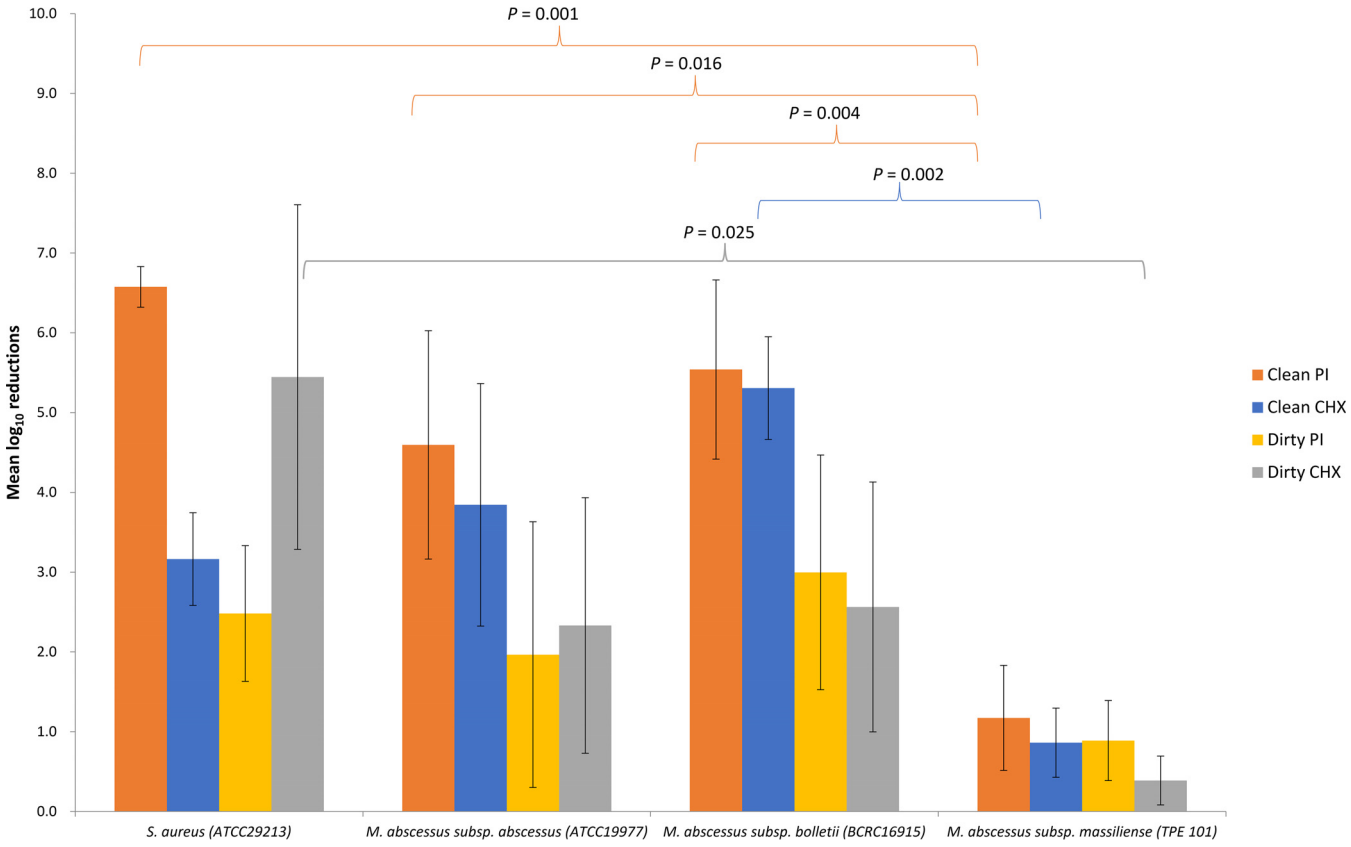


**FIG 2** Potencies of alcoholic PI and CHX by quantitative carrier testing (EN 14563) against different strains of *M. abscessus* expressed as the mean logarithmic reductions of CFU. The error bars indicate SD.

bottles, to be contaminated by *M. abscessus* in a manner similar to that in which an outbreak of postinjection *M. abscessus* infection was linked to the contamination of the benzalkonium chloride used to disinfect the skin prior to articular steroid injection (25). In contrast, based on the suspension results, multiuse bottles of PI are unlikely to permit the growth of *M. abscessus*, although its activity did appear to be attenuated by organic material and on a two-dimensional surface.

Chlorhexidine gluconate is a nonvolatile, slowly bactericidal agent (26, 27). In susceptible bacteria, it collapses the membrane potential, and membrane disruption is followed by leakage of intracellular constituents. Its mechanism of action is concentration dependent. Higher concentrations of CHX cause coagulation of cytoplasmic proteins and nucleic acids, which is also slowly lethal (27). One of its main attributes is residual antimicrobial activity, which is beneficial in skin antiseptics used for catheter care but not necessarily in hand hygiene or wound care (18, 27). Another benefit of CHX over PI is the absence of skin staining, which is an important consideration in cosmetic surgery. The combination of CHX and an alcohol, with the alcohol providing rapid bactericidal effect, has gradually replaced PI as the first-line skin antisepsis in the last 2 decades (18, 19), at a time coinciding with increasing reports of outbreaks of nontuberculous mycobacterial skin and soft tissue infections after surgery, particularly cosmetic surgery (1).

CHX's general lack of activity against mycobacteria, particularly *Mycobacterium tuberculosis*, has been exploited in sputum decontamination to improve isolation rates (28, 29). However, CHX cannot be assumed to spare all mycobacteria, since it demonstrated highly mycobactericidal effects ($>$6 LRs) even in the presence of sputum in both suspension and carrier tests against *M. smegmatis*, and the MICs for some mycobacterial strains are on the order of those for CHX-sensitive Gram-positive cocci (27, 30, 31). The marginally better activity of CHX in the surface carrier tests for standard strains of *M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *abscessus* compared to the suspension test in our study may be due to the increased length of exposure before neutralization (120 s instead of 30 to 90 s in the suspension test), the lack of dilution of 2% chlorhexidine-75% alcohol in the surface carrier test (4,000 mg/liter versus 800 mg/liter in the suspension test), and the effect of the coformulated alcohol, which was allowed to evaporate in the surface carrier test but not in the suspension test. However, there was really no difference in the activity of CHX assayed (LRs all $<$1) by suspension or carrier tests against *M. abscessus* subsp. *massiliense* (TPE 101).

This dominant clone of *M. abscessus* subsp. *massiliense* (TPE 101) emerged in 2010, causing skin and soft tissue infections in northern and central Taiwan after invasive procedures, with a peak attack rate in 2012, whose common source was finally traced to a brand of contaminated ultrasonography transmission gel (8). At our institution, this coincided with implementation of the Joint Commission International-endorsed use of chlorhexidine in central vascular catheter infection prevention bundles in 2010. In fact, the pivotal case that led to identification of the ultrasonography gel as the point source of the outbreak was a patient diagnosed with metastatic pancreatic adenocarcinoma, who had received an ultrasound-guided tunneled central vascular catheter in the operating room in anticipation of chemotherapy and developed TPE 101 bacteremia within 24 h. The investigation revealed that both used and unopened sonography gels were also contaminated by nonfermentative Gram-negative bacilli (NFGNB) and *Candida* species (8). However, neither the above-mentioned patient nor the other cases receiving ultrasound-guided procedures who developed *M. abscessus* subsp. *massiliense* procedural site infections were concomitantly infected by these NFGNB or yeasts. Hence, we hypothesized that inadequate mycobactericidal decontamination of the skin after ultrasonography may have led to the emergence of *M. abscessus* subsp. *massiliense* TPE 101.

This hypothesis of antiseptic failure effectively leading to selective pressure for TPE 101 was supported by our findings (Fig. 2). Of the tested *M. abscessus* strains, including the less frequently isolated *M. abscessus* subsp. *bolletii* and the *M. abscessus* subsp. *abscessus* ATCC 19977 strain, both not implicated in skin and soft tissue infections at

our institution, CHX achieved LRs of >3 under clean and >2 under dirty conditions by carrier testing, possibly qualifying it as mycobacteriostatic. However, CHX LRs were uniformly <1 against TPE 101 regardless of the presence or absence of an organic load. This intraspecies differential resistance to CHX was statistically significant (Fig. 2). Intraspecies differential resistance to CHX has been demonstrated before by a transmission electron microscopy (EM) study on glutaraldehyde-resistant *Mycobacterium chelonae* strains compared to a reference strain of *M. chelonae*, NCTC 946 (32). Fraud et al. were able to demonstrate significant cell wall alterations (by EM analysis and the dramatic loss of lipids) observed in *M. chelonae* NCTC 946 spheroplasts at low CHX concentrations of 25 to 100 mg/liter, which resulted in cytosolic protein precipitation and cell death of the NCTC 946 strains, but not in their two glutaraldehyde-resistant *M. chelonae* strains, which required much higher concentrations of CHX (500 mg/liter) to induce the same effects, ostensibly because the permeability barrier had been altered to a lesser extent (32).

By the surface carrier test, TPE 101 also demonstrated high resistance to PI. The possible mechanisms for the enhanced CHX and PI resistance phenotype of this outbreak strain, such as decreased cell wall permeability or "rough colony" morphology, deserve further characterization by EM studies, as described above (32–34). Furthermore, TPE 101 was typed as ST48, differing by only 1 of 7 multilocus sequence type (MLST) loci (*murC* gene) from the globally successful clone ST23, and belonging to the same clonal complex 3 as ST23 (8). ST23 has been responsible for an epidemic of postsurgical infections involving at least 2,032 cases across 63 hospitals in Brazil (BRA100) and two respiratory outbreaks among cystic fibrosis cohorts in the United States and the United Kingdom (9, 11, 12). BRA100 has been shown to tolerate high concentrations of glutaraldehyde (up to 7%), which is a common disinfectant for endoscopic or heat-intolerant surgical equipment, and other clinical strains of *M. abscessus* subsp. *massiliense* have demonstrated *in vitro* resistance to quaternary ammonium compounds (35, 36). The resistance of these epidemic strains to skin antiseptics such as CHX and PI has not been previously determined, yet it is highly likely that outbreak strains of *M. abscessus* subsp. *massiliense*, including ST23 and ST48, share multiple disinfectant resistance mechanisms that facilitate their adaptation to health care settings.

The results of *S. aureus* bactericidal suspension and carrier tests performed in this study are in agreement with published studies (15, 16). Although there have been several studies on the activities of PI and CHX against other mycobacterial species, we found only one comparable study on the mycobactericidal activities of PI and CHX against *M. abscessus* (30, 37, 38). In that study, one *M. abscessus* strain was tested using the suspension method. Nevertheless, similar to our suspension results, PI was shown to be rapidly mycobactericidal even at dilutions to 0.05% for 30 s for their *M. abscessus* clinical isolate, as well as for standard strains of *Mycobacterium avium*, *Mycobacterium kansasii*, and *M. tuberculosis*. Although CHX activity was not tested against their *M. abscessus* clinical isolate, they also showed a lack of CHX activity against *M. tuberculosis* H37Rv, *M. kansasii* ATCC 12478, and *M. avium* ATCC 15769 (37). The conclusion from this and other published studies by this group on other mycobacterial strains was that PI remains a useful antiseptic against mycobacteria, albeit with an expected reduction of activity with an organic load and when tested on surrogate or skin surfaces (39, 40).

The limitations of this study include the interreplicate variability, which could have been reduced by increasing the replicates (currently tested in triplicate), and the testing of only two well-established skin antiseptics and not of newer, potentially more mycobactericidal antiseptics. CHX's activity may be improved at higher concentrations, so it would be worth testing commercial formulations of 4% and 20% CHX. The strengths of this study include the use of two methods to assess antiseptic activity (since antiseptics act differently on planktonic and surface-adherent microorganisms, yielding discrepancies between suspension and carrier tests), realistic contact times, and the use of an organic load to mimic the skin and biofilms (30). In addition, both

antiseptics studied were alcohol based to avoid the previous criticism that the differences between CHX and PI may be solvent related (41).

Nevertheless, these *in vitro* findings will need to be corroborated by skin surface (*ex vivo*) and clinical data to determine their clinical impact. Of note, the various randomized clinical trials showing superiority of CHX over PI documented a decrease of infections by mostly bacteria (and *Candida* species) only; mycobacteria were absent from all reports, due to either rarity or omission (17, 18, 42). It may thus be prudent for future clinical trials of CHX and PI to consider obtaining cultures specifically for recovering mycobacteria, since they may be overlooked by routine culture methods and there is poor evidence to assume that the broad-spectrum antimicrobial activity of these two common antiseptic preparations encompass pathogenic mycobacteria.

The principal conclusion from this work is that alcohol-based 2% CHX is insufficient to prevent, and may actually facilitate, health care-associated infections with epidemic *M. abscessus* strains. The observed growing preference for using CHX as the first-line antisepsis in central vascular catheter placement and surgery might feasibly contribute to the increasing frequency of *M. abscessus* isolated from the bloodstream and surgical sites (24). More studies are needed to establish the best practice for skin antisepsis if mycobacterial infections are also to be prevented and to establish the virulence traits conferring the epidemic potential of *M. abscessus* subsp. *massiliense* ST48 and ST23 strains.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** The organisms studied were *M. abscessus* subsp. *abscessus* (ATCC 19977), *M. abscessus* subsp. *bolletii* (BCRC 16915), and *M. abscessus* subsp. *massiliense* (TPE 101, ST48, clonal complex 3) and *S. aureus* (ATCC 29213). Subculture and manipulation of the test organisms were kept to a minimum. Suspensions of all the test organisms were prepared and frozen in 1-ml aliquots at −80°C until required.

**Skin antiseptics tested.** The mycobactericidal activities of the two most common skin disinfectants, Sindine antiseptic solution (alcoholic) containing 10% PI with 0.7 ml/1 ml 95% alcohol (Sinphar Pharmaceutical Co. Ltd., Taiwan) and Easy Antiseptic Liquid 2% (alcoholic) containing 2% CHX gluconate and 75% alcohol (2% CHX; Panion & BF Biotech Inc., Taiwan), were assessed over concentrations of 125 to 4,000 mg/liter (PI) and 25 to 800 mg/liter (CHX) (corresponding to dilutions of 1:25 to 1:800), respectively.

**Suspension test.** The methods for preparing the test suspension and performing the disinfectant tests have been previously described in detail (43, 44). Briefly, bacteria were harvested from blood/Mueller-Hinton agar, added to moistened glass beads, and shaken for 5 min. Ten milliliters of double-distilled water was added, agitated, and left to settle for 30 min. The supernatant was removed to a second sterile bottle and left to settle for a further 2 h. Following sonication at 50 to 60 Hz for 10 min, the bacterial suspension in distilled water was adjusted turbidimetrically to 0.5 McFarland standard; 100 $\mu$l of the supernatant was added to 900 $\mu$l of the disinfectant at room temperature for contact times of 30, 60, and 90 s, thus spanning realistic in-use contact times. After the required contact time, 10 $\mu$l was removed to 990 $\mu$l of the neutralization/recovery system and serially diluted to $10^{-2}$. A combination of 3% Asolectin from soybean, 10% Tween 80, and 0.3% sodium thiosulfate in double-distilled water was used to inactivate or neutralize the antiseptic solution according to published results and our validation tests (45). Ten microliters of the neat solution and subsequent dilutions were plated on Mueller-Hinton agar, and surviving colonies were enumerated following appropriate incubation for 3 to 5 days at 35°C. The results of the test were expressed as the LR, which is the $\log_{10}$ value of the counts after exposure to the test antiseptic (Na) subtracted from the $\log_{10}$ value of the counts after exposure to the control (Nc). The experiments were performed in triplicate. An average (mean) LR of greater than 5 was considered biocidal.

**Quantitative carrier test.** The European standard EN 14563, dedicated to testing products used in the medical area for mycobactericidal activity, was established in 2008 (46, 47). The potencies of alcoholic PI and CHX against the three mycobacterial and the reference *S. aureus* isolates was evaluated under "dirty" and "clean" conditions in accordance with EN 14563 with the following minor modifications.

Briefly, a bacterial suspension yielding $1.5 \times 10^9$ to $5 \times 10^9$ CFU/ml was freshly prepared, homogenized, and used within 2 h (46). One milliliter of interfering substances (0.3 g/liter bovine albumin under clean conditions or a mixture of 3 ml/liter sheep erythrocytes and 3.0 g/liter bovine albumin under dirty conditions) was mixed with 9 ml of the bacterial suspension, and 0.05 ml of this mixture was pipetted and evenly spread on the inoculation square of a frosted glass carrier. The carrier was maintained at 35°C for 60 min ± 10 s. After drying, the test antiseptic solution (CHX or PI) or distilled water (control) was applied to cover the entire surface of the inoculation square using a sterile cotton swab in the manner that preoperative skin is disinfected. At the end of 2 min of contact time to simulate recommended practice with the test antiseptic or control, the carrier was transferred into a neutralizing solution containing glass beads. The bacteria were dislodged from the surface by shaking. The number of surviving bacteria in each sample was determined, and the reduction was calculated and expressed as

the LR as described above. The experiments were conducted three times, and an average (mean) LR was obtained for each test condition.

**Statistical methods.** Statistical analysis was performed using SPSS Statistics software, version 21 (IBM Corp.). The statistical method was based upon analysis of variance of the mean LRs partitioned into components attributable to differences in concentration (dilutions), exposure time, bacterial strain, and choice of treatment (PI or CHX). Pairwise differences among the different experimental and control groups were detected by the Bonferroni method. Two-sided *P* values of less than or equal to 0.05 were considered significant.

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We declare no conflicts of interest.

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