Germicidal Activity against Carbapenem/Colistin-Resistant Enterobacteriaceae Using a Quantitative Carrier Test Method

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ABSTRACT Susceptibility to germicides for carbapenem/colistin-resistant Enterobacteriaceae is poorly described. We investigated the efficacy of multiple germicides against these emerging antibiotic-resistant pathogens using the disc-based quantitative carrier test method that can produce results more similar to those encountered in health care settings than a suspension test. Our study results demonstrated that germicides commonly used in health care facilities likely will be effective against carbapenem/colistin-resistant Enterobacteriaceae when used appropriately in health care facilities.

KEYWORDS carbapenem-resistant Enterobacteriaceae, Klebsiella pneumoniae carbapenemase, colistin-resistant Enterobacteriaceae, mcr-1, germicides, disinfectants, antiseptics, efficacy

Carbapenem-resistant Enterobacteriaceae (CRE) have broad resistance to most ß-lactam antibiotics and are a growing worldwide problem (1). CRE infections are difficult to treat, have a substantial mortality (1), and are involved in health care-associated outbreaks via contaminated environmental surfaces and medical equipment (2). Colistin-resistant Enterobacteriaceae isolates carrying mcr-1 are a global health concern, since colistin is often a last-line antibiotic used to treat CRE (3). Furthermore, a recent study from China described a hospital outbreak caused by MCR-1-producing Klebsiella pneumoniae with potential spread of mcr-1 via the hospital environment (4).

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Although there is currently no strong scientific evidence demonstrating that clinical use of disinfectants/antiseptics is associated with selection of antibiotic-resistant organisms, some studies have described reduced susceptibility to disinfectants (e.g., quaternary ammonium compounds [QAC]) and antiseptics (e.g., chlorhexidine) as well as cross-resistance (e.g., benzalkonium chloride/quinolones) (5–7). Susceptibility to germicides (e.g., disinfectants, antiseptics) for carbapenem- or colistin-resistant Enterobacteriaceae is poorly described (7, 8). In this study, we assessed the efficacy of multiple germicides against these emerging antibiotic-resistant pathogens because of global clinical and public health concerns.

Three species of Klebsiella pneumoniae carbapenemase (KPC)-producing Enterobacteriaceae, including clinical isolates (9) of K. pneumoniae (Kp11), Enterobacter cloacae (Ec12), and a strain of Escherichia coli (ATCC BAA-2340), as well as E. coli carrying mcr-1 (MRSN 388634), were studied. Multiple different types of germicides, including 5 high-level disinfectants/chemical sterilants, 7 low- and intermediate-level disinfectants (2 dilutions of sodium hypochlorite), 8 antiseptics, and 1 disinfectant/antiseptic, were tested (Table 1). The germicides were prepared according to the manufacturer’s instructions, and germicides requiring dilution were made fresh each day =3 h before use. Germicides were stored at room temperature and tested within the expiration date. Sterile distilled water was utilized in the experiments. Hard water was prepared for
| Germicide name                      | Manufacturer, location            | Active ingredient                                      | Formulation tested | Classification        | KPC E. coli | KPC K. pneumoniae | KPC E. cloacae | MCR-1 E. coli |
|------------------------------------|----------------------------------|--------------------------------------------------------|--------------------|-----------------------|-------------|-------------------|----------------|---------------|
| Purell Advanced instant hand sanitizer | GOJO, Akron, OH                  | 70% ethanol                                            | Undiluted          | Antiseptic            | 4.6         | 3.5               | 4.1            | 4.2           |
| Betadine solution                  | Purdue Products L.P., Stamford, CT | 10% povidone-iodine/1% titratable iodine               | Undiluted          | Antiseptic            | 3.4         | 2.9               | 3.5            | 3.9           |
| Solution of hydrogen peroxide 3% USP | Medichoice, Mechanicsville, VA    | 3% hydrogen peroxide                                    | Undiluted          | Antiseptic            | 2.2         | 3.5               | 3.2            | 1.7           |
| Medicated Soft 'N Sure Soft Care Defend | Steris Corp., St. Louis, MO       | 0.5% triclosan                                          | Undiluted          | Antiseptic/handwash   | 2.3         | 2.9               | 3.3            | 3.8           |
| Avagard                            | Diversey, Inc., Charlotte, NC    | 1% chloroxylenol                                        | Undiluted          | Antiseptic/surgical hand scrub | 2.6         | 3.8               | 3.7            | 1.8           |
| Scrub-Stat 2%                      | Ecolab, St. Paul, MN              | 2% chlorhexidine gluconate solution, 61% ethyl alcohol | Undiluted          | Antiseptic/surgical hand scrub/handwash | 3.4         | 3.5               | 3.4            | 4.1           |
| Scrub-Stat 4%                      | Ecolab, St. Paul, MN              | 4% chlorhexidine gluconate solution                     | Undiluted          | Antiseptic/handwash   | 3.1         | 4.0               | 3.5            | 4.4           |
| Isopropyl rubbing alcohol 70% USP | Medichoice, Mechanicsville, VA    | 70% isopropyl alcohol                                   | Undiluted          | Antiseptic/disinfectant | 4.6         | 4.0               | 4.9            | 4.4           |
| Austin's A-1 bleach 1:10           | James Austin Company, Mars, PA     | 5.25% sodium hypochlorite                               | 1:10 dilution      | Disinfectant          | 4.9         | 5.6               | 5.9            | 4.4           |
| Austin's A-1 Bleach 1:50           | James Austin Company, Mars, PA     | 5.25% sodium hypochlorite                               | 1:50 dilution      | Disinfectant          | 4.9         | 3.5               | 3.4            | 4.4           |
| Vesphene Ile                       | Steris Corp., St. Louis, MO       | 5.20% o-phenylphenol, 7.66% p-tertiary amylphenol       | 1:128 dilution     | Disinfectant          | 4.9         | 5.6               | 5.8            | 4.4           |
| Hydrogen peroxide cleaner disinfectant | Clorox Company, Oakland, CA       | 1.4% hydrogen peroxide                                  | Undiluted          | Disinfectant          | 4.9         | 5.6               | 5.8            | 4.4           |
| Lysol disinfectant spray A-456 II disinfectant cleaner | Reckitt Benckiser, Parsippany, NJ | 58% ethanol, 0.1% QAC<sup>b</sup>                        | Undiluted          | Disinfectant          | 4.9         | 5.6               | 5.8            | 4.4           |
| Super Sani-Cloth wipe              | Ecolab, St. Paul, MN              | 21.7% QAC<sup>c</sup>                                   | 1:25 dilution      | Disinfectant          | 4.9         | 3.5               | 4.7            | 4.4           |
| Prime Sani-Cloth wipe              | PDI, Orangeburg, NY               | 55% isopropyl alcohol, 0.5% QAC<sup>d</sup>             | Undiluted<sup>f</sup> | Disinfectant          | 4.9         | 5.2               | 5.8            | 4.4           |
| Cidex OPA                          | Advanced Steril. Prod., Irvine, CA | 0.55% ortho-phthalaldehyde                              | Undiluted          | High-level disinfectant | 4.8         | 5.6               | 5.9            | 4.4           |
| Cidex                              | Advanced Steril. Prod., Irvine, CA | 2.4% glutaraldehyde                                    | Undiluted          | High-level disinfectant/chemical sterilant | 2.4         | 4.8               | 3.4            | 2.0           |
| Oxycide                            | Ecolab, St. Paul, MN              | 27.5% hydrogen peroxide                                 | 1:43 dilution      | High-level disinfectant/chemical sterilant | 4.9         | 5.6               | 5.8            | 4.4           |
| Revital-Ox Resort                  | Steris Corp., Mentor, OH          | 5.8% peroxyacetic acid                                  | Undiluted          | High-level disinfectant/chemical sterilant | 5.2         | 5.9               | 5.8            | 4.4           |
| S40 Sterilant                      | Steris Corp., Mentor, OH          | 35% peracetic acid                                      | Undiluted          | High-level disinfectant/chemical sterilant | 4.9         | 5.6               | 5.8            | 4.4           |

<sup>a</sup>Test condition of 10<sup>6</sup> test organisms with 5% FCS and 1 min contact time.

<sup>b</sup>QAC (quaternary ammonium compounds): alkyl (C<sub>14</sub> 50%, C<sub>12</sub> 40%, C<sub>16</sub> 10%) dimethyl benzyl ammonium saccharinate 0.1%.

<sup>c</sup>QAC: octyl decyl dimethyl ammonium chloride 6.51%, dioctyl dimethyl ammonium chloride 2.604%, didecyl dimethyl ammonium chloride 3.906%, alkyl (C<sub>14</sub> 50%, C<sub>12</sub> 40%, C<sub>16</sub> 10%) dimethyl benzyl ammonium chloride 8.66%.

<sup>d</sup>QAC: n-alkyl (C<sub>12</sub> 68%, C<sub>14</sub> 32%) dimethyl ethyl benzyl ammonium chlorides 0.25%; n-alkyl (C<sub>14</sub> 60%, C<sub>12</sub> 30%, C<sub>16</sub> 5%, C<sub>18</sub> 5%) dimethyl benzyl ammonium chloride 0.25%.

<sup>e</sup>QAC: didecyl dimethyl ammonium chloride 0.61%.

<sup>f</sup>Extract from cloth. We did not quantify QAC adhering to the cloth that is used to apply that particular germicide and may release smaller amounts of active QAC (17), but our data were not affected by this possibility because the extract was tested.
The disc-based quantitative carrier test method was used to assess the bactericidal activity of chemical germicides according to the method of Sattar et al. (10, 11). Brushed stainless steel discs (1 cm in diameter, 0.7 mm in thickness; Muzeen and Blythe Ltd., Winnipeg, Canada) were used as carriers. A 10-μl inoculum containing ~10⁶ test organisms with 5% fetal calf serum (FCS) was placed on each disc and dried in a vacuum desiccator for 2 h. After drying, each carrier was placed in a plastic vial with the inoculated side up. The dried inoculum was entirely covered by 50 μl of the test germicide for 1 min at room temperature (~20°C). A 9.95-ml eluent with neutralizer was added into each carrier holder to dilute and neutralize the germicide. Serial dilutions of the eluates were filtered to evaluate the bacterial viability and achieve countable numbers. The membrane filters of appropriate serial dilutions were placed on sheep blood agar plates and incubated for 24 to 48 h at 37°C and counted (i.e., CFU determined). Based on our ability to count CFU per plate, we used counts of 500 CFU for plates with confluent growth and 300 CFU for plates with colony numbers too numerous to count (TNTC). This was done in order to calculate means of multiple experimental runs. Three replicates were performed for each organism and each germicide. Three carrier controls were quantitated during each experiment in the manner described above. Compared to mean carrier control counts, the log₁₀ reduction of the test organism for each germicide was calculated.

The efficacy of germicides, along with active ingredient, product name, and classification, against test organisms is summarized in Table 1. Overall, most germicides reached at least a 3-log₁₀ reduction (20/22 [91%] for KPC K. pneumoniae, 22/22 [100%] for KPC E. cloacae, 18/22 [82%] for KPC E. coli, and 19/22 [86%] for MCR-1 E. coli). Furthermore, all germicides, except for two products (1% chlorhexidine gluconate plus 61% ethyl alcohol and 3% hydrogen peroxide) against MCR-1 E. coli, demonstrated at least a 2-log₁₀ reduction for these pathogens even in challenging test conditions (5% FCS and 1 min exposure time).

Under conditions of 10³ inoculum of MCR-1 E. coli with 5% FCS and 1 min contact time for 1% chlorhexidine gluconate plus 61% ethyl alcohol and 3% hydrogen peroxide, 1.6-log₁₀ and 0.7-log₁₀ reductions, respectively, were observed. Additionally, with a 10⁶ inoculum of MCR-1 E. coli with 5% FCS and 5 min contact time for 3% hydrogen peroxide, a 3.6-log₁₀ reduction was achieved.

The present study assessed a broad range of germicides against carbapenem/colistin-resistant Enterobacteriaceae, including high-level disinfectants used for semicritical items, disinfectants for noncritical items, and antiseptics for patients, which are commonly used in health care facilities, using the disc-based quantitative carrier test method that can produce results more similar to those actually encountered in health care settings than a suspension test (10).

Although a specific quantitative microbial bioburden in the health care environment linked to a specific increased risk for health care-associated infections has not been defined, several studies demonstrated that epidemiologically important pathogens on environmental surfaces were <72 CFU/Rodac in precleaning/postcleaning (12) or 61 CFU/room when hospital rooms were disinfected with quaternary ammonium compounds (13). Moreover, CRE surface contamination was infrequent and generally associated with only low levels (mean 5 CFU/120 cm² of contaminated surface) in an academic hospital in the United States (14). There is no standard level of germicidal efficacy for environmental surfaces, but most germicides tested are likely to be clinically effective (>3-log₁₀ reduction with a margin of safety) against carbapenem/colistin-resistant Enterobacteriaceae when used appropriately. Germicidal activity against colistin-resistant Enterobacteriaceae, except for a few germicides with challenging conditions (5% FCS and 1 min exposure time), was similar to activity against CRE. Many studies of health care disinfectants demonstrated microbial reduction against health
care-associated pathogens, with a contact time of ~1 min as a realistic condition of health care use, although some of the Environmental Protection Agency (EPA)-registered disinfectants used in this study have an EPA registration claim of >1 min contact time (15). It is also essential to facilitate best disinfection/antiseptic practice, since health care-associated outbreaks have often been caused by inadequate use of disinfectants/antiseptics for the environmental surfaces and medical equipment (2).

This study has the following limitations. First, given some TNTC and confluent growth cultures, our germicidal activity should be considered a maximum level. We assessed germicidal activity under different conditions for germicides that presented a relatively low germicidal levels (i.e., a <2-log\textsubscript{10} reduction). Second, germicides with high surface tension (e.g., 0.55% orthophthalaldehyde [Cidex OPA]) may have affected the results because of the difficulty in spreading out their inoculum on discs. Third, the phenotypic and genotypic resistances to germicides were not evaluated.

EPA assessments of germicidal activity for EPA-registered disinfectant products against multidrug-resistant organisms other than methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus do not exist (16). Furthermore, bacterial activity based on suspension tests of health care disinfectants has been reported from manufacturers and published literature, but their activity may be less applicable to actual clinical use (10, 11). Thus, our data based on a quantitative carrier test with a realistic condition of clinical use can mimic an actual health care environment and clinical application. Further investigations for germicidal activity against carbapenem/colistin-resistant Enterobacteriaceae under different conditions (e.g., inoculum, exposure time, with/without 5% FCS) and for phenotypic and genotypic resistances to germicides (e.g., MIC, specific resistance gene) may be necessary.

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