



## Major Article

## Efficacy testing of novel chemical disinfectants on clinically relevant microbial pathogens

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Nosocomial infection  
VRE  
MRSA

**Background:** There has been a dramatic increase in the number of hospital-acquired infections, which is linked to the pandemic of multidrug resistance. Clinical environments provide an ideal reservoir for the growth, proliferation, and transmission of pathogenic organisms, including bacterial and yeast species. Consequently, the need for improved, effective disinfectants is of paramount importance.

**Methods:** Studies were conducted to assess the efficacy of chemical disinfectants—peracetic acid and triameen—on microbial strains. Testing included the assessment of antimicrobial and antispore activity of disinfection solutions performed on a range of clinical isolates that pose a high risk for patient morbidity in clinical settings.

**Results:** Both chemical disinfectants successfully inactivated all test strains, with peracetic acid showing a greater level of antimicrobial activity. *Escherichia coli* proved most susceptible when assessed by the Kirby disk diffusion, suspension, and medical suspension assays with the greatest reduction in cell viability achieved. Antibiotic-resistant *Enterococcus* and *Staphylococcus aureus* strains showed greatest resistance to both disinfectants.

**Discussion and conclusions:** Test chemicals show potential to act as intermediate-level disinfectants inactivating vegetative microorganisms and bacterial spores on clinically relevant strains where they show potential as a preventative measure in relation to nosocomial infections.

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There has been a dramatic increase in the number of hospital-acquired infections (HAIs) in recent years, with unacceptable levels of morbidity and/or mortality. This pandemic is a direct result of antibiotic overuse; its subsequent bacterial resistance; and as a consequence, multidrug resistance.<sup>1</sup> Furthermore, clinical settings provide an ideal environment for the growth and proliferation of infectious organisms due to the close proximity of patients, high person content, and general nature of infection treatment. The risk of infection and occurrence of nosocomial disease is also related to medical-surgical procedures with the introduction of pathogenic organisms such as vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Candida* species from contaminated invasive devices, surfaces, and personnel.<sup>2</sup> The persistence of these organisms and their spore and/or biofilm counterparts means that the risk of infection

is a constant threat. HAIs are estimated to cost medical institutes billions per year and health care organizations have a legal responsibility to lower or eliminate the risk of HAI occurrence.<sup>3</sup> Therefore, the prevention of hospital infections presents a major challenge that is an emergency and of utmost importance.<sup>4</sup> The introduction of effective disinfection products was and remains vital in the battle against the spread of pathogenic species, cross-resistance, and nosocomial disease prevention. A variety of biocide products are available for clinical disinfection with moderate to insufficient antimicrobial ability.<sup>4</sup> These biocides usually contain 1 or more chemicals such as alcohols, phenols, iodine, and chlorine, which have been used for hundreds of years,<sup>5</sup> and more recently peracetic acid.<sup>6</sup> During recent years, the European Union has set requirements or standards regarding the antimicrobial activity of disinfectants used in medical settings.<sup>4</sup> Furthermore, the Centers for Disease Control and Prevention has established guidelines that recommend hospitals to thoroughly clean and disinfect environmental and medical equipment surfaces on a regular basis as a preventive measure. To achieve this recommendation and to lower the incidence of HAIs, continued efforts to improve manual disinfection of surfaces are needed.<sup>6</sup> Therefore, the aim of this study was to assess the potential of current and

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novel chemical disinfectants on clinically relevant microbial strains. Testing was conducted in accordance with the relevant suspension, medical suspension, and carrier assays for disinfectants, surfaces, bacterial spores, and medical areas. Test solutions contained varying concentrations of either peracetic acid or triameen as active ingredients. Peracetic acid is a powerful oxidant capable of oxidizing the outer cell membranes of microorganisms based on the transfer of electrons to the microbial cell, thus rendering the cell inactive.<sup>2</sup> Triameen is a fatty amine derivative that displays potential as a chemical disinfectant for use in reducing the transmission of pathogenic microorganisms. To the authors' knowledge, this constitutes the first study outlining the effectiveness of both disinfection solutions on strains isolated and relevant to medical settings.

## METHODS

### Test chemicals

The antibacterial agents used in this study were pure biocides used as disinfectant alone or in commercial brands. The concentrations described are the concentration of the active component and include the concentration used following each manufacturer's instructions.

### Bactericidal and yeasticidal suspension tests

Suspension tests were conducted per the methods of the European guidelines for bacterial yeast strains for the testing of chemical disinfectants that form a homogeneous physically stable preparation in hard water and are used in food, industrial, domestic, and institutional areas. Isolated strains and *Candida albicans* (ATCC 10231), *Candida krusei* (ATCC 14243), *S aureus* (ATCC 25923), *E coli* (ATCC 25922), VRE, and MRSA were used for suspension testing. MRSA and VRE isolates were sourced from a blood culture at the National University of Ireland, Galway Hospital. Bacterial test suspensions were prepared by seeding sterile nutrient broth with an isolated colony and incubating under rotary conditions (125 rpm) for 12 hours at 37°C. Yeast suspensions were prepared by seeding sterile Sabouraud broth (Cruinn Diagnostics, Dublin, Ireland) with an isolated colony and incubating under rotary conditions (125 rpm) for 16 hours at 32°C. Cell counts were adjusted to 10<sup>10</sup> and 10<sup>9</sup> bacterial and yeast cells per milliliter, respectively, using sterile saline solution. Chemical test solutions were prepared per the manufacturer's instructions for use onsite and at a concentration above and below this working concentration. Before testing, all reagents are equilibrated to the test temperature of 20°C using the water bath. Subsequently, 8 mL test product was transferred to a sterile container with 1 mL sterile water. Afterward, 1 mL microbial suspension containing 1 × 10<sup>10</sup> bacterial cells was added. Additionally, 1 mL interfering substance (3.0 g/L bovine serum albumin) (Sigma, Wicklow, Ireland) was added and incubated for 0–30 minutes with mixing in a 20°C water bath. At set intervals of 5, 15, and 30 minutes, 1 mL test mixture was transferred into a tube containing 8 mL neutralizer (30 g/L polysorbate 80 + 3 g/L lecithine/l- $\alpha$ -phosphatidylcholine from egg yolk) (Sigma) and 1 mL sterile water. Samples were mixed and incubated in the water bath for 5 minutes. After neutralization, 100  $\mu$ L bacterial suspension was transferred onto agar plates in triplicate and incubated at 37°C for 24 hours. Surviving cells of treated organisms were counted to determine the level of bacterial inactivation following exposure to the test solutions compared with the untreated control. For compliance with this test, test chemicals must achieve a 10<sup>5</sup> bacterial cell and 10<sup>4</sup> yeast cell reduction in treatment times <5 minutes and <15 minutes, respectively.

### Medical suspension test

This test is a quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants for instruments used in medical areas. It is a suspension conducted with the addition of sheep erythrocytes (3 ml/L) to a 3.0 g/L bovine serum albumin interfering solution to simulate soiled medical conditions. All strains outlined above were treated in the presence of the interfering bovine serum albumin and sheep erythrocyte solution. Following exposure to the test chemicals, the disinfection reaction was neutralized as previously described. For compliance with this test, test chemicals must achieve a 10<sup>4</sup> (99.99%) bacterial cell and 10<sup>3</sup> (99.9%) yeast cell reduction in treatment times <5 minutes and <15 minutes, respectively.

### Antimicrobial test for medical surfaces

The European standard for medical surfaces outlines a test procedure to quantify the antimicrobial activity (bactericidal and/or yeasticidal) of chemical disinfectants used in food, industrial, domestic, and institutional areas and was used as a guidance document for test chemicals in this study. Stainless steel squares were submerged into the test solution at a concentration of 0.2% to allow for adherence of the chemical onto the surface; sterile water was used as a control. A test suspension of the selected microorganisms in the presence and absence of the interfering substance (ie, 3.0 g/L bovine serum albumin) was inoculated onto test stainless steel surfaces in triplicate for 30 minutes. Subsequently, the steel material was transferred into the neutralization medium to stop the reaction. The number of surviving organisms recovered from the surface was determined by standard spread plating. The number of viable organisms on the treated surface was compared with the untreated control to determine the log reduction of microbial strains.

### Antisporicidal activity

A quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic, and institutional areas was used. *Bacillus pumilus* (ATCC 14884) and *Bacillus cereus* (ATCC 11778) spores were produced per the method of Garvey et al<sup>7</sup> and stored at –20°C when not in use. For compliance with this test, test chemicals must achieve a 10<sup>4</sup> reduction of spores in treatment times <60 minutes in the presence of an interfering substance at 20°C. Specifically, 1 mL 10<sup>8</sup> spore suspension was added to 8 mL test chemical with 1 mL 3.0 g/L bovine serum albumin and allowed to react for up to 60 minutes at 20°C. Following treatment, the reaction was neutralized as previously described and 100- $\mu$ L samples were spread on nutrient agar plates in triplicate and stored at 37°C for 24 hours to establish a viable cell count.

### Kirby-Bauer disk diffusion assay

The bacterial organisms were grown on Mueller-Hinton (Cruinn Diagnostics) agar in the presence of various antimicrobial-impregnated filter paper disks. *Candida* species were grown on Sabouraud broth and plated on Sabouraud agar. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit the test organism. Agars were prepared per manufacturer's instructions in deionized water at room temperature and poured to a depth of 4 mm. Immediately before inoculation, media was checked to ensure it was moist but free of water droplets on the agar surface and the Petri dish lids. The test

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