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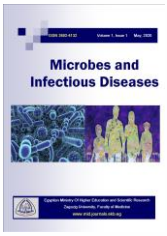

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Original article

Hospital disinfectant efficacy testing: Experimental evidence towards the requirement of common international regulatory framework, the case of Costa Rica

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ABSTRACT

Background: Costa Rica, as many developing countries around the world, lacks a specific regulatory framework for hospital disinfectant efficacy testing. The aim of this study is to evaluate the efficacy of disinfectant samples used in healthcare facilities of Costa Rica to provide experimental evidence to encourage the definition of public health polices in this matter. **Methods:** The determination of efficacy for disinfectant samples were based on Association of Official Agricultural Chemists (AOAC) use-dilution methods 955.15 (*Staphylococcus aureus* ATCC 6538) and 964.02 (*Pseudomonas aeruginosa* ATCC 15442) with minor modifications. **Results:** Ineffective disinfectant samples intended for their use in healthcare centers were identified. Experimental data indicates that disinfectants tested have lower activity against Gram-negative bacteria than Gram-positive bacteria. **Conclusions:** Despite most analyzed disinfectant samples are effective, there are products that did not satisfy performance criteria. The study gives experimental data that are useful to encourage the definition of a regulatory frame for hospital disinfectant efficacy testing in Costa Rica.

Introduction

Bacterial infections are a main concern to public health systems due the spreading of multidrug resistant strains. This is an important issue in healthcare facilities where patients, clinical personal and visitors can get infections if there are not good cleaning and disinfectant practices [1-3]. The propagation of this pathogens can increase mortality and morbidity of patients due secondary infections [4,5]. Furthermore, secondary bacterial infections can prolong the hospital stay and can hinder the clinical management of patients, this can be translated in higher cost for health care systems [6].

The efficacy of disinfectants used in medical installations is a recognized critical factor to limit the spreading of pathogenic microorganisms [2]. In consistence with this fact, countries as United States and members of European Union have been set specific quality standards for disinfectants to be approved as hospital grade product by regulatory instances [7,8]. Nevertheless, not all countries have

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this class of regulations. For instance, Costa Rica does not have specific quality or regulatory requirements for disinfectants used in healthcare facilities despite backgrounds related to the spreading of pathogenic bacteria in hospital environments that endangered the life of patients [9-11]. Therefore, the definition of specific regulatory requirements for the efficacy testing of disinfectants that claims hospital grade efficacy is an element that can be useful to protect patients and healthcare staff against the acquisition of bacterial diseases in medical installations.

Another point of concern is the fact that low efficacy disinfectants can enhance resistance of pathogenic bacteria against active principles of this products [12,13]. This can compromise the effectiveness of cleaning and disinfection tasks in hospital installations. Regional deficiencies in this matter could have worldwide implications considering the possibility of easily spreading of resistant strains through international means of transportation [14].

Considering the exposed backgrounds, the efficacy of disinfectants for use in healthcare centers of Costa Rica was evaluated to demonstrate the necessity of specific regulations for the efficacy testing of hospital grade disinfectants used in the country. Furthermore, results can contribute to highlight the requirement of guidelines for disinfectant efficacy testing.

Methods

Disinfectants samples

Screened disinfectants for hard surfaces, destinated to their use in healthcare facilities according to manufacturer, were obtained from respective institutional chemical warehouses or were purchased during 2020 or 2021. The disinfectants have quaternary ammonium compounds (QACs), aldehydes, alcohols, chelating agents and tensioactives (amine oxides and ethoxylated alchols) among their active antimicrobial ingredients. The products are intended to use in not critical areas (areas other than surgery units, intensive care units, isolation units, parenteral and enteral nutrition preparation areas, and parenteral drug preparation units) of hospitals, clinics, and primary health facilities. At least one gallon of each product was used for the analysis of its efficacy. Recipients containing the products kept the seals without any opening or damage until the assays. Disinfectants were analyzed before expiration date indicated by manufacturer. **Table 1** summarizes the labeled composition of the examined products with details about preparation for use and contact time with solid surfaces.

Disinfectant efficacy testing

The assays were based on AOAC use-dilution methods 955.15 (*Staphylococcus aureus* ATCC 6538) and 964.02 (*Pseudomonas aeruginosa* ATCC 15442) with minor modifications. The mentioned methods have been taken as reference considering that passing these tests are mandatory to approve claims of hospital grade for disinfectants according to Environmental Protection Agency (EPA) of United States [7]. The experimental procedures are described in the following sections.

Inoculation of steel cylinders (carriers)

For this step, 24 hours cultures of test bacteria incubated at 32.5 ± 2.5 °C in tryptic soy agar (BD Difco, United States) plates were used. Using sterile loop, colonies of the test bacteria were transferred to tubes containing 9 mL of sterile sodium chloride (Sigma-Aldrich, United States) solution (SSC) (0.9 % w/v). Then, the bacterial density of the tube was adjusted adding SSC to get an inoculum with a transmittance of 25 ± 1 % at 580 nm, which is equivalent to $7x10^8$ CFU/mL (95 % confidence interval (95 % CI): $4x10^8$-$9x10^8$ CFU/mL) and $6x10^8$ CFU/mL (95 % CI: $4x10^8$-$8x10^8$ CFU/mL) for *Staphylococcus aureus (S. aureus)* and *Pseudomonas aeruginosa* (*P. aeruginosa*), respectively. A liquid culture of each strain was prepared by transferring 2 mL of SSC inoculum to a flask containing 100 mL of sterile tryptic soy broth (TSB) (BD Difco, United States). The flask was incubated at 32.5 ± 2.5 °C for 48 hours. After incubation time, any superficial pellicle was removed. To prepare work bacterial suspensions, 70 mL of *S. aureus* culture were combined with 10 mL of sterile TSB or 20 mL of *P. aeruginosa* culture were mixed with 60 mL of sterile TSB. Then, sterile 80 steel cylinders (external diameter: 8 ± 1 mm, internal diameter: 6 ± 1 mm, length: 10 ± 1 mm) were aseptically transferred to each of these bacterial suspensions and were left to stand for 15 minutes. After inoculation time, cylinders were aseptically transferred to petri dishes in vertical position (10 cylinders per petri dish) containing filter paper (Whatman N°3, Whatman, United States) and were incubated at 32.5 ± 2.5 °C to dryness for an hour. With this procedure was possible to get bacterial charge of $1x10^6$-$1x10^7$ CFU

per cylinder for both test strains according to plate counting.

Disinfectant activity evaluation

Sixty inoculated cylinders were individually transferred to tubes containing 10 mL of disinfectant prepared according to instructions given by the manufacturer. The cylinders were stood in the disinfectant for the labeled contact period. For those disinfectants without any indication of contact period, 10 minutes of stand period of the cylinders in the disinfectant were used. This contact time was based on prescribed contact period approved by EPA for most of the registered hospital disinfectants [15]. After expose the cylinders to disinfectant, they were placed in tubes containing 20 mL of TSB supplemented with Tween 80 (40 g/L) and soy lecithin (5.0 g/L) (Sigma-Aldrich, United States). This media was used as disinfectant neutralizing broth (for those disinfectant with aldehydes or EDTA, TSB was also supplemented with sodium thiosulfate (6 g/L) (Sigma-Aldrich, United States) or magnesium sulphate heptahydrate (5 g/L) (Sigma-Aldrich, United States), respectively). The tubes with cylinders exposed to disinfectant were incubated at 32.5 ± 2.5 °C for 72 hours, after that time, they were visually inspected to detect growth as the presence of turbidity, debris, or floating pellicles. To confirm the identity of the microorganism in tubes positive for growth, their content was used to inoculate mannitol salt agar or *Pseudomonas* agar plates for *S. aureus* and *P. aeruginosa*, respectively. From negative tubes, 20 % of them were randomly chose to inoculate mannitol salt agar (BD Difco, United States) or *Pseudomonas* agar (BD Difco, United States) plates to confirm the absence of viable colonies of test bacteria. Plates were incubated at 32.5 ± 2.5 °C for 48 hours. No more than three or six tubes with *S. aureus or P. aeruginosa* inoculated cylinders had to show growth, respectively. Product samples that did not satisfy these performance criteria were considered as ineffective for its use in healthcare facilities [7].

Test controls

Test controls were done based on AOAC use-dilution methods 955.15 (S*taphylococcus aureus* ATCC 6538) and 964.02 (*Pseudomonas aeruginosa* ATCC 15442) with modifications [7]. To ensure the sterility of cylinders at the beginning of each assay, at least three of them were transferred to tubes containing 20 mL of neutralizing broth, the absence of growth was visually corroborated after 72 hours of incubation at 32.5 ± 2.5 °C. The capability of the neutralizing broth to neutralize residual quantities of disinfectants in the cylinders and promote bacterial growth was evaluated by transferring three sterile cylinders previously exposed to disinfectant to tubes containing 20 mL of neutralizing broth. Then, 100 CFU of the test bacterium were added to the tubes with the cylinders and they were incubated for 48 hours at 32.5 ± 2.5 °C, after this period, the tubes had to show growth. The proper inoculation of cylinders was evaluated transferring at least ten cylinders previously immerged in work bacterial suspensions to tubes containing 20 mL of neutralizing broth, bacterial growth was confirmed after 48 hours incubation period at 32.5 ± 2.5 °C After incubation time of control tubes, their content was used to inoculated mannitol salt agar or *Pseudomonas* agar plates to confirm the presence or absence of growth for the test bacteria. Plates were incubated at 32.5 ± 2.5 °C for 48 hours.

Table 1. Labeled composition and conditions of use of the analyzed disinfectants.

Disinfectant number	Components [A]	Dilution and contact period
1	Glutaraldehyde, didecyldimethylammonium chloride (DDAC), ethoxylated alcohol, isopropanol, preservative, lavender fragrance	Product ready to apply on hard surfaces. Contact period: Not indicated
2	DDAC, ethoxylated alcohol, propanol, ethanol, amine oxide, preservative, lavender fragrance	Product ready to apply on hard surfaces. Contact period: Not indicated
3	Amonium quaternary compounds, ethoxylated alcohol, isopropanol, amine oxide, preservative, lavender fragrance	Product ready to apply on hard surfaces. Contact period: Not indicated
4	Alkyldimethylbenzylammonium chloride (ADBAC), DDAC, ethoxylated alcohol, ethanol, amine oxide, preservative, lavender fragrance	Product ready to apply on hard surfaces. Contact period: Not indicated.
5	Glutaraldehyde, DDAC, ethoxylated alcohol, ethanol, lavender fragrance.	Product ready to apply on hard surfaces. Contact period: Not indicated
6	Fourth generation ammonium quaternary compounds mixture, ethoxylated alcohol, ethylenediaminetetraacetic acid (EDTA), isopropanol, floral fragrance	Concentrate solution: 0.5 L are combined with 3.5 L of water to get work solution to be applied in solid surfaces. Contact period: 10 minutes
7	Fourth generation ammonium quaternary compounds mixture, ethoxylated alcohol, ethylenediaminetetraacetic acid (EDTA), ethanol, lavender fragrance	Concentrate solution: 0.5 L are combined with 3.5 L of water to get work solution to be applied in solid surfaces. Contact period: 10 minutes
8	Isopropanol, ethanol, 1-methoxy-2-propano, C10-16 alkyldimethylbenzylammonium chloride, DDAC, Diethylene glycol butyl ether, colorant, fragrance	Concentrate solution: 4 mL are combined with 1 020 mL of water to get work solution to be applied in solid surfaces. Contact period: 3 minutes

[A]Active antimicrobial ingredients are underlined.

Results

The results are summarized in **table (2),** according to the shown data three of the tested disinfectant did not demonstrate an acceptable performance. These disinfectants had limitations of effectiveness specially against *P. aeruginosa*, only one of them had issues to satisfy acceptance criteria against *S. aureus*. All tested disinfectants had quaternary ammonium salts in their composition, however, they also have numerous active principles that make difficult to associate effectiveness with chemical composition. Interestingly, with exception of disinfectant 3, all products showed higher positive results for *P. aeruginosa* in comparison to *S. aureus*.

Table 2. Results obtained for the evaluation of the efficacy of the disinfectant samples.

Disinfectant	Test organism [A]	Positive cylinders [B]	Negative cylinders [C]	Compliance to performance criteria [D]
1	*S. aureus*	1	59	Yes
	P. aeruginosa	6	54	
2	*S. aureus*	0	60	No
	P. aeruginosa	12	48	
3	*S. aureus*	2	58	Yes
	P. aeruginosa	1	59	
4	*S. aureus*	6	54	No
	P. aeruginosa	15	45	
5	*S. aureus*	1	59	Yes
	P. aeruginosa	2	58	
6	*S. aureus*	0	60	No
	P. aeruginosa	10	50	
7	*S. aureus*	0	60	Yes
	P. aeruginosa	3	57	
8	*S. aureus*	2	58	Yes
	P. aeruginosa	4	56	

[A]Test strains: *S. aureus* (ATCC 6538) and *P. aeruginosa* (ATCC 15442). [B]Bacterial growth observed in culture media tubes with transferred inoculated cylinders exposed to disinfectant. [C]Bacterial growth not observed in culture media tubes with transferred inoculated cylinders exposed to disinfectant. [D]No more than three or six tubes with *S. aureus* or *P. aeruginosa* inoculated cylinders had to show growth, respectively [7].

Discussion

Good practices in cleaning and disinfection in healthcare facilities are fundamental factors to reduce the spreading of bacterial pathogens. This applies for both, critical and no critical environments that include floors, waiting rooms and medical offices. In this sense, a proper application of disinfectant and the use of effective products are requirements to enhance reductions in microbial charges and to minimize the propagation of harmful microorganism [16-19]. Therefore, an adequate quality control of disinfectant through efficacy testing is a measure that promotes the effectiveness of cleaning and disinfection actions in health care facilities [20]. Regulatory frameworks for the standardization of efficacy testing can contribute to reach a better performance in these preventive actions [21].

According to results, most analyzed samples of disinfectant products were effective. However, experimental results also demonstrate the use of ineffective disinfectants in healthcare facilities of Costa Rica. Most of disinfectants showed lower efficacy against *P. aeruginosa*, a microorganism used as a model of pathogenic Gram-negative bacteria in the assays. This observation is a concerning point considering the high percentage of multidrug-resistant bacteria corresponding to Gram-negative cells [22]. Furthermore, it is important to highlight the reports

of recent hospital outbreaks due this kind of pathogen in Costa Rica healthcare facilities [23].

One possible explanation for the lower activity of evaluated disinfectants against *P. aeruginosa* in comparison to *S. aureus* is the chemical composition of evaluated disinfectants. The main active principle in the products are quaternary ammonium compounds (QACs) and these biocides are less active against Gram-negative than Gram-positive bacteria [24,25]. Moreover, QACs based disinfectants that are ineffective could act as selective pressure factor to facilitate the growth and the spreading of Gram-negative bacteria which have antibiotic resistance factors including efflux pumps [25,26]. Thus, the development of additional products based in other main active components could be recommended to have more alternatives to reduce Gram-negative germs in solid surface of healthcare facilities.

Another point of interest is that similar products could have important differences in the efficacy testing results. For instance, disinfectants 6 and 7 have almost the same composition but only one is effective against both test bacteria. This could be a consequence of failures in quality of reagents used during formulation, manufacturing or storing of the product. Therefore, we recommend the consideration of these factors for disinfectant manufacturers and personal in charge of its application.

Interestingly, two disinfectants (disinfectants 1 and 5) have glutaraldehyde in their composition. This compound is mainly used for the disinfection of hospital instrumental as bronchoscopes, endoscopes, and surgical instruments rather than applicated as a solid surface disinfectant. This active ingredient is recognized as factor that can compromise occupational health of healthcare personnel [27]. This finding suggests the necessity of regulatory requirements definition not only for antimicrobial performance but also for chemical constitution of disinfectants to avoid unnecessary exposition of patients, visitors, and healthcare personnel to dangerous substances.

The identification of ineffective disinfectants in healthcare facilities of Costa Rica has major relevance at local level, however, this kind of findings must call attention of national and international public health authorities considering the possibility of global propagation of pathogenic bacteria facilitated by high traveling rates [14,28]. Thus, definition of standardized methods for efficacy testing of disinfectants used in healthcare facilities among different global regions is a measure for the prevention of infections that must be encourage.

It is important to highlight that this study has been carried out on a low quantity of disinfectants. This has been caused by the lack of an official or governmental list of products registered as hospital disinfectants. There is uncertainty about how many products in the market claims hospital grade. However, the existence of ineffective products and potential risks are facts that have been proved.

The lack of an official list of products registered as hospital disinfectants has introduced a limitation to this research corresponding to the reduced number of active antimicrobial ingredients in disinfectants tested that mainly are QACs, aldehydes, alcohols and tensioactives as amine oxides and ethoxylated alcohols. Despite is not mandatory to satisfy minimal criteria to claim hospital disinfectant grade according to EPA [29], the evaluation of antifungal and antiviral activity of disinfectants used in healthcare facilities can be useful in further research to get a better understanding of their helpfulness to protect patients and healthcare staff.

Conclusion

Despite majority of tested hospital disinfectant samples satisfy efficacy performance criteria, ineffective disinfectants were also identified. The use of these products could promote limitation in the effectiveness of cleaning and disinfection measures in health facilities of Costa Rica. The results of the study support the necessity of regulations in the country to ensure the quality of disinfectants used in healthcare facilities to prevent the spreading of pathogenic bacteria and related infections on patients, health workers and visitors.

Contributors

All authors conceived the idea of the study and participated in the experimental design. All authors contributed to interpreting the information and writing the paper. All authors reviewed and approved the final version of the manuscript.

Conflict of interest

The authors report no conflict of interest.

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