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Pilot study on the antibacterial activity of hydrogen peroxide and silver ions in the hospital environment

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Parole chiave: Perossido d'idrogeno, argento, disinfettante, ambiente ospedaliero, superfici

Abstract

Background. *Nosocomial environmental contamination plays an important role in the transmission of several health care-associated pathogens. Control of surfaces contamination can reduce the risk of cross-infection in hospitals. The aim of our study is to evaluate the disinfectant effectiveness of hydrogen peroxide and silver ions, against nosocomial multidrug-resistant strains, when it's used directly on surfaces.*

Methods. *Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 15442 and the same multidrug-resistant clinical isolates were selected to study the effectiveness of the disinfectant used in suspension or on the clean and dirty surface.*

Results. *Regarding the suspension activity test, the hydrogen peroxide and silver ions resulted effective after 5 min for ATCC strains and after 10 min for multidrug-resistant isolates; about the surface activity test, its action resulted after 10 min for ATCC strains and after 15 min for multidrug-resistant isolates. Moreover, it was more effective when used in the absence or in presence of a low concentration of biological materials.*

Conclusions. *In a complex environment such as hospital wards, to have a disinfectant notoriously effective but more easy and quick to use would be an useful solution to treat small surfaces occasionally contaminated by biological materials.*

Introduction

In the hospital environment, the inadequate disinfection procedures are an important public health problem for the serious repercussions in terms of morbidity and mortality, on organizational and economic system of the hospital and on the quality of the services provided.

Although a possible source of nosocomial pathogens is the patient's endogenous flora, an estimated 20% to 40% of health care associated infections has been attributed to cross-infection via the hands of health care workers, contaminated by direct contact with the patient or indirectly by touching contaminated environmental surfaces (1). The contaminated surfaces make an important contribution to the transmission of *Clostridium difficile*, vancomycin-resistant Enterococci, methicillin-resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *norovirus* (2), having the ability to survive in the dry-surface environment.

The resistance to disinfectant treatments becomes important in determining the prominence of a microorganism as agent

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responsible for nosocomial infections. Some pathogens, as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, survive for prolonged periods of time in the environment (3) and result associated to surface contamination in hospital rooms and/or health care worker hands (4). Recently new systems for the sanitation of the environment have been introduced in hospitals. The combined formulation of hydrogen peroxide and silver ions is much more powerful than hydrogen peroxide alone and can provide a long lasting effective disinfectant residual (5, 6). In particular, the addition of silver ions avoids the formation of biofilms produced by nosocomial strains (*e.g.*, *Staphylococcus aureus* methicillin-resistant) (7). This disinfectant is currently used in aerosolized form and requires expensive and sophisticated automated equipment, long contact times and forced ventilation of the hospital room after its use (8).

The aim of this research is to study the disinfectant effectiveness of combined formulation of hydrogen peroxide and silver ions, against nosocomial multidrug-resistant strains, when it's used directly on surfaces.

Materials and methods

Study design

For this study a solution of hydrogen peroxide (5%) and silver ions (0.1%) was employed. The evidence of the antibacterial activity was carried out according to the methods UNI EN 1040:2006 (9) and CEN EN 1276 (10) for the suspension activity test, according to the method WI 216028 (11) for the surface activity test.

The following four strains were tested: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442 and the same multidrug-resistant clinical isolates. After 24-hours growth on TSA (Tryptic Soy Agar) medium at 37°C, each isolate was suspended in 10 ml of sterile diluent (1g/1 tryptone and 8.5 g/1 of NaCl) as to achieve an inoculum of 10^8 cfu/ml. From this suspension, serial decimal dilutions were set up to 10 cfu/ml.

One milliliter of each bacterial suspension was added to 9 ml of disinfectant solution and to 9 ml of sterile distilled water, for control. To study the interference of the organic material bactericidal activity, two other solutions were set up, one containing 0.3 g/l and other 3 g/l of bovine serum albumin (BSA) (Sigma, Gillingham, UK), simulating the conditions of clean and soiling, respectively. Overall, 24 solution dilutions and 1 control were tested for each microorganism.

Suspension activity test

The action of the disinfectant was verified after 5, 10, 20 and 30 min of contact. Aliquots of 1 ml of each solution and their respective dilutions were neutralized by 5 min of contact with sodium thiosulfate (5g/1) and seeded in duplicate for inclusion in TSA medium. After incubation at 37°C for 24 hours, the germicidal effect (EG) was calculated using the following formula: EG = Log Nc - Log Nd (Nc = number of cfu/ml of control solution; Nd = number of cfu/ml of test solutions). In accordance with the European guidelines (9,10), the disinfectant is considered effective when EG was ≥ 5.

Surface activity test

Sterile stainless steel discs (Apex Laboratories, Sanford, NC, USA) with a diameter of 10 mm were inoculated with 10 ml of each suspension and dried at 37°C for 60 min. After drying, 0.1 ml of each solution were inoculated on the surface of each disk.

At the end of the contact time, each disk was immersed for 5 min in a flask containing 10 ml of neutralizing solution and glass beads. After a vigorous mixing to suspend the survived bacterial cells, 1 ml of each suspension was inoculated in duplicate in

TSA medium. After incubation at 37°C for 24 hours, the germicidal effect was calculated using the following formula: EG = Log Nc - Log Nd, where Nc and Nd result by applying the formula Log [($a + a^1$) / 2] x 10/d (a and a^1 are the colony-forming units recovered, d is the dilution factor).

In accordance with the European guidelines (11), the disinfectant was considered effective when EG was ≥ 4.

Results

Suspension activity test

The disinfecting action of hydrogen peroxide and silver ions resulted effective after 5 min for ATCC strains and after 10 min for multidrug-resistant isolates.

Regarding to the addition of BSA, in the presence of 0.3 g/l the disinfectant appears effective after 5 min of contact with ATCC strains, and after 10 min with multidrug-resistant isolates. In the presence of 3 g/l of BSA, the required contact time becomes 10 min for the ATCC strains and 20 min for multidrug-resistant isolates.

Surface activity test

The disinfecting action resulted effective after 10 min for ATCC strains and after 15 min for multidrug-resistant isolates.

Regarding to the addition of BSA, in the presence of 0.3 g/l the disinfectant appears effective after 10 min of contact with ATCC strains, and after 15 min with multidrug-resistant isolates. In the presence of 3 g/l of BSA, the required contact time becomes 20 min for the ATCC strains and 30 min for multidrug-resistant isolates.

Discussion

The airborne hydrogen peroxide, either in the form of vapour or dry mist, is known to be an effective method of disinfection of the hospital environment. Similarly, silver ions are efficiently used in several medical applications and they are able to block the formation of biofilms reducing the risk of infections (5).

Some authors have shown that the combined formulation of hydrogen peroxide and silver ions has a synergic action but it is generally used in aerosolized form by preprogrammed equipment (12). In these cases, an adequate contact time (proportional to the size of the area to be disinfected) and the need to empty the room before treatment are the key for the effectiveness of this process. These needs are not always applicable in emergency situations or departments, therefore the availability of a disinfectant notoriously effective but more easy and quick to use would be a useful solution to treat small surfaces occasionally contaminated by biological materials (*e.g.*, spillage of blood, urine).

Our study examined two typical hospital-acquired conditions that may interfere with the activity of a disinfectant: the surface contamination by biological materials and the pollution by multidrug-resistant nosocomial strains, often difficult to eradicate by common disinfectants. The choice to operate on multidrug-resistant strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* comes from the fact that they are endemic bacteria in many hospitals worldwide and cause a high morbidity and mortality rates, especially in intensive care unit patients (13).

No difference regarding the effectiveness of disinfectant against two species of examined pathogens was noted in this study. In agreement with the literature data (13), the action of the disinfectant was lower against multidrug-resistant strains if compared to ATTC strains, and lower in the surface activity tests than the suspension activity tests. In this regard, some authors have highlighted that the resistance of microorganisms to disinfectants

is frequently associated with the presence of biofilms on surfaces (14). Since our study is a preliminary investigation, this aspect has not yet been addressed, so in the near future we plan to investigate the impact of the environmental conditions on the effectiveness of the disinfectant, such as the temperature change, different surface type, presence and absence of biofilms.

Conclusions

This disinfectant might be introduced in nosocomial routine as a valid support to use directly on surfaces, especially in cases of emergency. However, further studies are needed to improve its performance in the presence of contamination by organic substance and multidrug-resistance strains.

Riassunto

Studio pilota sull'attività antibatterica del perossido di idrogeno e ioni argento in ambiente ospedaliero

Introduzione. La contaminazione dell'ambiente ospedaliero svolge un ruolo importante nella trasmissione degli agenti patogeni associati ad infezioni nosocomiali. Il controllo della contaminazione delle superfici può ridurre il rischio di *cross-infection*. Questo studio ha lo scopo di valutare l'efficacia del disinfettante perossido di idrogeno e ioni d'argento utilizzato su superfici nei confronti di ceppi nosocomiali multiresistenti di provenienza umana.

Metodi. Sono stati impiegati ceppi di *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeru*ginosa ATCC 15442 e gli stessi isolati multiresistenti di origine clinica. L'efficacia del disinfettante è stata valutata sia in sospensione sia sulle superfici, in condizioni di assenza o presenza di materiale biologico.

Risultati. Per quanto riguarda il test di attività in sospensione, il disinfettante si è rivelato efficace dopo 5 min di contatto per i ceppi ATCC e dopo 10 min per gli isolati multiresistenti; per l'attività in superficie, la sua azione è stata efficace dopo 10 min per i ceppi ATCC e dopo 15 min per gli isolati multiresistenti. Inoltre, il disinfettante è stato più efficace quando utilizzato in assenza o in presenza di basse concentrazioni di materiale biologico.

Conclusioni. In un ambiente complesso come quello ospedaliero, avere a disposizione un disinfettante efficace e di facile impiego potrebbe rappresentare una soluzione utile per trattare piccole superfici occasionalmente contaminate da materiale biologico.

References

1. Weber DJ, Rutala WA, Miller MB, Huslage K, Sickbert-Bennett E. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, *Clostridium difficile*, and Acinetobacter species. Am J Infect Control 2010; **38**: S25-33.
2. Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. Infect Control Hosp Epidemiol 2011; **32**: 687-99.
3. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? a systematic review. BMC Infect Dis 2006; **6**: 130.
4. Wilson APR, Hayman S, Folan P, et al. Computer keyboards and the spread of MRSA. J Hosp Infect 2006; **62**: 390-2.
5. Shuval Hillel, Rachel Yarom, Rivka Shenman. An innovative method for the control of Legionella infections in the hospital hot water systems with a stabilized hydrogen peroxide-silver formulations. Int J Infect Control 2009, v5:i1.
6. Silvestry-Rodriguez N, Sicairos-Ruelas EE, Gerba CP, Bright KR. Silver as a disinfectant. Rev Environ Contam Toxicol 2007; **191**: 23-45.
7. Edwards-Jones V. Antimicrobial and barrier effects of silver against methicillin-resistant *Staphylococcus aureus*. J Wound Care 2006; **15**: 285-90.
8. Fu TY, Gent P, Kumar V. Efficacy, efficiency and safety aspects of hydrogen peroxide vapour and aerosolized hydrogen peroxide room disinfection systems. J Hosp Infect 2012; **80**: 199-205.
9. UNI EN 1040:2006. Disinfettanti chimici e antisettici - Prova in sospensione quantitativa per la valutazione dell'attività battericida di base dei disinfettanti chimici e antisettici - Metodo di prova e requisiti (Fase 1).
10. BS EN 1276:2009 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity of chemical

disinfectants and antiseptics used in food, industrial, domestic and institutional areas. Test method and requirements (phase 2, step 1).
11. CEN/TC 216/WI 216028, September 1998 - Chemicals Disinfectants and Antiseptics – Quantitative surface test for the evaluation of bactericidal and/or fungicidal activity of Chemical Disinfectants used in food, industrial, domestic and institutional areas – Test Method and Requirements.
12. Holmdahl T, Lanbeck P, Wullt M, Walder MH. A head-to-head comparison of hydrogen peroxide vapor and aerosol room decontamination systems. Infect Control Hosp Epidemiol 2011; **32**: 831-6.
13. Hardy KJ, Gossain S, Henderson N, et al. Rapid recontamination with MRSA of the environment of an intensive care unit after decontamination with hydrogen peroxide vapour. J Hosp Infect 2007; **66**: 360-8.
14. Vestby LK, Møretrø T, Langsrud S, Heir E, Nesse LL. Biofilms forming abilities of Salmonella are correlated with persistence in fish meal-and feed factories. BMC Vet Res 2009; **5**: 20.

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