Bactericidal activity of 3 cutaneous/mucosal antiseptic solutions in the presence of interfering substances: Improvement of the NF EN 13727 European Standard?

Évaluation de l'activité bactéricide de 3 antiseptiques cutanéomuqueux en présence de substances interférentes : peut-on compléter la norme NF EN 13727 ?

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Abstract

Objective. – There is no standard protocol for the evaluation of antiseptics used for skin and mucous membranes in the presence of interfering substances. Our objective was to suggest trial conditions adapted from the NF EN 13727 standard, for the evaluation of antiseptics used in gynecology and dermatology.

Methods. – Three antiseptic solutions were tested in vitro: a chlorhexidine-benzalkonium (CB) combination, a hexamidine-chlorhexidine-chlorocresol (HCC) combination, and povidone iodine (P). The adaptation of trial conditions to the standard involved choosing dilutions, solvent, and interfering substances. The activity of solutions was assessed on the recommended strains at concentrations of 97% (pure solution), 50%, and 10% (diluted solution), and 1%. A logarithmic reduction ≥ 5 was expected after 60 seconds of contact, to meet requirements of bactericidal activity.

Results. – HCC did not present any bactericidal activity except on *P. aeruginosa* at a concentration of 97%. P was not bactericidal on *E. hirae* at any concentration and on *S. aureus* at 97%. CB had the most homogeneous bactericidal activity with a reduction > 5 log on the 4 bacterial strains at concentrations of 97%, 50% and 10%.

Conclusion. – Adapting the NF EN 13727 standard allowed assessing the 3 tested solutions: only CB was bactericidal in dirty conditions. This study proved the possibility of validating antiseptic choice in vitro, in current practice conditions, for adjunctive treatment of skin and mucous membranes disorders, primarily of bacterial origin or with a potential of superinfection.

Keywords: Antisepsis; Chlorhexidine; Benzalkonium; Povidone iodine

Résumé

Objectifs. – L'évaluation des antiseptiques à visée cutanéomuqueuse, en présence de substances interférentes, ne fait l'objet d'aucun protocole normatif. Notre objectif était de proposer des conditions d'essai adaptées de la norme NF EN 13727 pour l'évaluation d'antiseptiques utilisés en gynécologie et dermatologie.

Méthode. – Trois antiseptiques : une association chlorhexidine-benzalkonium (CB), une association hexamidine-chlorhexidine-chlorocrésol (HCC) et la povidone iodée (P) ont été testés in vitro. L'adaptation des conditions d'essai de la norme a concerné le choix des dilutions, du diluant et des substances interférentes. L'activité des produits a été évaluée sur les souches de la norme aux concentrations de 97 % (utilisation pure), de 50 % et 10 % (utilisation diluée) et de 1 %. Pour répondre aux exigences de bactéricidie, une réduction logarithmique ≥ 5 était attendue pour 60 secondes de contact.

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Résultats. – HCC n'avait pas d'activité bactéricide à l'exception de la concentration à 97 % sur *P.aeruginosa*. P n'avait pas d'activité bactéricide sur *E. hirae*, quelle que soit la concentration testée, ni sur *S. aureus* à 97 %. CB avait l'activité bactéricide la plus homogène avec réduction > 5 log sur les 4 souches bactériennes à 97 %, 50 % et 10 %.

Conclusion. – L'adaptation de la norme NF EN 13727 permet de discriminer les 3 produits testés : seul CB était bactéricide en conditions de saleté. Ce travail souligne la possibilité de valider le choix des antiseptiques in vitro dans des conditions représentatives de la pratique, dans le traitement d'appoint des affections cutanéomuqueuses primitivement bactériennes ou susceptibles de se surinfecter.

Mots clés : Antisepsie ; Chlorhexidine ; Benzalkonium ; Povidone iodée

1. Introduction

Topical antimicrobials are used in therapeutic and/or prophylactic indications, especially in case of infected or likely to be infected wounds. The current recommendations for controlling the spread of multiresistant bacteria and the preservation of microbial ecosystems suggest to limit or discontinue using topical antibiotic preparations and focus on the contribution of antiseptics [1–4]. Many formulations including the active principle of antiseptics (chlorhexidine, iodine or chlorine derivatives, quaternary ammonium compounds) are currently available. They aim to control cutaneous or mucocutaneous microbial colonization resulting in a reduction of the transient or commensal flora [5]. Recommending the use of antiseptics to prevent or treat mucocutaneous infections is based primarily on in vitro tests carried out according to French and European standards [6]. The sensitivity of various agents to interfering substances is taken into account for these tests. The presence of many organic (proteins) or inorganic (electrolytes, divalent cations) substances found in biological fluids such as blood, exudates or pus led to decrease or completely inhibit in vitro [2–7] and in vivo [7–12] activity of active ingredients and marketed solutions. Interfering substances are frequently present and this should be considered for the medical use of antiseptics and the conditions of use. This is why antiseptic activity should be evaluated according to the latest standards without (phase 1) and with interfering substances (phase 2) to be as close as possible to routine practice conditions. The NF EN 13727 standard [13] was revised in 2012; it suggests of four in vitro protocols in the presence of interfering substances, depending on the solution indication: hygienic hand rub and hygienic hand washing and rub, and surgical hand washing and rub, disinfection of medical devices, surface disinfection. However, there is currently no standard protocol for the evaluation of mucocutaneous antiseptics, whether in the specific context of wound antisepsis or for the local treatment of some infections (cutaneous, vaginal, oral, etc.).

This is why we conducted this study to assess, according to standard NF EN 13727 recommendations, the 3 mucocutaneous antiseptics commonly used in gynecology and dermatology for wound antisepsis. The selected solutions contained different active ingredients or combinations (chlorhexidine, benzalkonium chloride, povidone iodine, hexamidine, chlorocresol) to assess the discriminatory nature of the test conditions.

2. Method

2.1. Solutions used

The antiseptics tested are described below with their composition in active substances (INN, french acronym DCI):

- HCC (Cytéal®): hexamidine diisethionate 0.10%, chlorhexidine gluconate 0.5% (20% solution), chlorocresol 0.3% (Batch G 03045). Indications: cleaning infected or likely to be secondarily infected skin and mucous membranes. The solution can be used as a liquid soap: pure or diluted at 1:10, followed by intensive rinsing;
- P (Dermal Betadine®): povidone iodine (PVPI) 10% (batch 316136). Indications: adjunctive treatment of infected or likely to be secondarily infected skin and mucous membranes, and surgical field skin antisepsis. The solution can be used to scrub skin or diluted at 1:10 with sterile water or saline to wash wounds, and at 2% with sterile saline for wound irrigation;
- CB (Dermobacter®): chlorhexidine digluconate 0.2% benzalkonium chloride 0.5% (batch CH106). Current indications: cleaning and adjunctive treatment of infected or likely to be secondarily infected skin and mucous membranes. The solution can be used pure on skin or diluted at 1:10 when used for mucous membranes. Intensive rinsing should follow the application.

2.2. Bacterial strains

Four bacterial strains were used, as recommended by the NF EN 13727 standard [13]:

- *Staphylococcus aureus* CIP 4.83 (ATCC 6538);
- *Pseudomonas aeruginosa* CIP 103467 (ATCC 15442);
- *Escherichia coli* CIP 54117 (NCTC 10538);
- *Enterococcus hirae* CIP 58.55 (ATCC 10541).

The strains were provided by the Paris Pasteur Institute and kept in compliance with the NF EN 12353 standard [14].

2.3. Trial protocol

The test conditions corresponding to hygienic hand washing and rub, and surgical hand washing and rub were selected

and/or adapted according to the indications of tested antiseptics and to the NF EN 13727 standard specifications [13]. The test strains included Gram+ (*S. aureus* and *E. hirae*) and Gram– (*E. coli* and *P. aeruginosa*) bacteria. Bacterial suspensions were prepared in tryptone salt at a concentration ranging from 1.5×10^8 and 5.0×10^8 CFU (colony forming units)/mL, for a final test concentration ranging between 1.5×10^7 and 5.0×10^7 CFU/mL.

The solutions were tested:

- pure: the highest solution concentration used in the test for use on the skin was 97% according to the method and recommendations of the NF EN 13727 standard;
- and diluted: the highest solution concentration used in the test for mucocutaneous use was 50% as recommended by the NF EN 13727 standard.

A solution concentration of 10% corresponding to the usual recommendations for the use of the 3 solutions was also tested.

The 1% concentration was considered as non-active solution to be tested according to standard requirements.

The 97% concentration (volume/volume: V/V) was tested according to NF EN 13727 standard specifications by considering a direct application (pure solution use). Dilutions of 50%, 10%, and 1% (V/V) were prepared in hard water for application of solutions in diluted form. Hard water, as recommended, is a saline solution including magnesium chloride, calcium chloride, and sodium bicarbonate and simulates dilution with water from the water supply system, with a hardness of 30 °F. Divalent cations play a considerable role in the antiseptic activity and are therefore considered as potentially interfering substances [15].

Interfering substances, for dirty conditions, were a mixture of 3 g/L bovine serum albumin (Sigma Aldrich, Saint-Quentin Fallavier, France) and 3 mL of erythrocytes (BioMérieux, Crapone, France).

The tests were performed according to the NF EN 13727 standard, at a temperature of 20 ± 1 °C and for a contact duration of 60 ± 5 s because it was common to the 2 washing procedures: hand rub and hygienic hand washing and rub, and surgical hand washing and rub.

The absence of toxic effect of experimental conditions and neutralizer, and the validation of the dilution neutralization step were checked as specified by the NF EN 13727 standard [13].

Bacterial counts were made in TS agar (BioMérieux, Crapone, France) after incubation for 48 h at 36 ± 1 °C. The value of counts after trial expressed in decimal logarithms were subtracted from the initial values to obtain the log reductions. The upper limit of count defined by the standard induces a lower limit in terms of log reduction (< in the results tables). The lower limit of count defined by the standard induces an upper limit in terms of log reduction (> in the results tables). In every case, the experimental conditions (i.e. initial inoculum concentration and count conditions) allowed detecting log reductions ≥ 5. The solution was considered as bactericidal at concentrations allowing to reduce the bacterial count equal to or greater than 5 log of the initial number (before test), or in our test conditions: 97% for cutaneous use (pure), 50% and 10% for application to mucous membranes (diluted), after 1 minute of contact. The test had to include an inactive concentration, at 1% in our study.

3. Results

The following parameters were tested to interpret test results according to the NF EN 13727 standard [13]:

- bacteria inoculum count prior to contact ranging between 1.5×10^7 and 5.0×10^7 CFU/mL;
- no impact of the experimental conditions;
- no toxic effect of neutralizer;
- validation of the dilution-neutralization step.

The results showed that the HCC solution containing a combination of 3 active ingredients (hexamidine diisethionate, chlorhexidine digluconate, and chlorocresol) had no bactericidal activity in the defined conditions, especially in the presence of interfering substances (Table 1). A greater than 5-log reduction of *P. aeruginosa* was observed only at a 97% concentration (pure solution).

The P solution (povidone iodine) had a greater bactericidal activity than the HCC solution (Table 2). However, it was not active against *E. hirae* at any concentration, and not active

Table 1
Bactericidal activity of hexamidine, chlorhexidine, chlorocresol (HCC) expressed as a logarithmic reduction at the test concentrations of 97% (pure), 50%, and 10% (diluted) and 1% (inactive).
Activité bactéricide du produit hexamidine, chlorhexidine, chlorocrésol (HCC) exprimée en réduction logarithmique aux concentrations d'essai 97 % (utilisation pure), 50 % et 10 % (utilisation diluée) et 1 % (concentration inactive).

Tested strains	**Logarithmic reduction of microorganisms after contact with HCC**			
	Pure	Diluted		Inactive
	97%	50%	10%	1%
P. aeruginosa	**> 5.40**	< 2.02	< 2.02	< 2.02
E. coli	< 1.93	< 1.93	< 1.93	< 1.93
S. aureus	< 2.12	< 2.12	< 2.12	< 2.12
E. hirae	< 1.72	< 1.72	< 1.72	< 1.72
Results	Not bactericidal	Not bactericidal	Not bactericidal	Not bactericidal

In bold font: logarithmic reduction ≥ 5 (bactericidal activity). <: lower limit of detectable logarithmic reduction.

Table 2
Bactericidal activity of povidone iodine (P) expressed as a logarithmic reduction at the test concentrations of 97% (pure), 50%, and 10% (diluted) and 1% (inactive).
Activité bactéricide du produit povidone iodée (P) exprimée en réduction logarithmique aux concentrations d'essai 97 % (utilisation pure), 50 % et 10 % (utilisation diluée) et 1 % (concentration inactive).

Tested strains	**Logarithmic reduction of microorganisms after contact with P**			
	Pure	Diluted		Inactive
	97%	50%	10%	1%
P. aeruginosa	**> 5.40**	**> 5.40**	**> 5.40**	2.66
E. coli	**> 5.30**	**> 5.30**	**> 5.30**	**> 5.30**
S. aureus	< 2.12	**5.38**	**> 5.49**	< 2.12
E. hirae	< 1.72	< 1.72	< 1.72	< 1.72
Results	Not bactericidal	Not bactericidal	Not bactericidal	Not bactericidal

In bold font: logarithmic reduction ≥ 5 (bactericidal activity). <: lower limit of detectable logarithmic reduction.

Table 3
Bactericidal activity of chlorhexidine, benzalkonium (CB) expressed as a logarithmic reduction at the test concentrations of 97% (pure), 50%, and 10% (diluted) and 1% (inactive).
Activité bactéricide du chlorhexidine, benzalkonium (CB) exprimée en réduction logarithmique aux concentrations d'essai 97 % (utilisation pure), 50 % et 10 % (utilisation diluée) et 1 % (concentration inactive).

Tested strains	**Logarithmic reduction of microorganisms after contact with CB**			
	Pure	Diluted		Inactive
	97%	50%	10%	1%
	> 5.42	**> 5.36**	**> 5.33**	< 1.98
E. coli	**> 5.27**	**> 5.27**	**> 5.27**	< 2.03
S. aureus	**> 5.39**	**> 5.43**	**> 5.43**	2.50
E. hirae	**> 5.13**	**> 5.10**	**> 5.10**	3.33
Results	**Bactericidal**	**Bactericidal**	**Bactericidal**	Not bactericidal

In bold font: logarithmic reduction ≥ 5 (bactericidal activity). <: lower limit of detectable logarithmic reduction.

against *S. aureus* at a concentration of 97%, while concentrations of 50% and 10% were active in the presence of interfering substances.

The CB solution containing a combination of 2 active principles (chlorhexidine digluconate and benzalkonium chloride) had a greater and more homogeneous bactericidal activity than the 2 other solutions tested, with a greater than 5 log reduction at a concentration of 97%, 50%, and 10% against the 4 tested bacteria (*P. aeruginosa, S. aureus, E. coli, E. hirae*) after 1 minute of contact in the presence of interfering substances simulating dirty conditions (Table 3).

4. Discussion

The tests were performed in compliance with the technical specifications of the NF EN 13727 standard [13], considering the use of tested solutions that include not only hand hygiene (required by the standard), but also pure and diluted solution use for mucocutaneous antisepsis. Thus, the 4 strains suggested by the bactericidal activity evaluation standard were used because of their representativeness in terms of spectrum and pathophysiological involvement [16]. Likewise, considering the conditions of use, a short contact time of 60 seconds was used. The method was adapted for the choice of test dilutions, for the type of solvent, and the presence of interfering substances. The solutions were tested at a concentration of 97%, as recommended by the standard for undiluted use; for diluted use, especially when used in contact with mucous membranes and/or damaged skin, dilutions were performed with hard water. In every case, adding a mixture of proteins and erythrocytes allowed us to consider the test was performed with "unfavorable" conditions for the solutions, but very representative of routine practice (dirty conditions).

We observed drastic differences among the tested solutions with these conditions. HCC was not bactericidal against the 4 strains tested and, therefore, did not meet the NF EN 13727 standard [13] requirements. This solution is a combination of molecules with antibacterial activity. Hexamidine is an aromatic diamidine essentially described as bacteriostatic on Gram-positive cocci. The bactericidal activity is slow [17], which could explain the lack of activity observed in test conditions (contact for 60 seconds). Reported data indicates bactericidal activity after 5 minutes of contact, at the solution concentration, but for hydroalcoholic solutions [18]. The sensitivity to organic matter is considered variable according to authors [18–20]. Chlorhexidine is associated with hexamidine at a final concentration of 0.1%. This concentration is considered to be bactericidal against *S. aureus* after contact for 1.5 minute [21]. The presence of interfering substances, whether organic or divalent cations, affects the activity [22–24]. Chlorocresol is also reported to be sensitive to organic materials, with 30% to 50% decreased activity depending on the strain [25]. The usually weak activity of the HCC solution could correspond to a limited intrinsic activity, given the short contact time and concentration of antiseptic active

principles. This could explain detecting activity only at a concentration of 97%.

The P solution does not meet the NF EN 13727 standard requirements [13] in terms of bactericidal activity. Povidone iodine, the active ingredient in solution P, is an iodophor, the role of which is to increase the solubility of iodine but also to reduce the equilibrium concentration of free molecular iodine, thus forming a reserve of iodine. Although iodine is supposed to be a Gram+ and Gram− bactericidal agent, we observed in these tests a weaker activity on Gram+ bacteria including *E. hirae*. Iodine is reduced in the presence of organic matter and the bactericidal activity time can then be delayed. The paradoxical effect observed against *S. aureus* should be compared with the observations of various authors. Berkelman et al. [26] reported that *S. aureus* could survive beyond a minute in a solution of concentrated PVPI but was destroyed after 15 seconds of contact with the solution diluted to 1/100. The peak activity was obtained at PVPI concentrations ranging from 0.1% (corresponding to a concentration of 10% in our trial) to 1%. This phenomenon could be related to the ratio of free iodine/iodide ion (I_2/I^-). High concentrations in I^- (the case of highly concentrated solutions) are correlated with low concentrations of non complex-bound and, therefore, active I_2; and the I_2 concentration increases with dilution [27–30] explaining the fact that povidone iodine should be used diluted. The presence of other agents, including detergent types, may also intervene in the weak activity observed at high concentration [31]. The sensitivity to interfering substances of the 2 active principles used the most frequently for skin antisepsis, namely chlorhexidine and povidone iodine, was also demonstrated by Cremieux et al. [11] in the presence of an albumin, yeast, and calcium chloride mixture. The same authors demonstrated the major role of protein and calcium chloride as interfering substances for other antiseptic agents [7,10].

Likewise, recent in vitro data suggests that the presence of organic matter decreases the activity of povidone iodine against pathogens isolated from burns [32].

CB was bactericidal against the 4 tested strains and, therefore, met the NF EN 13727 standard [13]. It is a combination of 2 compatible cationic agents: benzalkonium chloride, a surfactant with detergent activity of the quaternary ammonium family that is primarily bactericidal against Gram + and completes the action of chlorhexidine. The latter is considered bactericidal against Gram+ and Gram− bacteria. It should be noted that, in this solution, chlorhexidine is used at a concentration 2 times greater than the one indicated for HCC. The combination's observed activity may be related to various phenomena. The chemical compatibility between the 2 compounds allows maintaining the intrinsic activity of the 2 agents. The mechanisms of action described for the 2 compounds may lead to a potentiation: a bactericidal action of benzalkonium chloride and chlorhexidine digluconate by lesion of the cell wall and cytoplasmic membrane, and intracellular precipitation of proteins [33–36]. The important point is the preservation of this activity in test conditions, i.e., in the presence of divalent cations and high protein load. This was previously observed by Cabotin and Bertrand [12] who reported a reduced sensitivity of the combination to interfering substances compared to a 0.2% chlorhexidine solution. The authors relate this observation to the combination of chlorhexidine with benzalkonium chloride, known to improve bactericidal activity [31].

Our study results highlight a great variability of bactericidal activity for products with similar indications, depending on test conditions. The validation of proposed conditions, particularly in terms of interfering substances, is based on in vivo considerations. Antisepsis of healthy skin and mucous membranes or of wounds, even if it is preceded by a debridement, corresponds to an important protein load in contact with the solutions, which may lead to their partial inactivation in vivo. Thus, various in vivo study results demonstrate the significant differences of activity for agents or solutions considered bactericidal in vitro. Noorani et al. [37] conducted a literature review on preoperative antisepsis for clean surgery and their meta-analysis proves superiority of chlorhexidine over povidone iodine. The observed difference was not related to the chlorhexidine concentration of the type of solution (foam, aqueous, or alcoholic solutions), but its intrinsic activity. Scowcroft, in his review [38], came to similar conclusions. Adler et al. [39] focused on abdominal disinfection before amniocentesis and also reported a greater effectiveness of an alcoholic chlorhexidine solution at 2% compared to an aqueous povidone iodine solution at 10%.

These various observations could be related to a greater and/or faster inactivation of povidone iodine in routine conditions (application on healthy or damaged skin) compared with chlorhexidine. Some authors emphasize the greater persistence of chlorhexidine [40]. Our study results do not question the use and effectiveness of antiseptics on damaged skin, but stress the need and the opportunity to evaluate antiseptic solutions in vitro, in routine conditions, i.e. in the presence of organic matter and cations, with demonstration of bactericidal activity in a short time. Thus, the NF EN 13727 [13] technical recommendations could be amended by considering our suggestions: dilution in hard water, presence of interfering substances corresponding to dirty conditions. The recommended contact temperature of 20 °C was not changed, but additional tests at 30 or 32 °C could be suggested to stick closer to routine practice conditions. These suggestions would help better assess the in vitro activity and especially the expected therapeutic effectiveness of antiseptic cleaning and adjunctive treatment of primarily or potentially secondarily infected bacterial mucocutaneous disorders.

Disclosure of interest

S. Salvatico, C. Feuillolay and C. Roques declare that they have no conflict of interest concerning this article. Y. Mas and F. Verrière work for the Laboratoire Innotech International (medical advisor and medical director, respectively).

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S. Salvatico made the tests.
C. Feuillolay validated tests.
Y. Mas analyzed the results and approved the article.
F. Verrière validated the protocol and wrote the article.

C. Roques wrote the protocol, analyzed the results, drafted and approved the article.

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References

[1] Dire DJ, Coppola M, Karr JL. A prospective evaluation of topical antibiotics for uncomplicated soft-tissue lacerations. Acad Emerg Med 1995;2:4–10.

[2] Hood R, Shermock KM, Emerman C. A prospective, randomized pilot evaluation of topical triple antibiotic versus mupirocin for the prevention of uncomplicated soft tissue wound infection. Am J Emerg Med 2004;22: 1–3.

[3] Bonomo RA, Van Zile PS, Li Q, Shermock KM, McCormick WG, Kohut B. Topical triple-antibiotic ointment as a novel therapeutic choice in wound management and infection prevention: a practical perspective. Expert Rev Anti Infect Ther 2007;5:773–82.

[4] Plan national d'alerte sur les antibiotiques 2011–2016. Ministère de l'Emploi, du Travail et de la Santé. Novembre 2011 (www.sante.gouv.fr).

[5] Messager S, Goddard PA, Dettmar PW, Maillard JY. Determination of the antibacterial efficacy of several antiseptics tested on skin by an "ex-vivo" test. J Med Microbiol 2001;50:284–92.

[6] AFNOR–NF EN 14885 « Antiseptiques et désinfectants chimiques – Application des normes européennes relatives aux antiseptiques et désinfectants chimiques ». Février 2007.

[7] Crémieux A, Chevalier J, Dauriac H. Évolution de la concentration bactéricide de désinfectants en fonction de la présence de diverses substances interférentes. Rev Institut Pasteur Lyon 1975;8:187–97.

[8] Chantefort A, Druilles J. Activité bactéricide de quelques désinfectants en présence ou non de substances interférentes protéiques. Path Biol 1984;32:615–8.

[9] Gélinas P, Goulet J. Neutralization of the activity of eight disinfectants by organic matters. J Appl Bacteriol 1983;54:243–7.

[10] Guiraud-Dauriac H, Crémieux A. Inactivation par les protéines et les ions calcium des désinfectants en fonction de leur nature chimique et des espèces bactériennes. Pathol Biol 1984;32:611–4.

[11] Crémieux A, Bonnaveiro N, Chevalier J. Intérêt d'un exsudat standard dans l'étude de l'activité in vitro des antiseptiques. Pathol Biol 1987;35:887–90.

[12] Cabotin PP, Bertrand JC. Étude comparative de l'activité bactéricide de deux antiseptiques cutanés en solution aqueuse, en présence de substances interférentes. Nouv Dermatol 2004;23:236–8.

[13] AFNOR–NF EN 13727 « Antiseptiques et désinfectants chimiques – Essai quantitatif de suspension pour l'évaluation de l'activité bactéricide en médecine – Méthode d'essai et prescriptions (phase 2, étape 1) ». Juillet 2012.

[14] AFNOR–NF EN 12353 « Antiseptiques et désinfectants chimiques – Conservation des microorganismes d'essai utilisés pour la détermination de l'activité bactéricide (Legionella incluses), mycobactéricide, sporicide, fongicide et virucide (bactériophages inclus) ». Juin 2013.

[15] Crémieux A. Factors affecting the bactericidal action of disinfectants. Implication in the selection of resistant strains. Drugs Exp Clin Res 1986;12:899–903.

[16] Fleurette J, Freney J, Reverdy ME, editors. Antisepsie et désinfection. Paris: Eska; 1995.

[17] Hugo WB. Amidines. In: Hugo WB, editor. Inhibition and destruction of the microbial cell. Academic Press: London; 1971. p. 121–36.

[18] Crémieux A, Bonnaveiro N. Concepts actuels sur l'efficacité des antiseptiques. Application à Hexomédine® solution et Hexomédine® trancutanée. Abstr Derm Hebdo 1990;79:1–8.

[19] Buraud M, Campion G, Darbord JC. Activité antibactérienne et cytotoxicité de l'hexamidine et de divers alcools utilisés en association. J Pharm Clin 1988;7:573–81.

[20] Chevalier J, Crémieux A. Comparative study on the antimicrobial effects of hexomedine and betadine on the human skin flora. J Appl Bacteriol 1992;73:342–8.

[21] Sasatsu M, Shimizu K, Noguchi N, Kono M. Evaluation of antiseptics by the modified phenol coefficient method: sensitivity of methicillin-resistant *S. aureus*. Biol Pharm Bull 1994;17:136–8.

[22] Hugo WB, Russell AD. Evaluation of non-antibiotic antimicrobial agents. In: Hugo WB, Russell AD, editors. Pharmaceutical microbiology. Oxford: Blackwell Scientific Publications; 1992. p. 258–87.

[23] Hugo WB, Russell AD. Types of antimicrobial agents. In: Russell AD, Hugo WB, Ayliffe GAJ, editors. Principles and practice of disinfection, preservation and sterilization. 2nd ed. Oxford: Blackwell Scientific Publications; 1992. p. 7–88.

[24] Walker EM, Lowes JA. An investigation into in vitro methods for the detection of chlorhexidine resistance. J Hosp Infect 1985;6: 389–97.

[25] Klarmann EG, Shternov VA, Von Worwern JV. The germicidal action of halogen derivatives of phenol and resorcinol and its impairment by organic matter. J Bacteriol 1929;17:423–42.

[26] Berkelman RL, Holland BW, Anderson RL. Increased bactericidal activity of dilute preparations of povidone-iodine solutions. J Clin Microbiol 1982;15(4):635–9.

[27] Gottardi W. The influence of the chemical behaviour of iodine on the germicidal action of disinfectant solutions containing iodine. J Hosp Infect 1985;6(Suppl. A):1–11.

[28] Gottardi W. Iodine and disinfection: theoretical study on mode of action, efficiency, stability, and analytical aspects in the aqueous system. Arch Pharm (Weinheim) 1999;332(5):151–7.

[29] Rackur H. New aspects of mechanism of action of povidone-iodine. J Hosp Infect 1985;6(Suppl. A):13–23.

[30] Schubert R. Disinfectant properties of new povidone-iodine preparations. J Hosp Infect 1985;6(Suppl. A):33–6.

[31] Chevalier J, Corre J, Crémieux A. Evaluation of synergistic effects of three bactericidal agents associated in an antiseptic formulation. Pharm Acta Helv 1995;70:155–9.

[32] Gunjan K, Shobha C, Sheetal C, Nanda H, Vikrant C, Chitnis DS. A comparative study of the effect of different topical agents on burn wound infections. Indian J Plast Surg 2012;45(2):374–8.

[33] Lim KS, Kam PCA. Chlorhexidine – pharmacology and clinical applications. Anaesth Intensive Care 2008;36(4):502–12.

[34] Kodedova M, Sigler K, Lemire BD, Gaskova D. Fluorescence method for determining the mechanism and speed of action of surface-active drugs on yeast cells. BioTechniques 2011;50:58–63.

[35] Cheung HY, Wong MMK, Cheung SH, Liang LY, Lam YW, Chiu SK. Differencial actions of chlorhexidine on the cell wall of *Bacillus subtilis* and *E. coli*. PloS One 2012;7(5):e36659.

[36] Vijayakumar R, Kannan VV, Sandle T, Manoharan C. In vitro antifungal efficacy of biguanides and quaternary ammonium compounds against cleanroom fungal isolates. J Pharm Sci Tech 2013;66(3):236–42.

[37] Noorani A, Rabey N, Walsh SR, Davies RJ. Systematic review and meta-analysis of preoperative antisepsis with chlorhexidine versus povidone-iodine in clean-contaminated surgery. Br J Surg 2010;97:1614–20.

[38] Scowcroft T. A critical review of the literature regarding the use of povidone iodine and chlorhexidine gluconate for preoperative surgical skin preparation. J Perioper Pract 2012;22(3):95–9.

[39] Adler MT, Brigger KR, Bishop KD, Mastrobattista JM. Comparison of bactericidal properties of alcohol-based chlorhexidine versus povidone-iodine prior to amniocentesis. Am J Perinatol 2012;29:455–8.

[40] Macias JH, Arreguin V, Munoz JM, Alvarez JA, Mosqueda JL, Macias AE. Chlorhexidine is a better antiseptic than povidone iodine and sodium hypochlorite because of its substantive effect. Am J Infect Control 2013;41(7):634–7.