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DETERMINATION OF THE MICROBIAL CONTAMINATION OF DISINFECTANT AND ANTISEPTIC PRODUCED IN LUTH

[1]Mr. Ch. Kishore,[2]Mr. B. Chander,[3]Mr. D. Vijayapal
[1]Associate Professor,[2,3]Assistant Professor
[1,2,3]Department of Pharmaceutics
Vaagdevi Institute of Pharmaceutical Sciences, Bollikunta, Warangal. Telangana.

ABSTRACT

Antiseptics and disinfectants are crucial tools for lowering the population of germs and, therefore, the number of diseases. Antimicrobial activity testing is done using a variety of techniques, many of which are unstandardized, unvalidated, and lacking in suitable controls. In response to these concerns, a number of European Standards (EN) have been created that outline the test procedures for determining whether chemical disinfectants or antiseptic products possess the proper virucidal, fungicidal, yeasticidal, mycobactericidal, or tuberculocidal activity. The 17 ENs pertaining to the assessment of the previously indicated antibacterial activity of preparations intended for the medical field are briefly discussed in this narrative overview, together with current publications on the subject. Tests on suspension and carriers have been conducted in both unclean and clean environments to replicate medical settings. Furthermore, research on biocides for hand antisepsis, surfaces disinfection—including airborne disinfection—and medical device and textile disinfection has shown a broad variety of uses for these standards. It has been underappreciated how important normative papers are when examining the antibacterial activity of disinfectants and antiseptics to prevent infections. This narrative review identifies a research need and attempts to urge scientists to do antimicrobial activity testing in accordance with verified ENs. It also seeks to increase knowledge of the many standardized biocidal activity tests available in the medical field. We also take note of the newly created European Pharmacopoeia monograph, which pertains to evaluating antiseptics that are categorized as therapeutic items for bactericidal and fungicidal action.

I. INTRODUCTION

Microbial infections are one of the greatest public health problems today. There are many groups of micro-organisms that represent threats to human health. In particular, the increase in the number of infections caused by multi-drugresistant (MDR) bacteria has been emerging in recent years. The Review on Antimicrobial Resistance published in 2014 under the supervision of Prof. J. O'Neill indicated that MDR strains could be responsible for 10 million deaths in 2050 [1]. Besides, the report mentions several fatal micro-organism infections, such as tetanus, cholera, diarrhoea, tuberculosis, measles, HIV and malaria. The World Health Organization (WHO) has also emphasized this issue: in February 2017, it published a list of the most dangerous bacterial pathogens, which should be the priority of all current research and new therapeutic options [2]. Moreover, by publishing the Global Tuberculosis Report 2020,

the WHO has highlighted the multidrug resistance of mycobacteria and high mortality in tuberculosis [3]. The Centers for Disease Control and Prevention (CDC) has established a dedicated website concerning fungal infections [4], including fungal disease and antifungal resistance. According to the Johns Hopkins University COVID-19 Dashboard e which monitors SARS-CoV-2 infections e as of March 2022, over 466 million COVID-19 cases and over 6 million deaths have been reported worldwide [5]. Many articles have been published on the search for new chemical compounds and substances of natural origin as potential active compounds in future medicines. Methods for testing antimicrobial activity of these agents are well described mainly in the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. In addition to antimicrobial drugs, effective disinfectants and antiseptics that are applied in the proper way and situation can largely limit the development of diseases caused by microorganisms. These agents are important weapons to reduce the number of micro-organisms and thus to limit their spread and the number of infections caused by pathogenic bacteria, fungi and viruses. However, the knowledge of disinfectants and antiseptics is still an underestimated area related to the prevention of microbial infections. These preparations are widely used; however, information on the methods of their antimicrobial efficacy testing in the medical area is very limited. Normative test methods are not sufficiently disseminated. Recently, disinfectants and antiseptics have been at the forefront of the defence against the SARS CoV-2 virus, the cause of the COVID-19 pandemic. A number of guidelines, recommendations and procedures for conducting instrument and surface disinfection as well as hand antisepsis have been developed. While a multitude of disinfectants and antiseptics have appeared on the market, users must be aware that these preparations should have an appropriate biocidal activity, evaluated according to appropriate standards. Our review contains a set of normative documents and, at the same time, indicates the purpose for which the given standards should be applied. We provide the standards that should be met by new preparations with a specific antimicrobial spectrum, applied to given objects in the medical area. European Standards (ENs) have been developed by the European Committee for Standardization (CEN), Technical Committee 216 (CEN/TC 216), describing the test methods to determine whether a chemical disinfectant or antiseptic product has appropriate bactericidal, sporicidal, mycobactericidal or tuberculocidal activity; fungicidal or yeasticidal activity; or virucidal activity. The standard for chemical disinfectants and antiseptics, EN 14885:2018 [6], combines and presents the laboratory methods for testing chemical disinfectant and antiseptic products to support claims that they have specific antimicrobial activity appropriate for their intended application. In the case of infections and epidemics, to limit the transmission of pathogens, the use of disinfectant and antiseptic preparations that meet the requirements of ENs developed by CEN/TC 216/Working Group 1 e Human medicine for products used in the medical area, should be considered. These standards are applicable to products used in areas and situations where disinfection/antisepsis is medically indicated; in patient care, for example, in hospitals, healthcare facilities, dental clinics, schools, kindergartens

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and nurseries; as well as in service establishments such as laundries and kitchens that deliver products directly to patients. These products are intended for antiseptic use e hand disinfection (skin disinfection is not covered in ENs) or disinfection of medical equipment, surgical instruments, anaesthetic equipment, endoscopes, the surfaces of different objects and the walls and floors of patient rooms and other medical rooms. It must be highlighted that determination of the antimicrobial activity of disinfectants and antiseptics is carried out in a very different way from the determination of the antimicrobial activity of drugs such as antibiotics or antimicrobial chemotherapeutics. Moreover, the maximum contact time of disinfectants with micro-organisms allowed by EN is 1 h, while the determination of the activity of antimicrobial drugs lasts at least 18 h.

Phase 2 European standards concerning chemical disinfectants and antiseptics applied in the medical area

The basic antimicrobial activity of chemical disinfectants and antiseptics is determined according to phase 1 ENs. They do not define the areas in which disinfectants and antiseptics are used. Furthermore, these preparations should meet the biocidal activity tests carried out by the suspension method of phase 2, step 1 and the carrier method of phase 2, step 2 tests, allowing the product to be qualified for certain applications. In the case of phase 2 standards, CEN generally defines the areas where the application of the relevant standards is recommended. The tests of phase 2, step 1 involve adding a suitably prepared mixture containing test micro-organisms and loading substances to the product sample. The loading substances in the medical area tests are selected depending on the intended practical use of the product e to simulate clean conditions, a solution with bovine serum albumin (BSA) 0.30 g/L is used, while to stimulate dirty conditions, a mixture of BSA 3.00 g/L and sheep erythrocytes 3 mL/L is used. After a certain contact time between disinfectants/antiseptics and micro-organisms, a specific volume of the suspension is taken, the biocidal effect is neutralized, the number of survivors is determined and the degree of microbial reduction is calculated. The neutralization process is very important to differentiate microbiostatic from microbiocidal effects. The composition of the solutions able to neutralize the antimicrobial activity of disinfectants/antiseptics is given in normative documents. The tests of phase 2, step 2 are conducted on a properly prepared surface. Moreover, these tests simulate the conditions of practical use of a given product even more than phase 2, step 1 tests. For antiseptics, the practice-like test method are the hands of volunteers, while for disinfectants recommended for the medical area, frosted glass plates are used in tests for medical instrument and stainless-steel discs or homogeneous polyvinyl chloride plates are applied in nonporous surface tests. Micro-organisms are applied to a given surface, dried and treated with a disinfectant; then, the biocidal activity is neutralized, the micro-organisms are recovered from the surface, counted and the degree of their reduction is determined. In this narrative review, we tried to answer the question: how should be tested the antimicrobial efficacy of chemical disinfectants and antiseptics, the activity of which is critical in reducing bacterial, fungal and viral infections as well as epidemics. The presented comprehensive overview of the available normative documents, related literature from recent years, and the

author's commentary, give a full insight into the issue in question.

2. MATERIALS

Table I presents the phase 2 ENs concerning chemical disinfectants and antiseptics applied in the medical area. The number of publications in PubMed from 2017 to 2021, in which the researches carried out tests in accordance with the given standards, has also been included. These data show how few investigations have been conducted with validated methods in accordance with the normative documents. It is worth noting that in the standardization area, in addition to European standards, the American Society for Testing and Materials (ASTM) plays a great role and has developed several thousand technical standards covering procedures for testing and classifying materials of all kinds, including disinfectants and antiseptics. ASTM standards are widely used all over the world, but not in European countries whose National Standards Bodies are affiliated to CEN. Furthermore, in addition to the normative documents, such as EN, a European Pharmacopoeia monography has been developed to determine the antibacterial and antifungal activity of antiseptics registered as medicinal products [7]. Pharmacopoeia editions become a kind of 'bible' for the pharmaceutical industry, and all pharmacopoeia monographies must be strictly followed to ensure that medicinal products on the market are therapeutically effective, safe and of good quality.

Determining bactericidal activity

EN 13727:2012 þ A2:2015 [8] is applicable to a wide range of products that can be used for hygienic and surgical handwash or hand rub, instrument disinfection by immersion and surface disinfection. According to this standard, a bactericide is a product that kills the following bacterial strains: Escherichia coli K 12 NCTC 10538 (for handwash products and hand disinfectants), Pseudomonas aeruginosa ATCC 15442, Staphylococcus aureus ATCC 6538, Enterococcus hirae ATCC 10541 and Enterococcus faecium ATCC 6057 (for disinfecting instruments at a temperature of 40 C). The product meets the requirements of the above standard if, under the specified test conditions, it reduces the number of bacteria by at least 5 log10, while for hygienic handwash products, the reduction must be at least 3 log10. Clean and dirty conditions may be used. This standard provides test conditions for antiseptics at contact times ranging from 30 to 60 s for hygienic handwash and hand rub disinfection and from 60 s to 5 min for surgical handwash and hand rub disinfection. The neutralization time had been set to 5 min; however, with short contact times, the test product could have not a bacteriostatic, but a bacteriocidal effect for a certain period of neutralization. Therefore, the neutralization time has been reduced to 10 1 s for preparations where the declared contact time is 10 min. Tyski et al. [9], assessing the possibility of reducing the neutralization time from 5 min to 10 s, examined 14 disinfectant and antiseptic products containing active substances from different chemical groups: alcohols, aldehydes, biguanides, quaternary ammonium compounds, phenols, amines derivatives and oxidizing agents. These products were tested according to EN 13727 as well as three other ENs, using the following test organisms: S. aureus ATCC 6538, P. aeruginosa ATCC 15442, E. coli NCTC 10538, E. coli ATCC 10536, E. hirae ATCC 10541, Candida albicans ATCC 10231 and Aspergillus brasiliensis (formerly Aspergillus niger) ATCC 16404. The biocidal activity of almost all tested

products was inhibited after 10 s of inactivation. Chojecka et al. [10] also proved that a 10-s neutralization time is sufficient to eliminate the residual activity of two tested products for hygienic hand rub disinfection with different ethanol content (89% and 70%). These results confirm that the contact time described in ENs can be determined precisely despite reducing the neutralization time from 5 min to as little as 10 s. Using EN 13727, the activity of a number of formulations of preparations intended for topical application in patients has been tested. Salvalico et al. [11] investigated bactericidal activity of three antiseptic preparations: (a) chlorhexidine gluconate 0.2% with benzalkonium chloride 0.5%; (b) a mixture of hexamidine diisethionate 0.10%, chlorhexidine gluconate 0.5%/20% solution, and chlorocresol 0.3%; and (c) povidone-iodine 10%. They tested these preparations at 97%, 50% and 10%, in dirty conditions, after a 60-s contact time. As a control, authors used 1% preparations, which did not meet the standard. Mixture (a) showed bactericidal activity (reduced cell counts of four bacterial strains >5 log10) in all three tested concentrations. Mixture (b) did not present bactericidal activity according to EN 13727, except for P. aeruginosa at a concentration of 97%. Mixture (c) was not bactericidal against E. hirae at any concentration and not bactericidal against S. aureus at 97%. Sahiner et al. [12] tested the bactericidal activity of five antiseptics e chlorhexidine digluconate 2%, povidone-iodine 7.5%, propan-2-ol 70%, hydrogen peroxide 3% and tincture of iodine 2% e in clean and dirty conditions after 1 and 5 min of contact. Only hydrogen peroxide 3% did not show bactericidal activity according to the standard. Propan-2-ol 70% and tincture of iodine 2% met EN 13727 after both contact times and both contamination conditions. Radischat et al. [13] investigated the influence of human wound exudate on the bactericidal efficacy of antiseptic agents e octenidine dihydrochloride, chlorhexidine digluconate, polyhexamethylene biguanide and povidone-iodine. The authors showed that the bactericidal activity of antiseptic preparations tested in the presence of human wound exudate is reduced compared with the activity determined directly in accordance with EN 13727 in the presence of an organic load e the clean and dirty conditions provided for this standard. The presence of the clinical micro-organisms (different species and micro-organism count) contaminating or colonizing the wound may also reduce the activity of antiseptics.

Table I
The European Standards concerning chemical disinfectants and antiseptics phase 2 applied in medical area

Biocidal range	EN number	Title	Ref.	No. of publications[b]
Bactericidal phase 2, step 1	13727:2012+A2:2015	Quantitative suspension test for the evaluation of bactericidal activity	[8]	7
Bactericidal phase 2, step 2[a]	1499:2013	Hygienic handwash	[22]	1
Bactericidal phase 2, step 2[a]	1500:2013	Hygienic hand rub	[24]	14
Bactericidal phase 2, step 2[a]	12791:2016+A1:2018	Surgical hand disinfection	[38]	10
Bactericidal phase 2, step 2	14561:2006	Quantitative carrier test for the evaluation of bactericidal activity for instruments	[46]	2
Sporicidal phase 2, step 1	17126:2018	Quantitative suspension test for the evaluation of sporicidal activity	[48]	1
Mycobactericidal phase 2, step 1	14348:2005	Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants for instruments	[50]	2
Mycobactericidal phase 2, step 2	14563:2008	Quantitative carrier test for the evaluation of mycobactericidal or tuberculocidal activity of chemical disinfectants used for instruments	[51]	2
Fungicidal phase 2, step 1	13624:2013	Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity	[57]	3
Fungicidal phase 2, step 2	14562:2006	Quantitative carrier test for the evaluation of fungicidal or yeasticidal activity for instruments	[59]	0
Virucidal phase 2, step 1	14476:2013+A2:2019	Quantitative suspension test for the evaluation of virucidal activity	[60]	12
Virucidal phase 2, step 2	16777:2018	Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity	[69]	1
Virucidal phase 2, step 2	17111:2018	Quantitative carrier test for the evaluation of virucidal activity for instruments used in the medical area	[70]	2
Bactericidal and yeasticidal phase 2, step 2	16615:2015	Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes (4-field test)	[74]	9
Bactericidal and yeasticidal phase 2, step 2[c]	13697:2015+A1:2019	Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity	[73]	3
Bactericidal, mycobactericidal and yeasticidal phase 2, step 2[a]	16616:2015	Chemical—thermal textile disinfection	[85]	0
Bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal[a]	EN 17272:2020	Methods of airborne room disinfection by automated process. Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities	[88]	0
Total microbiology	EN 14885:2018	Application of European Standards for chemical disinfectants and antiseptics	[6]	2

Determining sporicidal activity

Vegetative forms of bacteria are much more sensitive to chemical disinfectants and antiseptics than the persistent forms, namely bacterial spores. To address this issue, CEN has recently developed a specific standard for testing sporicidal activity in the medical area, namely EN 17126:2018 [48]. This suspension method (phase 2, step 1) is similar to Method 18

developed earlier by the German Association of Applied Hygiene (VAH). Another standard, EN 13704 [49], has been used to evaluate the sporicidal activity of chemical disinfectants, but it is only applicable to the preparations used in food, industrial, domestic and institutional areas e not the medical area. The sporicidal activity of products for surface, instrument and textile disinfection is evaluated according to EN 17126 [48] using the following strains: Bacillus subtilis ATCC 6633, Bacillus cereus CIP 105151 and Clostridium difficile R027 NCTC 13366 (currently, the valid name of these strictly anaerobic Grampositive rods is Clostridioides difficile). The standard defines two spectra of activity: sporicidal activity (against both Bacillus species) and sporicidal activity against C. difficile. C. difficile is one of the most commonly recognized causes of severe microbial diarrhoea. The number of cases caused by C. difficile infection has increased dramatically in recent years. Cases with Bacillus spp. strains are not frequent, but they can cause serious infections in humans and animals. In addition, Bacillus anthracis, classified as a biological weapon, is extremely dangerous, and therefore its spores could not be included in the standard. Hence, testing of the sporicidal activity of disinfectants is crucial. Clean and/or dirty conditions are used. The testing time is different: in the case of surface disinfection, it should be no longer than 15 min for products likely to come into contact with a person or applied to frequently touched surfaces or 60 min for products for other surfaces. However, the contact time of instruments and textile disinfection is no longer than 60 min. According to the manufacturers' recommendations, the test temperature is between 4 and 30 C for surface disinfection, between 20 and 70 C for instrument disinfection and between 20 and 80 C for textile disinfection. The neutralization time, which differentiates sporicidal from sporistatic effects, is usually 5 min. Preparation of Clostridioides and Bacillus spore stock suspension is described in the standard. The product demonstrates at least a 4 log10 reduction in spores count in concentrations and exposure times recommended by the manufacturer. The surface method (phase 2, step 2) for the evaluation of sporicidal activity has not been developed.

Determining mycobactericidal and/or tuberculocidal activity

Tuberculosis is one of the world's great public health threats; worldwide, it is one of the top causes of death, with an estimated >1.4 million people having died from tuberculosis in 2019 [3]. Drug-resistant Mycobacterium tuberculosis is of particular concern. According to the WHO, 3.3% of newly diagnosed tuberculosis cases worldwide in 2019 were infected with rifampicin-resistant or MDR M. tuberculosis (MDR/RR-TB) [3]. Although mycobacteria are bacteria, due to the specific cell envelope structures and the important role played in the field of public health, a quantitative suspension test [50] and a quantitative carrier test [51] have been developed by CEN to determine the mycobactericidal and tuberculocidal activity of chemical disinfectant preparations used for instruments in the medical area. This activity has been defined as the ability of the preparation to reduce the number of mycobacterial cells of the relevant test organisms: Mycobacterium avium ATC,C 15769 and Mycobacterium terrae ATCC 15755. However, due to the importance of disinfection processes against M. tuberculosis, whose MDR strains are extremely dangerous to humans, it was decided to determine tuberculocidal activity separately. Determination

of this property cannot be simply performed with M. tuberculosis because of its pathogenicity. Thus, the tuberculocidal activity is determined indirectly as the ability of the product to reduce the number of M. terrae ATCC 15755. Both mycobactericidal and tuberculocidal activity can be estimated in the presence of aggravating substances dedicated for clean condition (BSA 0.3 g/L) and/or dirty condition (BSA 3 g/L and 3 mL/L sheep erythrocytes).

Determining virucidal activity

There has been a pronounced increase in interest and demand for disinfecting preparations with virucidal activity. The recommendations and procedures that attempt to mitigate the COVID-19 pandemic necessitate the frequent use of such preparations. There are an abundance of preparations on the market, the producers of which have declared virucidal activity e including against SARS-CoV-2, although proper tests with the use of this virus have not always been carried out. Considering the viral particle structure, enveloped viruses are much more susceptible to chemical disinfectants and antiseptics than nonenveloped viruses. It should be emphasized here that SARSCoV-2 is an enveloped virus. The current standards for the determination of virucidal activity include several test organisms that are recommended: non-enveloped RNA viruses, namely poliovirus type 1, LSc 2ab from the picornavirus group and MNV strain S99 Berlin, and non-enveloped DNA viruses, namely adenovirus type 5 (Adenoid 75 strain, ATCC VR-5) and very small murine parvovirus (Crawford strain, ATCC VR-1346). Enveloped DNA viruses e modified vaccinia virus Ankara (MVA) ATCC VR-1508 strain or vaccinia virus Elstree strain e are also included as standard strains.

3. DISCUSSION&CONCLUSION

Antiseptics and disinfectants are essential for avoiding infections. Normative papers ensure that these preparations are effective. As a result, it is essential to create exact guidelines that address every aspect, including hand antisepsis, medical linens, device disinfection, surfaces of various items, and, if necessary, patient and other medical area walls and flooring. Preparations exhibiting suitable bactericidal, sporicidal, mycobactericidal or tuberculocidal, fungicidal or yeasticidal, or virucidal action may be available on the market, depending on the environment and the intended usage of a specific biocide. The normative texts—primarily ENs—remain crucial. Recently, there has been a widespread usage of antiseptic practices and antiseptics to stop the spread of SARS-CoV-2 and lessen the COVID-19 pandemic. Notable is the development of a unique European Pharmacopeia monograph that details the assessment of antibacterial and antifungal activity of antiseptic pharmaceuticals. The vast array of standard papers that have been created to evaluate a broad range of antimicrobial activity using surface-carrier and suspension testing guarantees that consumers have access to high-quality, efficient goods. Proper disinfection and antiseptic processes should only employ goods that adhere to proven methodologies specified in normative publications. After that, we will reduce harmful microorganisms to the levels required by the regulations, stopping the spread of illness.

REFERENCES

[1] Antimicrobial resistance: tackling a crisis for the health and wealth of nations. The review on

antimicrobial resistance chaired by Jim O'Neill December 2014. Available at: https://www.jpiamr.eu/wpcontent/uploads/2014/12/AMR-Review-Paper-Tackling-a-crisis-forthe-health-and-wealth-of-nations_1-2.pdf [last accessed November 2021].

[2] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;18:318e27.

[3] Global tuberculosis report 2020. Geneva: World Health Organization; 2020. Available at: https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf [last accessed November 2021].

[4] Centers for Disease Control and Prevention (CDC). Available at: https://www.cdc.gov/fungal/index.html [last accessed November 2021].

[5] Johns Hopkins University. Available at: https://www.arcgis.com/apps/dashboards/bda7594740fd40299423467b48e9ecf6 [last accessed September 2021].

[6] EN 14885:2018. Application of European Standards for chemical disinfectants and antiseptics. Brussels, Belgium: CEN-CENELEC Management Centre; 2018.

[7] European Pharmacopoeia 10.0 Ed. T.1. In: Monography: 5.1.11. Determination of bactericidal, fungicidal or yeasticidal activity of antiseptic medicinal products; 2020. p. 643e4. 07/2017:50111.

[8] EN 13727þA2. Chemical disinfectants and antiseptics e quantitative suspension test for the evaluation of bactericidal activity in the medical area e Test method and requirements (phase 2, step 1). Brussels, Belgium: CEN-CENELEC Management Centre; 2015. 2015.

[9] Tyski S, Bocian E, Grzybowska W. Reduction of the neutralisation time during antimicrobial activity testing of disinfectants according to European Standards. Rocz Panstw Zakl Hig 2013;64:111e5.

[10] Chojecka A, Tarka P, Kierzkowska A, Nitsch-Osuch A, Kanecki K. Neutralization efficiency of alcohol based products used for rapid hand disinfection. Rocz Panstw Zakl Hig 2017;68:389e94.

[11] Salvatico S, Feuillolay C, Mas Y, Verrie`re F, Roques C. Bactericidal activity of 3 cutaneous/mucosal antiseptic solutions in the presence of interfering substances: Improvement of the NF EN 13727 European Standard? Me´decine et maladies infectieuses 2015;45:89e94.

[12] S,ahiner A, Halat E, Algın Yapar E. Comparison of bactericidal and fungicidal efficacy of antiseptic formulations according to EN 13727 and EN 13624 standards. Turk J Med Sci 2019;49:1564e7.

[13] Radischat N, Augustin M, Herberger K, Wille A, Goroncy-Bermes P. Influence of human wound exudate on the bactericidal efficacy of antiseptic agents in quantitative suspension tests on the basis of European Standards (DIN EN 13727). Int Wound J 2020;17:781e9.

[14] Severing A-L, Rembe J-D, Koester V, Stuermer EK. Safety and efficacy profiles of different commercial sodium hypochlorite/ hypochlorous acid solutions (NaClO/HClO): antimicrobial efficacy, cytotoxic impact and physicochemical parameters in vitro. J Antimicrob Chemother 2019;74:365e72.

[15] Ciotti C, Ferraob B, Garriguesa I, Ne´romea S. Bacteria which are highly resistant to antibiotics are not resistant hydro-alcoholic products. Infect Dis 2021;51:77e80.