

HAND DISINFECTANTS AND THEIR ACTIVITY AGAINST CLINICAL ISOLATES OF *BORDETELLA PERTUSSIS*

Petra Uttlová^{1,2}, Jan Urban¹

¹National Reference Laboratory for Disinfection and Sterilization, National Institute of Public Health, Prague, Czech Republic

²Third Faculty of Medicine, Charles University, Prague, Czech Republic

SUMMARY

Objectives: The aim of the study was to investigate possible emergence of resistance to disinfectants in *Bordetella pertussis* strains isolated from patients with whooping cough in the Czech Republic in 2014 and 2015.

Methods: In an EN1500-based study, clean and dry fingertips of volunteers were always contaminated with one of the two clinical isolates of *B. pertussis*. Clinical isolates of *B. pertussis* were obtained from the National Reference Laboratory for Pertussis and Diphtheria, National Institute of Public Health (NIPH), Prague, Czech Republic. Dry and contaminated fingertips were immersed in 10 ml medium and then rubbed with the fingers for 1 minute. After that, the hands were treated with isopropanol 60% v/v or tested products, and then the fingertips were rubbed again into 10 ml of pure medium for 1 minute. The suspensions obtained were immediately diluted and plated on charcoal medium.

Results: Ethanol-based product A and propanol-based product B showed bactericidal activity after 30 s of contact. The confidence interval limit for product A and B was 0.12 and 0.19, respectively. Quaternary ammonium compound-based product C was found to be ineffective after 30 s of contact. The confidence interval limit for product C was 0.62.

Conclusion: Products A and B were assessed as effective against clinical isolates of *B. pertussis* in accordance with EN 1500. Quaternary ammonium compound-based product C did not comply with the requirements of EN 1500.

Key words: *Bordetella pertussis*, hygienic hand disinfection, European standard EN 1500, susceptibility, disinfectants

Address for correspondence: P. Uttlová, Centre for Epidemiology and Microbiology, National Reference Laboratory for Disinfection and Sterilization, National Institute of Public Health, Šrobárova 48, 100 42 Prague 10, Czech Republic. E-mail: petra.uttlova@seznam.cz

<https://doi.org/10.21101/cejph.a7141>

INTRODUCTION

Pertussis or whooping cough is a highly contagious disease of the respiratory tract and has been reported to be an important cause of morbidity and mortality in infants and children under 5 years of age worldwide (1, 2). After pertussis vaccination was introduced in the 1950s and the 1960s, a substantial drop in morbidity and mortality was observed in industrialised countries (3, 4). However, whooping cough cases have been on the rise in many countries over the last decade, which is attributed to increased susceptibility, weakened immunity, and adaptation of the bacteria or, possibly, to improved detection due to the availability of more advanced diagnostic tools (5, 6). As estimated by the World Health Organization (WHO), over 50 million cases of whooping cough occurred worldwide in 2011, with 300 thousand of these being fatal (7). The cause is the bacterium *Bordetella pertussis*, which is transmitted from human to human by direct contact, particularly via airborne droplets (8). The most recent study has reported a survival time of *B. pertussis* of 3–5 days on a dry surface. *B. pertussis* can survive 6 hours on skin, 5 days on clothes and 2 days on paper (9). The survival time can be even longer, varying with the amount of inoculum, sputum, and proteins (10). Therefore, indirect transmission of the disease can occur through fomites or the skin possibly

contaminated by secretions from the upper airways (11). In hospitals and other healthcare settings, antiseptics and disinfectants are widely used for the treatment of the skin or surfaces. These products contain various active ingredients, such as alcohols, phenols, iodine, chlorine, etc. These chemicals often have a wide antimicrobial activity. Unfortunately, there is less focus on the mechanism of action of these chemicals as compared to antibiotics, and few data are available in this regard (12). Proper disinfection of surfaces, fomites and the skin is vital. To date, no study has been published on the resistance of *B. pertussis* to disinfectants. The present study focuses on testing hand and skin disinfectants for antimicrobial activity in accordance with EN 1500 (13). Two clinical isolates of the bacterium *B. pertussis* were used in the tests.

MATERIALS AND METHODS

Study Participants

Twelve women and 8 men working in the healthcare sector were selected for the group of volunteers. The age range of the volunteers was 28–57 years. The average age of the volunteers was 41.5 years.

Interfering Substance

0.3 g of bovine serum (Bioveta, a.s., Ivanovice na Hané, Czech Republic) was dissolved in 100 ml of dilution solution (Tryptone, pancreatic digest of casein, Oxoid Limited, Hampshire, United Kingdom; sodium chloride (NaCl), Lach-ner, s.r.o., Neratovice, Czech Republic – hereinafter Lach-ner). The final concentration of bovine serum in the test procedure is 0.3 g/l.

Hard Water

Solution A: 19.84 g of magnesium chloride (MgCl₂) (Lach-ner) and 46.24 g of calcium chloride (CaCl₂) (Merck KGaA, Darmstadt, Germany) were dissolved in water and diluted to 1,000 ml. The solution was sterilised in an autoclave. Solution B: 35.02 g of sodium bicarbonate (NaHCO₃) (Lach-ner) was dissolved in water and diluted to 1,000 ml. The solution was sterilised by membrane filtration (Millipore Express PLUS, EMD Millipore Corporation, Burlington, USA). After that, 6.0 ml of solution A and 8.0 ml of solution B were mixed under sterile conditions, and the obtained solution was adjusted to a volume of 1,000 ml. When needed, pH was adjusted with a solution of sodium hydroxide (NaOH) (Lach-ner) at a concentration of about 40 g/l (1 mol/l) or a solution of hydrochloric acid (HCl) (Lach-ner) at a concentration of about 36.5 g/l (1 mol/l).

Test Products and Neutralization

Three commonly available products were selected to be tested under conditions simulating practical use. The ethanol-based product (designated as product A) is used undiluted (100% concentration) and is intended for hygienic and surgical hand disinfection. The active ingredient is ethanol (85.0 g/100 g product), and other ingredients are 2-butanone, 85% glycerol, 1-tetradecanol, 1-propanol, and purified water. The second product (designated as product B) is used undiluted (100% concentration) and is also intended for hygienic and surgical hand disinfection. The active ingredients are isopropanol (45.0 g/100 g), 1-propanol (30.0 g/100 g), and mecetronium ethylsulfate (0.2 g/100 g). Other ingredients are 85% glycerol, 1-tetradecanol, perfume, patent blue V 85%, and purified water. The third product based on quaternary ammonium salts (designated as product C) was diluted with hard water to 1% solution. The active ingredients of product C are quaternary ammonium compounds (QAC), benzyl-C8-18-alkyldimethyl, bromides (10.0 g/100 g aqueous solution). Product C is intended for superficial skin disinfection, hand disinfection, and disinfection of small wounds and scrapes. All three products showed antimicrobial activity in accordance with EN 1500 for hygienic hand disinfection using standard strains of *Escherichia coli*. These are typical products intended for hand disinfection or, possibly for skin or wound disinfection. Isopropanol 60% v/v (Lach-ner) was used as the reference product. Disinfectants A and B were neutralised with combination of Tween 80 (3 g/l) (Lach-ner), cysteine (1 g/l) (Sigma-Aldrich, Inc., Saint Louis, USA), histidine (0.5 g/l) (Merck KGaA, Darmstadt, Germany), and sodium thiosulphate (0.5 g/l) (Ing. Petr Švec – PENTA s.r.o., Praha, Czech Republic) incorporated in the diluent solution. Disinfectant C was neutralised with combination of polysorbate 80 (30 g/l) (Lach-ner), sodium dodecyl sulphate (4 g/l) (Sigma-Aldrich, Inc., Saint Louis, USA), and lecithin (3 g/l) (Carl Roth GmbH + Co. KG,

Karlsruhe, Germany) incorporated in the diluent solution. The test for the validation of neutralisation was performed according to European Standard EN 1500 (13).

Quantitative Suspension Test Method

Quantitative suspension tests were carried out using two selected clinical isolates of *B. pertussis*. Clinical isolates of *B. pertussis* were obtained from the National Reference Laboratory for Pertussis and Diphtheria, National Institute of Public Health (NIPH), Prague, Czech Republic. They were performed in accordance with European Standard EN 13727+A2 (14). EN 13727+A2 specifying that the minimum requirement for the determination of bactericidal activity is a 5 log reduction (lg R) of the bacterial count. If these conditions are met, we can proceed to the next step of testing, which is testing according to EN 1500 (13).

Products A and B were tested by a modified method for ready-for-use products in accordance with EN 13727+A2. An amount of 0.2 ml of a five-fold concentrated interfering substance was pipetted into a test tube and then 0.1 ml of a 10-fold concentrated test bacterial suspension was added. After mixing, the test tube was placed in a water bath for two minutes. Then, 9.7 ml of undiluted product A or B were added, and under control conditions, 9.7 ml of sterile water were added. The mixture was mixed again and then kept in a water bath for a selected contact time of 30 s or 60 s, respectively. Subsequently, 1 ml of the mixture was taken and pipetted into a test tube with 8 ml of neutralizing agent and 1 ml of sterile water. After neutralizing for 10 s, the suspensions were diluted and plated on a charcoal medium added with cephalaxin.

Product C was tested using the standard method in accordance with EN 13727+A2. 1.0 ml of an interfering substance and 1.0 ml of the test bacterial suspension were pipetted into a test tube. After mixing, the mixture was placed in a water bath for two minutes. After that, 8.0 ml of product C diluted with hard water to 1.25 times the actual test concentration was added to the mixture and, under control conditions, with 8.0 ml of sterile water. The mixture was mixed again and then kept in a water bath for a selected contact time of 30 s or 60 s, respectively. Subsequently, 1 ml of the mixture was taken and pipetted into a test tube with 8 ml of neutralizing agent and 1 ml of sterile water. After neutralizing for 10 s, the suspension was diluted and plated on a charcoal medium added with cephalaxin.

Artificial Contamination of the Hands

Artificial contamination of the hands was carried out using two selected clinical isolates of *B. pertussis*. The volunteers were contaminated with only one bacterial strain at a time in each experiment. The results obtained are therefore the average of the values obtained from the two strains used. This method was performed in accordance with European Standard EN 1500 (13).

Preparation and Application of the Bacterial Suspension

Two selected clinical isolates of *B. pertussis* were cultured at 36 ± 1 °C in 50 ml of Stainer-Scholte medium for five to seven days. Prior to the artificial hand contamination, the bacterial cultures were transferred to empty Petri plates. The hands were first washed with reference soap (*sapo kalinus*) for 1 min to remove resident hand flora and then dried with paper towels. The

fingertips were immersed in the bacterial culture prepared in Petri plates for five seconds. The wet fingertips were exposed to the air until dry, i.e., for about three minutes.

Experimental Procedure

When dry, the contaminated fingertips were immersed in 10 ml of Stainer-Scholte medium and then rubbed with the fingers for one minute. After that, the hands were treated with 3 ml of isopropanol 60% v/v, the reference product, rubbed into the skin for 30 s. This step was repeated after 30 s with another 3 ml of isopropanol 60% v/v, and thus the total time of rubbing was 60 s (with a total amount of the reference product of 6 ml). After that, the fingertips were rubbed into 10 ml of the neutralizing agent. Each test product was applied to the hands at a volume of 3 ml and then rubbed into the skin for 30 s. After that, the fingertips were rubbed into 10 ml of the neutralizing agent. The suspensions obtained were immediately diluted and plated on charcoal medium added with cephalixin.

Assessment of Results

The count of colony-forming units (CFU) was determined on each plate. The bacteria released from the contaminated fingertips were enumerated prior to and after the application of each test product. The logarithmic reduction (lg R) was determined from the ratio of the two resulting counts between log₁₀ pre-treatment minus log₁₀ post-treatment value. The results obtained after hygienic hand rubbing with the test product were compared to those after application of the reference product, isopropanol 60% v/v. To meet the efficacy conditions in accordance with EN 1500 (13), the results of the test product must be noninferior. The Wilcoxon's paired test was used to determine the significance level. Noninferiority is supposed when the Hodges-Lehmann upper 97.5% confidence limit for the individual difference in log₁₀ bacterial reductions between study products and the reference product is smaller than the agreed inferiority margin of 0.6.

RESULTS

The in vitro tests performed in accordance with EN 13727+A2 (quantitative suspension test for the evaluation of bactericidal activity in the medical area) have shown that products A, B, and C are significantly effective against the bacterium *B. pertussis*. Each of the test products achieved more than 5 log reduction in viable bacteria after a contact time of 30 s and 60 s (Table 1). Ethanol-based product A was tested at a 97% concentration. After 30 s of action, product A achieved on average a 5.47 log reduction in bacterial count and after 60 s of action, on average a 6.01 log reduction. Product B with the active ingredient propanol was also tested at a 97% concentration. After 30 s and 60 s of action, product B resulted in on average a 5.42 log and 5.99 log reduction, respectively. QAC-based product C was tested at a 1% concentration. When applied for 30 s and 60 s, product C showed on average a 5.57 log and 6.08 log reduction, respectively. A 5 log decrease met the conditions required for testing in the next step in accordance with EN 1500 (hygienic handrub). Products A, B, and C were tested in accordance with EN 1500. All three test products were compared to the reference product isopropanol 60% v/v. Product A achieving a 4.03 log reduction in viable bacteria was superior to the reference product whose application resulted in a 3.85 log reduction (Table 2). Product B responsible for a 4.01 log reduction in viable bacteria was superior to the reference product achieving a 3.94 log reduction (Table 2). Product C, the use of which led to a 3.70 log reduction in viable bacteria, was inferior to the reference product showing a 3.96 log reduction (Table 2). Based on the paired rank test for 20 subjects and one-sided Wilcoxon's paired test at a 0.025 significance level (97.5%), the confidence interval limit was established for each test product. The paired differences in lg R between the test product and the reference product are presented in decreasing order. The confidence interval limit for products A, B, and C are 0.12, 0.19, and 0.62, respectively (Table 3). The confidence interval values for

Table 1. Average log reductions in bacterial counts for individual test products depending on concentration and contact time in accordance with EN 13727+A2

Product (concentration)	Active ingredient	Log reduction (lg R) after 30 s			Log reduction (lg R) after 60 s		
		<i>B. pertussis</i>		Average	<i>B. pertussis</i>		Average
		Strain 1	Strain 2		Strain 1	Strain 2	
A (97%)	Ethanol	5.53	5.41	5.47	5.92	6.10	6.01
B (97%)	Propanol	5.39	5.44	5.42	6.05	5.93	5.99
C (1%)	QAC	5.68	5.46	5.57	6.13	6.02	6.08

QAC – quaternary ammonium compounds. The results obtained are the average of the values obtained from the two *B. pertussis* strains used.

Table 2. Bactericidal activity of products determined according to EN 1500

Product (concentration)	Active ingredient	Log reduction (lg R) achieved with reference product (isopropanol 60% v/v)			Log reduction (lg R) achieved with test product			Difference
		<i>B. pertussis</i>		Average	<i>B. pertussis</i>		Average	
		Strain 1	Strain 2		Strain 1	Strain 2		
A (100%)	Ethanol	3.90	3.80	3.85	4.15	3.91	4.03	-0.18
B (100%)	Propanol	3.97	3.91	3.94	4.07	3.96	4.01	-0.07
C (1%)	QAC	3.92	4.01	3.96	3.65	3.76	3.70	0.26

QAC – quaternary ammonium compounds. The results obtained are the average of the values obtained from the two *B. pertussis* strains used.

Table 3. Product critical values

Product (concentration)	Product critical value		
	<i>B. pertussis</i> strain 1	<i>B. pertussis</i> strain 2	Average
A (100%)	0.04	0.20	0.12
B (100%)	0.20	0.17	0.19
C (1%)	0.63	0.61	0.62

The product critical values were obtained based on the paired rank test for 20 subjects and one-sided Wilcoxon's paired test at a 0.025 significance level (97.5%).

products A and B are lower than the agreed inferiority margin of 0.6. It can therefore be said with confidence that both products are effective against *B. pertussis*. The confidence interval value for product C is higher than the agreed inferiority margin of 0.6. The product is therefore ineffective against *B. pertussis*.

DISCUSSION

This is the first study to investigate clinical isolates of *B. pertussis* and their sensitivity to hand-disinfectants. Appropriate hand hygiene is the most effective measure to prevent the spread of microbes that may cause disease both in the community and healthcare settings. Thus, the hands play a key role in transmission of infection in healthcare settings, the industry, and households (15). In a recent study, they pointed to high rates of noncompliance with hand hygiene. This study concluded that over 60% of medical staff in the intensive care unit at the local hospital do not maintain hand hygiene (16). Biocides generally have a broader spectrum of activity on bacteria as compared to antibiotics and in particular have more target sites, therefore, microbial resistance to biocides is rather rare (17, 18). Here we extend the previous paper of Uttlová et al. (19), who tested selected products in accordance with EN 13727+A2 (14) and EN 14561 (20). Four disinfectants with a wide range of uses were selected for the previous study. One alcohol-based hand disinfectant was included. As EN 14561 is a standard primarily intended to test tool disinfectants, the test product appeared to be inactive (19). Although bacteria are known not to have any acquired resistance to alcohols, it has not previously been shown that the most common bacteria in clinical medicine are equally inactivated in suspension tests (21). Therefore, in the following study, three hand disinfectants with various active ingredients were selected and tested in accordance with EN 1500 (13). First of all, the three products were tested in accordance with EN 13727+A2, i.e., using the first-degree testing by the quantitative suspension test method with protein contamination. The *in vitro* tests proved all test products (products A, B and C) to be active against both bacterial strains of *B. pertussis*. The obtained results between the used two strains were not different. The products were effective with the same result. Suspension tests, however, are not the critical part of the efficacy assessment of hand rubs. All three products achieved a more than 5 log reduction in viable bacteria after both 30 s and 60 s of action, which demonstrates bactericidal activity as required by EN 13727+A2. This met the conditions to proceed to the next step of testing according to EN 1500. In accordance with EN 1500, the preparations were tested after their application on the hands of volunteers in accordance with EN 1500, prescribing that the

test result obtained with the test product should not be statistically significantly inferior to the test result obtained with the reference product isopropanol 60% v/v. To compare the test product with the reference product for Ig R (reduction), the noninferiority test was used. The representative of Gram-negative bacteria commonly used in the tests in accordance with EN 1500 is *Escherichia coli*, but we replaced it by two different clinical isolates of the Gram-negative bacterium *Bordetella pertussis*. Clinical isolates of *B. pertussis* were obtained from the National Reference Laboratory for Pertussis and Diphtheria, National Institute of Public Health (NIPH), Prague, Czech Republic. When tested, the subjects had to adhere to strict rules and were thoroughly decontaminated after each test to prevent transmission of infection. All subjects received a booster dose of pertussis vaccine before testing. Products A and B showed higher bactericidal activity compared to the reference product. The confidence interval limits for products A and B were on average 0.12 and 0.19, respectively, and correspond to the paired rank test for 20 subjects and to the one-sided Wilcoxon's paired test at a 0.025 significance level (97.5%). These limit values of 0.12 and 0.19, respectively, for the difference in Ig R between the test product and the reference product are inferior to the agreed inferiority margin of 0.6. Therefore, the hypothesis can be accepted that the results obtained for products A and B are not statistically inferior to the result obtained for the reference product with a certainty of 97.5%. Therefore, when used for hygienic hand disinfection, products A and B have bactericidal activity against the bacterium *B. pertussis* and comply with EN 1500. The effectiveness of alcohol-based products has already been confirmed in a study conducted under practical conditions for hygienic hand disinfection. However, significant differences were found between these alcohol-based products (22). The products we tested did not show significant differences in efficacy, only the generally known slightly better effect of isopropanol was demonstrated (23). Product C showed lower bactericidal activity as compared with the reference product. The confidence interval limit of 0.62 for product C does not correspond to the paired rank test for 20 subjects and to the one-sided Wilcoxon's paired test at a 0.025 significance level (97.5%). The limit value of 0.62 for the difference in Ig R between the test product and the reference product is not inferior to the agreed inferiority margin of 0.6. Therefore, the hypothesis can be rejected that the result obtained for product C is not statistically inferior to the result obtained for the reference product with a certainty of 97.5%. When used for hygienic hand disinfection, after 30 s of action, product C did not comply with the requirements of EN 1500 and did not show bactericidal activity against the bacterium *B. pertussis*. Yet the definition of QAC resistance in Gram-negative bacteria is still not clear, but in a study focused on the QAC benzalkonium chloride (BC), the

BC-resistant isolates generally showed three- to five-fold increase in minimum inhibitory concentration values as compared to the BC-sensitive isolates (24). However, *Staphylococcus aureus* has been studied for resistance to QAC as a representative of Gram-positive bacteria. All *S. aureus* strains used in the study showed resistance to QAC and the genes for resistance to QACs were identified (25). Furthermore, studies on the use of QACs to control *Listeria* in the food industry are worth mentioning, where several studies have reported the occurrence of *Listeria* resistance to QACs. However, the effective concentrations found were lower than those used in practice (26). It is therefore possible that *B. pertussis* may also show increased resistance to QAC. However, concentrations higher than 1% cannot be used for hygienic hand disinfection testing of this product.

CONCLUSIONS

Based on the results obtained through this study products A and B were assessed as effective against clinical isolates of *B. pertussis* in accordance with EN 1500. Quaternary ammonium compound-based product C did not comply with the requirements of EN 1500 and was found to be ineffective. It was therefore found that alcohol-based products are more effective in eliminating the bacterium *B. pertussis* than the quaternary ammonium salt-based product. The development of possible resistance was not detected. It can therefore be safely assumed that alcohol-based products will continue to have bactericidal effects against this bacterial species, which is still plentifully circulating in the population.

Acknowledgements

We would like to thank the staff of the National Reference Laboratory for Pertussis and Diphtheria, National Institute of Public Health (NIPH), Prague, Czech Republic, for providing clinical isolates of *Bordetella pertussis*. We would like to thank all volunteers who participated in this study.

Funding

Supported by the Ministry of Health, Czech Republic – conceptual development of research organization (National Institute of Public Health – NIPH, 75010330).

Conflict of Interests

None declared

Adherence to Ethical Standards

Ethical approval was received from the Ethics Committee of the National Institute of Public Health, Prague, Czech Republic.

REFERENCES

1. Pertussis vaccines: WHO position paper - September 2015. Wkly Epidemiol Rec. 2015 Aug 28;90(35):433-58.
2. Calvert A, Heath PT. Pertussis. Medicine (UK). 2017;45(12):735-8.
3. Mooi FR, van Loo IH, King AJ. Adaptation of *Bordetella pertussis* to vaccination: a cause for its reemergence? Emerg Infect Dis. 2001;7(3 Suppl):526-8.
4. Roush SW, Murphy TV. Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. JAMA. 2007 Nov 14;298(18):2155-63.
5. de Greeff SC, Mooi FR, Westerhof A, Verbakel JMM, Peeters MF, Heuvelman CJ, et al. Pertussis disease burden in the household: how to protect young infants. Clin Infect Dis. 2010 May 15;50(10):1339-45.
6. Naninck T, Coutte L, Mayet C, Contreras V, Loch C, Le Grand R, et al. In vivo imaging of bacterial colonization of the lower respiratory tract in a baboon model of *Bordetella pertussis* infection and transmission. Sci Rep. 2018 Aug 16;8(1):12297. doi: 10.1038/s41598-018-30896-7.
7. Chen Z, He Q. Immune persistence after pertussis vaccination. Hum Vaccin Immunother. 2017 Apr 3;13(4):744-56.
8. Pinto MV, Merkel TJ. Pertussis disease and transmission and host responses: insights from the baboon model of pertussis. J Infect. 2017;74 Suppl 1:S114-9.
9. Mitscherlich E, Marth EH. Microbial survival in the environment: bacteria and rickettsiae important in human and animal health. Berlin: Springer-Verlag; 1984.
10. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis. 2006 Aug 16;6:130. doi: 10.1186/1471-2334-6-130.
11. Warfel JM, Beren J, Merkel TJ. Airborne transmission of *Bordetella pertussis*. J Infect Dis. 2012 Sep 15;206(6):902-6.
12. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999 Jan;12(1):147-79.
13. EN 1500. Chemical disinfectants and antiseptics - hygienic handrub - test method and requirements (phase 2/step 2). Brussels: European Committee for Standardization; 2013.
14. EN 13727+A2. Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2/step 1). Brussels: European Committee for Standardization; 2015.
15. Jumaa PA. Hand hygiene: simple and complex. Int J Infect Dis. 2005 Jan;9(1):3-14.
16. Alsubaie S, Maither Ab, Alalmaei W, Al-Shammari AD, Tashkandi M, Somily AM, et al. Determinants of hand hygiene noncompliance in intensive care units. Am J Infect Control. 2013 Feb;41(2):131-5.
17. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr. 2016 Apr;4(2):10.1128/microbiolspec.VMBF-0016-2015. doi: 10.1128/microbiolspec.VMBF-0016-2015.
18. Maillard JY. Resistance of bacteria to biocides. Microbiol Spectr. 2018 Apr;6(2). doi: 10.1128/microbiolspec.ARBA-0006-2017.
19. Uttlova P, Urban J, Melicherčíková V, Zavadilová J, Fabianová K. Susceptibility of clinical isolates of *Bordetella pertussis* to chemicals. Epidemiol Mikrobiol Immunol. 2018;67(3):122-8.
20. EN 14561. Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area - Test method and requirements (phase 2/step 2). Brussels: European Committee for Standardization; 2006.
21. Kampf G, Hollingsworth A. Validity of the four European test strains of prEN 12054 for the determination of comprehensive bactericidal activity of an alcohol-based hand rub. J Hosp Infect. 2003 Nov;55(3):226-31.
22. Kramer A, Rudolph P, Kampf G, Pittet D. Limited efficacy of alcohol-based hand gels. Lancet. 2002 Apr 27;359(9316):1489-90.
23. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999 Jan;12(1):147-79.
24. Sidhu MS, Sørum H, Holck A. Resistance to quaternary ammonium compounds in food-related bacteria. Microb Drug Resist. 2002;8(4):393-9.
25. Bragg R, Jansen A, Coetzee M, van der Westhuizen W, Boucher C. Bacterial resistance to Quaternary Ammonium Compounds (QAC) disinfectants. Adv Exp Med Biol. 2014;808:1-13.
26. Ortiz S, López V, Martínez-Suárez JV. Control of *Listeria monocytogenes* contamination in an Iberian pork processing plant and selection of benzalkonium chloride-resistant strains. Food Microbiol. 2014;39:81-8.

Received October 2, 2021

Accepted in revised form November 20, 2022