Scientific Bulletin. Series F. Biotechnologies, Vol. XXIV, No. 1, 2020
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

PRELIMINARY RESULTS ON BACTERIAL CROSS-RESISTANCE ASSESSMENT ASSOCIATED WITH POVIDONE IODINE USAGE

Getuța DOPCEA[1, 2], Alina Elena NANU[2], Ioan DOPCEA[2], Călina Petruța CORNEA[1], Camelia Filofteia DIGUȚĂ[1], Florentina MATEI[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd., District 1, Bucharest, Romania
[2]Eurofins Evic Product Testing Romania SRL, 64-66 Mărăşeşti Blvd, District 4, Bucharest, Romania

Corresponding author email: getuta.dopcea@gmail.com

Abstract

Povidone iodine (PVP-I) is a valuable, widespread antiseptic that could trigger bacterial cross-resistance to antibiotics when used at sub-lethal concentrations, although no acquired bacterial cross-resistance has been reported for PVP-I. In this study are assessed the changes in the antimicrobial susceptibility profile of Staphylococcus aureus and Staphylococcus epidermidis strains after five exposures to 0.3125 % PVP-I. For all Staphylococcus epidermidis tested strains the mean of the minimum inhibitory concentrations (MICs) and of the minimum bactericidal concentrations (MBCs) significantly decreased after repeated exposures to sub-lethal concentrations of PVP-I compared to the initial values. There was no significant change from baseline in mean results of the disk diffusion test of any antibiotic for the tests performed on the analysed strain.

Key words*: cross-resistance, Staphylococcus aureus, Staphylococcus epidermidis, povidone iodine, resistance.*

INTRODUCTION

Povidone iodine (PVP-I) is a valuable, widely used antiseptic in the healthcare industry.
Iodine compounds have immediate antimicrobial activity over a wide range of viruses, spores, bacteria, fungi and protozoa (Paulson, 1999). The antimicrobial effect of PVP-I is driven by cell wall penetration and binding capabilities with amino acids and unsaturated fatty acids leading to cell membrane disruption.
An extensive use of biocides at sub-lethal concentrations could trigger bacterial cross-resistance to antibiotics (Molina-González, 2014; Dopcea et al., 2019), thus still no acquired bacterial cross-resistance has been reported for PVP-I (Dopcea & Matei, 2018). Microorganisms of the genus *Staphylococcus* are one of the most numerous bacteria found on the human skin and they are an important source of healthcare-associated skin infections (Merezeanu, 2016).
In this study we aimed to assess the changes in the antimicrobial susceptibility profile of *Staphylococcus aureus* and *Staphylococcus epidermidis* when exposed to PVP-I.

MATERIALS AND METHODS

Four strains from ATCC were used: *Staphylococcus epidermidis* ATCC 12228 (MSSE), *Staphylococcus epidermidis* ATCC 51625 (MRSE), *Staphylococcus aureus* ATCC 43300 (MRSA) and *Staphylococcus aureus* ATCC 25923 (MSSA).
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of PVP-I (Alfa Aesar) for each tested strain were determined before and after exposure using a modified broth macrodilution method (CLSI, 2012, NCCLS 1999). Briefly, PVP-I was diluted into concentrations between 10% and 0.0195%. The inoculum suspension for each strain was adjusted to 1 x 10^6 CFU/ml and 10 µl of inoculum was added into the test tubes containing 2 ml of PVP-I diluted at different concentrations in Mueller-Hinton broth (MHB) (Tulip Diagnostics (P) Ltd.), also two control test tubes, a positive control tube containing

only PVP-I and a negative control tube containing only MHB, were inoculated. All the tubes were then incubated at 30°C for 24 h. The MIC was defined as the lowest concentration of PVP-I that completely inhibits growth of the tested strain in the tubes. For determination of MBC, 0.5 ml aliquots were plated on tryptic soy agar containing lecithin and polysorbate 80 (TSA+N) (Scharlau, Spain) from all tubes having no visible growth, followed by incubation at 37°C for 24 h. MBC was defined as the lowest PVP-I concentration corresponding to the TSA+N plate with no bacterial growth observed after incubation.

Antimicrobial susceptibility was determined for all strains before and after PVP-I exposure using the disk diffusion test (CLSI, 2006). The following antibiotics were used: penicillin (10 µg), gentamicin (10 µg), tetracycline (5 µg), ofloxacin (5 µg) and doxycycline (30 µg) (all from Becton Dickinson).

Five exposures variants of the strains to 0.3125% PVP-I were performed. Inoculum was produced from overnight culture and adjusted to 1 x 10^6 CFU/ml. Each exposure consisted of 1 ml Mueller Hinton Broth (MHB) (Accumix - Tulip Diagnostics), 1 ml PVP-I and 0.1 ml inoculum. The samples were kept for 24 h at 30ºC. All procedures were repeated two times for a total of three replicates.

Paired sample t test was used to compare MICs, MBCs, and the sizes of antibiotic zones of inhibition before and after exposure to PVP-I.

RESULTS AND DISCUSSIONS

The mean MICs and MBCs results are summarized in Table 1 and Table 2, respectively. Following all five exposure variants to PVP-I, the mean MICs and MBCs had the same values as before exposure for both *Staphylococcus aureus* strains, but for both *Staphylococcus epidermidis* strains a significant reduction in MICs and MBCs of PVP-I after exposure was recorded.

Table 1. Mean MICs results for all strains

	Baseline	After exposure to PVP-I
MSSE	0.3125%	0.1563%*
MRSE	0.3125%	0.1563%*
MRSA	0.3125%	0.3125%
MSSA	0.3125%	0.3125%

* Significantly different from baseline if p≤0.05

Table 2. Mean MBCs results for all strains

	Baseline	After exposure to PVP-I
MSSE	0.6250%	0.3125%*
MRSE	0.6250%	0.3125%*
MRSA	0.6250%	0.6250%
MSSA	0.6250%	0.6250%

* Significantly different from baseline if p≤0.05

The disk diffusion method results are summarized in Table 3 and their evolution between baseline and after PVP-I exposure for all tested strains are shown in Figure 1 for penicillin, Figure 2 for gentamicin, Figure 3 for tetracycline, Figure 4 for ofloxacin and Figure 5 for doxycycline.

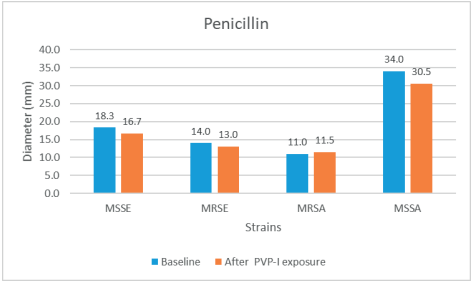

Figure 1. Disk diffusion method results on penicillin for all strains before and after exposure to PVP-I

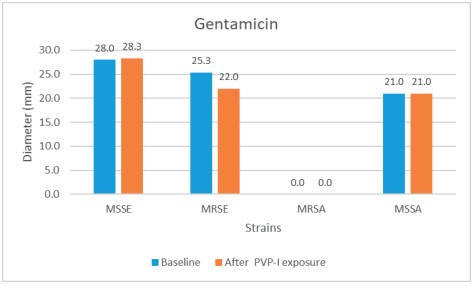

Figure 2. Disk diffusion method results on gentamicin for all strains before and after exposure to PVP-I

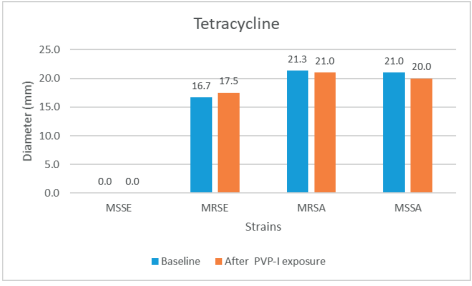

Figure 3. Disk diffusion method results on tetracycline for all strains before and after exposure to PVP-I

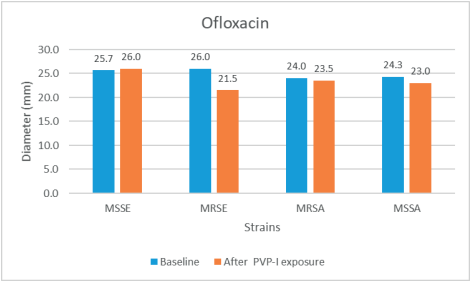

Figure 4. Disk diffusion method results on ofloxacin for all strains before and after exposure to PVP-I

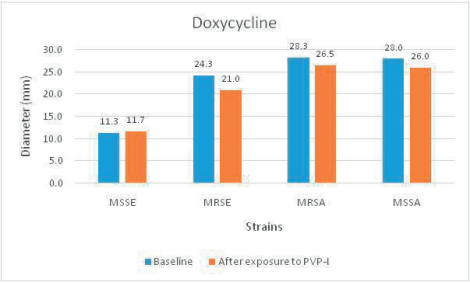

Figure 5. Disk diffusion method results on doxycycline for all strains before and after exposure to PVP-I

After the exposure to PVP-I a decrease in mean results of the disk diffusion tests compared to baseline ranging between 0.3 mm and 4.5 mm was shown. This decrease was observed on both *S. aureus* strains (MRSA and MSSA) for tetracycline, ofloxacin and doxycycline, and moreover for penicillin only on MSSA, also on *S. epidermidis* MRSE strain for penicillin, gentamicin, ofloxacin and doxycycline, however *S. epidermidis* MSSE strain showed a decrease only for penicillin.

There is no significant change from baseline in mean results of the disk diffusion test of any antibiotic for any strain (Table 3). For all four strains there was no clinical change in antimicrobial susceptibility to any of the antibiotics tested following exposure to PVP-I (Table 3). Dopcea et al. (2019) reported no clinical change in antimicrobial susceptibility to any of the antibiotics tested following exposure of the same strains to octenidine dihydrochloride, but when these strains were exposed to chlorhexidine digluconate a clinically decreased susceptibility was observed to penicillin and tetracycline for MSSA and gentamicin for MRSA.

Table 3. Disk diffusion method results for all strains before and after exposure to PVP-I

Penicillin
[Mean diameter (mm) ± SD]

	Baseline		After exposure to PVP-I	
MSSE	18.33 ± 0.15	R	16.67 ± 0.25	R
MRSE	14.00 ± 0.10	R	13.00 ± 0.28	R
MRSA	11.00 ± 0.00	R	11.50 ± 0.07	R
MSSA	34.00 ± 0.26	S	30.50 ± 0.21	S

Gentamicin
[Mean diameter (mm) ± SD]

	Baseline		After exposure to PVP-I	
MSSE	28.00 ± 0.20	S	28.33 ± 0.23	S
MRSE	25.33 ± 0.25	S	22.00 ± 0.71	S
MRSA	0.00 ± 0.00	R	0.00 ± 0.00	R
MSSA	21.00 ± 0.17	S	21.00 ± 0.00	S

Tetracycline
[Mean diameter (mm) ± SD]

	Baseline		After exposure to PVP-I	
MSSE	0.00 ± 0.00	R	0.00 ± 0.00	R
MRSE	16.67 ± 0.15	I	17.50 ± 0.07	I
MRSA	21.33 ± 0.15	S	21.00 ± 0.14	S
MSSA	21.00 ± 0.10	S	20.00 ± 0.14	S

Ofloxacin
[Mean diameter (mm) ± SD]

	Baseline		After exposure to PVP-I	
MSSE	25.67 ± 0.15	S	26.00 ± 0.10	S
MRSE	26.00 ± 0.20	S	21.50 ± 0.35	S
MRSA	24.00 ± 0.10	S	23.50 ± 0.21	S
MSSA	24.33 ± 0.06	S	23.00 ± 0.00	S

Doxycycline
[Mean diameter (mm) ± SD]

	Baseline		After exposure to PVP-I	
MSSE	11.33 ± 0.06	R	11.67 ± 0.12	R
MRSE	24.33 ± 0.12	S	21.00 ± 0.28	S
MRSA	28.33 ± 0.15	S	26.50 ± 0.21	S
MSSA	28.00 ± 0.17	S	26.00 ± 0.00	S

[S]Susceptible
[I]Intermediate
[R]Resistant
*Significantly different from baseline if p≤0.05

The usage of PVP-I as an antiseptic, even at sub-lethal concentration, does not lead to significant variations in antimicrobial susceptibility profile of the strains used in this study.

Although *Staphylococcus aureus* strains, and *Staphylococcus epidermidis* strains did not showed increased MIC and MBC values to PVP-I following exposure and no resistance to these strains was demonstrated, still PVP-I should be tested for resistance against other isolated strains from healthcare facilities.

Among other antiseptics PVP-I remains one of the best options for human skin disinfection in the healthcare facilities, due to its' effectiveness,

safety for use in humans and lack of induced resistance.

CONCLUSIONS

Both *S. epidermidis* strains became more susceptible to PVP-I following exposure to PVP-I.

There was no clinical change in antimicrobial susceptibility profile of any strain used in our study following exposure.

In the conditions of this study the antimicrobial susceptibility profile of all tested strains to penicillin, gentamicin, tetracycline, ofloxacin and doxycycline suffered variations, either to increase the susceptibility or to decrease the susceptibility, which were not statistically significant, compared to the initial values before PVP-I exposure.

Staphylococcal exposure to PVP-I, under the conditions of this study, did not change significantly the antimicrobial susceptibility profile.

REFERENCES

Clinical and Laboratory Standards Institute (CLSI) (2006). *Performance Standards for Antimicrobial Disk Susceptibility Tests*. M2-A9. Approved Standard - Ninth Edition.

Clinical and Laboratory Standards Institute (CLSI) (2012). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. M07-A9. Approved Standard - Ninth Edition.

Dopcea, G., & Matei, F. (2018). Review on Some Current Skin Antiseptics. *Scientific Bulletin. Series F. Biotechnologies*, Vol. XXII, ISSN 2285-1364, 147-158.

Dopcea, N. G., Dopcea, I., Nanu, A., Diguta, C., Matei, F. (2019). Resistance and cross-resistance in *Staphyloccocus* sp. after prolonged exposure to different antiseptics, *Journal of Global Antimicrobial Resistance*. ISSN2213-7165, https://doi.org/10.1016/j.jgar.2019. 10.021.

Merezeanu, N., Gheorghe, I., Popa, M., Lazăr, V., Banu, O., Bolocan, A., Grigore, R., Bertești eanu, S.V., Pântea, O. (2016). Phenotypic and Genotypic Investigation of Resistance and Virulence. Features of Methicillin Resistant *Staphylococcus aureus* Strains Isolated from Hospitalized Patients. *Romanian Biotechnological Letters*, Vol. 21, No. 3, 11591-11598.

Molina-González, D., Alonso-Calleja, C., Alonso-Hernando, A., Capita, R. (2014). Effect of sub-lethal concentrations of biocides on the susceptibility to antibiotics of multi-drug resistant *Salmonella enterica* strains. *Food Control*, Volume 40, 329-334.

NCCLS (1999). *Methods for Determining Bactericidal Activity of Antimicrobial Agents*. Approved Guideline M26-A.

Paulson, DS. (1999). *Handbook of topical antimicrobial testing and evaluation*. New York: Marcel Dekker, Inc.