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Short communication

Variations in Antibiotic Susceptibility Profile of *Staphylococcus aureus* after Povidone-Iodine Stress

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ABSTRACT

Background: *Staphylococcus aureus* is one of the major causes of nosocomial infection which is the most commonly identified agent responsible for skin and soft tissue infections. Cleaning of the skin before an intervention remarkably reduces the risk of infections. The aim of this study was to investigate the effects of povidone-iodine stress on the antimicrobial susceptibility pattern of *S. aureus* resistant subpopulation.

Methods: A 24 h fresh culture was prepared by *S. aureus* inoculation into the tube containing trypticase soy broth (TSB). Then an povidone–iodine resistant subpopulation of strain was selected by exposure to povidone-iodine stress with concentrations 3% and 10% (wt/vol). The resulting survived cells were maintained in 10 mL TSB. The Antibiotic susceptibility testing was performed on strains by disk diffusion method according to CLSI recommendations.

Results: Evaluation of antimicrobial susceptibility pattern revealed significant differences in zone of inhibitions between stressed and unstressed strains. *S. aureus* strains stressed at 3% povidone-iodine concentrations showed statistically smaller zone of inhibition against clindamycin, methicillin and chloramphenicol compared to unstressed strains. Although, 3% povidone-iodine stressed strains were become more susceptible to penicillin G and cephalothin.

Rifampicin, ciprofloxacin, methicillin and chloramphenicol in stressed strains showed a significant decrease in zone of inhibition at 10% povidone-iodine concentrations, and higher sensitivity to co-trimoxazole, cephalothin and cephalexin.

Conclusion: The observed changes in antimicrobial susceptibility patterns of *S. aureus* strains can contribute to povidone–iodine stresses. Although extend study on wider range of skin and wound associated pathogens necessitate to reach a comprehensive conclusion.

Introduction

Staphylococcus aureus is one of the major cause of nosocomial infections which is the most commonly identified agent responsible for skin and soft tissue infections.[1,2] *S. aureus* skin and soft tissue infections may initiate as minor boils or abscesses,[3] but it's may progress to severe infections when there is a breach such as wound that allows the organism access to adjoining tissues or the bloodstream.[4,5] Primary skin infections treatment consists of incising and draining the lesion and accompanied by antimicrobial drugs administration.[3,6] However, emerge of drug resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) made treatment of *S. aureus* infections as a serious challenge for public health.[7] Reports of MRSA isolation incidence from Iran shown a higher rate compared to the reports from neighboring countries in the Middle East which calls for a great attention to understand resistance mechanisms for preventing future outcome.[8]

Cleaning the skin before an intervention remarkably reduces the infection risk that emphasized the need for reliable and effective antiseptics.[9,10] Currently, several antiseptics are being used for this purpose including chlorhexidine and povidone-iodine.[11] Povidone–iodine solution

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(10% povidone-iodine; PVP-I) is Iodophor-containing products and broad-spectrum microbicides which commonly used for topical cleansing, wound care, and antisepsis.[3] Povidone-iodine acts by destroying microbial protein and DNA.[12] *In vitro* studies substantiate povidone-iodine effectiveness against *S. aureus*, especially MRSA strains.[9] There have been no previous reports of bacterial resistance to povidone–iodine preparations. However, previously the potential role of antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance by cross resistance mechanism has been shown.[13] The aim of this study was to investigate the effects of povidone–iodine stress on the antimicrobial susceptibility pattern of *S. aureus*.

Materials and Methods

Bacterial strains and stress conditions

S. aureus ATCC 25923 which is a methicillin-sensitive *Staphylococcus aureus* (MSSA) were used in this study obtained from the Iranian Research Organization for Science and Technology. A 24 h fresh culture (exponential phase) was prepared by *S. aureus* inoculation into the tube containing trypticase soy broth (TSB; Merck, Germany). Then a povidone–iodine resistant subpopulation of strain was selected from original population by exposure to povidone-iodine stress with concentrations 3% and 10% (wt/vol) in pH 7 for 5 minute at room temperature. The commercial stock of povidone-iodine 10% (100 mL contain 10 g povidone-iodine) provide by regional pharmacy and 100 mL povidone-iodine 3% aliquot was prepared from original stock. The resulting survived cells were harvested by centrifuged at 3,000 ×*g* for 15 min and followed re-suspended in a tube containing 10 mL TSB and incubated at 37 °C for an overnight. Adjusting the turbidity of bacteria suspension was accordance to 0.5 McFarland standard.

Antibiotic susceptibility testing

Cell suspensions containing stressed and normal bacterial cells were seeded on Mueller Hinton agar (Merck, Germany) plates using a sterile swab. The Antibiotic susceptibility testing was performed on strains by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations.[14] The followed antibiotic discs of erythromycin (15µg), penicillin G (10 U), gentamicin (10 µg), ciprofloxacin (5 µg), cefalexin, (30 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg), rifampicin (5 µg), clindamycin (2 µg), cephalothin (30 µg) and methicillin (25 µg) were used for screening of antimicrobial susceptibility patterns. The plates were incubated for 18 h at 37 ° C and then examined 4 times for the development of zones of inhibition.

Statistical analysis

Analysis was performed using SPSS™ software, version 21.0. Values were expressed as the mean ± standard deviation (continuous variables). Paired t-tests were used to analyze the results. The level of P <0.05 was considered as statistical significance.

Results

The results of antibiotic susceptibility testing revealed significant differences in zone of inhibitions between povidone–iodine resistant subpopulation and unstressed *S. aureus* strains. The variation in zone of inhibitions was seen almost toward all tested antibiotics; however, the significant differences in zone of inhibition were observed against nine of tested antibiotics. The mean of inhibition zones for stressed and unstressed strains are presented in Table 1.

Table 1. Mean zone of inhibition for stressed and unstressed *S. aureus* strains.

Antibiotics	Mean of zone size (mm)		
	Stressed with povidone–iodine		Unstressed
	3%	10%	
Rifampicin	25±3	21±3[a]	25±1
Penicillin G	36±2[a]	32±3	32±1
Clindamycin	19±1[a]	22±1	21±1
Gentamicin	18±2	20±2	19±1
Erythromycin	22±2	20±1	22±1
Co-trimoxazole	20±1	24±1[a]	21±1
Ciprofloxacin	24±2	20±2[a]	24±1
Cephalothin	34±2[a]	34±1[b]	30±1
Cephalexin	23±2	25±1[b]	22±1
Methicillin	11±3[a]	10±1[b]	19±1
Chloramphenicol	20±1[b]	25±2[b]	32±1

[a]Compared to unstressed strain differences was statistically significant (P <0.05); [b] Compared to unstressed strain differences was statistically significant (P <0.001).

S. aureus strains stressed at 3% povidone–iodine concentrations showed statistically smaller zone of inhibition against clindamycin, methicillin and chloramphenicol compared to unstressed strains. Although, at 3% povidone–iodine stress *S. aureus* strains were become more susceptible to penicillin G and cephalothin.

Rifampicin, ciprofloxacin, methicillin and chloramphenicol showed a significant decrease in zone of inhibition at 10% povidone–iodine concentrations compared to unstressed strains. Moreover, 10% povidone–iodine stress were mostly associated with higher sensitivity to co-trimoxazole, cephalothin and cephalexin.

Discussion

Biocide agents usage have very long history and bacterial adaptation and resistance to biocides is not a new phenomenon.[15] Previous conducted studies demonstrated some evidences in confirm of this phenomenon.[15] Biocidal activity can be affected by various factors such as concentration, period of contact, pH, temperature, the presence of interfering material, and the types, numbers, location, and condition of microorganisms.[16]

Observations of current study revealed that *S. aureus* can adaptively growing at two povidone–iodine concentrations, 3%, and 10% (wt/vol). This finding is consistent those of some previous reports which stated bacterial isolates with biofilm forming ability may able to survive in commercially manufactured povidone–iodine solutions or decreased susceptibility to iodine.[17]

To survive in the environment, bacteria will do all in their power to respond a harmful stresses. Stress like exposed to a wide range of antibiotics and biocides that could act as a selective pressure for the development and isolation of resistant isolates by several mechanisms.[16] There is no previous data on effects of povidone–iodine stress against bacterial strains; however, several authors reports adverse consequence of environmental stresses on bacterial isolates, including antibiotic susceptibility changes.[18-20] Our results show that povidone–iodine stresses causes significant changes in antibiotic susceptibility patterns in tested strain. Biocide and antibiotic action have some similarities and differences. These similarities include the penetration of cationic agents, entry by passive diffusion, membrane damaging, similar morphological changes and a shared target sites.[16] Some biocides can induce efflux even though they are not substrates. So cross resistance may be an explanation for susceptibility decreased to antibiotics in our findings.[16] Some authors claimed that widespread use of biocides in healthcare setting, industrial, and other facilities may contributes to the overall rate of drug resistance.[16,21] Previously a link has been shown between insusceptibility to quaternary ammonium compounds (QACs) and resistance to ampicillin and penicillin in clinical isolates of human and food-related staphylococci.[16]

The basic mechanism of povidone–iodine action expressed by multiple modes of action that include the disruption of microbial metabolic pathways and alteration in structural components of cell membranes.[22] Although the exact mechanism of antibiotic susceptibility changes in povidone–iodine resistant subpopulation is unknown, the alterations of cell membranes structure or short-term phenotypic changes could be explanation for these findings. In support of this, previous works indicate that microorganisms may adapt to environmental stresses by several mechanism including alterations in the cell envelope, membrane permeability, porin synthesis overexpression of multi-gene components or operons, and alteration of antibiotic target sites.[17]

In our findings, toward some tested antibiotics povidone–iodine stressed strains show higher susceptibility than unstressed strains. Previously, McMahon *et al.* noted increasing in antibiotic susceptibility of stressed *E. coli*, *S. enterica* serovar Typhimurium, and *S. aureus* strains.[20] Respond to environmental stresses may followed by stimulate the expression of specific genes which may give a uniform properties to stressed cells. The well knowing example is heat-shock proteins (HSPs) that are produced when bacteria are subjected to a wide range of physicochemical agents.[23] However, this matter afford further studies with the aiming to investigate protein profile alterations and signaling mechanisms of stressed strains.

Conclusion

In summary the most of antibiotic susceptibility variations were tend to decreasing sensitivity, which may be a great concern for dealing with povidone–iodine resistant subpopulation. Regarding to results, one of the considerable observation that can effect on course of antibiotic therapy in povidone–iodine resistant subpopulation was such a significant sensitivity reduction toward methicillin. The observed changes in antimicrobial susceptibility pattern of *S. aureus* strains can contribute to povidone–iodine stress; although, a great deal remains to determine the exact mechanism of antibiotics susceptibility variation. Moreover, extend study on wider range of skin and wound associated pathogens necessitate to reach a comprehensive conclusion.

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Conflict of interests

The authors claim that there is no conflict of interest.

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