COMPARATIVE EVALUATION OF THE EFFECTS OF ANTISEPTIC AGENTS ON ISOLATES FROM COMPLICATED GUNSHOT WOUNDS (IN VITRO)

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https://doi.org/10.71066/FTTMK.2.22

Abstract

The increasing prevalence of antimicrobial resistance among microorganisms associated with complicated gunshot wounds highlights the need for effective local antiseptic treatment. The aim of this study was to comparatively evaluate the in vitro antimicrobial activity of commonly used antiseptic agents against Gram-positive clinical isolates obtained from complicated gunshot wounds. Clinical isolates of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Streptococcus pyogenes* were examined. The antimicrobial activity of povidone-iodine, decamethoxin, dioxidine, chlorhexidine, Cutasept, and miramistin was evaluated using a serial dilution method at concentrations ranging from 12.5% to 75.0% of the original commercial preparations. The minimum concentration capable of inhibiting microbial growth was determined for each antiseptic–microorganism combination. Considerable differences in susceptibility were observed among both the bacterial species and the antiseptic agents tested. Povidone-iodine demonstrated the highest overall antimicrobial activity, inhibiting most isolates at the lowest concentrations examined. Particularly high susceptibility to povidone-iodine was observed in *S. aureus*, *S. epidermidis*, and *E. faecalis*. Decamethoxin, chlorhexidine, and Cutasept also showed antimicrobial activity, although generally at higher concentrations, whereas dioxidine and miramistin were less effective against some of the tested isolates. The findings demonstrate that the antimicrobial efficacy of antiseptic agents against microorganisms isolated from complicated gunshot wounds is both agent- and species-dependent. Determination of minimum effective concentrations may therefore support the rational selection of topical antiseptic agents and contribute to optimizing the local treatment of infected wounds.

Keywords: antiseptics; complicated gunshot wounds; antimicrobial activity; povidone-iodine; Gram-positive bacteria; antimicrobial resistance

Introduction

The use of antiseptic agents is one of the key components of the local treatment of infected wounds, particularly in the context of the increasing prevalence of antimicrobial resistance among clinically relevant microorganisms. This issue is of particular importance in the management of complicated gunshot wounds, which are characterized by extensive microbial contamination, substantial tissue damage, the presence of foreign bodies, and a high risk of purulent-septic complications. Unlike systemic antibacterial agents, antiseptics are applied directly to the affected area, thereby providing a high local concentration of the active substance while minimizing the risk of systemic adverse effects. Their antimicrobial activity is mediated through disruption of cell membrane integrity, protein denaturation, induction of oxidative processes, or inhibition of essential microbial metabolic pathways.

In current clinical practice, a wide range of antiseptic agents is used for the local treatment of wounds, with povidone-iodine, chlorhexidine digluconate, decamethoxin, dioxidine, miramistin, hydrogen peroxide, and other agents being among the most commonly used. The choice of a specific antiseptic is determined by the characteristics of the wound process, the expected

spectrum of microorganisms, the stage of wound healing, and the particular clinical situation. This choice is especially important in the management of complicated gunshot wounds, which are characterized by polymicrobial communities, biofilm formation, and the circulation of multidrug-resistant nosocomial strains.

For a long time, the development of resistance to antiseptic agents was considered unlikely compared with resistance to antibiotics. However, recent studies indicate a gradual decrease in the susceptibility of certain clinical isolates to some antiseptics, particularly following prolonged exposure, exposure to subinhibitory concentrations, or bacterial growth within biofilms. Therefore, determining the antiseptic susceptibility of isolates recovered from complicated gunshot wounds represents an important area of current research, as it provides a rationale for selecting the most effective agents for local therapy and enables assessment of the potential risk of developing antiseptic tolerance.

One of the most extensively studied and widely used antiseptic agents is povidone-iodine (PVP-I). It is a water-soluble complex of polyvinylpyrrolidone and iodine, in which the polymer acts as a carrier and ensures the gradual release of active iodine into the aqueous environment. This equilibrium between bound and free iodine helps maintain stable antimicrobial activity while reducing the tissue-irritating effects associated with elemental iodine (Bigliardi et al., 2017).

The povidone-iodine complex was first synthesized in 1956 by Harry A. Shelanski and Margaret V. Shelanski and is currently included among the antiseptic agents recommended by European guidelines for the local treatment of gunshot wounds, stab wounds, and bite wounds (Babalska et al., 2021). Its antimicrobial effect is mediated by the rapid penetration of free iodine into microbial cells, where it oxidizes proteins, nucleic acids, and membrane lipids, resulting in irreversible damage to essential cellular structures and subsequent microbial cell death. Povidone-iodine exhibits an exceptionally broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains, as well as fungi, protozoa, and both enveloped and non-enveloped viruses. With prolonged contact, it also demonstrates activity against certain bacterial spores.

An important advantage of povidone-iodine is its ability to disrupt established bacterial and fungal biofilms both under in vitro conditions and in experimental models designed to closely mimic clinical settings (Lepelletier et al., 2020).

The efficacy of povidone-iodine has been confirmed by numerous experimental studies demonstrating its activity against a broad spectrum of pathogens, including bacteria, fungi, viruses, bacterial spores, protozoa, and amoebic cysts (Bigliardi et al., 2017). In particular, a 10% povidone-iodine solution exhibits rapid bactericidal activity against clinical isolates of Staphylococcus aureus, irrespective of their methicillin susceptibility. According to experimental data, a bactericidal effect against both methicillin-susceptible S. aureus (MSSA) and methicillin-resistant S. aureus (MRSA) was achieved within 15–60 seconds of exposure to the antiseptic. Furthermore, comparative in vitro studies have demonstrated greater efficacy of povidone-iodine against MRSA than chlorhexidine, with povidone-iodine retaining its antimicrobial activity even against strains exhibiting reduced susceptibility to chlorhexidine (Lepelletier et al., 2020).

Of particular importance is the fact that, despite more than a century and a half of clinical use of iodine-based preparations, no convincing evidence has been obtained for the development of acquired or cross-resistance of microorganisms to this antiseptic. This represents an important advantage of povidone-iodine over several other topical antimicrobial agents (Bigliardi et al., 2017).

Dioxidine, a synthetic quinoxaline di-N-oxide derivative, occupies an important place among topical antimicrobial agents. Its bactericidal activity is mediated by inhibition of DNA synthesis and disruption of nucleic acid metabolism in bacterial cells. The drug has been used for several decades in the treatment of purulent-inflammatory conditions, including infected wounds and soft tissue infections. Dioxidine exhibits a broad spectrum of antimicrobial activity, including activity against multidrug-resistant strains, and experimental studies suggest that its efficacy may increase

under anaerobic conditions due to enhanced generation of reactive oxygen species (ROS). Moreover, bacterial DNA damage has been shown to occur even at concentrations below the minimum inhibitory concentration (MIC) (Zharkova et al., 2023). However, the clinical use of dioxidine is limited by its potential toxicity, particularly its ability to cause damage to the adrenal cortex, which is of particular concern in pediatric practice (Ali et al., 2021).

Available literature data indicate that dioxidine exhibits greater activity against Gram-negative bacteria than against Gram-positive bacteria, including Staphylococcus aureus. Eradication of certain Gram-positive isolates, such as S. epidermidis, requires substantially higher concentrations of the drug than those required to inhibit the growth of Gram-negative microorganisms. It should be noted that some of these isolates also exhibited resistance to fluoroquinolones. Despite certain structural similarities between dioxidine and other quinoxaline derivatives, no convincing evidence of cross-resistance between dioxidine and fluoroquinolones has been reported to date. Moreover, according to pharmacological recommendations, dioxidine is considered appropriate for use in cases where other antibacterial agents have demonstrated insufficient efficacy (Zharkova et al., 2023; Publishing Group “GEOTAR-Media,” 2015).

Decamethoxin is another antiseptic agent currently used in the treatment of infected wounds. Its antimicrobial activity is based on its interaction with phospholipid components of the microbial cytoplasmic membrane, resulting in disruption of membrane structural organization, increased membrane permeability, loss of cellular homeostasis, and subsequent bacterial cell death (Osmanov et al., 2020). According to the prescribing information, decamethoxin exhibits a broad spectrum of antibacterial and antifungal activity and is indicated for the topical treatment of infectious and inflammatory conditions. In vitro studies have demonstrated its high activity against pandrug-resistant clinical isolates, as well as its ability to inhibit the growth of bacterial strains with a pronounced biofilm-forming capacity (Kolesnyk et al., 2024).

Chlorhexidine is one of the classical topical antiseptic agents and has been widely used in medical practice for several decades. Its antimicrobial activity is associated with damage to the bacterial cytoplasmic membrane, resulting in disruption of the membrane potential, leakage of intracellular contents, and subsequent cell death (Kuyyakanond & Quesnel, 1992). At the final stage of its bactericidal action, cytoplasmic coagulation and precipitation of complexes with phosphorylated compounds occur (Poppolo Deus & Ouanounou, 2022).

At the same time, increasing evidence suggests the development of cross-resistance between chlorhexidine and certain antibiotics or other antiseptic agents. The major mechanisms underlying this phenomenon are thought to include modification of the bacterial outer membrane, alterations in lipopolysaccharide structure, and activation of cellular defense systems. For example, vancomycin-resistant Enterococcus faecium isolates have been shown to exhibit reduced susceptibility to chlorhexidine, while cross-resistance between chlorhexidine and colistin has been reported in Klebsiella pneumoniae. In Pseudomonas stutzeri, resistance to chlorhexidine has been associated with reduced susceptibility to gentamicin, polymyxin, triclosan, ampicillin, and benzalkonium chloride (Abbood et al., 2023).

Among the antiseptic agents widely used in Ukraine, miramistin also occupies an important place. It is a cationic surfactant with antibacterial, antifungal, and antiviral activity. Its mechanism of action is based on the electrostatic interaction of positively charged regions of the molecule with negatively charged components of microbial membranes. Following insertion of the hydrophobic portion of the molecule into the lipid bilayer, membrane structure becomes destabilized, membrane permeability is disrupted, receptor systems are impaired, and intracellular contents are released. At high concentrations, miramistin can cause complete membrane disruption accompanied by micelle formation. Some studies also suggest that miramistin may interact with bacterial DNA (Osmanov et al., 2020).

Thus, current evidence indicates that, despite the wide range of antiseptic agents available, their activity against clinical isolates may vary considerably depending on the microbial species, biofilm-forming capacity, and the presence of antimicrobial resistance mechanisms. Therefore, a comparative in vitro assessment of the susceptibility of microorganisms isolated from

complicated gunshot wounds to the most commonly used antiseptic agents is highly relevant and of considerable practical importance. Such studies provide a basis for the rational selection of topical antiseptic therapy, enable assessment of the potential efficacy of individual antiseptic agents, and facilitate the identification of possible trends toward reduced susceptibility among clinically significant pathogens.

Materials and Methods

Clinical specimens were obtained from patients with complicated gunshot wounds who were undergoing inpatient treatment in the surgical departments of hospitals in Uzhhorod, Ukraine. Samples were collected from the wound surface using sterile FLmedical transport systems (Italy) and subsequently transported to the laboratory in accordance with current requirements for microbiological examination.

Following the isolation and identification of pure cultures, clinically significant Gram-positive isolates were selected for further analysis, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Streptococcus pyogenes*.

Commercial antiseptic preparations widely used in clinical practice were evaluated for antimicrobial activity: decamethoxin (Dekasan, Yuria-Pharm LLC, Ukraine), povidone-iodine (Betadine, EGIS Pharmaceuticals PLC, Hungary), dioxidine (Dioxidine, Farmak JSC, Ukraine), Cutasept (BODE Chemie GmbH, Germany), chlorhexidine (Pharmaceutical Factory LLC, Ukraine), and miramistin (Miramistin, Darnitsa Pharmaceutical Company PJSC, Ukraine).

The susceptibility of clinical isolates to the antiseptic preparations was determined using a serial dilution method at working concentrations corresponding to 12.5%, 25.0%, 37.5%, 50.0%, 62.5%, and 75.0% of the original commercial preparation. Fresh 24-hour microbial cultures were used to prepare standardized inocula in sterile physiological saline. The minimum antiseptic concentration at which inhibition of growth of the tested isolate was observed was used as the evaluation criterion. For each microbial species, the minimum concentration exhibiting antimicrobial activity was determined.

The results were analyzed by comparing the minimum effective concentrations of the tested antiseptic preparations against clinical isolates of different microbial species. This approach enabled a comparative assessment of the antimicrobial activity of the individual antiseptics and identification of the preparations capable of inhibiting bacterial growth at the lowest concentrations.

Results and Discussion

Comparative analysis of antiseptic activity against Gram-positive clinical isolates revealed substantial differences in the minimum concentrations of the tested agents required to inhibit microbial growth. Overall, povidone-iodine exhibited the lowest threshold concentrations at which bacterial susceptibility was observed, indicating high antimicrobial activity across the tested microbial species (Table 1).

Among Staphylococcus aureus isolates, povidone-iodine demonstrated the highest activity, with some strains showing susceptibility at concentrations as low as 12.5%. Chlorhexidine also exhibited relatively high activity, inhibiting bacterial growth at concentrations starting from 37.5%. The minimum effective concentration of Cutasept was 50%. In contrast, decamethoxin and miramistin exhibited antimicrobial activity only at substantially higher concentrations (62.5%), whereas dioxidine was the least active agent against S. aureus, with susceptibility of some isolates observed only at the highest concentration tested (75%).

Staphylococcus epidermidis isolates exhibited considerably greater susceptibility to most of the antiseptic agents tested. The lowest concentrations at which antimicrobial activity was observed were 12.5% for both povidone-iodine and dioxidine. Decamethoxin inhibited bacterial growth at a concentration of 37.5%, whereas the minimum effective concentration of Cutasept was 50%. These findings indicate a high susceptibility of S. epidermidis to topical antiseptic agents, particularly iodine-based preparations.

Among Enterococcus faecalis isolates, povidone-iodine again demonstrated the highest antimicrobial activity, with susceptibility observed at concentrations as low as 12.5%. Cutasept inhibited bacterial growth at a concentration of 37.5%, whereas decamethoxin and dioxidine exhibited activity only at concentrations of 50% or higher. Notably, E. faecalis isolates recovered as monocultures were completely resistant to dioxidine, whereas among isolates recovered from polymicrobial associations, some strains exhibited susceptibility to this agent at a concentration of 50%.

The lowest susceptibility among the Gram-positive microorganisms tested was observed for Streptococcus pyogenes isolates. The minimum effective concentrations of povidone-iodine and dioxidine were 50%, whereas decamethoxin and Cutasept exhibited antimicrobial activity only at a concentration of 62.5%, indicating that higher concentrations of these agents were required to achieve a bactericidal effect.

Thus, among the antiseptic agents tested, povidone-iodine exhibited the lowest minimum concentrations required to inhibit the growth of the majority of Gram-positive clinical isolates. Dioxidine demonstrated similarly high activity against Staphylococcus epidermidis. In contrast, decamethoxin, Cutasept, and particularly miramistin required substantially higher concentrations to exert antimicrobial activity, whereas dioxidine was the least effective agent against Staphylococcus aureus and Enterococcus faecalis isolates.

Table 1. Minimum concentrations of antiseptic agents at which antimicrobial activity against clinical isolates of Gram-positive microorganisms was observed

Microorganism	Povidone-iodine	Decamethoxin	Dioxidine	Chlorhexidine	Cutasept	Miramistin
Staphylococcus aureus	12.5%	62.5%	75%	37.5%	50%	62.5%
Staphylococcus epidermidis	12.5%	37.5%	12.5%	–	50%	–
Enterococcus faecalis	12.5%	50%	50%	–	37.5%	–
Streptococcus pyogenes	50%	62.5%	50%	–	62.5%	–

Conclusions

The present study demonstrated that clinical isolates of Gram-positive microorganisms recovered from complicated gunshot wounds exhibited varying levels of susceptibility to the antiseptic agents tested. Povidone-iodine showed the highest antimicrobial activity, inhibiting the growth of the majority of isolates at the lowest concentrations tested. Decamethoxin, chlorhexidine, and Cutasept also exhibited pronounced antimicrobial activity; however, higher concentrations were generally required to achieve a comparable inhibitory effect. Dioxidine and miramistin demonstrated the lowest activity against some of the microorganisms tested.

The findings support the relevance of comparative assessment of the susceptibility of clinical isolates to antiseptic agents and highlight the importance of determining their minimum effective concentrations when selecting topical therapy for infected gunshot wounds. These results may provide an experimental basis for further studies aimed at optimizing the use of antiseptic agents in the management of wound infections.

Acknowledgements

The authors gratefully acknowledge the support of the CEEPUS program, which enabled student and staff mobility and thereby made the joint research and publication possible.

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