In vitro efficacy of various topical antimicrobial agents against multidrug-resistant bacteria

ABSTRACT
Aim: To evaluate the in vitro efficacy of nine topical antimicrobial agents frequently used in our burn centre against five multidrug-resistant bacterial strains isolated from burn wounds of our patients.

Material and methods: A simple and reproducible in vitro model was used to evaluate the effects of the following topical antimicrobials: 1% silver sulfadiazine, neomycin with bacitracin, 1% acetic acid, 0.2% nitrofurazone, 1% acetic acid with 1% silver sulfadiazine, Acticoat®, Aquacel®Ag, Atrauman®Ag, and Ialugen®Plus. Survival of five bacteria planktonic forms (Pseudomonas aeruginosa, methicillin-resistant Staphylococcus aureus, Staphylococcus haemolyticus, Enterococcus faecalis, and Escherichia coli) was evaluated 24-hours after application.

Results: The highest efficacies against all strains were found with 1% silver sulfadiazine, 1% silver sulfadiazine + 1% acetic acid, Acticoat®, Aquacel®Ag and Ialugen®Plus. The combination of neomycin with bacitracin in ointment form and Atrauman®Ag were absolutely ineffective.

Conclusions: Topical antimicrobial agents play an important role in the treatment of burns, but they should be used according to the efficacy against bacterial strains in wounds. Thanks to our results we can modify the spectrum of topical antimicrobials used in our burn centre. In the future, we would like to examine the efficacy of the topical agents using an in vitro biofilm model.

INTRODUCTION
Improvements in burn wound care have led to longer survival and potentially an extended hospital stay. The most likely cause of death is an infectious complication after surviving the initial burn insult and resuscitation period[1]. It is now estimated that approximately 75% of the mortality following thermal injuries is directly related to infection[2,3,4]. Burn patients are at high risk for infections caused by nosocomially-acquired multidrug-resistant organisms because of generalised humoral and cellular immunodeficiency, gastrointestinal translocation of microorganisms, prolonged hospital stay, and invasive diagnostic and therapeutic procedures[2,5]. The standard of care at specialised burn centres worldwide is early excision of necrotic tissues and grafting, which results in decreased mortality. However, the benefit of topical antimicrobial agents is that they can be used at all levels of care. Another benefit of topical antimicrobials is that they can be applied directly to the site of colonisation or infection and can be used for both prophylaxis and treatment of burn wound infections[6]. According to 43% of burn specialists worldwide, the antimicrobial activity in a burn dressing is considered to be essential[7]. Bacteria with resistance to multiple systemic antimicrobials are increasing in prevalence, which raises the concern for a subsequent increase in resistance to topical antimicrobial agents[6,8,9]. In the last decades, one of the most commonly applied topical agents for partial-thickness burns has been silver sulfadiazine[10,11]. Despite the possible but uncommon side effects attributed to silver sulfadiazine[9,12], this agent is also a reference standard therapy in many studies.

The most frequently used topical agent in our burn centre is a combination of 1% silver sulfadiazine cream and 1% acetic acid solution. In addition to our own clinical experience, several studies have affirmed the efficacy of this solution[13-17]. According to the European Wound Management Association position document [Management of wound infection (2006)], the selection of antimicrobial agents to reduce or eradicate micro-organisms must be influenced by the specificity and efficacy of the agent. However, the decision for
selection of a particular topical antimicrobial agent in our burn centre has so far been mostly empiric. The common standard is to take a swab from the burn wound at admission and at least once per week, after which the sample is sent to the microbiological laboratory for evaluation of wound flora and susceptibility testing. The results inform us of bacterial susceptibility to most of the systemic antibiotics, but not to topical ones. From the high variety of topical antimicrobials only the minimal inhibition concentration of nitrofurazone and bacitracin are routinely tested in our laboratory so far.

**AIM**

The aim of this study was to evaluate and compare the efficacy of nine topical antimicrobials and investigate whether the multidrug-resistant bacteria isolated from burns of our hospitalised patients are still susceptible against the most frequently applied agents in our burn centre.

**MATERIAL AND METHODS**

Multidrug-resistant bacteria isolated in the year 2011 from burn wounds of patients hospitalised in the Bratislava Burn Centre were used to create a bacterial collection stored in the research laboratory of the Institute of Microbiology. We tested the susceptibility of five bacterial strains \[Pseudomonas aeruginosa\], methicillin-resistant \[Staphylococcus aureus\] (MRSA), \[Staphylococcus haemolyticus\], \[Enterococcus faecalis\], and \[Escherichia coli\] in planktonic form against nine topical antimicrobials (Table 1) using the modified quantitative method originally introduced by Hammond A. et al. [18]. In comparison with the original method, solutions and impregnated dressings were also tested in addition to ointments.

We placed three sterile 6-mm cellulose disks on a petri dish with Luria Bertani agar (LB-agar), and 10 μl bacterial suspension containing \(10^2\)-\(10^3\) colony forming units (CFU) was dropped on each disk (Figure 1). The surface of the dish was covered with a sterile gauze square (5 x 5 cm) saturated with topical antimicrobials, or with one of the commercially produced impregnated dressings. From each cream/ointment, 1 g was uniformly spread on the gauze. From solutions, 2-ml aliquots were dropped to saturate the gauze. A sterile gauze square without topical antimicrobials was used as a control.

A sterile small glass petri dish was placed on the gauze to maintain direct contact between the agent and the inoculated disks (Figure 2). The dishes were incubated at 37°C for 24 hours, after which the gauze squares were removed and each disk transferred into a sterile tube containing 1 ml phosphate buffered saline (PBS). The tubes were vigorously vortexed three times for 2 minutes to detach bacteria from the disks. Suspended cells were serially diluted four times (a 10-fold dilution was used) in PBS and 10-μl aliquots of each dilution were inoculated on LB agar plates. These plates were incubated at 37°C for 16 hours and the numbers of CFU were counted (Figure 3). The final result recorded represented \(\geq 10^8\) CFU/disk if the bacterial growth was so massive that the number of CFU obtained was uncountable even in the highest dilution.

**RESULTS**

Using five multidrug-resistant bacterial strains in planktonic form our results demonstrated strong bactericidal action of five topical antimicrobial agents: 1% silver sulfadiazine, 1% silver sulfadiazine with 1% acetic acid, Acticoat®, Aquacel®Ag, and Ialugen®Plus. In all of these samples tested no bacterial growth was detected 24 hours after their application. The same bactericidal effect against MRSA, S. haemolyticus, and P. aeruginosa was found with 1% acetic acid. On the other hand, this agent had only a bacteriostatic effect against E. coli and E. faecalis. Nitrofurazone in a 0.2% concentration

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<tr>
<th>Table 1: List of the tested topical antimicrobial agents</th>
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<td>Solutions</td>
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<td>1% Acetic Acid</td>
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<td>1% Silver Sulfadiazine</td>
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<td>1% Silver Sulfadiazine with 1% Acetic Acid</td>
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<td>Ialugen®Plus</td>
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<td>1% silver sulfadiazine, 0,2% natrii hyaluronas</td>
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was highly effective as well, with the exception against *P. aeruginosa* where no effect was observed. On the other hand, neomycin with bacitracin and Atrauman®Ag were found ineffective against all of the bacteria tested, as we observed the same bacterial growth using these agents as in the control samples with sterile gauze only. The results are shown in Figure 4.

**DISCUSSION**

Bacterial resistance belongs to the most severe problems in modern medicine. Some authors suggest that resistance against topical antimicrobials increases similar to resistance seen against systemic antibiotics[19]. Animal and clinical studies are performed to determine the efficacy of topical antimicrobials in vitro. While studies on patients are focused on comparisons between new agents and standard therapy[12,20,21], in vitro models are designed to test more agents[1,19,22,23]. To show the efficacy of topical antimicrobials against bacterial flora from the wounds of patients it is necessary to establish a standard in vitro model. This is important to test and compare the efficacy of different forms (solutions, ointments, etc.) of agents. We proved that a modification of the method introduced by Hammond A. et al. (2011) provides this possibility. Our in vitro method for testing and comparing antimicrobial efficacy is simple and reproducible, and the quantitative analysis of the surviving microbial inoculum is fully sufficient to demonstrate the efficacy of the tested agents. The majority of results obtained in our study are in concordance with results of other already published studies. For instance, we demonstrated resistance of *P. aeruginosa* to 0,2% nitrofurazone, which, on the other hand, had a bactericidal effect on the rest of the bacterial strains tested. These findings are similar to those of Conly et al.[24]. We also found high efficacy of 1% silver sulfadiazine, despite it having been the most frequently used topical antimicrobial in our burn centre for many years and that evidence for bacterial resistance to silver has been reported[25-27]. Similarly high efficacy of this agent was demonstrated by Koo et al.[28]. In addition to our results, the findings of Ryssel et al.[13-16] have shown a bacterio-
We demonstrated that the topical antimicrobial agents of our tests were performed in vitro on planktonic bacteria in concordance with results published by other groups. We demonstrated excellent efficacy of five topical antimicrobials in preventing wound infection; however, all of our tests were performed in vitro on planktonic bacteria only, and further research on bacterial biofilms and in the clinical setting will be necessary.

**CONCLUSIONS**

We demonstrated that the topical antimicrobial agents most frequently used in our burn centre (1% silver sulfadiazine and 1% acetic acid) have high efficacy against multidrug-resistant bacteria isolated from burned wounds of our patients.

**Implication for clinical practice**

The use of topical antimicrobials is very important not only according to usual practice but also according to the establishment of their efficacy in vitro. Even if we were not able to test all varieties of bacteria from wounds of our patients, we have tested the efficacy of the most regularly used agents against resistant strains of bacteria can help burn specialists to prevent and treat burn wound infection more effectively.

Further research

We are currently performing tests on bacteria in a biofilm form of growth to find out the efficacy of the tested agents in burns treated several hours/days after injury. This testing will also be expanded to a larger number of clinical bacterial strains.

References


[11] Stand O, San Miguel L, Rowan S, Sahlqvist A. Comparison of silver-coated dressing (Acticoat®), chlorhexidine acetate 0.5% (Bactigrass®), and fusidic acid 2% (Fucidin) for topical antibacterial effect in methicillin-resistant Staphylococci-contaminated, full-skin-thickness rat burn wounds. Burns 2005 Nov: 31:7-874.


[21] Stand O, San Miguel L, Rowan S, Sahlqvist A. Comparison of silver-coated dressing (Acticoat®), chlorhexidine acetate 0.5% (Bactigrass®), and fusidic acid 2% (Fucidin) for topical antibacterial effect in methicillin-resistant Staphylococci-contaminated, full-skin-thickness rat burn wounds. Burns 2005 Nov: 31:7-874.


