Burn Wound Infection Susceptibilities To Topical Agents: The Nathan’s Agar Well Diffusion Technique

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ABSTRACT Originally established in 1977 and revised in 1989, Nathan’s Agar Well Diffusion (NAWD) is currently the most successful technique used in evaluating the efficacy of topical antimicrobial agents against various bacterial isolates of burn wound infections.

Methods: In the interest of gathering more up-to-date information, 126 gram-positive and 79 gram-negative bacterial isolates were tested against the following topical antimicrobial agents: silver sulfadiazine (1% Silvadene cream, Hoechst/Marion Roussel, Inc.), mafenide acetate (Sulfamylon, Bertex Pharmaceuticals), furacin (Nitrofurazone, Roberts Pharmaceutical Corp), 2% mupirocin (Bactroban, SmithKline Beecham) Polymixin B/Bacitracin (E. Fougera & Co.), Silvadene-Nystatin (1% Silvadene/100,000 U/G Nystatin), Modified Dakins (sodium hypochlorite [NaOCl] 0.025%) and silver nitrate (AgNO3). Using a 6-mm biopsy punch, Mueller-Hinton agar plates were prepared by “punching out” eight equally spaced wells, and then inoculating with a 0.5 McFarland Standard suspension of each bacterial isolate. The wells were then filled with the topical antimicrobial agents and the plates incubated at 37°C for 22 to 24 hours. Susceptibilities were then determined by measuring the zones of inhibition for each topical agent.

Results: The data clearly shows that except for Poly B/Bacit and Modified Dakins the remaining six antimicrobial agents were extremely effective against both the gram-positive and gram-negative isolates. Among them, silver sulfadiazine was 100% effective against the gram-positives (P ≤0.001), and 92.4% effective against the gram-negatives. Mafenide acetate was equally effective against both groups of organisms, at 97% (P=0.001). Cost assessment reveals that silver sulfadiazine ($72.48 per application) is more cost-effective when used twice daily than mafenide acetate and furacine ($162.40 and $123.74 per application, respectively). Furacine, on the other hand, was very effective against the gram-negatives. Mupirocin susceptibility for both groups was 88.9% and 91.1%, respectively. The responses to Silvadene-Nystatin and silver nitrate were about the same for each group (see Table 1).

Conclusion: It is recommended, when considering a topical agent for a gram-positive infection, that silver sulfadiazine should be considered first, followed by furacine, with mafenide acetate as a back-up. On the other hand mafenide acetate should be the first line of defense against gram-negative infectious agents, with silver sulfadiazine as the back-up agent.

The age of topical antibacterial therapy for infections reached its zenith before the age of antiseptics. Joseph Lister’s (1867) hallmark discovery of the effects of topical carbolic acid application developed a new approach to infection.1 Dakins2,3 came out with his solution of 0.5% sodium hypochlorite (NaOCl) to combat infection, which was widely used during WWI in war wounds and burns. Fleming’s4 discovery of penicillin in 1929 and study on the toxicity of Dakin’s solution put topical therapy on the back burner. Halstead’s5 famous quote during the age of antiseptics “A wound which has been irrigated with solutions of carbolic acid, corrosive sublimate or other disinfectant labors under the disadvantage of a more or less extensive area of superficial necrosis” might have brought a death knell to the use of topical antibacterials.6

Moncrief and Lindberg were the first to employ mafenide acetate on burn wound infections (1966).6 Silver nitrate entered the therapeutic arena about the same time (Bader, 1966).7 In 1968, Charles Fox introduced a new topical antibacterial called silver sulfadiazine.8 In the burn arena, prior to the advent of topical antibacterial agents, the overall mortality rate in a typical burn population would be reported a 38% to 45%. However after the use of topical antimicrobial therapy the overall mortality was reduced to 14% to 24%.9

This enhanced survival was probably due to a susceptibility assay developed at the Cincinnati Shriners Burns Hospital in 1978 by Nathan and his colleagues10 called Nathan’s Agar Well Diffusion Assay (NAWD). This assay has become the “gold standard” among many burn centers throughout the world.

In 1990, Strock et al.11 showed that three of four major topical agents assayed Bactroban, mafenide acetate and silver sulfadiazine were far more superior in vitro and in vivo in treating burn wound infections than Poly B/Bacitracin/Neomycin for gram-positives. Mafenide acetate, Bactroban, and silver sulfadiazine were as effective for the gram-negatives, but mafenide acetate heads the list.

In 1991, Heggers et al.12 provided evidence that sodium hypochlorite (NaOCl) at a concentration of 0.025% was equally antibacterial and lacked the tissue toxicity that the original Dakins solution elicited.4 Based on these data, our unit decided to reassess the efficacy of

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using the NAWD assay to provide therapeutic information regarding the susceptibility of infecting pathogens in burn wound infections.

**MATERIALS AND METHODS**

One hundred twenty-six gram-positive and 79 gram-negative organisms were isolated from burned patients and identified by MicroScan. Isolated colonies were transferred into 3 ml of bacteriostatic water to make a bacterial suspension equivalent to a 0.5 McFarland Standard.

Along with each group of organisms tested, three different ATCC strains of *Pseudomonas aeruginosa#27853, Escherichia coli#25922* and *Staphylococcus aureus#29213* were also tested to assure quality control. An eight-well antimicrobial agent-filled Mueller-Hinton Agar Plate (MHA) without bacterial inoculation served as negative control. NAWD has been recognized since its original presentation as the only Topical Antimicrobial Assay by the American Burn Association (ABA).

By sterile technique, eight 6-mm wells were made on a standard 150 mm MHA (Figure 1). Each bacterial isolate was inoculated onto the MHA plate by dipping a cotton swab into the suspension and streaking over the surface of the plates so as to create a confluent lawn of bacterial growth.

Each topical antimicrobial agent was introduced into each well as labeled. There were eight agents used:

1. 1% Silvadene cream (silver sulfadiazine).
2. 1% Sulfamylon cream (mafenide acetate).
3. 0.2 Nitrofurazone ointment (furacin).
4. Bactroban ointment (2% mupirocin).
5. Poly B/Bacitracin 500 units.
6. Silvadene-Nystatin cream (a 50/50 preparation of 1% Silvadene and 100,000 U/G of Nystatin).
7. Modified Dakins-sodium hypochlorite (NaOCl) 0.025%
8. 0.5% silver nitrate

After 22 to 24 hours of incubation at 37°C, the susceptibility was determined by measuring in mm the diameter of the zones of inhibition around each antibacterial well. Cleared zones with diameters greater than or equal to 10 mm are considered susceptible for the organism tested.

The efficacy of fresh liquid NaOCl (0.025%) and liquid Poly B (10,000 units), Bacitracin (500 units) at the same concentration of the ointment was examined. The pharmacy prepared fresh NaOCl and each product was tested for its effectiveness against several of the organisms tested using a Minimum Inhibition Concentration (MIC). A 0.5 MacFarland standard was inoculated to 5 ml of 0.025% NaOCl and 5 ml of Polymyxin B/Bacitracin (Poly B/Bacit) and incubated...
overnight at 37°C and then plated on McConkey Agar to examine survival for gram-negatives. Sheep blood agar was used to examine the survival of the gram-positives.

### Cost Analysis of Topical Antimicrobials

We calculated the daily cost of medication required for a 1-m² size burn area. The cost was based on the actual wholesale price (AWP) cost of the largest container available for each medication. We determined that one pound (454 gm) of cream or ointment was needed to apply a thin layer of cream or ointment to a 1-m² area of fine mesh gauze. Four liters of solution were needed to soak four 50-ply burn dressings 46 cm × 46 cm to cover approximately a 1-m² area. The list price for 1 L of sterile water for irrigation was $7.13; it was $7.49 for sterile normal saline for irrigation. The cost of the dressing was not factored into this equation.

### Statistical Analysis

All topical susceptibility responses were assessed using the Welch version of the t-test which is considered more reliable. Significance was considered when the \( P \) value was 0.05 or less.
RESULTS

General overall susceptibility.

A. Gram-Positives: It is evident from the responses of the organisms tested that the following topical agents were very effective against the gram-positives tested. Silver sulfadiazine was the most efficient topical agent, at 100% (P<0.001) effective, followed by furacin, mafenide acetate, and silver nitrate (98.6%, 97.6% and 96.8%, respectively). There is little significant difference between the effectiveness of these agents. Mupirocin, on the other hand, showed a 12% decrease over the response that Strock et al. reported (NS).11

The modified Dakins and the Poly B/Bacit ointment showed a significant reduction (P<0.05) in their effectiveness in the NAWD method (Table 1).

B. Gram-Negatives: The topical agents that were most effective against the gram-negatives, in order of effectiveness, were mafenide acetate (P<0.001), silver sulfadiazine, mupirocin, Silvadene-Nystatin, silver nitrate, and furacin (97.5%, 92.4%, 91.1%, 84.8%, 83.5% and 75%, respectively). Similar results were noted for Poly B/Bacit ointment and Modified Dakins, and were comparable to the gram-positive response (Table 2).

The major strains of staphylococci studied for significant response to the topical agents were S. aureus, E. epidermidis and S. haemolyticus (Tables 3 and 4). As for the responses of the remaining isolates, while some showed a significant response, the data is not presented.

Tables 5 and 6 present the significant response of silver sulfadiazine and mafenide acetate on the gram-negatives compared to the other topical agents. Mafenide acetate was the most effective against P. aeruginosa (P<0.001). For freshly prepared Modified Dakins (0.025%) and Poly B/Bacit, inoculated with gram-positive and gram-negative isolates, no-growth was noted at 24 and 48 hours. Using these same solutions with the NAWD technique, the zones of inhibition ranged from 13 to 23 mm for the Modified Dakins against both gram-positive and gram-negative isolates. However, the zones of inhibition for Poly B/Bacit ranged from 0 mm for E. faecalis to 20 mm for E. coli. Cost assessment reveals that silver sulfadiazine ($72.48) is more reasonable when employed twice daily than mafenide acetate ($162.40 and $123.74, respectively). The actual cost of the agents required to keep dressings wet and because larger volumes are needed to soak the 50-ply dressings. The cost breakdown in descending order is presented in Table 7.

CONCLUSION

Our results show that even though the NAWD technique has not been approved by the College of American Pathologists or the National Committee of Clinical Laboratory Standards (NCCLS), it has provided a wide range of choices for topical therapeutic application for the localized infected burn wound. For 20 years of application, the topical antimicrobial agents have generated some resistant strains of gram-positive staphylococci for Bactroban and pseudomonads for furacin. However, as Strock et al.13 and Heggars et al.12 have reported, the morbidity and mortality of severely burned patients was reduced significantly since the infections were effectively controlled by the proper choice of topical therapeutic agents in combination with systemic treatment. Our current data clearly shows that adjunctive topical therapy, along with standard systemic therapy, has been responsible for the reduction in mortality. In 1999, we admitted 277 acute-burn patients with an average total body surface area (TBSA) of 30% to 50% with a subsequent mortality rate of 3.5%. In the year 2000, we admitted 255 patients with an average TBSA of 35% to 55%, with a subsequent mortality rate of 2.4%. Of the 16 deaths for both years, only four were caused by sepsis. Therefore, this current millennium data suggests that the Modified NAWD...
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Table 7 Cost Breakdown for Topical Agents Used in Burn Treatment (Based on Twice-daily Dressing Changes)

<table>
<thead>
<tr>
<th>Product</th>
<th>Unit cost (AWP)</th>
<th>Frequency per day</th>
<th>Cost/m²/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mupirocin Ointment</td>
<td>22 gm = $36.85</td>
<td>2</td>
<td>$1,520.90</td>
</tr>
<tr>
<td>Mafenide acetate 8.5% Cream</td>
<td>454 gm = $117.44</td>
<td>2</td>
<td>$234.88</td>
</tr>
<tr>
<td>Nitrofurazone Ointment</td>
<td>454 gm = $98.11</td>
<td>2</td>
<td>$196.22</td>
</tr>
<tr>
<td><em>Silver Nitrate 0.5% in Sterile Water for Irrigation</em></td>
<td>1 liter = $9.85</td>
<td>4</td>
<td>$157.60</td>
</tr>
<tr>
<td><em>Sodium Hypochlorite 0.25% in Normal Saline for Irrigation</em></td>
<td>1 liter = $7.54</td>
<td>4</td>
<td>$120.64</td>
</tr>
<tr>
<td>Silver sulfadiazine 1% Cream</td>
<td>400 gm = $31.93</td>
<td>2</td>
<td>$72.48</td>
</tr>
<tr>
<td><em>Polymyxin B/Bacitracin and Nystatin 1:1 Ointment</em></td>
<td>454 gm = $19.65</td>
<td>2</td>
<td>$39.30</td>
</tr>
<tr>
<td>Polymyxin B/Bacitracin Ointment</td>
<td>28.35 gm = (1 oz)</td>
<td>2</td>
<td>$30.00</td>
</tr>
<tr>
<td>(10,000 Units/Polymyxin, 500 Units Bacitracin)</td>
<td></td>
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</table>

Although the NCCLS has not examined the efficacy of the NAWD technique, it is considered to be the “gold standard,” accepted by the American Burn Association. It has withstood the test of time as a means to determine topical susceptibility and provides definitive information regarding topical antimicrobial therapy.10-17

**REFERENCES**


**Acknowledgments**

We would like to acknowledge our Graphic Arts Department; Ms. Sandra Baxter, our medical illustrator, who provided timely and precise illustrations of our data, and Mr. Lewis Mihlin, Jr., and Ms. Tina Garcia for their photographic expertise.

Funded in part by the James W. McLaughlin Fellowship Fund and Shriners Hospital for Children, Burns Hospital, Clinical Microbiology Department.

*Presented in part at the 101st General Meeting of the American Society for Microbiology, May 20-24, 2001.*