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Comparative antibacterial activity of topical antiseptic eardrops against methicillin-resistant *Staphylococcus aureus* and quinolone-resistant *Pseudomonas aeruginosa*

Cha Kyung Youn [a,b,1], Sook-Jin Jang [c,1], Eu-Ri Jo [a], Ji Ae Choi [c], Ju-Hwan Sim [a], Sung Il Cho [a,*]

[a] Department of Otolaryngology-Head and Neck Surgery, Chosun University School of Medicine, Gwangju, South Korea
[b] Division of Natural Medical Science, Chosun University, Gwangju, South Korea
[c] Department of Laboratory Medicine, Chosun University School of Medicine, Gwangju, South Korea

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ABSTRACT

Objective: Aural irrigation using antiseptic solutions can be an effective medical treatment of chronic suppurative otitis media (CSOM) owing to the increasing prevalence of antibiotic-resistant CSOM infections. In the present study, we compared the antimicrobial activities of 100% Burow's solution, 50% Burow's solution, 2% acetic acid, vinegar with water (1:1), and 4% boric acid solution against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *S. aureus* (MSSA), quinolone-resistant *Pseudomonas aeruginosa* (QRPA), and quinolone-susceptible *P. aeruginosa* (QSPA) *in vitro*.
Methods: We examined the antimicrobial activities of five antiseptic solutions against MRSA, MSSA, QRPA, and QSPA. The antimicrobial activities of the solutions were calculated as a percentage of the surviving microorganisms by dividing the viable count in each antiseptic solution with that in control. The time (D_{10} value) required for each of the five solutions to inactivate 90% of the microorganism population was also investigated.
Results: Burow's solution exhibited the highest antimicrobial activity and the lowest D_{10} value against MRSA, MSSA, QRPA, and QSPA, followed by 2% acetic acid, vinegar with water (1:1), and 4% boric acid solution.
Conclusion: Our results indicate that Burow's solution has the most potent activity against bacteria including antibiotic-resistant strains. Twofold dilution of the solution is recommended to avoid ototoxicity.

1. Introduction

Chronic suppurative otitis media (CSOM) is one of the most common diseases of the ear. The usual causative organisms of CSOM include *Staphylococcus aureus* and *Pseudomonas aeruginosa* [1]. Topical treatment with antibiotics or antiseptics is more effective than systemic antibiotic treatment in CSOM [2]. However, topical antibiotics are not effective against pathogens of CSOM such as methicillin-resistant *S. aureus* (MRSA) and quinolone-resistant *P. aeruginosa* (QRPA). Since the prevalence of MRSA and QRPA infections has been increasing in CSOM [3,4], treatment with topical antiseptics is useful especially in outpatient clinics. Antiseptic solutions have the additional advantages of being inexpensive and they do not induce bacterial resistance. 100% Burow's solution, 50% Burow's solution, 2% acetic acid, vinegar with water (1:1), and 4% boric acid solution have been reported to be effective in the treatment of CSOM [5–8]. This study aimed to compare the bactericidal activities of these five antiseptic solutions against MRSA, methicillin-susceptible *S. aureus* (MSSA), QRPA, and quinolone-susceptible *P. aeruginosa* (QSPA) and to guide in the selection of topical antiseptics.

2. Materials and methods

2.1. Solution preparation

To evaluate the antimicrobial and therapeutic effects of antiseptic solutions, 100 and 50% Burow's solutions, 2% acetic

* Corresponding author at: Department of Otolaryngology-Head and Neck Surgery, Chosun University Hospital, 365 Pilmun-daero, Dong-gu, Gwangju 61453, South Korea. Tel.: +82 62 220 3207; fax: +82 62 225 2702.
E-mail address: chosi@chosun.ac.kr (S.I. Cho).
[1] These authors contributed equally to this work.

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acid, vinegar with water (1:1), and 4% boric acid solution were prepared with sterilized water. Burow's solution was manufactured according to the protocol described in British Pharmacopoeia 2009 and was used at 100 and 50% strengths for the experiment. Acetic acid (2%; Junsei chemical Co., Tokyo, Japan) was used. Commercially available vinegar (Ottogi corp., Seoul, South Korea) was used by diluting with water in a 1:1 ratio. Boric acid (Sigma, Saint Louis, MO, USA) was diluted to 4%.

2.2. Antimicrobial assay

For the antimicrobial assay, two gram-positive bacteria, MRSA clinical isolate from antibiotic susceptibility testing for patients with CSOM otorrhea and MSSA reference strain (ATCC 29213), and two gram-negative bacteria, QRPA clinical isolate from antibiotic susceptibility testing for patients with CSOM otorrhea and QSPA reference strain (ATCC 27853) were used.

Bacteria strains were grown in LB agar plates (Difco Laboratories, Sparks, MD, USA) at 37 °C for 24 h. The colonies were then suspended in sterilized saline and the turbidities of the suspensions were equivalent to McFarland 1.0 (10^8–10^9 colony-forming units/mL). The culture suspension (50 μL) was inoculated into 5 mL each of sterilized saline buffer as control, Burow's solution (100 and 50%), acetic acid (2%), vinegar with water (1:1), and boric acid (4%). The samples were incubated at room temperature and 100 μL of each was collected at 5, 10, 30, 60, and 120 min. Dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) of the samples were prepared and 0.1 mL of each solution was spread on duplicated LB agar plates. The plates were incubated at 37 °C for 24 h. The number of colonies formed on the agar plates was counted. The antimicrobial activity of each solution was expressed as a percentage of the surviving microorganisms by dividing the viable count in each antiseptic solution with that in the control (saline buffer). In addition, the sensitivity of a microorganism to a solution was expressed as D_{10} value (decimal reduction time). The D_{10} value is the time required to inactivate 90% of the population or to reduce the microorganism population to 1/10 its original number. The results are shown as mean values of six replicate samples.

3. Results

3.1. Bactericidal activities of five solutions against MRSA and MSSA

The bactericidal activities of 100% Burow's solution, 50% Burow's solution, 2% acetic acid, vinegar with water (1:1), and 4% boric acid solution against MRSA and MSSA were compared. Table 1 and Fig. 1 show the bactericidal activities against MRSA. These bacteria did not survive at 5 and 60 min in 100% Burow's solution and 50% Burow's solution, respectively. MRSA survival was inhibited completely at 120 min by both 2% acetic acid and vinegar with water (1:1). However, 4% boric acid solution did not completely inhibit the survival of MRSA at these time periods. Table 2 shows the bactericidal activities against MSSA. The survival of these bacteria was completely inhibited at 5, 60, 60, and 120 min by 100% Burow's solution, 50% Burow's solution, 2% acetic acid, and vinegar with water (1:1), respectively. However, 4% boric acid solution did not completely inhibit the survival of MSSA at these time periods. The survival rates of MRSA and MSSA were the lowest in the Burow's solution-treated groups.

Table 1
Antimicrobial activities of five solutions against MRSA.

	Survival rate (mean %, $n=6$) MRSA					
	0 min	5 min	10 min	30 min	60 min	120 min
Burow's solution (100%)	100	0.0				
Burow's solution (50%)	100	89.8	65.8	35.5	0.0	
Acetic acid (2%)	100	86.7	73.4	56.6	42.4	0.0
Vinegar:Water (1:1)	100	90.1	83.4	74.2	50.3	0.0
Boric acid (4%)	100	98.0	91.2	84.8	80.8	75.3

3.2. Bactericidal activities of the five solutions against QRPA and QSPA

The bactericidal activities of 100% Burow's solution, 50% Burow's solution, 2% acetic acid, vinegar with water (1:1), and 4% boric acid solution against QRPA and QSPA were compared. Tables 3 and 4 show the bactericidal activities against QRPA and QSPA. 100% Burow's solution, 50% Burow's solution, 2% acetic acid, and vinegar with water (1:1) completely inhibited QRPA survival at 5 min (Fig. 2). At 60 min, 4% boric acid solution inhibited the survival of QRPA and QSPA. These results indicate that 100% Burow's solution, 50% Burow's solution, 2% acetic acid, and vinegar with water (1:1) are more effective against QRPA and QSPA than 4% boric acid solution.

3.3. D_{10} values for the five solutions against MRSA and MSSA

The time required to inactivate 90% of the microorganism population (D_{10} value) was compared for 100% Burow's solution, 50% Burow's solution, 2% acetic acid, vinegar with water (1:1), and 4% boric acid solution. The D_{10} value of 100% Burow's solution, 50% Burow's solution, 2% acetic acid, vinegar with water (1:1), and 4% boric acid solution against MRSA was 0.11 ± 0.04, 7.29 ± 0.59, 18.04 ± 1.91, 20.50 ± 1.99, and 100.30 ± 13.34 min, respectively (Table 5). The D_{10} value of 100% Burow's solution, 50% Burow's

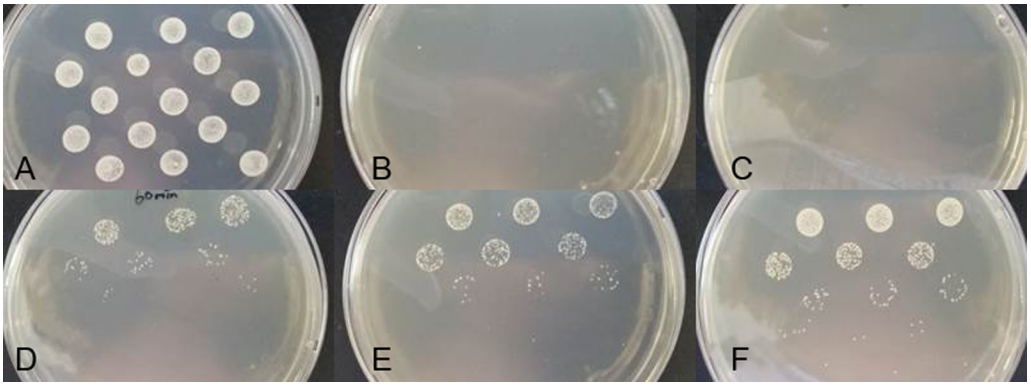

Fig. 1. Bactericidal activities of five solutions against MRSA at 60 min. Representative pictures of (a) control, (b) 100% Burow's solution, (c) 50% Burow's solution, (d) 2% acetic acid, (e) vinegar with water (1:1), and (f) 4% boric acid solution are shown. The survival rate of MRSA was the lowest in Burow's solution-treated groups (b and c).