EXPERIMENTAL STUDY

Acta Orthop Traumatol Turc 2013;47(4):281-285
doi:10.3944/AOTT.2013.3114

Comparison of the efficiency of different antibiotic irrigation solutions in decontamination of allografts contaminated with *Staphylococcus aureus*

İbrahim KAYA, İbrahim SUNGUR, Murat YILMAZ, Filiz PEHLİVANOĞLU, Kadriye KARTYAŞAR, Gönül ŞENGÖZ

Department of Orthopedics and Traumatology, Haseki Training and Research Hospital, İstanbul, Turkey

Objective: The purpose of this study was to investigate the local antibacterial efficacy of saline, rifampicin, gentamicin, high-concentration fusidic acid and low-concentration fusidic acid in the decontamination of allografts contaminated with *Staphylococcus aureus*.

Methods: Fifty-five sterile, fresh-frozen femoral heads obtained from the bone bank were contaminated with methicillin-sensitive ATCC 25923 *Staphylococcus aureus*. Samples were divided into groups and debrided with high-pressure (80 psi) pulse lavage using a saline, rifampicin irrigation solution (50 mg/l), gentamicin irrigation solution (50 mg/l) and low- (50 mg/l) or high-concentration (500 mg/l) fusidic acid irrigation solution for 30 seconds from a distance of 10 cm. After irrigation, allografts were incubated in the culture and developed colonies were counted. Mean±standard deviation (min-max) values were calculated. The differences between the four irrigation groups were evaluated using the Kruskal-Wallis variance analysis and groups were compared two at a time using the post-hoc Mann-Whitney U test.

Results: No colonization was detected with the exception of one allograft in the rifampicin irrigation group. The gentamicin irrigation group had similar results as the high-concentration fusidic acid irrigation group and both results were superior to those of the saline and low-concentration fusidic acid irrigation groups (p=0.010 and 0.004, respectively). The low- and high-concentration fusidic acid irrigation groups were similar and were not shown to have superior results than saline irrigation group.

Conclusion: Rifampicin irrigation solution was the most effective in the decontamination of allografts previously contaminated with *Staphylococcus aureus*. Gentamicin, high-concentration fusidic acid, low-concentration fusidic and saline irrigation solutions may also be used respectively, according to their effectiveness.

Key words: Contaminated bone; fusidic acid; irrigation; *Staphylococcus aureus*.

Infection risk in contaminated open fractures is reported as 13 to 50% and is directly proportional to bacterial colony counts. The purpose of debridement and irrigation of open fractures and soft tissue injuries is to prevent bacterial contamination at the injury site.[1-3]

Different studies investigating the effectiveness of irrigation solutions with antibiotics or various antiseptics for the decontamination of open wounds and contaminated bones have been reported[4-9] with inconsistent results.[2,5-9]

Correspondence: İbrahim Kaya, MD. Haseki Eğitim ve Araştırma Hastanesi, Ortopedi ve Travmatoloji Kliniği, Adnan Adıvar Cad., 34073, Fatih, İstanbul, Turkey.
Tel: +90 212 - 529 44 00 e-mail: drikaya27@hotmail.com

Submitted: November 13, 2012 **Accepted:** May 2, 2013

Available online at
www.aott.org.tr
doi:10.3944/AOTT.2013.3114
QR (Quick Response) Code:

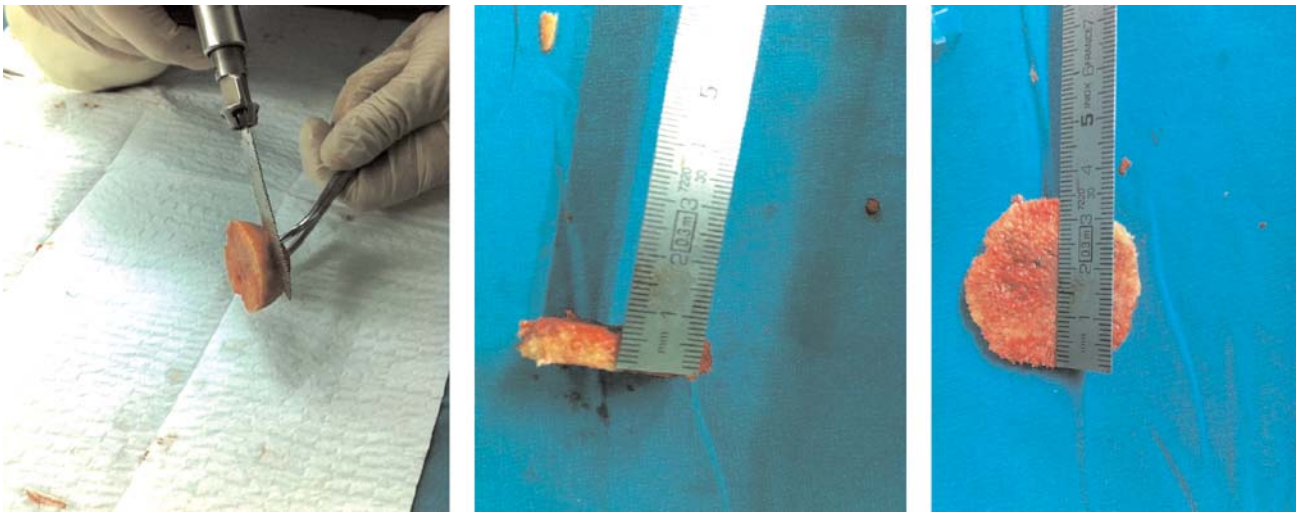

Fig. 1. **(a-c)** Preparation of the bone sample. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Despite the different results on decontamination with low- and high-pressure pulse lavage, recently there have been an increasing number of studies reporting that high-pressure pulse lavage is quite effective in bacterial decontamination of both open fractures and soft tissue injuries.[3,10-14]

The purpose of this study was to compare the effect of various antibiotic irrigation solutions used in pomade and local wound care for their local bactericide activity to saline irrigation for the decontamination of contaminated allografts.

Materials and methods

This study included 55 fresh femoral heads obtained from arthroplasty operations for primary coxarthrosis and femoral neck fractures. Specimens were immediately transported to the clinic, following pathologic and microbiologic sampling, and stored in a -86°C medical deep freezer (Sanyo® Medical Freezer MDF; Panasonic Corp., Osaka, Japan). Samples with aerobic culture (+) at 48 hours, were macroscopically abnormal, had pathological fractures, rheumatologic disease or dislocated hips were excluded.

When a sufficient number of samples with adequate laboratory tests were collected, femoral heads were divided into 3.5×3.5×0.6 cm pieces using a power saw in the operating theater, following sterile dressing. Fifty-five bone specimens were obtained (Fig. 1).

Bone samples were contaminated in the microbiology laboratory. A trypticase soy broth medium of 4 McFarland standard and prepared with methicillin-sensitive *Staphylococcus aureus* control strain provided from Istanbul University KUKENS laboratory was used for the contamination process.

Bone specimens previously established as sterile were kept in contamination solution for 5 hours (vortexed every 10 minutes) at room temperature. Contaminated samples were transported to the operation room in 100 cc sterile containers for irrigation with five different solutions. Although various techniques for the contamination process, including rubbing samples directly or with contaminated cotton sticks against the operation room floor or have been described in the literature, there is no standard procedure.[15]

Samples were kept in a solution with a standard microorganism concentration (4 McFarland) at room temperature for a minimum of 5 hours in the microbiology laboratory to provide homogeneous contamination.

Contaminated bone specimens were randomly divided into 5 groups of 11. The first group was washed with sterile saline, the second group with rifampicin solution (50 mg/l), the third group with gentamicin

Table 1. Average colony counts of groups (mean±standard deviation).

Saline	Gentamicin irrigation solution	Low-concentration fusidic acid irrigation solution	High-concentration fusidic acid irrigation solution	P value
3445.4±1206.9	1709.0±1608.3	5927.2±4903.3	3369.6±2931.3	0.015

solution (50 mg/l), the fourth group with low-concentration fusidic acid (50 mg/l; 100 times minimal inhibitor concentration (MIC)), and the fifth group with high-concentration fusidic acid (500 mg/l; 1,000 times MIC). Contaminations were performed using 80 psi high-pressure pulse lavage (Tava Surgical® lavage pump set; Tava Surgical, Ventura, CA, US) from a distance of 10 cm for 30 seconds.[10]

Following irrigation in the operating theater in sterile conditions, bone specimens were transported to the laboratory in 100 cc sterile containers and covered completely with the sterile trypticase soy broth liquid medium. Specimens were vortexed every 10 minutes to let the bacteria contained in the bone to pass to the liquid medium and kept there for 30 minutes. 0.01 ml samples obtained from each bone bullion were incubated in 5% sheep blood agar for 18 to 24 hours at 35 to 37ºC. At the end of 18 to 24 hours, the number of bacterial colony forming units (CFUs) was measured.

All data were expressed as mean±standard deviation and median (min-max). Because there was no colonization except for one in rifampicin irrigation group, this group was excluded from analysis. For the other four groups, multiple comparisons were made using the Kruskal-Wallis variance analysis test. Post-hoc comparisons were performed with the Mann-Whitney U test. P values of <0.05 were considered significant. The Statistical Package for Social Sciences (SPSS) for Windows 15.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

In the rifampicin group, there was growth in only one specimen. The number of counted colonies was 600. The efficiency of rifampicin solution in decontamination was significantly higher than the other groups (p=0.000).

Significant differences were found between the growing colony counts of the other four groups (p=0.015) (Table 1 and Fig. 2). Irrigation with gentamicin solution was significantly superior to those of irrigation with low-concentration fusidic acid and sterile saline solution (p=0.004 and p=0.010, respectively) and superior to irrigation with high-concentration fusidic acid solution with no significance (p=0.171).

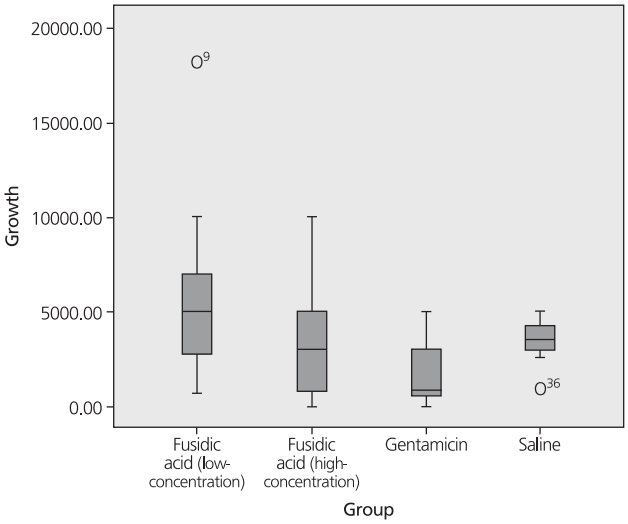

Fig. 2. Colony counts according to groups.

Results of irrigation with low- and high-concentration fusidic acid solutions were similar with each other (p =0.116) and no statistically significant difference was detected when compared with the results of irrigation with saline solution (p=0.171 and p=0.797, respectively) (Table 2).

Discussion

This study suggests that rifampicin irrigation solution is the most effective for the decontamination of bone. Rifampicin is an antibiotic which inhibits the beta subunit of RNA polymerase enzyme and shows bactericidal effect by blocking mRNS synthesis.[16] Rifampicin containing irrigation solutions are known to be used for local decubitus wound care. Hirn et al.[17,18] rinsed bone specimens contaminated with coagulase negative staphylococci with sterile saline, cefuroxime axetil and rifampicin solutions. They compared their results with low-pressure pulse lavage with sterile saline solution and stated that rifampicin washing is more effective. While rifampicin was more effective than cefuroxime,

Table 2. Comparison of groups two at a time.

	P value
Saline – gentamicin solution	0.010
Saline – low-concentration fusidic acid solution	0.171
Saline – high-concentration fusidic acid solution	0.797
Gentamicin solution – low-concentration fusidic acid solution	0.004
Gentamicin solution – high-concentration fusidic acid solution	0.171
Low-concentration fusidic acid solution – high-concentration fusidic acid solution	0.116

they stated that they should be used in combination for serious infections considering the high possibility of resistance development to rifampicin. A high risk of resistance development after irrigation with rifampicin solutions restricts its use in clinical practice.

In another study, Hirn et al.[19] compared the results of simple soaking of 140 femoral heads in cefuroxime and rifampicin solutions with pulse lavage irrigation. They concluded that soaking in antibiotic solutions is not more effective than pulse lavage.

Gentamicin has a wide use in orthopaedic surgery parenterally both in the pre- and postoperative stages, locally as a pumice, or by adding to bone cement, especially in arthroplasty surgery.[20] It shows its bactericidal effect through the inhibition of the mRNA.[21]

In our study, the irrigation with gentamicin solution group had superior results to those of the low-concentration fusidic acid and saline solution irrigation group and similar results to the high-concentration fusidic acid irrigation group.

Fusidic acid is a bacteriostatic antibiotic which inhibits protein synthesis at the ribosomal elongation phase whereas it can show bactericidal effects in high concentrations.[22,23] Oral fusidic acid is used alone or in combination with other antibiotics for *Staphylococcus aureus* infections as maintenance treatment following intravenous glycopeptide antibiotics.[22]

Drugeon et al.[24] compared the efficacy efficiency of fusidic acid against methicillin-sensitive *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* with various antibiotics. They determined synergistic activity in three of four combinations an antagonistic activity in one combination. Neut et al.[25] added fusidic acid and clindamycin in addition to gentamicin to cement in increased methicillin-resistant septic joint arthroplasty. They reported that the addition of fusidic acid was more effective than the addition of clindamycin.

In a study using rats, Ersoz et al. reported that adding fusidic acid to cement and parenteral teicoplanin is more effective than parenteral teicoplanin alone for tibial osteomyelitis, although the difference was not statistically significant.[26] In our study, the results of the low- and high-concentration fusidic acid irrigation groups were similar, but no statistically significant superior effect was determined compared to the saline irrigation group.

In Hirn et al's[27] study on the efficacy of the saline irrigation technique alone, 55 fresh frozen human femoral heads were contaminated with *S. aureus* and *Bacillus Spp.* and later washed in saline solution and antibiotic solution (cefuroxime axetil) by simple shaking and high-pressure saline irrigation. Colony counts proved 75% culture (-) in the high-pressure saline irrigation group. This rate was 10% in the saline bath and 20% in the antibiotic saline bath groups.

Furthermore, it is difficult to establish the efficacy of pressure irrigation with mechanical washing versus antibacterial activity during decontamination. Despite the antibacterial effect, mechanical irrigation is more important.[28-30]

The literature is unclear on how agents show their systemic effect and reach their effective concentration. Antibiotics with systemic effect are partly time-dependent and partly dependent to concentration. These pharmacodynamic properties specify drug dosage and intervals.

The antibiotics used in this study are locally effective (eye/ear drops, pomade form) agents and with systemic bactericidal effects are known to be provided with local use.

Specimens are in touch with the antibiotics only during irrigation which may raise suspicions about the efficacy of antibiotics. However, the difference in the results of the rifampicin and saline irrigation group prove the efficacy of the antibiotics.

Studies have investigated whether high-pressure irrigation damages the organic bone matrix and thus decelerates bone healing or transport microorganisms on the bone and soft tissues to deeper tissues. For this reason, some authors suggest using high-pressure lavage in gross contaminated wounds. Our study was limited in that low-pressure lavage is being investigated as well.[9,13,14,31-33]

An additional limitation of our study was that it was not an experimental study executed on *in vivo* live bone. Instead, lavage was carried out on allografts.

In conclusion, rifampicin appears to be the most effective irrigation solution followed by gentamicin, high-concentration fusidic acid, low-concentration fusidic acid and saline irrigation solution in the decontamination of bone infected with staphylococcus strain. Rifampicin resistance is an important argument against its widespread use. As rifampicin constitutes the main stay of tuberculosis treatment which is frequent in our country, resistance may lead to an increase in number of more complicated tuberculosis patient (MDR tuberculosis). However, it should be taken into consideration that other antibiotics may also develop resistance.

Conflicts of Interest: No conflicts declared.

References

1. Gustilo RB, Mendoza RM, Williams DN. Problems in the management of type III (severe) open fractures: a new classification of type III open fractures. J Trauma 1984;24:742-6.
2. Gustilo RB, Merkow RL, Templeman D. The management of open fractures. J Bone Joint Surg Am 1990;72:299-304.
3. Bhandari M, Adili A, Schemitsch EH. The efficacy of low-pressure lavage with different irrigating solutions to remove adherent bacteria from bone. J Bone Joint Surg Am 2001;83-A:412-9.
4. Conroy BP, Anglen JO, Simpson WA, Christensen G, Phaup G, Yeager R, et al. Comparison of castile soap, benzalkonium chloride, and bacitracin as irrigation solutions for complex contaminated orthopaedic wounds. J Orthop Trauma 1999; 13:332-7.
5. Burd T, Conroy BP, Meyer SC, Allen WC. The effects of chlorhexidine irrigation solution on contaminated bone-tendon allografts. Am J Sports Med 2000;28:241-4.
6. Hooe W, Steinberg B. Management of contaminated bone grafts: an experimental *in vitro* study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996;82:34-7.
7. Soyer J, Rouil M, Castel O. The effect of 10% povidone – iodine solution on contaminated bone allografts. J Hosp Infect 2002;50:183-7.
8. Tarbox BB, Conroy BP, Malicky ES, Moussa FW, Hockman DE, Anglen JO, et al. Benzalkonium chloride. A potential disinfecting irrigation solution for orthopaedic wounds. Clin Orthop Relat Res 1998;(346):255-61.
9. Owens BD, White DW, Wenke JC. Comparison of irrigation solutions and devices in a contaminated musculoskeletal wound survival model. J Bone Joint Surg Am 2009;91:92-8.
10. Kural C, Kaya I, Yilmaz M, Demirbaş E, Yücel B, Korkmaz M, et al. A comparison between three irrigation methods in the debridement of contaminated bovine cancellous bone and the effect of duration of irrigation on the efficiency of debridement. Acta Orthop Traumatol Turc 2009;43:359-65.
11. Svoboda SJ, Bice TG, Gooden HA, Brooks DE, Thomas DB, Wenke JC. Comparison of bulb syringe and pulsed lavage irrigation with use of a bioluminescent musculoskeletal wound model. J Bone Joint Surg Am 2006;88:2167-74.
12. Anglen JO, Apostoles S, Christensen G, Gainor B. The efficacy of various irrigation solutions in removing slime-producing *Staphylococcus*. J Orthop Trauma 1994;8:390-6.
13. Lee EW, Dirschl DR, Duff G, Dahners LE, Miclau T. High-pressure pulsatile lavage irrigation of fresh intraarticular fractures: effectiveness at removing particulate matter from bone. J Orthop Trauma 2002;16:162-5.
14. Draeger RW, Dirschl DR, Dahners LE. Debridement of cancellous bone: a comparison of irrigation methods. J Orthop Trauma 2006;20:692-8.
15. Crowley DJ, Kanakaris NK, Giannoudis PV. Irrigation of the wounds in open fractures. J Bone Joint Surg Br 2007;89: 580-5.
16. Calfee DP. Rifamycins. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's principles and practices of infectious diseases. 7th ed. Philadelphia: Churchill Livingstone; 2010. p. 403-17.
17. Liu JW, Chao LH, Su LH, Wang JW, Wang CJ. Experience with a bone bank operation and allograft bone infection in recipients at a medical centre in southern Taiwan. J Hosp Infect 2002;50:293-7.
18. Hirn M, Laitinen M, Pirkkalainen S, Vuento R. Cefuroxime, rifampicin and pulse lavage in decontamination of allograft bone. J Hosp Infect 2004;56:198-201.
19. Hirn M, Laitinen M, Vuento R. Pulse lavage washing in decontamination of allografts improves safety. Chir Organi Mov 2003;88:149-52.
20. Kittinger C, Marth E, Windhager R, Weinberg AM, Zarfel G, Baumert R, et al. Antimicrobial activity of gentamicin palmitate against high concentrations of *Staphylococcus aureus*. J Mater Sci Mater Med 2011;22:1447-53.
21. Gilbert DN, Leggett JE. Aminoglycosides. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's principles and practices of infectious diseases. 7th ed. Philadelphia: Churchill Livingstone; 2010. p. 359-84.
22. Wang JL, Tang HJ, Hsieh PH, Chiu FY, Chen YH, Chang MC, et al. Fusidic acid for the treatment of bone and joint infections caused by meticillin-resistant *Staphylococcus aureus*. Int J Antimicrob Agents 2012;40:103-7.
23. Mandell LA. Fusidic acid. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's principles and practices of infectious diseases. 7th ed. Philadelphia: Churchill Livingstone; 2010. p. 355-7.
24. Drugeon HB, Caillon J, Juvin ME. *In-vitro* antibacterial activity of fusidic acid alone and in combination with other antibiotics against methicillin-sensitive and -resistant *Staphylococcus aureus*. J Antimicrob Chemother 1994;34:899-907.
25. Neut D, Hendriks JG, van Horn JR, Kowalski RS, van der Mei HC, Busscher HJ. Antimicrobial efficacy of gentamicin-loaded acrylic bone cements with fusidic acid or clindamycin added. J Orthop Res 2006;24:291-9.
26. Ersoz G, Oztuna V, Coskun B, Eskandari MM, Bayarslan C, Kaya A. Addition of fusidic acid impregnated bone cement to systemic teicoplanin therapy in the treatment of rat osteomyelitis. J Chemother 2004;16:51-5.
27. Hirn MY, Salmela PM, Vuento RE. High-pressure saline washing of allografts reduces bacterial contamination. Acta Orthop Scand 2001;72:83-5.
28. Gross A, Bhaskar SN, Cutright DE, Beasley JD 3rd, Perez B. The effect of pulsating water jet lavage on experimental contaminated wounds. J Oral Surg 1971;29:187-90.
29. Dirschl DR, Wilson FC. Topical antibiotic irrigation in the prophylaxis of operative wound infections in orthopedic surgery. Orthop Clin North Am 1991;22:419-26.
30. Bahrs C, Schnabel M, Frank T, Zapf C, Mutters R, von Garrel T. Lavage of contaminated surfaces: an *in vitro* evaluation of the effectiveness of different systems. J Surg Res 2003;112:26-30.
31. Bhandari M, Schemitsch EH, Adili A, Lachowski RJ, Shaughnessy SG. High and low pressure pulsatile lavage of contaminated tibial fractures: an *in vitro* study of bacterial adherence and bone damage. J Orthop Trauma 1999;13:526-33.
32. Hassinger SM, Harding G, Wongworawat MD. High-pressure pulsatile lavage propagates bacteria into soft tissue. Clin Orthop Relat Res 2005;(439):27-31.
33. Boyd JI 3rd, Wongworawat MD. High-pressure pulsatile lavage causes soft tissue damage. Clin Orthop Relat Res 2004; (427):13-7.