Comparison of the Sterilization Efficiency of 3 Disinfectants for Dropped Anterior Cruciate Ligament Grafts

A Systematic Review and Meta-analysis

Xianyue Shen,* MD, Yanguo Qin,*[†] MD, PhD, Jianlin Zuo,[‡] MD, PhD, Tong Liu,[‡] MD, PhD, and Jianlin Xiao,^{†‡} MD, PhD

Investigation performed at The Second Hospital, Jilin University, Changchun, China

Background: The inadvertent contamination of anterior cruciate ligament (ACL) grafts can occur if they are accidentally dropped on the floor during ACL reconstruction. There has been no meta-analysis conducted to compare the sterilization efficiency of the different disinfectants used on dropped ACL grafts.

Purpose: To compare the sterilization efficiency of 3 disinfectants to decontaminate ACL grafts as necessary.

Study Design: Systematic review.

Methods: A systematic literature review was performed using the MEDLINE, Embase, and Cochrane Library databases. All studies reporting the management of dropped or contaminated grafts were considered for this meta-analysis.

Results: A total of 7 studies meeting inclusion criteria were identified from a literature search. The pooled results of this metaanalysis indicated that the rate of positive cultures of ACL grafts dropped on the operating room floor was 44.9% and that the commonly contaminated microbes were staphylococci and bacilli. The meta-analysis results indicated that the sterilization efficiency of a 4% chlorhexidine solution was superior to an antibiotic solution (odds ratio [OR], 0.17 [95% CI, 0.05-0.57]; P = .004) and a 10% povidone-iodine solution (OR, 0.04 [95% CI, 0.01-0.20]; P < .0001). Further, the antibiotic solution was superior to the 10% povidone-iodine solution (OR, 0.20 [95% CI, 0.07-0.55]; P = .002).

Conclusion: The results of our meta-analysis demonstrated that staphylococci and bacilli were the most common contaminants on dropped ACL grafts and that decontamination using a 4% chlorhexidine solution more reliably disinfected ACL grafts. This information can help to guide surgeons as regards appropriate remedial measures.

Keywords: sterilization; graft; anterior cruciate ligament (ACL); disinfectant; contamination

Injuries to the anterior cruciate ligament (ACL) are common, especially among athletes. ACL reconstruction is the most common surgical intervention performed in the knee joint, with 45.1 procedures per 100,000 people annually, and this incidence is increasing annually.³ The inadvertent contamination of ACL grafts can occur if they are accidentally dropped on the floor during ACL reconstruction, which might lead to significant complications. About 25% of all fellowship-trained sports medicine surgeons have reported at least 1 accidental contamination of an ACL graft, leading to a management dilemma for surgeons.¹⁶

Although the incidence of dropped ACL grafts is unknown, the implantation of a contaminated ACL graft in the knee joint may cause severe septic arthritis. It has been reported that the incidence of septic arthritis after using contaminated grafts during ACL reconstruction is 0.51% to 0.6%.^{25,33} Previous studies have proposed some remedies for when ACL grafts are accidentally dropped on the operating room floor, including attempted sterilization of the dropped graft, harvesting another autograft from the contralateral knee, and the use of an allograft; all of these options carry additional risks to the patients.^{8,20,24} Disinfecting the contaminated graft would appear to be the first choice. However, there are limited data available concerning the decontamination efficiency of commonly used disinfectants on contaminated grafts. Meanwhile, there has been no meta-analysis conducted to compare the

The Orthopaedic Journal of Sports Medicine, 9(5), 23259671211002873 DOI: 10.1177/23259671211002873 © The Author(s) 2021

This open-access article is published and distributed under the Creative Commons Attribution - NonCommercial - No Derivatives License (https://creativecommons.org/ licenses/by-nc-nd/4.0/), which permits the noncommercial use, distribution, and reproduction of the article in any medium, provided the original author and source are credited. You may not alter, transform, or build upon this article without the permission of the Author(s). For article reuse guidelines, please visit SAGE's website at http://www.sagepub.com/journals-permissions.

decontamination efficiency of different disinfectants on dropped ACL grafts.

This study aimed to provide guidelines for surgeons by comparing the efficiency of 3 disinfectants commonly available in the operating room, a 4% chlorhexidine solution, an antibiotic solution, and a 10% povidone-iodine solution, and identifying the most effective way to disinfect ACL grafts that fell on the operating room floor. We hypothesized that the 4% chlorhexidine solution, antibiotic solution, and 10% povidone-iodine solution would have equivalent efficacy and that using any of them would provide effective sterilization in cases of dropped ACL grafts during surgery.

METHODS

Search Strategy and Eligibility Criteria

This study was registered with PROSPERO, an international database of prospectively registered systematic reviews (CRD42020205369). A systematic review was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. On May 1, 2020, an electronic literature search was performed using the MEDLINE, Embase, and Cochrane Library databases. The search terms used were as follows: *anterior cruciate ligament* and ACL combined with graft contamination, dropped graft, and sterilization. No restrictions were imposed on the date of publication.

All novel studies evaluating the management of dropped or contaminated grafts were eligible for inclusion in this review. The study exclusion criteria were as follows: non-English language publications, nonexperimental studies, noncomparative studies, and studies unrelated to ACL grafts.

Titles and abstracts were independently reviewed by 2 authors (X.S. and T.L.) to ensure that the selected articles met the inclusion criteria. Any disagreements regarding study inclusion and data were resolved by discussion and consensus involving a senior reviewer (Y.Q.).

Data Extraction

The same 2 reviewers independently extracted all relevant data and imported them into a spreadsheet (Excel 2019; Microsoft), which was then reviewed by another senior reviewer (J.Z.). The imported data included the study's country of origin, study design, sample types, sample size, source of contamination, decontamination management protocol, and outcomes.

Methodological Quality Assessment

The included studies' methodological quality was assessed using the criteria of the modified Methodological Index for Non-Randomized Studies (MINORS).¹⁷ Similar to the original MINORS, the modified MINORS criteria included 12 items, with 2 additional items proposed for future in vitro studies. The total score of the methodological quality assessment was 24. The quality of studies was rated as follows: high quality with 19-24 points, moderate quality with 13-18 points, and low quality with <12 points. All included articles were independently assessed by 2 reviewers (X.S. and J.X.), and any disagreements regarding the quality assessment were resolved by discussion and consensus involving a senior reviewer (J.Z.). The purpose of the quality assessment in this meta-analysis was to evaluate and describe the study quality and design characteristics of all included studies.

Statistical Analysis

Review Manager 5.3 software (Cochrane Collaboration) was used for the meta-analysis of the extracted data. The primary outcome of interest was the odds ratio (OR) of positive cultures. A random-effects model was used to evaluate the random variables and I^2 statistic to evaluate the data for heterogeneity among studies and confirm the appropriateness of pooling among groups, with >50% regarded as significantly heterogenic. The OR, with a 95% CI, was calculated for dichotomous outcomes. If no events occurred in 2 groups or no comparison between 2 groups could be made within a study, those studies were excluded from this part of the meta-analysis.

RESULTS

Study Characteristics

The literature search yielded a total of 423 articles. After removing duplicate studies and applying exclusion criteria, 30 studies were selected for a full-text review. Based on eligibility criteria, 23 of these studies were excluded after a full-text review, and the remaining 7 articles were ultimately included.^{2,4,10,12,19,21,24} A flowchart of the study selection process is shown in Figure 1.

[†]Address correspondence to Yanguo Qin, Department of Orthopedics, The Second Hospital of Jilin University, Changchun, Jilin, China (email: qinyg@jlu. edu.cn) and Jianlin Xiao, Department of Orthopedics, China-Japan Union Hospital of Jilin University, Changchun, Jilin, China (email: xiaojianlin10@jlu.edu. cn).

^{*}Department of Orthopedics, The Second Hospital, Jilin University, Changchun, China.

[‡]Department of Orthopedics, China-Japan Union Hospital, Jilin University, Changchun, China.

Final revision submitted November 15, 2020; accepted December 16, 2020.

One or more of the authors has declared the following potential conflict of interest or source of funding: This work was financially supported by the National Natural Science Foundation of China (No. 81601908) and the Outstanding Youth Fund of the Department of Science and Technology of Jilin Province (No. 20180520108JH). AOSSM checks author disclosures against the Open Payments Database (OPD). AOSSM has not conducted an independent investigation on the OPD and disclaims any liability or responsibility relating thereto.

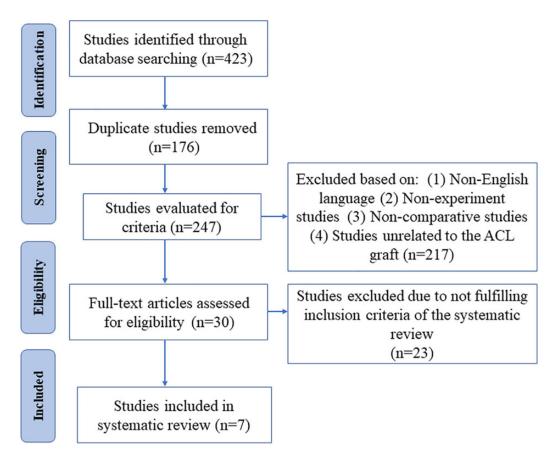


Figure 1. Flowchart describing the literature search and review process. ACL, anterior cruciate ligament.

Lead Author (Year)	Country	Study Design	Graft Type	Modified MINORS Score
Molina ¹⁹ (2000)	USA	Controlled trial	ACL specimens removed from patients undergoing total knee arthroplasty	16
Plante ²⁴ (2013)	USA	Controlled trial	Hamstring tendons harvested from patients	14
Badran ² (2016)	Egypt	Controlled trial	Hamstring tendon autograft specimens	19
Parker ²¹ (2008)	USA	Controlled trial	Fresh-frozen bone-patellar tendon-bone grafts from screened donors	21
Cooper ¹⁰ (1991)	USA	Controlled trial	Bone–patellar tendon–bone grafts harvested from fresh-frozen cadaveric knees	16
Barbier ⁴ (2015)	France	Controlled trial	Hamstring tendon autografts from patients	22
$Goebel^{12}$ (1994)	USA	Controlled trial	Bone–patellar tendon–bone grafts harvested from adult California White rabbits	16

TABLE 1 Characteristics of Included Studies a

^aACL, anterior cruciate ligament; MINORS, Methodological Index for Non-Randomized Studies.

Quality Assessment According to the Modified MINORS

The 7 eligible articles were evaluated according to the modified MINORS quality assessment. The methodological quality score of the included studies ranged from 14 to 22. All included studies were considered to be of moderate or high quality. Individual study characteristics, sterilization procedures, and quality assessment scores of all 7 included studies are summarized in Table 1.

The main disinfectants used in these 7 studies to decontaminate ACL grafts included a 4% chlorhexidine solution (173 samples in 5 studies), an antibiotic solution (158 samples in 5 studies), a 10% povidone-iodine solution (143 samples in 4 studies), saline (30 samples in 1 study), and a sodium hypochlorite solution (25 samples in 1 study). The

	4% Chlorhexidine solution		Antibiotic solution		Odds Ratio			Odds Ratio		
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% Cl	Year		M-H, Fixed, 95% Cl	
Cooper. DE 1991	0	0	3	10		Not estimable	1991			
Goebel MF.1994	0	8	8	8	55.7%	0.00 [0.00, 0.20]	1994	←		
Molina. ME 2000	1	50	3	50	20.4%	0.32 [0.03, 3.18]	2000			
Parker. RD 2008	0	0	0	0		Not estimable	2008			
Plante. MJ 2012	1	30	1	30	6.7%	1.00 [0.06, 16.76]	2012			
Barbier O. 2014	2	25	0	0		Not estimable	2014			
Badran. MA 2016	0	60	2	60	17.2%	0.19 [0.01, 4.11]	2016			
Total (95% CI)		173		158	100.0%	0.17 [0.05, 0.57]				
Total events	4		17							
Heterogeneity: Chi ² = 5.41, df = 3 (P = 0.14); I ² = 45% 0.002 0.1 1 10 500									500	
Test for overall effect: Z = 2.86 (P = 0.004) Favours 4% Chlorhexidine solution Favours Antibiotic solution									000	

Figure 2. Forest plot depicting the sterilization efficiency of the 4% chlorhexidine solution versus the antibiotic solution. M-H, Mantel-Haenszel.

decontamination methods included immersion for a variable duration of time, mechanical agitation and serial dilution, pulsed lavage, and a soak/rinse combination. Additionally, 6 studies^{2,4,10,12,19,24} reported the incidence of positive cultures of microbes after disinfecting the dropped ACL grafts with different disinfectants, while 1 study²¹ reported the number of colony-forming units after culturing. The ACL grafts in 1 study¹² were harvested from rabbits, while the grafts used in the rest of the studies^{2,4,10,19,21,24} were from humans. All 7 of these studies mainly evaluated the sterilization efficiency of different disinfectants on contaminated ACL grafts (Appendix Table A1).

Culture Outcomes

Overall, 5 studies 2,4,10,19,24 reported the culture results of ACL grafts that fell on the operating room floor and remained there for 15 seconds or 3 minutes. The rate of contamination ranged from 23.3% to 60.0%, and the pooled rate of positive cultures was 44.9% (92/205). The other 2 studies^{12,21} reported the simulation of microbial species and the number of microorganisms on the operating room floor based on the culture results. In these 7 studies, the ACL grafts dropped on the operating room floor were most commonly contaminated with staphylococci (Staphylococcus aureus and Staphylococcus epidermidis) and bacilli (Appendix Table A1). The contaminated ACL grafts treated with the antibiotic solution were mainly positive for staphylococci, bacilli, Clostridium, Acinetobacter, and diphtheroids. Simultaneously, staphylococci and bacilli were present on ACL grafts treated with the 10% povidone-iodine solution and staphylococci and gram-negative bacilli on grafts treated with the 4% chlorhexidine solution. The pooled results showed that the positive culture rates of disinfected ACL grafts with the 4% chlorhexidine solution, antibiotic solution, and 10% povidone-iodine solution were 2.3% (4/173), 10.8% (17/158), and 21.0% (30/143), respectively.

Comparison of 4% Chlorhexidine Solution Versus Antibiotic Solution

Among the 7 included studies, 4 studies^{2,12,19,24} compared the sterilization efficiency between the 4% chlorhexidine

solution and an antibiotic solution on ACL grafts dropped on the operating room floor. The pooled results indicated that there was a significant difference in the incidence of positive cultures between the 2 groups, with the 4% chlorhexidine solution displaying superior decontamination in comparison with the antibiotic solution (OR, 0.17 [95% CI, 0.05-0.57]; P = .004). Heterogeneity was low, with almost all variations in the effect size attributed to random sampling errors ($I^2 = 45\%$; P = .14) (Figure 2).

Comparison of 10% Povidone-Iodine Solution Versus Antibiotic Solution

Only 3 small studies^{2,12,19} evaluated the decontamination efficiency between the 10% povidone-iodine solution and antibiotic solution, but 1 of them¹² was not assessed because it reported that both the 10% povidone-iodine solution and the antibiotic solution were 100% ineffective. There was no heterogeneity among the results of these 3 studies ($I^2 = 0\%$; P = .97), and the results were analyzed through a fixed-effects model. The pooled results revealed that the antibiotic solution showed superior decontamination to the 10% povidone-iodine solution (OR, 0.20 [95% CI, 0.07-0.55]; P = .002) (Figure 3).

Comparison of 4% Chlorhexidine Solution Versus 10% Povidone-Iodine Solution

There were 4 studies^{2,4,12,19} that compared the decontamination efficiency between the 4% chlorhexidine solution and 10% povidone-iodine solution on ACL grafts dropped on the operating room floor. There was heterogeneity between the results of each study ($I^2 = 66\%$; P = .03), and the results were analyzed through a random-effects model, which showed no statistical significance between the 2 groups (OR, 0.09 [95% CI, 0.01-0.96]; P = .005). Based on the sensitivity analysis results, heterogeneity arose mainly from the Barbier et al⁴ study. After excluding this study, no heterogeneity was found ($I^2 = 0\%$; P = .44). After excluding the Barbier et al⁴ study, the pooled results showed that there was a significant difference between the 2 groups (OR, 0.04 [95% CI, 0.01-0.20]; P < .0001) (Figure 4).

	Antibiotic so	olution	10% Providone-iodine	solution		Odds Ratio		Odds		Ratio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% CI	Year		M-H, Fixed, 95% Cl		
Cooper. DE 1991	3	10	0	0		Not estimable	1991				
Goebel MF.1994	8	8	8	8		Not estimable	1994				
Molina. ME 2000	3	50	12	50	56.5%	0.20 [0.05, 0.77]	2000		_		
Parker. RD 2008	0	0	0	0		Not estimable	2008				
Plante. MJ 2012	1	30	0	0		Not estimable	2012				
Barbier O. 2014	0	0	1	25		Not estimable	2014				
Badran. MA 2016	2	60	9	60	43.5%	0.20 [0.04, 0.95]	2016				
Total (95% CI)		158		143	100.0%	0.20 [0.07, 0.55]					
Total events	17		30								
Heterogeneity: Chi ² = 0.00, df = 1 (P = 0.97); l ² = 0%								100			
Test for overall effect: Z = 3.10 (P = 0.002)								0.01	0.1 Favours Antibiotic solution	Favours 10% Providon	100 ne-iodine solution

Figure 3. Forest plot depicting the sterilization efficiency of the 10% povidone-iodine solution versus the antibiotic solution. M-H, Mantel-Haenszel.

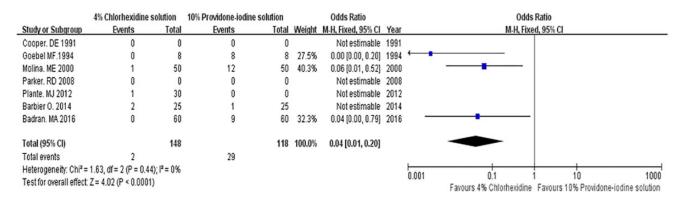


Figure 4. Forest plot depicting the sterilization efficiency of the 4% chlorhexidine solution versus the 10% povidone-iodine solution. M-H, Mantel-Haenszel.

DISCUSSION

The present systematic review and meta-analysis revealed 2 main findings. One, the rate of accidentally dropped ACL grafts that resulted in positive bacterial cultures was 44.9% and predominantly consisted of staphylococci and bacilli. Two, comparing the sterilization efficiency of 3 different disinfectants, the 4% chlorhexidine solution showed excellent decontamination on the dropped ACL grafts, with the lowest positive culture rate of only 2.3\%, followed by the antibiotic solution of 10.8% and the 10% povidone-iodine solution of 21.0%.

The inadvertent contamination of ACL grafts during ACL reconstruction has led to surgical complications, and there is currently no consensus in the literature to support the most appropriate management.¹³ If ACL grafts are dropped onto the operating room floor, proper and effective disinfection methods should be used to ensure low incidence of complications and the preservation of ACL grafts. Previous studies have reported some differences in the contamination rates of bone autografts intentionally dropped onto the operating room floor. Alomar et al¹ conducted a laboratory study in which 69 fresh osteochondral autografts were dropped onto the operating room floor, and they found that the rate of contamination was 42%; the most common microorganisms were

Staphylococcus epidermidis (24.1%) and bacilli (20.7%). As part of an experiment, Hirn et al¹⁵ rubbed 60 femoral head specimens on the operating room floor and found coagulase-negative staphylococci and bacilli as the most prevalent microorganisms. In another study, Bruce et al⁷ found that the contamination rate of dropped osteo-articular fragments was 70%, with coagulase-negative staphylococci as prevalent microorganisms.

The present meta-analysis revealed that the contamination rate of grafts was 44.9%, and the 2 most common microorganisms identified from the dropped ACL grafts were staphylococci and bacilli, which is consistent with previously reported operating room microbial profiles.³¹ Similar to this study's results, Barbier et al⁴ reported that the risk of contamination of a hamstring graft dropped on the floor was up to 40%. However, in a previous experimental study, Burd et al⁸ deliberately dropped tendon grafts on the operating room floor and found that the contamination rate was variable. They reported that the contamination rate was 60% for bone and tendons, while it was about 10% for bone alone. This reminds us that the susceptibility to contamination of bone fragments and ACL grafts is different, and dropped bone fragments may be prone to a higher risk of contamination. Nevertheless, we should interpret these results cautiously, as positive bacterial culture results do not necessarily indicate septic arthritis after ACL

reconstruction in clinical practice. Also, Hantes et al¹⁴ conducted a prospective study and found no association between graft contamination and postoperative infection.

Although several experimental studies have investigated the management of ACL graft contamination,^{14,21,24} to our knowledge, there is no meta-analysis that has comprehensively reviewed these studies to date. In existing studies, disinfectants available in the operating room, including a chlorhexidine solution, antibiotic solution, and iodophor solution of variable concentrations, are usually the first choices. For instance, in 2011, Bauer et al⁶ conducted a study to compare the effectiveness of 5 different disinfectant methods on experimentally contaminated bone grafts. Although they proved that both dry iodophor and chlorhexidine produced excellent sterilization effects, the latter killed all live cells within the bone graft. Similarly, Bruce et al⁷ demonstrated that povidone-iodine and 4% chlorhexidine gluconate were the most effective disinfectants for dropped osteoarticular bone fragments, but they did not recommend 4% chlorhexidine gluconate as an effective decontaminant because it decreased chondrocyte cell viability. Liu et al¹⁸ found that the use of 2% chlorhexidine significantly reduced the cell viability of osteoblasts, fibroblasts, and myoblasts and permanently stopped cell migration regardless of exposure time. It seems that chlorhexidine can kill the living cells of a bone graft, but its effect on ACL grafts is unclear, and it is essential to guide graft sterilization clinically.

Chlorhexidine belongs to the bisbiguanide class of antiseptics, which is cytotoxic to fibroblasts and negatively affects cell proliferation. Previous studies^{13,28} have shown that chlorhexidine does not affect the biological structure of tendon grafts. This effect on tendon grafts may be caused by the difference in porosity in which bone porosity allows easy ingress of detergents to the interior of bone. Further research is needed in the future to clarify the possible effects of these disinfectants on viable tendon cells. Also, it is important to note that no viable cells remain after the incorporation of typical ACL grafts.

There is only 1 systematic review that has comprehensively discussed the treatment strategies for intraoperative ACL graft contamination,¹⁷ and its authors noted that they believed that chlorhexidine is the best disinfectant for dropped tendon grafts. However, their investigation included different concentrations of detergents or sterilization techniques, and thus, there were no comparable data available to perform a meaningful meta-analysis. An antibiotic solution and povidone-iodine (10%) solution are 2 other common and easily available disinfectants. A povidone-iodine (10%) solution is an effective antimicrobial remedy generally used for various purposes, including prophylactic disinfection and wound irrigation. Sover et al²⁹ recommended using a 10% povidone-iodine solution to sterilize bone grafts but indicated that there must be sufficient exposure time according to the degree of contamination. However, Stanford et al³⁰ demonstrated that the exposure of contaminated grafts to a 10% povidone-iodine solution for 30 minutes resulted in incomplete sterilization.

In the present study, the dropped ACL grafts treated with the antibiotic solution had a 10.8% positive culture

rate, which showed a better disinfection effect than the 10% povidone-iodine solution. Similarly, Yaman et al³⁴ demonstrated that keeping contaminated graft materials in an antibiotic solution for specific periods yielded favorable outcomes. However, the ingredients of the antibiotic solutions included in the present study are not consistent; they consisted mainly of gentamicin, clindamycin, polymyxin, and bacitracin. Although vancomycin was proven to be an effective antibiotic for disinfecting ACL grafts,^{22,23} none of the studies included vancomycin. Schüttler et al²⁷ used a porcine tendon model and demonstrated that 100%of the tendon contaminated by Staphylococcus epidermidis was effectively disinfected with vancomycin after 20 minutes. Perez-Prieto et al²² performed a prospective controlled study and found that ACL graft harvest and manipulation caused bacterial contamination in 14% of cases but that contamination can be eliminated entirely after a soak in a vancomycin solution. Consistent with Perez-Prieto et al, another controlled observational study with 1585 patients by Phegan et al²³ concluded that presoaking of hamstring grafts with topical vancomycin during ACL reconstruction might reduce the postoperative infection rate. However, Deijkers et al¹¹ believed that irrigating contaminated grafts with an antibiotic solution had no benefit because the exposure time was not sufficient enough to make antibiotics effective.

Although the pooled results of the studies in the present meta-analysis showed that the antibiotic solution had an inferior sterilization effect on ACL grafts, we speculate that the difference is because of the inconsistent sensitivity of different microorganisms to antibiotics. Therefore, we believe that antibiotics sensitive to staphylococci and bacilli may be more suitable for the disinfection of dropped ACL grafts. Of course, further research should focus more on comparing the decontamination efficiency of vancomycin, 4% chlorhexidine, and 10% povidone-iodine.

Limitations

There are several limitations to this systematic review and meta-analysis. First, there was little uniformity across all studies in reporting cleaning methods after ACL grafts were contaminated. Additional procedures were performed in all the included studies, and thus, the effect of cleaning methods such as mechanical washing and rinsing could not be assessed. Second, the graft choice was variable among the included studies: A hamstring tendon autograft was used in 3 studies, while a bone-patellar tendon-bone graft was used in 3 other studies and ACL specimens in 1 more study. It has been proven that different grafts have different mechanical properties and clinical prognoses.^{9,26} However, in the present meta-analysis, we considered that the varied graft choice might have a negligible effect on the contamination event caused by the graft's falling on the operating room floor. Moreover, we should also acknowledge the presence of bone in bone-patellar tendon-bone grafts.

A third limitation is that the components of the antibiotic solutions among the included studies were varied, and this aggregation may overlook some important information. That said, it is challenging to collect efficient data because of the large difference in antibiotics involved in the studies, and no studies used vancomycin, which has been shown to be effective in reducing ACL infection rates.³² Fourth, the endpoint of the included studies was positive culture growth. However, it is noteworthy that positive culture results do not necessarily indicate that a clinical infection will develop.^{5,14} Data from these in vitro laboratory results could be extrapolated to clinical practice, but caution should be exercised. Although the present meta-analysis did not address the long-term infection risk associated with reimplanting contaminated grafts in humans, we believe that the culture-positive growth from the surgical environment could be considered a reasonable indicator for the risk of future infections.

CONCLUSION

The present systematic review and meta-analysis results demonstrated that the contamination of dropped ACL grafts during ACL reconstruction occurred at a relatively high rate, with staphylococci and bacilli being the most common microorganisms in dropped ACL grafts. Decontamination using a 4% chlorhexidine solution reliably disinfected ACL grafts that dropped on the operating room floor, with a disinfection rate of 97.7%. Based on the current data, we recommend disinfecting with a 4% chlorhexidine solution and reimplanting ACL grafts. This information can guide sports medicine surgeons to decide on appropriate remedial measures in similar dilemmas.

REFERENCES

- Alomar AZ, Somily AM, Alraiyes TM, Bin Nasser AS, Aljassir FF. Quantification analysis of the intraoperative bacterial contamination rate and level in osteochondral autografts. *Am J Sports Med.* 2016;44(3): 761-766.
- Badran MA, Moemen DM. Hamstring graft bacterial contamination during anterior cruciate ligament reconstruction: clinical and microbiological study. *Int Orthop*. 2016;40(9):1899-1903.
- Bansal A, Lamplot JD, VandenBerg J, Brophy RH. Meta-analysis of the risk of infections after anterior cruciate ligament reconstruction by graft type. Am J Sports Med. 2018;46(6):1500-1508.
- Barbier O, Danis J, Versier G, Ollat D. When the tendon autograft is dropped accidently on the floor: a study about bacterial contamination and antiseptic efficacy. *Knee*. 2015;22(5):380-383.
- Barriga A, Diaz-de-Rada P, Barroso JL, et al. Frozen cancellous bone allografts: positive cultures of implanted grafts in posterior fusions of the spine. *Eur Spine J*. 2004;13(2):152-156.
- Bauer J, Liu RW, Kean TJ, Dennis JE, Petersilge W, Gilmore A. A comparison of five treatment protocols for contaminated bone grafts in reference to sterility and cell viability. *J Bone Joint Surg Am.* 2011; 93(5):439-444.
- Bruce B, Sheibani-Rad S, Appleyard D, et al. Are dropped osteoarticular bone fragments safely reimplantable in vivo? *J Bone Joint Surg Am.* 2011;93(5):430-438.
- 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(2):241-244.
- Chen W, Li H, Chen Y, Jiang F, Wu Y, Chen S. Bone–patellar tendon– bone autografts versus hamstring autografts using the same suspensory fixations in ACL reconstruction: a systematic review and metaanalysis. *Orthop J Sports Med*. 2019;7(11):2325967119885314.

- Cooper DE, Arnoczky SP, Warren RF. Contaminated patellar tendon grafts: incidence of positive cultures and efficacy of an antibiotic solution soak. An in vitro study. *Arthroscopy*. 1991;7(3):272-274.
- Deijkers RL, Bloem RM, Petit PL, Brand R, Vehmeyer SB, Veen MR. Contamination of bone allografts: analysis of incidence and predisposing factors. *J Bone Joint Surg Br.* 1997;79(1):161-166.
- Goebel ME, Drez D Jr, Heck SB, Stoma MK. Contaminated rabbit patellar tendon grafts: in vivo analysis of disinfecting methods. *Am J Sports Med.* 1994;22(3):387-391.
- Han Y, Giannitsios D, Duke K, Steffen T, Burman M. Biomechanical analysis of chlorhexidine power irrigation to disinfect contaminated anterior cruciate ligament grafts. *Am J Sports Med*. 2011;39(7): 1528-1533.
- Hantes ME, Basdekis GK, Varitimidis SE, Giotikas D, Petinaki E, Malizos KN. Autograft contamination during preparation for anterior cruciate ligament reconstruction. *J Bone Joint Surg Am*. 2008;90(4): 760-764.
- Hirn MY, Salmela PM, Vuento RE. High-pressure saline washing of allografts reduces bacterial contamination. *Acta Orthop Scand*. 2001; 72(1):83-85.
- Izquierdo R Jr, Cadet ER, Bauer R, Stanwood W, Levine WN, Ahmad CS. A survey of sports medicine specialists investigating the preferred management of contaminated anterior cruciate ligament grafts. *Arthroscopy*. 2005;21(11):1348-1353.
- Khan M, Rothrauff BB, Merali F, Musahl V, Peterson D, Ayeni OR. Management of the contaminated anterior cruciate ligament graft. *Arthroscopy*. 2014;30(2):236-244.
- Liu JX, Werner J, Kirsch T, Zuckerman JD, Virk MS. Cytotoxicity evaluation of chlorhexidine gluconate on human fibroblasts, myoblasts, and osteoblasts. J Bone Joint Infect. 2018;3(4):165-172.
- Molina ME, Nonweiller DE, Evans JA, Delee JC. Contaminated anterior cruciate ligament grafts: the efficacy of 3 sterilization agents. *Arthroscopy*. 2000;16(4):373-378.
- Nagda SH, Altobelli GG, Bowdry KA, Brewster CE, Lombardo SJ. Cost analysis of outpatient anterior cruciate ligament reconstruction: autograft versus allograft. *Clin Orthop Relat Res.* 2010;468(5): 1418-1422.
- Parker RD, Maschke SD. Mechanical agitation and serial dilution: an option for anterior cruciate ligament graft sterilization. *J Knee Surg*. 2008;21(3):186-191.
- Perez-Prieto D, Portillo ME, Torres-Claramunt R, Pelfort X, Hinarejos P, Monllau JC. Contamination occurs during ACL graft harvesting and manipulation, but it can be easily eradicated. *Knee Surg Sports Traumatol Arthrosc.* 2018;26(2):558-562.
- Phegan M, Grayson JE, Vertullo CJ. No infections in 1300 anterior cruciate ligament reconstructions with vancomycin pre-soaking of hamstring grafts. *Knee Surg Sports Traumatol Arthrosc.* 2016;24(9): 2729-2735.
- Plante MJ, Li X, Scully G, Brown MA, Busconi BD, DeAngelis NA. Evaluation of sterilization methods following contamination of hamstring autograft during anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthrosc.* 2013;21(3):696-701.
- Pogorzelski J, Themessl A, Achtnich A, et al. Septic arthritis after anterior cruciate ligament reconstruction: how important is graft salvage? Am J Sports Med. 2018;46(10):2376-2383.
- Salem HS, Varzhapetyan V, Patel N, Dodson CC, Tjoumakaris FP, Freedman KB. Anterior cruciate ligament reconstruction in young female athletes: patellar versus hamstring tendon autografts. *Am J Sports Med*. 2019;47(9):2086-2092.
- Schüttler KF, Scharm A, Stein T, et al. Biomechanical and microbiological effects of local vancomycin in anterior cruciate ligament (ACL) reconstruction: a porcine tendon model. *Arch Orthop Trauma Surg.* 2019;139(1):73-78.
- Sobel AD, Hohman D, Jones J, Bisson LJ. Chlorhexidine gluconate cleansing has no effect on the structural properties of human patellar tendon allografts. *Arthroscopy*. 2012;28(12):1862-1866.
- Soyer J, Rouil M, Castel O. The effect of 10% povidone-iodine solution on contaminated bone allografts. J Hosp Infect. 2002;50(3): 183-187.

- Stanford R, Solomon M, Levick M, Kohan L, Bell S. Sterilization of contaminated bone-tendon autografts using 10% povidone-iodine solution. *Orthopedics*. 1999;22(6):601-604.
- Suzuki A, Namba Y, Matsuura M, Horisawa A. Bacterial contamination of floors and other surfaces in operating rooms: a five-year survey. J Hyg (Lond). 1984;93(3):559-566.
- 32. Vertullo CJ, Quick M, Jones A, Grayson JE. A surgical technique using presoaked vancomycin hamstring grafts to decrease the risk of

infection after anterior cruciate ligament reconstruction. *Arthroscopy*. 2012;28(3):337-342.

- Wang C, Lee YH, Siebold R. Recommendations for the management of septic arthritis after ACL reconstruction. *Knee Surg Sports Traumatol Arthrosc.* 2014;22(9):2136-2144.
- Yaman F, Unlu G, Atilgan S, Celik Y, Ozekinci T, Yaldiz M. Microbiologic and histologic assessment of intentional bacterial contamination of bone grafts. *J Oral Maxillofac Surg.* 2007;65(8):1490-1494.

APPENDIX

TABLE A1

Disinfectants, Sterilization Methods, and Outcomes of Included Studies^a

Lead Author (Year)	Contamination Method	Disinfectants	Cleaning Method	Outcome Evaluation	No. of Positive Cultures/ Total: Types of Microorganisms
Molina ¹⁹ (2000)	Dropped on the floor for 15 s	 Untreated Antibiotic solution (40 mg of neomycin sulfate and 200,000 U of polymyxin B sulfate in 1000 mL of sterile saline) 10% povidone-iodine solution 4% chlorhexidine solution 	Immersion for 90 s	Culture	 29/50: Staphylococcus, Bacillus 3/50: Bacillus, Clostridium 12/50: Bacillus, Staphylococcus 1/50: gram-negative rods
Plante ²⁴ (2013)	Dropped on the floor adjacent to the surgical field for 15 s	Uncontaminated graft after harvest	Soak for 3 min	Culture	 7/30: S aureus, viridans streptococci 10/30: S aureus 7/30: S aureus, nonaureus staphylococci 9/30: S aureus 1/30: nonaureus staphylococci 1/30: Bacillus
Badran ² (2016)	Dropped on the floor adjacent to the surgical field for 15 s	 Untreated 10% povidone-iodine solution 4% chlorhexidine solution Bacitracin solution (50,000 U/L of normal saline) 	Immersion for 3 min	Culture	 30/60: S epidermidis 9/60: S epidermidis, S aureus 0/60: NG 2/60: S epidermidis, Acinetobacter
Parker ²¹ (2008)	Determined bacterial flora on the operating room floor and contaminated in a bacterial suspension for 15 s	 Untreated Antibiotic solution (166.66 U/mL of polymyxin B and 16.66 U/mL of bacitracin) Pulsatile lavage with antibiotic solution Mechanical agitation and serial dilution with antibiotic solution 	Soak/pulsatile lavage/ mechanical agitation and serial dilution for 15 min	Semiquantitative culture	
Cooper ¹⁰ (1991)	Dropped on the operating room floor for 3 min	• Untreated	Soak/rinse for 15 min	Culture	 6/10: diphtheroids, Bacillus 3/10: S epidermidis, diphtheroids
Barbier ⁴ (2015)	Dropped on the operating room floor for 15 s	 Untreated 4% chlorhexidine gluconate solution 10% povidone-iodine solution 0.5 g/100 mL sodium hypochlorite solution 	Immersion for 15 min	Culture	 10/25: Staphylococcus 2/25: Aerococcus sanguinicola, S aureus, S epidermidis 1/25: S capitis 4/25: S hominis, S capitis, S warneri
Goebel ¹² (1994)	Contaminated for 20 s each with 2 different species of coagulase- negative staphylococci	• 10% povidone-iodine solution	Soak for 30 min	Culture	 6/6: coagulase-negative staphylococci 8/8: Staphylococcus 8/8: S capitis, coagulase- negative staphylococci 0/8: NG

^aCFU, colony-forming unit; NG, no growth.