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Major article

Microbial contamination of surgical instruments used for laparotomy

Yuhei Saito RN, MS [a,b,*], Hiroyoshi Kobayashi MD, PhD [b], Yushi Uetera MD, PhD [a], Hiroshi Yasuhara MD, PhD [a], Takumi Kajiura PhD [b,c], Takashi Okubo MD, PhD [b]

[a] Surgical Center, The University of Tokyo Hospital, Tokyo, Japan
[b] Division of Infection Prevention and Control, Tokyo Healthcare University Postgraduate School, Tokyo, Japan
[c] Research and Development Division, Yoshida Pharmaceutical Co, Ltd, Tokyo, Japan

Key Words:
Surgical site infections
Intraoperative contamination
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Sterilization
Aseptic technique

Background: The aim of this study was to determine the risk of contamination of surgical instruments according to the type of instrument and the surgical procedure.

Methods: Microbiologic examination was conducted on 140 pairs of forceps used in 24 elective laparotomies. These included 60 pairs of tissue forceps and 80 pairs of DeBakey forceps. Microbes on their surface were recovered using a membrane filter method. Adenosine triphosphate assay was also performed simultaneously in each pair of forceps.

Results: A total of 66 strains of microbes was recovered from 44 collected instruments (31%), with microbial counts ranging from 0 to 296 colony-forming units. Among the recovered microbes, gram-negative cocci were dominant. The remaining microbes included 6 strains of gram-positive rods and 4 strains of gram-negative rods. The most common organism was *Staphylococcus epidermidis*, followed by *S hominis* and *S warneri*. Residual adenosine triphosphate was not correlated with the number of recovered microbes.

Conclusion: Surgical instruments tend to be contaminated during operations by microbes that inhabit the skin and organs. Surgical instruments could act as fomites for the pathogens of surgical site infection even if the surgical field is not apparently contaminated, through application of appropriate practices adhering to surgical site infection guidelines.

Infection that occurs at the operative site is known as *surgical site infection* (SSI).[1] SSIs have various adverse effects on patients who undergo surgery, such as unfavorable postoperative complications, need for additional treatment of SSI, prolonged hospital stay, and even mortality.[2,3] Substantial research has been conducted to prevent SSI, and, as a result, recommendations have been published as guidelines for SSI.[1,4] In these guidelines, sterilization of surgical instruments is recommended as one of the fundamental and classical measures against SSI. If instruments were microbially contaminated, it would lead to increased SSI incidence.[5] Therefore, instruments are decontaminated and sterilized between surgical procedures to prevent cross transmission. However, in spite of sterilization, surgical instruments remain one of the most important sources of SSI. They can be contaminated during surgical procedures through contact with resident skin flora, which recover several hours after preoperative skin preparation, or through contact with microbes in the digestive tract such as stomach, duodenum, and colon. Surgical instruments might act to spread microbes over the surgical field.

Previous studies have examined the microbial contamination of surgical instruments in central sterile supply departments, showing a relatively high incidence of contamination with high microbial counts.[6-10] However, these results do not necessarily indicate that contamination of instruments occurred intraoperatively because the possibility of postoperative contamination outside the operating room was not excluded. These studies only investigated the amount of microbes as the load for sterilization, and the authors did not discuss the possible intraoperative transmission of microbes, which might cause SSI via surgical instruments.

There are a limited number of references regarding the intraoperative contamination of surgical instruments as a risk for SSI. Furthermore, little attention has been paid to intraoperative contamination in most of the studies and guidelines, although cleaning and sterilization are always recommended.[1,3,4,11] There

* Address correspondence to Yuhei Saito, RN, MS, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail address: saitoyu-nur@h.u-tokyo.ac.jp (Y. Saito).

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may be an assumption that instruments will remain sterile throughout the operation because reprocessing of surgical instruments including sterilization is a well-established practice.

The aim of this study was to determine the risk of contamination of surgical instruments according to the type of instrument and the surgical procedure. This is key in determining the mechanisms of SSI through elucidating the relationship between SSI and contamination of surgical instruments during operations.

METHODS

Sixty pairs of tissue forceps and 80 pairs of DeBakey forceps were examined in the present study (Table 1). They were suspected to be important candidates as fomites in the development of SSI because they are used in almost all operations and are frequently in contact with skin and other organs, compared with other instruments. Tissue forceps are mainly used to grasp skin and DeBakey forceps to grasp organs or mucous membrane. DeBakey forceps were used with relatively high frequency, particularly for hepatobiliary operations and tended to be used in longer operations.

The operations were randomly selected from scheduled laparotomies during the period of November to December 2008. They included 7 gastrectomy, 6 colectomy, 6 hepatectomy, 2 pancreatoduodenectomy (PD), and 3 abdominal aortic aneurysm (AAA) repair operations. Laparoscopic operations were excluded from this study. The number of forceps according to operation time was 49 for short operations (less than 4 hours), 67 for intermediate-length operations (4-8 hours), and 24 for long operations (more than 8 hours). Gastrointestinal anastomosis was performed in gastrectomy, colectomy, and PD. This might cause microbial contamination by transmitting microbes inhabiting the transected intestinal lumen to the forceps, the surgeons' gloves, and the surgical field. On the contrary, anastomosis was not performed in hepatectomy and AAA repair.

Cephazolin was administered 1 hour before skin incision in patients undergoing gastrectomy, hepatectomy, PD, and AAA repair, and cefmetazole was administered in the same way to those undergoing colectomy. Among a total of 24 patients in this study, 2 had a history of iodine allergy, and 22 did not. Those 2 patients underwent preoperative skin preparation using chlorhexidine, and povidone-iodine was used in the other 22 patients. The 2 patients with iodine allergy underwent colectomy and PD, respectively.

Either of 2 types of preoperative skin preparation was performed in the present study, ie, paint-only technique and scrub-and-paint technique. With the paint-only technique, antiseptic is applied with cotton balls, drawing concentric circles over the patient's skin at least 3 times before draping and skin incision. This technique was used in gastrectomy and colectomy. With the scrub-and-paint technique, the patient's skin is scrubbed with surgical scrub solution and dried by blotting with a towel, followed by application of antiseptic with the paint technique as described above. This technique was used in hepatectomy, PD, and AAA repair.

Recovery of microbes

The forceps were collected at the end of surgery with an aseptic technique to assess bacterial contamination. Immediately after collection, they were immersed individually in 100 mL phosphate-buffered saline with 0.05% Polysorbate 80 solution in a sterilized polyethylene bag. The bag was agitated on a shaker at 150 rpm for 30 minutes. Next, 50 mL of the solution was sampled from the bag and filtered through a 0.45-μm membrane filter (Milliflex; Merck Millipore, Billerica, MA). The filters were put on tripticase soy agar plates and cultured at 32.5°C ± 2°C for 3 to 5 days; then, the numbers of colonies were counted, and the microbial count per instrument was determined. Microorganisms were identified by Gram's stain and using Microorganism Identification Test Kits; API STAPH, API 20 STREP, API 20NE, and API C AUX (SYSMEX bioMérieux Co, Ltd, Tokyo, Japan).

Table 1
Characteristics of surgical procedures and instruments

	Number of surgical instruments		
	Tissue forceps	DeBakey forceps	Number of operations
Surgical procedure			
Gastrectomy	14	16	7
Colectomy	24	25	6
Hepatectomy	12	24	6
Pancreatoduodenectomy	4	8	2
Abdominal aortic aneurysm repair	6	7	3
Operation time, hours			
<4	24	25	8
4-8	28	39	12
>8	8	16	4

Recovery factor

The recovery factor was determined to estimate the original number of microbes on the instruments. We utilized 12 forceps contaminated artificially with *Bacillus subtilis* (American Type Culture Collection 6633) spore solution (*Bacillus subtilis* 6633 Eiken; Eiken Chemical Co, Ltd, Tokyo, Japan). First, 50 μL of the spore solution adjusted to approximately 10^8 colony-forming units (CFU)/mL was dropped onto the surface of the forceps and stainless test pieces. Next, they were dried for 30 minutes at room temperature and put in separate sterile trays. After adding 100 mL phosphate-buffered saline with 0.05% Polysorbate 80 solution, the trays were agitated on a shaker for 30 minutes at 150 rpm. Next, 1 mL of the sampled solution was incubated on tripticase soy agar plates at 32.5°C ± 2°C for 48 hours, and the number of colonies was counted and the recovery factor calculated. Approximately 2.0×10^3 CFU of *B subtilis* were recovered from the surface of forceps, whereas 2.9×10^3 CFU were recovered from the spore solution. The recovery factor of instruments, which was calculated as 0.69, was factored into the final data.

Adenosine triphosphate assay

The amount of adenosine triphosphate (ATP) remaining on the surface of surgical instruments was measured to assess gross contamination of the instruments, which might be utilized as an indicator of the frequency of use or degree of tissue contact with the instruments. First, 100 μL of the sampled solution described above was put directly into the test tube of the ATP assay kit (LuciPac W; Kikkoman, Chiba, Japan) instead of putting a swab soaked in the solution. Next, the test tube was assembled as it was before, and ATP was measured using a luminometer (Lumitester; Kikkoman) in relative light units (RLU).

Statistical analysis

For statistical analysis, Pearson χ^2 test, Fisher exact test, and simple regression analysis were performed using JMP6.0 (SAS Institute, Cary, NC). A P value less than .05 was considered significant.

RESULTS

Microbial counts and identification

Forty-four of 140 pairs of forceps (31.4%) were found to be contaminated with microbes in a total of 24 laparotomies (Table 2). They included 13 tissue forceps and 31 DeBakey forceps. Frequency of a positive culture test result was significantly higher in DeBakey forceps than in tissue forceps (38.8% vs 21.7%, respectively, $P = .031$, Pearson χ^2 test).

Regarding the type of surgical procedure, the frequency of positive culture was highest in PD (41.7%), followed by AAA repair (38.5%), gastrectomy (36.7%), colectomy (32.7%), and hepatectomy (19.4%). According to the range of colony counts of 0, 1 to 10, 10 to 100, and > 100, the numbers of microbes recovered from the forceps were 19, 7, 4, 0 in gastrectomy; 33, 10, 5, 1 in colectomy; 29, 6, 0, 0 in hepatectomy; 7, 4, 1, 0 in PD; and 8, 3, 1, 1 in AAA repair, respectively. There was no significant difference in the frequency of positive culture among the types of surgical procedure ($P = .45$, Pearson χ^2 test). The numbers of forceps with positive culture were 32 of 91 (35.2%) in the anastomosis group and 12 of 49 (24.5%) in the nonanastomosis group, with no significant difference in positive culture rate between these 2 groups ($P = .25$, Fisher exact test).

The numbers of forceps with positive culture were 16 of 49 (32.7%) for short operations, 20 of 67 (29.9%) for intermediate-length operations, and 8 of 24 (33.3%) for long operations, with no significant difference among these 3 groups ($P = .93$, Pearson χ^2 test). The rate of positive culture did not increase as the duration of operation became longer.

The number of forceps with positive culture was 28 of 91 (30.8%) in the cephazolin group and 16 of 49 (32.7%) in the cefmetazole group, with no significant difference in positive culture rate between these 2 groups ($P = .85$, Fisher exact test). The number of forceps with positive culture was 43 of 132 (32.6%) in the povidone-iodine group and 1 of 8 (12.5%) in the chlorhexidine group, with no significant difference in positive culture rate between these 2 groups ($P = .44$, Fisher exact test). The number of forceps with positive culture was 27 of 79 (34.2%) in the paint-only group and 17 of 61 (27.9%) in the scrub-and-paint group, with no significant difference in positive culture rate between these 2 groups ($P = .47$, Fisher exact test).

Microbes recovered from surgical instruments included gram-positive cocci and rods, gram-negative rods, and fungi (Table 3). The most common organism recovered from instruments was *Staphylococcus epidermidis* (from 15 pairs of forceps), followed by *S hominis* (7 pairs), *S warneri* (5 pairs), and *Kocuria varians/rosea* (5 pairs). Approximately 62.1% of the recovered microbes were gram-positive cocci, 9.1% were gram-positive rods, and 6.1% were gram-negative rods. The number of strains of gram-positive cocci was 8 in gastrectomy, 21 in colectomy, 8 in hepatectomy, 5 in PD, and 11 in AAA repair. The number of strains of gram-positive rods recovered was 5 in gastrectomy and 1 in colectomy, and the number of strains of gram-negative rods recovered was 3 in gastrectomy and 1 in PD. The number of strains of fungi recovered was 2 in gastrectomy and 1 in colectomy. *Staphylococcus* species were found on both tissue forceps and DeBakey forceps and in all types of surgical procedures. Although *Enterococcus faecalis* and *E durans* were also found on both tissue forceps and DeBakey forceps, they were recovered only in colectomy cases. *Stenotrophomonas maltophilia* was recovered from tissue forceps only in PD cases.

Regarding the types of microbial species, 29 strains of gram-positive cocci were recovered in the paint-only group and 24 strains in the scrub-and-paint group, and 6 strains of gram-positive rods and 3 strains of fungi were recovered in only the paint-only group.

Table 2
Frequency of microbial contamination of surgical instruments according to type of instrument

Surgical instrument	Colony count (CFU) 0	1-10	11-100	>100	Positive culture rate (%)*
Tissue forceps	47 (78.3)	9 (15.0)	4 (6.7)	0 (0.0)	21.7
DeBakey forceps	49 (61.3)	21 (26.3)	7 (8.8)	3 (3.8)	38.8
Total	96 (68.6)	30 (21.4)	11 (7.9)	3 (2.1)	31.4

CFU, Colony-forming units.
NOTE. Numbers in parentheses represent proportion (%) of forceps with each range of colony count.
*$P = .031$, Pearson χ^2 test.

Table 3
Number of microbes recovered from forceps according to type of surgical procedure and instrument

Microbes	Instrument TF	DF	Total
Gram-positive cocci			
Staphylococcus sp	11	30	41
Kocuria varians/rosea	1	4	5
Micrococcus sp		1	1
Enterococcus sp	1	1	2
Streptococcus salivarius		1	1
Unidentified	1	2	3
Gram-positive rods			
Unidentified	1	5	6
Gram-negative rods			
Stenotrophomonas maltophilia	1		1
Unidentified		3	3
Fungi			
Candida utilis		1	1
Unidentified	1	1	2
Total	17	49	66

DF, DeBakey forceps; *TF*, tissue forceps.

Amount of ATP

ATP was detected from all collected instruments, with a mean value of 4,130 RLU, ranging from 6 to 41,073 RLU. There was no significant difference in RLU values between tissue forceps and DeBakey forceps or among the 5 types of procedures.

Forceps with higher residual ATP did not necessarily have more microbes on their surface (Fig 1). The correlation coefficient was 0.21, which was not statistically significant. The forceps included in the group with residual ATP below 100 RLU had a markedly lower contamination rate; only 1 of 12 pairs of forceps was microbially contaminated.

DISCUSSION

Our results demonstrated that intraoperative contamination of surgical instruments was not infrequent in spite of various means of preventing SSI, such as administration of antibiotics, preoperative skin preparation, and intraoperative aseptic technique. In general, the surgical field is considered to remain aseptic during operation.[12] However, microbes gradually recover in the surgical field and could cause microbial contamination of sterilized surgical instruments.[13,14] The relatively high incidence of contamination of surgical instruments sheds light on the mechanisms of SSI development. There is a possibility that skin drapes and meticulous surgical skills may be far more important for decreasing the risk of SSI than we previously considered. Gentle surgical maneuvers should be recommended even more strongly as basic practice.

Previous research has suggested that microbial contamination of surgical instruments occurs during surgical procedures.[6-10]

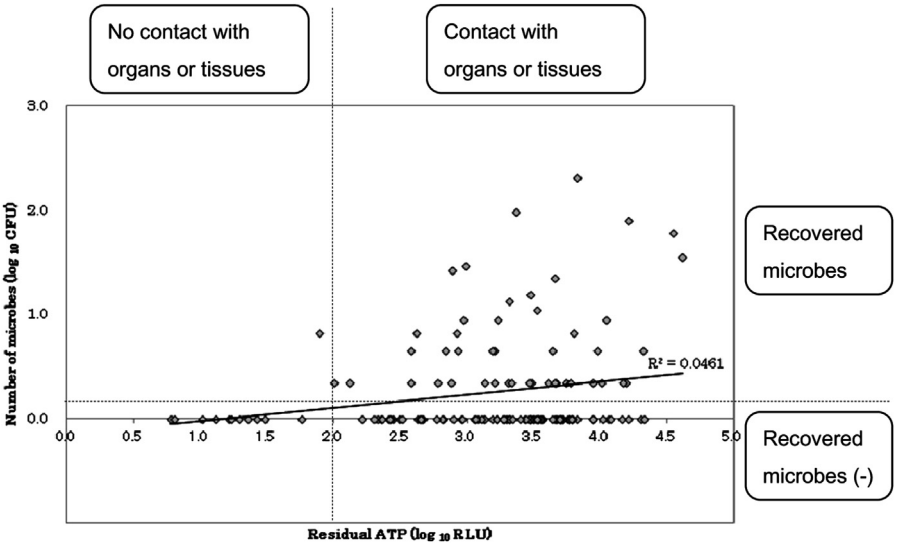

Fig 1. Number of microbes and residual ATP on surgical instruments. Positive culture reflects microbial transmission to forceps from organs or tissues of the patients. Forceps with high relative light units (RLU) were considered to have had more contact with organs or tissues, whereas others had little or no contact. This is supported by the higher incidence of positive culture of high RLU forceps compared with that of other forceps.

However, the precise mechanisms of contamination have not been elucidated because the authors were more concerned about the bioburden of surgical instruments before sterilization for the next use at operation. They did not assume that the surgical instruments were contaminated in the surgical field or refer to it when it did occur. Our results strongly suggest that surgical instruments are frequently contaminated during operations.

The spectrum of microbial species identified in the present study was similar to that of SSI pathogens identified in previous studies.[4,15] Our data suggested a close relationship between contamination of surgical instruments and the development of SSI, which accords with evidence that endogenous microbial flora are potential pathogens of SSI.[4] It is reported that microbial contamination of surgical instruments led to increased SSI incidence.[5] If surgical instruments were contaminated with endogenous microbial flora, it would also lead to increased risk of SSI. Microbial flora on patients might be transmitted via surgical instruments throughout the surgical field and become potential pathogens of SSI.

Previous studies reported that the rate of SSI varies depending on the operative procedure category.[16-18] The contamination rate of surgical instruments in the present study was relatively constant regardless of the procedure, being 31.4%, which appeared to conflict with previous studies. However, our results were not inconsistent with those studies because the existence of microbes on the surface of surgical instruments does not necessarily result in the development of SSI. The risk of SSI is affected by both the virulence of microbes and the resistance of host patients, as conceptualized previously.[4]

Another important finding of our study is that surgical instruments were contaminated through not only direct contact but also indirect contact. Some tissue forceps were contaminated with microbial species that inhabit the digestive tract, in spite of the fact that they were thought to have no direct contact with the source of microbial species. In general, tissue forceps have no direct contact with organs because instruments are selected by surgeons according to their specific purpose in that phase of the operation; tissue forceps are utilized exclusively for dealing with skin, whereas DeBakey forceps are used for other purposes throughout the operation. Some studies have demonstrated bidirectional transmission of microbes between hands and inanimate objects.[19,20] These data indicate that microbes are transported to the forceps from their source, without any direct contact with the source. The recovery of *Enterococcus* species in colorectal surgery may support our speculation. It also suggests that surgical gloves may play a role as fomites in the surgical field by transportation of microbes.

The results of ATP assay could not provide relevant information as a screening test for microbial contamination. This finding accorded with the results of previous studies.[21] Most of the forceps we examined were visually contaminated with blood and subcutaneous or visceral fat, which are considered to have ATP molecules within and around them. The reaction between ATP and luciferin was considered to be a factor in the results of the ATP assay.[21] However, we believe it can be utilized as an indicator to assess the events of direct contact with organs or tissues. Analysis of data related to the relationship between residual ATP and the number of microbes on surgical instruments showed that there was a threshold around the value of 2.0-$\log_{10}$ RLU, at which microbes begin to be recovered (Fig 1). The frequency of positive culture was relatively low below this threshold, whereas it was higher in instruments with higher residual ATP. These data suggested that instruments with residual ATP of 100 or more RLU had contact with the patient's organs or tissues.

Our study has a few limitations. One is that we did not follow the postoperative clinical course of the study patients because we only focused on intraoperative contamination of forceps. The population of patients in the present study was too small to discuss the relation between SSI rate and the contamination of surgical instruments.[22,23] Further study should be conducted to examine the relationship between intraoperative contamination of surgical instruments and the development of SSI.

Another limitation of our study is that not all the microbes on surgical instruments, including biofilm-forming microbes, might have been recovered. More types and greater numbers of microbes might be recovered using other recovery methods or culture conditions such as longer culture times. For example, *S aureus* and *Escherichia coli*, the major pathogens of SSI, were not identified in the present study.[4,15] However, this was reasonable considering the identification rate in a previous study.[14] It was also suggested that more than 85% of SSI was caused by pathogens that do not need longer culture times, and there were no microbes listed as pathogens that need longer culture times.[4] We believe that the influences

of biofilm and longer culture times are negligible because of the identical positive culture rates regardless of the operation time.

As another example related to microbial recovery, the microbial species identified in our study were limited to aerobes because only aerobic culture was adopted for incubation. However, the results of anaerobic microbes might have had only a limited influence on our conclusion because the rate of SSI development caused by anaerobes is low.[15,24] There was potential existence of viable but nonculturable bacteria, which might have been detected using 16S ribosomal RNA or DNA detection methods. However, the suitability of these methods has not been established because of limited reports on determining the pathogens of SSI. Therefore, we would not have been able to conclude whether viable but nonculturable bacteria can cause SSI, even if we had obtained positive results from these methods. Future studies are needed to investigate the relation between the taxonomy in the contamination on forceps and the pathogens of SSI.

CONCLUSION

Our results demonstrated that surgical instruments tend to be contaminated during operation by microbes that inhabit the superficial or deep layers of the skin and internal organs. It is likely that surgical instruments could act as fomites for microbes including pathogens of SSI, even if the surgical field is not apparently contaminated.

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