

Authors

Norihiro Kameda
MSN, RN
Faculty doctoral student, Graduate School
of Nursing, Chiba University.

Junko Nishio
BScMLT
Laboratory Technologist, Graduate School
of Nursing, Chiba University.

Toshiko Ogawa
RN, PhD
Senior Lecturer, Graduate School of
Nursing, Chiba University.

Shinobu Okada
RN, PhD
Professor, Graduate School of Nursing,
Chiba University.

Corresponding author

Norihiro Kameda
MSN, RN
Faculty doctoral student, Graduate
School of Nursing, Chiba University.
norihiro0430@chiba-u.jp

Intestinal bacterial contamination of surgical instruments used for wound closure during intestinal surgery

Keywords: surgical site infections, microbiological contaminations, surgical instruments, intestinal surgeries

Background

A surgical site infection (SSI) is defined by the Centers for Disease Control and Prevention (CDC) as an infection occurring at the operative site within specific time frames, depending on the nature of the surgical procedure¹. SSI incidence differs among surgeries, and intestinal surgery is one of those with a high SSI incidence. In Japan, from 2008 to 2010, SSI incidence rates for colon and rectal surgery were 15.0 and 17.8 per cent, respectively². SSI results in prolonged hospital stays and additional medical expenses^{3,4}. (3) Therefore, SSI prevention is particularly important for improving patient outcomes.

Perioperative surgical site contamination with endogenous or exogenous bacteria is the main cause of SSI, explaining why the SSI incidence in intestinal surgery is higher than in other surgeries. Although the most common bacteria that cause SSI live on skin, such as *Staphylococcus aureus* and coagulase-negative staphylococci (CNS), bacteria of intestinal origin, such as *Enterococcus* species., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *K. oxytoca* and *Acinetobacter* species., have also been isolated from SSIs developed after intestinal surgeries¹. Surgical incision and operating site contamination with intestinal bacteria is a particularly problematic issue during intestinal surgery.

Previous research suggests that surgical instruments transmit SSI-causing pathogens⁵. Thus, operating theatre staff should manage surgical instruments appropriately, distinguish contaminated instruments from clean instruments and change gloves periodically to keep the operating field as clean as possible.

Using clean instruments for wound closure is important because the subcutaneous adipose tissue is vulnerable to infection. Therefore, preparing sterilised surgical instruments specifically for wound closure is recommended to reduce SSI risk. In a prospective study in which 397 patients underwent colorectal surgeries⁶, the exchange of surgical instruments for dermal suturing was associated with a lower incisional SSI rate (odds ratio, 0.276). In a comparison of three types of wound closures (no irrigation, irrigation only and irrigation plus surgical instrument exchange), the SSI incidence was the lowest in the irrigation plus exchange group, followed by the irrigation and the no irrigation groups (2.1, 6.5 and 12.0 per cent, respectively; $p = 0.034$). Therefore, using sterilised instruments for wound closure is effective in reducing incisional SSIs⁷.

In Japan, instruments not used for intestinal manipulation are considered clean, even if they were used for prior skin incisions, and they are still used for wound closure after being kept in a clean area. Koh⁸

reported that experienced nurses spent less time on the final count (count of all instrumentation, gauzes and swabs introduced into the sterile field throughout the surgery) and encountered fewer interruptions during the count than novices, indicating better task management performance. Bacteria generated from the operating field might contaminate surgical instruments in the clean area, and the surgical nurses' experience could influence this. Microbiological evaluation of these surgical instruments is necessary for the use of sterilised surgical instruments for wound closure to be accepted as a standard procedure.

In this study, we aimed to evaluate the degree and nature of contamination of surgical instruments used for wound closure during intestinal surgery and to validate the relationship between surgical features and surgical staff member's years of experience.

Methods

Study facility

We conducted this study at a teaching hospital in Japan where standard SSI-prevention measures were being practiced. This included maintaining the operating room environment at a positive pressure compared to the adjacent rooms and filtering the air through high-efficiency particulate air filters. An antimicrobial agent was administered 60 minutes preoperatively and every three hours during surgery. Hair was clipped with surgical clippers if necessary, and the patient bathed on the day prior to surgery. Surgical instruments used for intestinal manipulation were kept separate from clean instruments and not used for wound closure. Staff exchanged surgical gloves after intestinal manipulation.

Targeted surgeries

We included only scheduled intestinal surgical procedures accompanying abdominal operations in our study. We collected samples for microbiological examination from surgical instruments used during colon surgery (COLN) and rectal surgery (REC). We also collected samples from surgical instruments used during hepatectomy (BILI-O) and pancreatic surgery (BILI-PD) as an anastomosis between the biliary duct and small intestine accompany these surgeries.

Surgical staff

We collected data concerning the gender and years of experience of the scrub nurses and surgeons participating in the targeted surgeries.

Targeted instruments

Seven instruments, including two pairs of hooked forceps, flat hooks and needle holders, and one pair of surgical scissors were examined. Table 1 displays targeted instruments and their usage. Upon commencement of surgery, a package of these instruments was opened for skin incision, kept separated from the operating field in the clean area and used only for operation in the

clean area, if necessary (not used for intestinal manipulation), during the surgery. We collected these surgical instruments for microbiological examination immediately prior to wound closure. Because we had collected surgical instruments that were supposed to be used for wound closure, sterile instruments prepared by the researcher were used instead for wound closure of the target surgeries.

Microbiological examination

Immediately following surgery, the seven instruments were placed into clean plastic bags and washed three times with 20 ml of sampling solution (10.1 g Na_2HPO_4 , 0.4 g KH_2PO_4 , 1.0 g Triton X-100 made up to a final volume of 1000 ml with distilled water). We recovered each sampling solution after washing and filtered 60 ml through a 0.45- μm membrane filter (47 mm diameter). The membrane filters were placed on a sterilised pad containing 2 ml of trypticase-soy broth (Difco), cultured under microaerobic conditions for 24 hours and then under aerobic conditions for 24 hours at 37°C. We counted the colonies growing on the filters and stained using Gram stain. All isolates from clean instruments and the predominant isolates from unclean instruments were identified

Table 1: Target instruments and their usage

Instrument	Use
surgical scissors (Cooper scissors)	• blunt peeling and cutting of tissue • incision of abdominal wall, fascia and intestinal tract • cutting sutures
hooked forceps	• gripping a variety of things (instead of using the fingers), such as tissue, gauze, suture needle
flat hook	• separating muscles and tissues obstructing surgeon's view
needle holder (Matchu needle holder)	• gripping and carrying suture needle when suturing organs or surgical wounds

biochemically (VITEK2® Compact; Sysmex bioMérieux) or by mass spectrometry (MALDI Biotyper®). We compared the isolates in cases where both instruments were used for the same operation.

Statistical analysis

The Pearson χ^2 test was used for comparing contamination among surgical procedures and risk factors among surgical procedures. One-way analysis of variance (ANOVA) compared patient age, operation duration and the amount of bleeding during surgery. The Pearson correlation coefficient was used to analyse the relationship between the logarithmic value of the bacterial count and years of experience of the surgical staff. We used IBM SPSS® Statistics 23.0 for statistical analysis. $P < 0.05$ was considered to be significant.

Ethical considerations

The study site's Ethical Review Committee approved this study as

did the ethics committee of the Graduate School of Nursing, Chiba University (approval number 26-74). Data collection only proceeded after obtaining consent from patients and staff of the target surgeries. Written informed consent was sought from the surgical team (to gain access to use the surgical instruments) and from patients involved in the surgeries to allow publication of their data. Information pertaining to the patients' privacy was kept confidential.

Results

Summary of the targeted surgeries and surgical staff

Table 2 summarises instrument data collected from 31 intestinal surgeries. The surgeries included BILI-O, BILI-PD, COLN and REC cases – 10 (32.3 per cent), 10 (32.3 per cent), 6 (19.6 per cent) and 5 (16.1 per cent), respectively. A statistically significant difference in operation duration among the procedures

was determined ($p = 0.00$). BILI-PD surgeries were the longest operations and COLN were the shortest.

Surgical instrument contamination

We obtained samples from 217 surgical instruments and isolated bacteria from 190 (87.6 per cent) instruments. The amount of detected bacteria ranged from 2.0 to 1.7×10^4 CFU (colony forming units).

The degree of contamination of each instrument was categorised according to the logarithmic value of detected numbers of bacteria as follows:

1. no detection (-), not contaminated
2. $\log_{10}\text{CFU} < 1.5$ ($\pm$), slightly contaminated
3. $1.5 \leq \log_{10}\text{CFU} < 2.5$ (+), mildly contaminated
4. $2.5 \leq \log_{10}\text{CFU} < 3.5$ (++), moderately contaminated
5. $\log_{10}\text{CFU} \geq 3.5$ (+++), severely contaminated.

Table 2: Summary of the targeted operations

			Surgical procedure				p*
		(N = 31)	BILI-O	BILI-PD	COLN	REC	
Gender (n)	Male	21 (67.7%)	6 (19.4%)	2 (22.6%)	3 (9.7%)	5 (16.1%)	0.32
	Female	10 (32.3%)	4 (12.9%)	3 (9.7%)	3 (9.7%)	-	
Age (Mean $\pm$ SD)		65 $\pm$ 15.2	62.4 $\pm$ 14.9	67.1 $\pm$ 8.4	60.7 $\pm$ 19.9	64.2 $\pm$ 15.0	0.45
Wound classification (n)	Class II	31 (100%)					
ASA-PS (n)	1	5 (16.1%)	2 (6.5%)	2 (6.5%)	-	1 (3.2%)	0.28
	2	23 (74.2%)	8 (25.8%)	8 (25.8%)	4 (12.9%)	3 (9.7%)	
	3	3 (9.7%)	-	-	2 (6.5%)	1 (3.2%)	
Risk index (n)	0	2 (6.5%)	-	-	2 (6.5%)	-	0.00
	1	27 (87.1%)	10 (32.3%)	10 (32.3%)	2 (6.5%)	5 (16.1%)	
	2	2 (6.5%)	-	-	2 (6.5%)	-	
Operation length in minutes (Mean $\pm$ SD)		374.6 $\pm$ 114.3	405.5 $\pm$ 24.1	456.7 $\pm$ 18.5	226.0 $\pm$ 31.9	327.0 $\pm$ 53.7	0.00
Bleeding (ml)		882.9 $\pm$ 793.7	1239.0 $\pm$ 363.7	523.3 $\pm$ 289.0	523.3 $\pm$ 289.0	840.0 $\pm$ 108.7	0.34

* $P < 0.05$ was considered to be significant.

Table 3: Instrument contamination level and comparison of instrument contamination by surgical procedure

Type of surgery	Instrument	n	Degree of contamination*				
			–	±	+	++	+++
BILI-O	surgical scissors	10	0	2	6	1	1
	hooked forceps	20	0	12	6	2	0
	flat hook	20	3	6	10	0	1
	needle holder	20	1	10	6	1	2
	BILI-O total	70	4 (5.7%)	30 (42.9%)	28 (40.0%)	4 (5.7%)	4 (5.7%)
BILI-PD	surgical scissors	10	2	2	4	2	0
	hooked forceps	20	4	8	5	3	0
	flat hook	20	5	9	6	0	0
	needle holder	20	3	16	1	0	0
	BILI-PD total	70	14 (20.0%)	35 (50.0%)	16 (22.9%)	5 (7.1%)	0 (0.0%)
COLN	surgical scissors	6	1	0	3	1	1
	hooked forceps	12	2	7	3	0	0
	flat hook	12	3	5	3	1	0
	needle holder	12	3	4	3	2	0
	COLN total	42	9 (21.4%)	16 (38.1%)	12 (28.6%)	4 (9.5%)	1 (2.3%)
REC	surgical scissors	5	0	0	3	1	1
	hooked forceps	10	0	7	1	2	0
	flat hook	10	0	4	5	1	0
	needle holder	10	0	6	4	0	0
	REC total	35	0 (0.0%)	17 (48.6%)	13 (37.1%)	4 (11.4%)	1 (2.8%)
Total instruments		217	27 (12.4%)	98 (45.2%)	69 (31.8%)	17 (7.8%)	6 (2.8%)

* Degree of contamination:
– (no contamination)

± (slightly contaminated)
+ (mildly contaminated)

++ (moderately contaminated)
+++ (severely contaminated).

Table 3 shows the degree of contamination of each instrument in the four types of surgical procedures investigated. We observed contamination in more than two of the seven instruments in all surgeries, and contamination of all instruments was observed in 18 (58.1 per cent) cases. The chance of instrument contamination in BILI-O and REC was 94.2 and 100 per cent, respectively, and this chance was significantly greater than that observed in BILI-PD and COLN (80.0 and 78.5 per cent, respectively; $p = 0.004$). The ratio of severely contaminated (+++) instruments

was the greatest in BILI-O (5.7 per cent). In COLN and REC, 9.5 and 11.4 per cent of instruments, respectively, were moderately contaminated (++). Severely and moderately contaminated instruments accounted for 11.4, 11.9 and 14.3 per cent of instruments in BILI-O, COLN and REC, respectively, but they accounted for only 7.1 per cent of instruments in BILI-PD. There was no bacterial isolation in 20.0 and 21.4 per cent of instruments used in BILI-PD and COLN, respectively. Conversely, we did not isolate any bacteria in 5.7 per cent of instruments used in BILI-O

and 0.0 per cent of instruments used in REC.

Detected bacterial species

We isolated 627 bacteria from the surgical instruments. The most frequently isolated species were *Staphylococcus* species (206 isolates, 32.9 per cent), including *Staphylococcus aureus* (17 isolates, 2.7 per cent) and CNS (189 isolates, 30.1 per cent). *Bacillus* species (159 isolates, 25.4 per cent) and *Enterococcus* species (47 isolates, 7.5 per cent) were also frequently isolated. Gram negative rods, such as *Escherichia coli*, *Pseudomonas*

Table 4: Bacterial species of 627 isolates derived from surgical instruments

Bacteria	Number of isolates
coagulase-negative staphylococci (CNS)	189 (30.1%)
<i>Staphylococcus aureus</i>	17 (2.7%)
<i>Bacillus</i> species	159 (25.4%)
<i>Enterococcus</i> species	47 (7.5%)
<i>Escherichia coli</i>	12 (1.9%)
<i>Pseudomonas aeruginosa</i>	13 (2.1%)
<i>Klebsiella pneumoniae</i>	14 (2.2%)
<i>Klebsiella oxytoca</i>	2 (0.3%)
<i>Acinetobacter baumannii</i>	2 (0.3%)
Other bacteria	172 (27.4%)
Total	627 (100%)

aeruginosa, *Klebsiella pneumoniae*, *K. oxytoca* and *Acinetobacter baumannii* were also detected at 12 (1.9 per cent), 13 (2.1 per cent), 14 (2.2 per cent), 2 (0.3 per cent) and 2 (0.3 per cent) isolates, respectively (see Table 4).

Most of the isolated bacteria were organisms that cause SSI. Figure 1 presents detection of SSI causative bacteria during each surgical procedure. CNS was detected in almost all cases. It was detected in

nine cases (90.3 per cent) of BILI-O and BILI-PD, five cases (83.3 per cent) of COLN and five cases (100 per cent) of REC. *Enterococcus* species were the second most frequently isolated bacteria. They were found in four cases (40.0 per cent) of BILI-O and BILI-PD, three cases (50.0 per cent) of COLN and one case (20.0 per cent) of REC. *S. aureus* was detected in six cases – two cases (20.0 per cent) of BILI-O and BILI-PD, one case (17 per cent) of COLN and one case of REC. *E. coli* was also detected in six cases – four cases (50.0 per cent) of BILI-O, one case (16.7 per cent) of COLN and one case (20.0 per cent) of REC. *E. cloacae* was detected in five cases – two cases (20.0 per cent) of BILI-PD, two cases of COLN (33.3 per cent) and one case of REC (20.0 per cent) – and *P. aeruginosa* was isolated in one case of BILI-O.

The bacteria detected were divided into two groups according to their

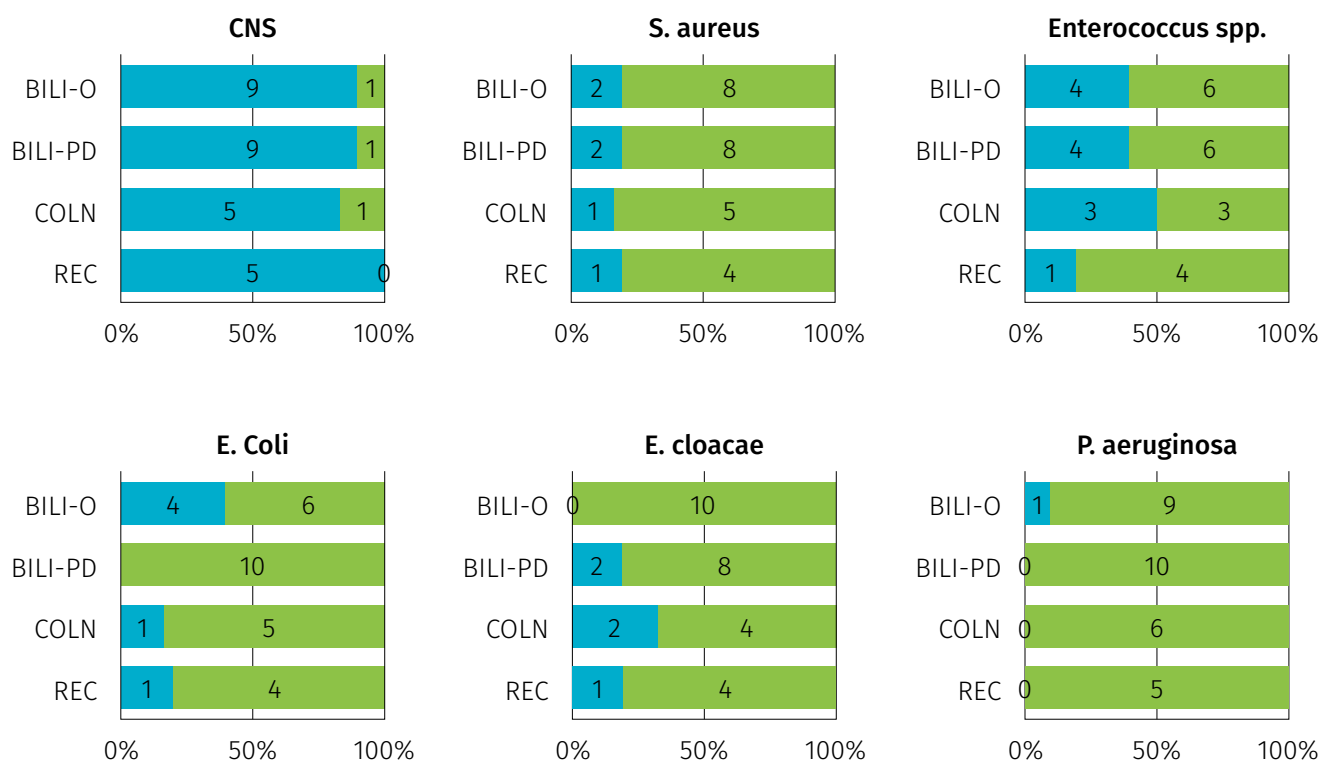


Figure 1: SSI causative bacteria and the number of cases in which they were detected on any of the seven clean instruments used in the four types of surgery (Blue indicates bacteria detected, green indicates bacteria not detected.)

habitats – skin-derived bacteria were CNS, *S. aureus* and other Gram positive cocci while intestine-derived bacteria were *Enterococcus* species, *E. coli*, *P. aeruginosa*, *Lactobacillus* species, *Bacillus* species and other Gram positive and Gram negative rods. In this study, depending on the characteristics of the environment to which the surgical instruments were exposed and the detected bacterial load, *Bacillus* species and other Gram positive rods were considered to be derived from the patient's intestine even if they were also isolated from the environment.

An analysis of the total bacterial load on the seven targeted instruments in 31 surgeries showed that the amount of intestine-derived bacteria exceeded the number of skin-derived bacteria in 18 (58.1 per cent) of the 31 cases. The cases with the highest cumulative loads in all seven instruments were a BILI-O case (No. 24) with 3.5×10^4 CFU intestine-derived bacteria and an REC case (No. 31) with 8.1×10^2 CFU skin-derived bacteria.

Relationship between years of experience and instrument contamination

The mean number of years experience of the surgical staff performing the 31 targeted surgeries were 4.8 ± 4.3 years for scrub nurses and 25.6 ± 10.8 years for surgeons. Thirteen female and seven male scrub nurses and 15 male surgeons were involved in the targeted surgeries. Of these, eight scrub nurses and six surgeons participated in two or more surgeries. A comparison of cases of the same surgical type in which the same nurse participated showed that bacterial amounts varied. For example, nurse O participated in three BILI-PD cases and the log values of the bacteria detected were 3.5, 2.4 and 0.7.

The relationship between the years of experience of surgical staff participating in each surgery and the total amount of bacteria of the seven targeted instruments expressed as logarithmic values was analysed. The correlation coefficients between the years of experience of the scrub nurses and surgeons and the number of bacteria were 0.1 and 0.14, respectively, and no significant correlation was detected ($p = 0.59$ and $p = 0.44$, respectively). Even for intestine-derived bacteria alone, correlation coefficients between the years of experience of the surgical staff and the number of bacteria were 0.07 and 0.19, respectively, and no significant correlation was found ($p = 0.71$ and $p = 0.30$, respectively).

Discussion

Bacterial contamination of the surgical site necessarily precedes SSI development¹. Therefore, using contaminated surgical instruments for wound closure contaminates the surgical site, increasing the SSI risk.

In this study, we examined surgical instruments that were supposed to be used for wound closure in intestinal operations. We observed bacterial contamination in 87.6 per cent of instruments. The isolated bacteria included *Enterococcus* species, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *K. oxytoca*, *A. baumannii* and *Bacillus* species, apparently derived from intestinal tracts. We isolated *Enterococcus* species and *E. coli* in 38.7 and 19.4 per cent of cases, respectively. These bacteria have also been frequently identified as causes of SSI by surveillance of National Healthcare Safety Network, from 2006 to 2007⁹. Of particular interest, we detected *P. aeruginosa* in one case of upper intestinal surgery (BILI-O). It was reported that *P. aeruginosa* was found in gastrointestinal flora and its detection rate increases with an

increased length of hospital stay. Results from our study suggest that these SSI causative bacteria might contaminate the surgical wound when instruments for wound closure become contaminated with these bacteria.

We divided the isolated bacteria into skin-derived and intestine-derived bacteria. The amount of intestine-derived bacteria outweighed skin-derived bacteria in 58.1 per cent of cases and surgical instruments were more heavily contaminated with intestine-derived bacteria than skin-derived. During intestinal surgery in Japan, surgical instruments that are in direct contact with the intestinal tract are isolated from other clean instruments to prevent droplet and contact transmission of intestinal bacteria to the clean instruments re-used for wound closure¹⁰. The results of our study suggest that bacteria in operating fields are transmitted to other surgical instruments during the surgery even if they are separated from operating fields as clean instruments and are not used for intestinal manipulation.

Intestinal bacteria could be transmitted by droplets splashed from the operating field or through contact by operating room personnel. It has been reported that droplet splash of invisible droplets could occur during surgery. A study investigating the exposure of face-shield masks to blood during surgery revealed traces of blood on 151 of 330 face-shield masks, unnoticed by 97.2 per cent of participants¹¹. Similarly, invisible droplets generated from the opened intestinal lumen may contaminate clean instruments. These droplets probably contain a large amount of intestinal bacteria, thereby resulting in severe contamination of surgical instruments.

In addition to droplet transmission, direct contact transmission by operating room personnel might occur. In a study of 941 patients undergoing surgeries because of upper and lower digestive tract diseases, the frequency of glove changes was reported as an independent factor to reduce SSI risk¹². This study supports our opinion that bacterial transmission by direct contact occurs during surgery. As with droplet transmission, the incidence of contact transmission increases with an increase in bacterial load in the operating field.

This possibility is supported by our finding that the ratio of severely and moderately contaminated instruments used in COLN and REC surgeries with relatively shorter duration were similar to that of severely and moderately contaminated instruments used in BILI-PD. Generally, lower gastrointestinal tract surgeries have a heavier bacterial load than upper gastrointestinal tract surgeries¹³. Thus, in surgeries involving organs where the bacterial load is generally heavy, bacteria are more likely to disperse into the surrounding environment. Heavier contamination of the instruments contaminated with intestine-derived bacteria rather than skin-derived bacteria also suggests that droplet or contact transmission from intestinal tracts might cause severe contamination.

Contamination of surgical instruments with skin-derived bacteria, including *Staphylococcus* species, was expected because the targeted instruments might have been used for skin incision before sampling. *S. aureus* and *CNS* were detected in 19.4 and 90.3 per cent of cases, respectively. Although the degree of contamination by skin-derived bacteria was lower than contamination by intestine-derived bacteria in 18 cases, the

surgical instruments in some cases were highly contaminated with skin-derived bacteria. As reported previously, *S. aureus* and *CNS* can cause SSI by contaminating skin incision sites^{14,15}. Thus, the reduction of contamination with skin-derived bacteria must also be considered.

We predicted that instruments in surgeries where less experienced scrub nurses were involved would be more contaminated because of inappropriate handling of surgical instruments due to a lack of experience, which increases the chance of bacterial spread. However, there was no correlation between the years of experience of the scrub nurses and the number of colonies detected. Rather, our results suggest that surgical instrument contamination is determined by the type of surgery, and contamination cannot be completely avoided when opening the intestine is part of the surgical procedure.

Bacterial contamination of surgical instruments during surgical procedures has already been observed in a previous investigation⁵. The positive culture rate in our study (87.6 per cent) was three times higher than that reported in a previous study (31.4 per cent). Isolated bacteria were almost similar to those observed in our study, although more Gram positive and Gram negative rods were isolated in our study. In the previous study, samples were collected from two pairs of forceps only, and gastrectomy and abdominal aortic aneurysm repair were included. Thus, differences in targeted instruments and surgeries and culture conditions might be a possible explanation for the higher positive culture rate of Gram positive and Gram negative rods in our study.

Previous studies did not consider what the targeted surgical instruments were used for and where

they were kept during the surgery^{5,16}. Our study focussed on the surgical instruments to be used for wound closure. The fact that they were heavily contaminated indicates the risk of contamination of surgical wounds by surgical instruments. We have provided direct bacteriological evidence to support the necessity of using sterilised surgical instruments for wound closure.

Conclusion

This study examined microbiological contaminations of the surgical instruments used in 31 intestinal surgeries accompanying manipulation of the gastrointestinal tract. We revealed that instruments used for wound closure were contaminated not only with skin-derived bacteria but also with intestine-derived bacteria. Some instruments were heavily contaminated, and their use for wound closure might cause incisional SSI. The degree of contamination was not affected by the years of experience of the scrub nurses and surgeons involved in the surgery. Our results indicate that a new set of sterilised surgical instruments should be prepared for wound closure to minimise SSI risk.

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