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Effects of Disinfection Methods, Antimicrobial Sensitivity, and Environmental Conditions on Bacterial Contamination of Surgical Instruments During Canine Ovariohysterectomy

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ABSTRACT

Surgical site infections (SSIs) pose a threat to animal health and potentially compromise recovery after surgical procedures. Antimicrobial resistance (AMR) also exacerbates SSI. Therefore, preventive measures are needed to reduce the risk of SSI during surgery, as well as surveillance of bacteria and antibiotic susceptibility. The present study aimed to observe the risk factors and prevention of SSI in canine ovariohysterectomy (OH), bacterial sensitivity to antibiotics commonly used in veterinary practice, and assess the association of surgical room environment and bacterial contamination. Nine mongrel dogs underwent OH procedures, with surgical instruments (thumb forceps, scissors, and hemostatic forceps) subjected to three intraoperative disinfection methods (70% alcohol, 10% povidone-iodine, or no treatment). Each of the surgical instruments on each disinfection method was swabbed at three points in time (0, 30, and 60 minutes) intraoperatively. Environmental conditions (humidity and temperature) of the surgical room were monitored at each sampling interval. The bacterial identification was performed using conventional microbiological methods. The identified bacteria were tested for antimicrobial susceptibility to doxycycline, enrofloxacin, cefadroxil, and gentamicin using the Kirby-Bauer method. Results indicated that 70% alcohol reduced bacterial contamination in two-thirds (2/3) of replicates, compared to 10% povidone-iodine (0/3) and untreated control (0/3). The bacteria that were identified were *Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus aureus*, and *Klebsiella* spp. Most of the bacteria identified were susceptible to all antibiotics tested, except *Klebsiella* spp., which displayed resistance to gentamicin and cefadroxil, while doxycycline and enrofloxacin remained susceptible. The bacterial contamination also might be supported by the humidity and temperature of the surgical room, which is above the standard requirement. The present study findings highlighted the importance of standardized intraoperative disinfection protocols, surveillance of antimicrobial susceptibility, and environmental control in veterinary surgical procedures.

Keywords: Antimicrobial resistance, Ovariohysterectomy, Small animal, Surgical instrument, Surgical site infection

INTRODUCTION

Ovariohysterectomy (OH) or spaying is a routine surgery performed in small animal practices. In addition to controlling the population, ovariohysterectomy has been performed to reduce the probability of health disturbances related to reproductive problems and hormonal imbalance, such as dystocia, mammary tumors, and pyometra (Guest et al., 2023; Bertero et al., 2024). Ovariohysterectomy is considered a major surgery because it requires a celiotomy. Therefore, the OH procedure is prone to microbial contamination that can cause infection at the surgical site, especially in the cases of insufficient surgical preparation and poor aseptic techniques, which are known as surgical site infections (SSI; Rezaei et al., 2025).

Surgical Site Infections are a significant concern in veterinary surgery. Surgical Site Infections can occur during clean surgical procedures in cats and dogs, which account for approximately 2.5% and 48% of the procedures, respectively. The reported SSI rates for OH and orchidectomy are 2.2% and 5.7%, respectively (Turk et al., 2015; Stetter et al., 2021). Surgical site infections increase morbidity and mortality in both veterinary and human surgical procedures. Moreover, SSI increases costs owing to prolonged medication (Stetter et al., 2021). Treatment of surgical site infection generally requires antibiotics (Aboderin et al., 2024). Thus, the types of bacteria and their sensitivity to certain antibiotics should be assessed.

There are several risk factors for SSI in clean surgical procedures, such as the surgical environment, sterilization procedure, surgical instruments, drapes, surgical gowns, surgical gloves, and anesthesia duration (Beal et al., 2000; Owusu et al., 2022; Dyer et al., 2024). In addition to technical issues, factors that can lead to SSI in animals include the American Society of Anesthesiologists (ASA) status, age, and physical status (Turk et al., 2015; Stetter et al., 2021).

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Surgical instruments are the main cause of SSI; therefore, sterilization before surgery and maintaining sterility during the surgical procedure are paramount to reducing the risk of SSI. While autoclaving is the standard preoperative sterilization method for surgical instruments, maintaining the sterility of surgical instruments poses a challenge. In Indonesia, some small animal clinics use intraoperative disinfection methods, whereby surgical instruments are soaked in antiseptic solutions, such as 70% alcohol or 10% povidone-iodine, to reduce bacterial contamination. The disinfection and sterilization of surgical instruments using isopropyl alcohol 70% has been reported in mice (Keen et al., 2010). Another study conducted by Razzaq et al. (2019) used several substances, including povidone-iodine, to sterilize surgical instruments in laboratory settings. However, the effectiveness of intraoperative disinfection methods, specifically during canine OH, remains poorly documented.

Therefore, the present study aimed to evaluate the effectiveness of intraoperative disinfection methods using 70% alcohol and 10% povidone iodine in reducing bacterial contamination of surgical instruments (thumb forceps, scissors, and hemostatic forceps) during canine OH. In addition, the present study aimed to identify bacterial species contaminating surgical instruments at different time points (0, 30, and 60 min) during surgery, evaluate the bacterial susceptibility to commonly used veterinary antibiotics in Indonesian animal clinics (cefadroxil, doxycycline, enrofloxacin, and gentamicin), and assess the association between surgical room environmental conditions (humidity and temperature) and bacterial contamination.

MATERIALS AND METHODS

Ethical approval

The present study and sampling process were approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, East Java, Indonesia (certificate number: No. 10-KEP-FKHUB-2025).

Study period and collection of samples

The experiment was conducted between May and August 2025. Nine mongrel dogs were included in the present study and randomly divided into three treatment groups with three dogs in each group. The treatment groups included an alcohol-based disinfectant group (Alcohol), a povidone-iodine disinfectant group (Povidone), and a control group without disinfectant treatment (Without). For each dog, three types of surgical instruments were evaluated, including thumb forceps, scissors, and hemostatic forceps. Bacterial contamination was assessed at three time points during the surgical procedure, namely at 0 min, 30 min, and 60 min. The sample size was selectively determined with ethical responsibility following the replacement, reduction, and refinement principles to minimize animal use. Thus, the present study design generated 81 samples from nine dogs, three instruments, and three time points. Prior to surgery, each dog underwent health screening, including physical examination, anthelmintic treatment, and hematological examination by a licensed veterinarian. Hematological examination was performed using a Veterinary Hematology analyzer Rayto® RT-7600 (Rayto Life and Analytical Science Co., Ltd., China). The health screenings were performed to confirm the health of the dogs for anesthesia and surgery. The health status of the animals was evaluated based on the physical status classification of ASA, which provides a standardized method for assessing pre-anesthetic patient status and communicating perioperative risk. The classification of ASA included the following categories. The ASA I includes healthy and normal patients with no underlying disease. The ASA II describes patients with mild systemic disease with no functional limitations. The ASA III refers to patients with severe systemic disease with functional limitations. The ASA IV describes patients with severe systemic disease that is a constant threat to life. The ASA V refers to moribund patients not expected to survive 24 h with or without surgery (Portier and Ida, 2018). The surgical procedure was conducted in the Laboratory of Surgery and Radiology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, East Java, Indonesia, while bacterial identification was conducted in the Laboratory of Microbiology and Immunology, Universitas Brawijaya, Malang, East Java, Indonesia.

The surgical instruments that were used for surgery were autoclaved using GEA LS35-LJ (GEA® Medical, Indonesia) for 30 minutes at 121°C according to the standard sterilization procedure. The nine dogs were allocated into three treatment groups (n = 3 per group), with each dog receiving only one intraoperative treatment protocol on the surgical instruments. In the alcohol group, all three surgical instruments (thumb forceps, scissors, and hemostatic forceps) were placed in a sterile Nierbekken bowl (250 ml) containing 150 ml of 70% ethyl alcohol (OneMed®, Indonesia) when not in active use during surgery. In the povidone-iodine group, instruments were placed in a sterile 250 ml Nierbekken bowl containing 150 ml of 10% povidone-iodine (OneMed®, Indonesia). In the control group, the instruments received no intraoperative disinfection and were directly placed on the surgical drape when not in use. All instruments were soaked for a minimum of 15 seconds before reuse and dried using sterile gauze. The surgical instruments were swabbed using a Transport Swab Sterile (OneMed®, Indonesia) with 1.5 ml of NaCl 0.9% at 0 min

(just before the first incision), 30 min, and 60 min of canine OH surgery. Transport swabs containing samples were maintained at ambient room temperature (20-25°C) and transported within 2 hours from the Laboratory of Veterinary Surgery and Radiology to the Laboratory of Microbiology and Immunology in order to preserve bacterial viability, prevent overgrowth, and ensure the sample integrity during the transportation process according to the microbiological specimen handling protocol (Miller et al., 2018). Other parameters collected during sample collection were the environmental conditions, which included the humidity and temperature of the surgical room and the health status of the animal. The humidity and temperature of the surgical room were measured using a Notale® Hygrometer Thermometer Humidity Meter NTL-HM370 (Notale®, Indonesia), with manufacturer-stated accuracy of ±1°C for temperature and ±5% for relative humidity. The device was wall mounted at height approximately 1 meter from surgical table and positioned approximately 2 meters from surgical table. The humidity and temperature were recorded at each sampling time point. The health status of the animals was measured using hematological parameters and according to the health signs published by ASA.

Bacterial identification

Samples from transport swabs were transferred to 2 ml of Brain Heart Infusion Broth (BHIB; Merck Millipore, Germany) and incubated in an incubator (Memmert GmbH, Germany) at 37°C for 24h for the initial enrichment. After incubation, each sample was subjected to Gram staining to observe the bacterial morphology and Gram reaction. After the initial growth in the BHIB, a loopful of each BHIB sample was streaked on an object glass and subjected to Gram staining, as a preliminary Gram stain provided initial information about bacterial morphology and Gram reaction in the BHIB. The samples from BHIB were then sub-cultured into Nutrient Agar (NA; Merck Millipore, Germany) plates by streaking and incubated at 37°C for 24h to obtain well-isolated bacterial colonies. Each colony type was selected and Gram-stained separately. The samples were examined under a light microscope at 1000x magnification with oil immersion to determine the bacterial morphology (cocci or bacilli) and Gram reaction. Gram-positive bacteria were indicated by purple staining, whereas Gram-negative bacteria were indicated by red/pink staining. Selective media inoculation was performed based on Gram staining results. For Gram-positive cocci, samples containing Gram-positive cocci were inoculated into Mannitol Salt Agar (MSA; Merck Millipore, Germany) and incubated in an incubator 37°C for 24h. Catalase and coagulase tests were used to confirm the presence of *Staphylococcus aureus*. Samples with gram-positive bacilli were inoculated into Blood Agar (BA; Oxoid®, UK) and incubated at 37°C for 24h to assess the colony morphology and hemolytic patterns on BA. Endospore staining was also performed. Further differentiation between *Bacillus* species was achieved through inoculation into MSA and the Voges-Proskauer test. Samples containing Gram-negative bacteria, inoculation was performed on the MacConkey Agar (MCA; Merck Millipore, Germany) and Triple Sugar Iron Agar (TSIA; Merck Millipore, Germany), followed by incubation at 37°C for 24h, suspected. Enterobacteriaceae were further evaluated using Indole, Methyl Red, Voges-Proskauer, and Citrate tests (IMViC). The urease test was conducted to confirm the presence of *Klebsiella* spp. Following incubation on the primary isolation media, single well-isolated colonies were sub-cultured on a plate prior to antimicrobial susceptibility testing. Parallel inoculation was performed on MCA and MSA if the Gram-positive and Gram-negative bacteria were observed in the same sample. Bacterial identification was performed according to the standard protocols described by Cappuccino and Sherman (2019).

Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on purified bacterial isolates on representative bacterial isolates selected from positive samples collected at the 60-minute time point for each instrument across all treatment groups. The antibiotics selected for testing in the present study were commonly used in small animal veterinary practice, namely, cefadroxil, doxycycline, enrofloxacin, and gentamicin. Identified bacterial colonies were inoculated into Mueller-Hinton Broth (MHB) and incubated at 37°C for 24h. The resulting bacterial suspension was adjusted to match the 0.5 MacFarland turbidity standard (1.5×10^8 CFU/mL). The standardized inoculum was then uniformly streaked across the entire surface of Mueller-Hinton Agar (MHA) plates. Antibiotic discs (Oxoid®, UK) containing cefadroxil (30 µg), doxycycline (30 µg), enrofloxacin (5µg), and gentamicin (10 µg) were aseptically placed into the inoculated agar using sterile forceps. The plates were then incubated at 37°C for 24h. The diameter of the zone of inhibition surrounding each antibiotic disk was measured in millimeters using a calibrated ruler. The measurements were interpreted according to the zone diameter. Isolates were classified as susceptible, intermediate, or resistant based on the zone diameter interpretive criteria established by the Clinical and Laboratory Standards Institute (CLSI, 2020). For enrofloxacin (5 µg), the isolates were classified as susceptible (≥ 23 mm), intermediate (17-22 mm), or resistant (≤ 16 mm). For cefadroxil (30 µg), the breakpoints were defined as susceptible (≥ 18 mm), intermediate (15-17 mm), or resistant (≤ 14 mm). Doxycycline (30 µg) interpretive criteria were susceptible (≥ 14 mm), intermediate (11-

13 mm), or resistant (≤ 10 mm). Gentamicin (10 µg) breakpoints were susceptible (≥ 15 mm), intermediate (13-14 mm), or resistant (≤ 12 mm).

Statistical analysis

The data obtained were quantitatively analyzed using IBM® SPSS Statistics 25. The effectiveness of the three surgical instruments soaked in 10% povidone iodine, 70% alcohol, and without treatment was analyzed using the chi-square test. The environmental data (temperature and humidity) were summarized using descriptive analysis.

RESULTS

Animal status

The OH procedure in the present study has not indicated any functional impairment in hematocrit (HCT) or total red blood cell (RBC) count parameters. Overall, the hematological findings confirm that the animals remained clinically stable throughout the study period, with no evidence of hematological disorders related to the intervention. The hematological data for each dog are indicated in Table 1.

According to the hematological data, four dogs (Dog 1, 4, 5, and 8) indicated elevated total white blood cell (WBC) parameters (6-17 × 10^3/µL), with values of 20.7 × 10^3/µL, 18.2 × 10^3/µL, 19.7 × 10^3/µL, and 19.2 × 10^3/µL, respectively. Dog 7 was only slightly within the normal limit (17.4 × 10^3/µL). Dog 6 indicated a decrease in hemoglobin (HGB) and platelet (PLT) below the normal standard (11.9-18 g/dL) and (200-500 10^3/µL), respectively. All of the dogs indicated no sign of anemia. Nonetheless, the hematological results of all dogs were classified as ASA I status, which is clinically healthy at the time of evaluation.

Table 1. Haematological parameters of dogs in the present study from May to August 2025

Dog	WBC (10^3/µL)	RBC (10^6/µL)	HGB (g/dL)	HCT (%)	PLT (10^3/µL)	ASA Status
Dog 1	20.7	7.12	14.8	46.9	460	ASA I
Dog 2	13.5	6.69	13.6	43.9	292	ASA I
Dog 3	10.37	6.64	14.5	42.9	529	ASA I
Dog 4	18.2	6.48	14.1	44	433	ASA I
Dog 5	19.7	5.97	13.6	40	262	ASA I
Dog 6	11.5	5.47	11.8	35	165	ASA I
Dog 7	17.4	7.65	17.3	53.3	410	ASA I
Dog 8	19.2	5.65	13.5	38.4	223	ASA I
Dog 9	6.5	5.65	15.5	39.6	222	ASA I
Standard value*	6-17	4.95-7.87	11.9-18.9	35-57	200-500	

Standard value is adopted from Brooks et al. (2020). WBC: White blood cell, RBC: Red blood cell, HGB: Haemoglobin, HCT: Haematocrit, PLT: Platelet, ASA: American Society of Anaesthesiologists

Presence, identification, and antibiotic sensitivity of bacteria

Bacterial identification revealed that disinfection with 70% alcohol resulted in substantially lower bacterial counts compared to 10% povidone iodine treatment and the untreated group across all three surgical instruments that were evaluated (thumb forceps, scissors, and hemostatic forceps). A detailed summary of the bacterial presence across surgical instruments for each treatment and observation time point is provided in Table 2.

Table 2. Presence of bacteria on surgical instruments in the present study from May to August 2025

Treatment	Dog	Thumb forceps			Scissors			Haemostatic forceps		
		0 min	30 min	60 min	0 min	30 min	60 min	0 min	30 min	60 min
Alcohol 1	Dog 1	-	-	+	-	-	-	-	-	-
Alcohol 2	Dog 2	-	-	-	-	-	-	-	-	-
Alcohol 3	Dog 6	+	+	+	+	+	+	+	+	+
Povidone 1	Dog 8	+	+	+	+	+	+	+	+	+
Povidone 2	Dog 4	+	+	+	+	+	+	+	+	+
Povidone 3	Dog 7	+	+	+	+	+	+	+	+	+
Without 1	Dog 3	+	+	+	+	+	+	+	+	+
Without 2	Dog 5	+	+	+	+	+	+	+	+	+
Without 3	Dog 9	+	+	+	+	+	+	+	+	+

Numbers 1, 2, and 3 represent replicates within each treatment group. (-) Negative bacterial contamination, (+) Positive bacterial contamination.

The results, as indicated in Table 2, illustrate the variation in bacterial contamination among the treatment groups. In the 70% alcohol treatment group, two replicates (Alcohol 1 and Alcohol 2) indicated effective bacterial reduction, with bacterial contamination in alcohol 2 completely absent at three time points (0, 30, and 60 min). Alcohol 1 demonstrated negative bacterial contamination, except for a single positive result on the thumb forceps at 60 min. In the third replicate (Alcohol 3), however, bacterial contamination was observed on all instruments at three time points, similar to the 10% povidone-iodine and untreated groups. The present study revealed that 70% alcohol decreased bacterial contamination in most replicates, even though not all subjects in this treatment group experienced the same outcomes.

Chi-square analysis indicated a correlation between the treatment and the presence of bacteria ($p < 0.05$). *Staphylococcus aureus* and *Bacillus cereus* were identified on the surgical instruments soaked in 70% alcohol. The bacteria found in 10% povidone-iodine were *Bacillus cereus* and *Bacillus megaterium*. The bacteria found in untreated instruments were *Bacillus cereus*, *Bacillus megaterium*, and *Klebsiella* spp. A total of 13 bacterial isolates were obtained from samples and submitted to antimicrobial testing. Each row in Table 3 indicates the susceptibility results of an individual isolate. *Bacillus cereus* (n = 89) was the most commonly isolated bacterium, followed by *Bacillus megaterium* (n = 32), *Staphylococcus aureus* (n = 1), and *Klebsiella* spp. (n = 1). The antimicrobial sensitivity of each bacterium detected in the surgical instruments is indicated in Table 3.

The antimicrobial susceptibility results in Table 3 indicated that most bacterial isolates were susceptible to all the antibiotics tested. *Staphylococcus aureus* obtained from 70% alcohol-treated thumb forceps with inhibition zone diameters ranging from 23 mm for gentamicin, 32 mm for cefadroxil, 35 mm for enrofloxacin, and 37 mm for doxycycline. *Bacillus cereus,* which was mostly found in all types of instruments, indicated susceptibility to all antibiotics tested, ranging from 17 mm to 40 mm. *Klebsiella* spp., however, displayed resistance to gentamicin and cefadroxil (0 mm), while doxycycline (25 mm) and enrofloxacin (27 mm) remained susceptible.

Table 3. Antibiotic susceptibility of bacteria found in each treatment group against gentamicin, cefadroxil, doxycycline, and enrofloxacin

Treatment	Dog	Instrument	Time point	Bacteria	Antibiotic susceptibility			
					Gentamicin 10 µg	Cefadroxil 30 µg	Doxycycline 30 µg	Enrofloxacin 5 µg
Alcohol 70%	Dog 1	Thumb forceps	60 min	*Staphylococcus aureus*	23 mm (S)	32 mm (S)	37 mm (S)	35 mm (S)
Alcohol 70%	Dog 6	Thumb forceps	60 min	*Bacillus cereus*	23 mm (S)	23 mm (S)	30 mm (S)	33 mm (S)
Alcohol 70%	Dog 6	Scissors	60 min	*Bacillus cereus*	30 mm (S)	30 mm (S)	38 mm (S)	36 mm (S)
Alcohol 70%	Dog 6	Haemostatic forceps	60 min	*Bacillus cereus*	24 mm (S)	40 mm (S)	27 mm (S)	38 mm (S)
Povidone iodine 10%	Dog 8	Thumb forceps	60 min	*Bacillus cereus*	18 mm (S)	21 mm (S)	17 mm (S)	25 mm (S)
Povidone iodine 10%	Dog 7	Scissors	60 min	*Bacillus cereus*	21 mm (S)	35 mm (S)	28 mm (S)	31 mm (S)
Povidone iodine 10%	Dog 7	Haemostatic forceps	60 min	*Bacillus cereus*	24 mm (S)	38 mm (S)	30 mm (S)	33 mm (S)
Without treatment	Dog 5	Thumb forceps	60 min	*Bacillus cereus*	23 mm (S)	40 mm (S)	31 mm (S)	33 mm (S)
Without treatment	Dog 5	Thumb forceps	60 min	*Bacillus megaterium*	29 mm (S)	35 mm (S)	30 mm (S)	31 mm (S)
Without treatment	Dog 3	Scissors	60 min	*Bacillus megaterium*	23 mm (S)	37 mm (S)	23 mm (S)	33 mm (S)
Without treatment	Dog 3	Scissors	60 min	*Bacillus cereus*	23 mm (S)	33 mm (S)	23 mm (S)	33 mm (S)
Without treatment	Dog 3	Haemostatic forceps	60 min	*Bacillus cereus*	22 mm (S)	30 mm (S)	21 mm (S)	30 mm (S)
Without treatment	Dog 3	Haemostatic forceps	60 min	*Klebsiella spp.*	0 mm (R)	0 mm (R)	25 mm (S)	27 mm (S)

(S): Susceptible, (R): Resistant, (I): Intermediate.

Surgical room humidity and temperature

The room temperature and humidity that were assessed during each surgery were recorded to observe the relationship between bacterial contamination and humidity and temperature, as indicated in Table 4. The humidity of the surgery room was between 48 and 68% and fluctuated from 0 to 60 minutes of the sampling process. The temperature was between 22°C and 25°C; unlike humidity, the temperature was rather stable across all the treatment and sampling times. The identical environmental measurements observed between certain treatment groups were caused by some surgeries occurring simultaneously in the same surgical room. Therefore, the temperature and humidity data were not independent.

Table 4. Humidity and temperature in the surgery room during each surgery from May to August 2025

Treatment	Humidity (%)			Temperature (˚C)		
	0	30	60	0	30	60
Alcohol 1	51	50	65	23	22.8	25.3
Alcohol 2	51	50	65	23	22.8	25.3
Alcohol 3	59	62	62	23	24	25
Povidone 1	64	65	62	23	24	24
Povidone 2	56	53	51	23	23	22
Povidone 3	59	62	62	23	24	25
Without 1	48	68	64	23	23	24
Without 2	56	53	51	23	23	22
Without 3	64	65	62	23	24	24

DISCUSSION

Surgical site infections can occur due to several factors, and surgical instruments can be attributed to factors that could lead to SSI. The health status of the animals in the present study was assessed using ASA. All dogs that underwent OH were categorized as ASA I, and the correlation between ASA status and contamination of bacteria could not be evaluated statistically due to the lack of variation between each dog. However, according to a study conducted by Cavalli et al. (2025), there was a correlation between the incidence of SSI in soft tissue surgery and each ASA status. A low risk of SSI was found in ASA I and II patients, and the higher the ASA, the more significant the risk of SSI (Sheretz et al., 1992; Tresson et al., 2023).

Preventing SSI and choosing the appropriate antibiotic for treatment after surgery are prominent strategies for combating Antimicrobial Resistance (AMR; Aboderin et al., 2024). The prevention of contamination of surgical instruments before and during surgery is also required to avoid the incidence of wound contamination. The present study found that 70% alcohol significantly reduces the presence of bacteria compared to 10% povidone-iodine and no treatment. Alcohol is a substance that denatures proteins, whereas iodine or iodophors are oxidized or substituted by free iodine. Alcohol demonstrates bactericidal activity against Gram-positive and Gram-negative bacteria through protein denaturation and disruption of the cell. Iodine-based compounds indicated variable efficacy depending on bacterial cell wall composition (Nye and Thieman Mankin, 2024). However, according to Table 2, the effectiveness of 70% alcohol was not consistent, as indicated in Alcohol 3, which was similar to 10% povidone-iodine and the untreated control groups. The instability of the results might be caused by the presence of organic matter, which reduces the efficacy of alcohol (Rutala and Weber, 2016). Moreover, alcohol has a limited contact time because of rapid evaporation and no residual antimicrobial activity (Nye and Thieman Mankin, 2024). Therefore, the inconsistent bacterial reduction outcomes observed with Alcohol 3 treatment highlight that although 70% alcohol can be effective as an intraoperative disinfectant, the antimicrobial efficacy of alcohol-based disinfectant relies on contact time and the presence or absence of organic matter.

Another aspect that should be addressed to combat AMR is bacterial identification and antimicrobial susceptibility testing, which are important for selecting appropriate antibiotic therapies. In this present study, *Bacillus cereus* was the predominant bacterium isolated from surgical instruments. The predominance of *Bacillus cereus* as the primary contaminant is also consistent with that of Owusu et al. (2022), who found that *Bacillus cereus* was the major contaminant of surgical instruments. *Bacillus cereus* is a spore-forming bacterium that is omnipresent in the environment. Due to its ability to survive standard disinfection procedures through spore formation, *Bacillus cereus* has been implicated in postoperative wounds (Esmkhani and Shams, 2022). The environmental origin of contamination is also supported by the presence of *Bacillus megaterium,* as the *Bacillus* genus consists of spore-forming dust-borne bacteria (Lang-Yona et al., 2025). Therefore, both *Bacillus cereus* and *Bacillus megaterium* might have been present in the operating room through personnel movement or air circulation. *Staphylococcus aureus* and *Klebsiella* spp. are pathogenic bacteria that are frequently associated with SSI in animal surgery, particularly in immunocompromised patients or those undergoing prolonged surgical procedures (Turk et al., 2014; Gulaydin et al., 2024).

The antimicrobial susceptibility testing in the present study indicated that *Bacillus cereus, Bacillus megaterium,* and *Staphylococcus aureus* were susceptible to the four antibiotics tested (Table 3) with inhibition zones above the susceptibility breakpoints according to CLSI (2020). A previous study conducted by Gulaydin et al. (2024) also found that *Staphylococcus aureus* was sensitive to gentamicin, enrofloxacin, and doxycycline but resistant to beta-lactam antibiotics. Moreover, *Bacillus cereus* has also been reported to be susceptible to gentamicin and tetracycline but resistant to beta-lactam antibiotics (Mohammadi et al., 2023). *Bacillus megaterium* is susceptible to beta-lactam antibiotics, gentamicin, and tetracycline (Agersø et al., 2018). *Klebsiella* spp. isolated in the present study however,

indicated complete resistance towards gentamicin and cefadroxil by the absence of an inhibition zone around those antibiotics, while doxycycline and enrofloxacin remained susceptible (Table 3). Gulaydin et al. (2024) reported that *Klebsiella pneumoniae*, which is found in small animal surgery, was resistant to tetracycline, yet susceptible to gentamicin and enrofloxacin. The resistance pattern observed in the present study highlights the emerging threat of multidrug-resistant gram-negative bacteria in veterinary surgery. Therefore, it is important to conduct antimicrobial susceptibility testing to select the appropriate antibiotic to achieve successful treatment of illness.

The operating room environment, particularly temperature and humidity, represents an important risk factor for SSI development (Hammond et al., 2023). To minimize this risk, the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE, 2020) recommends that surgical rooms be maintained at a temperature between 20°C and 24°C, with a relative humidity level between 20% and 60%. The bacterial contamination might be caused and supported by the humidity and temperature records of the surgical room, which are above the standard requirements.

Furthermore, this study faced several limitations, including a relatively small sample size and variable disinfection and organic matter, which we did not observe. The antimicrobial susceptibility testing in this study did not incorporate standard bacterial control strains in this present study. Though some studies (Zúniga-Moya et al., 2016; Owusu et al., 2022) did not comprise the American type culture collection reference strain. Additionally, environmental monitoring device to assess the temperature and humidity in the present study is limited to a single wall-mounted device. Multiple sensors need to be employed and placed at various locations to provide better information on spatial environmental variability. The environmental independence between treatment groups also represents a limitation of this present study. Some surgeries from different treatment groups were conducted simultaneously in the same surgical room, resulting in shared environmental conditions. Consequently, the observed differences in bacterial contamination cannot be attributed solely to the efficacy of the disinfectant solutions, as environmental confounders were not controlled between groups.

CONCLUSION

The present study revealed that surgical instruments soaked in 70% alcohol had lower bacterial contamination than those soaked in 10% povidone iodine or without treatment. Nevertheless, this result should be approached with caution because the treatment groups shared the same environmental conditions, as some surgeries were performed concurrently in the same operating room. Four bacterial species were identified in the present study, which are *Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus aureus*, and *Klebsiella* spp., and the most frequent bacteria encountered were *Bacillus cereus*. Most bacteria were susceptible to the antibiotics tested, except for *Klebsiella* spp., which were resistant to gentamicin and cefadroxil. The mentioned findings regarding intraoperative disinfection efficacy, bacterial species distribution, and antimicrobial susceptibility suggest the significance of sterilization techniques and control of the surgical room environment for surgical procedures that appear to be low risk. The study findings on intraoperative disinfection methods, bacterial contamination, antimicrobial susceptibility patterns, and environmental factors underline the importance of disinfection practices and environmental conditions during surgical procedures, even in surgeries that are considered low risk, such as OH. Further studies are needed to observe effective intraoperative disinfection protocols while ensuring environmental independence between treatment groups and to continue the surveillance of antimicrobial susceptibility of bacteria in veterinary surgery. Such efforts will support surgical outcomes for patients in veterinary medicine.

DECLARATIONS

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Authors' contributions

Esti Dhamayanti and Siti Kurniawati conceptualized the study and designed the experiments. Esti Dhamayanti, Salsabila Marva Yanita Putri, and Vistarina Amadora Yuscrates conducted sampling and microbial analysis. Esti Dhamayanti and Siti Kurniawati conducted data analysis. All authors participated in writing the manuscript and checked the final edition of the manuscript.

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Competing interests

The authors declare no conflict of interest.

Ethical considerations

All authors have checked the ethical issues aspects, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy. No AI tools were used for writing and preparing the present manuscript.

Availability of data and materials

The data supporting the findings of the present study are available upon reasonable request from the corresponding authors.

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