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Comparison of bacterial profile and antibiotic susceptibility isolated from surgical site after ventral midline and lateral flank approaches of ovariohysterectomy in queens and bitches

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Abstract: The aim of the study was to identify the bacterial profile and antimicrobial susceptibility from surgical site cultures in queens and bitches undergoing ovariohysterectomy via ventral midline and lateral flank approaches. Healthy 22 queens and 21 bitches were assigned randomly either ventral midline or lateral flank approach for routine ovariohysterectomy. Surgical site samples were collected before the surgery and on the 1st, 2 nd ,and 3 rd days after the surgery for microbiological analysis. A total of 70 different strains were isolated from 50 (29%) of 172 samples. In both queens and bitches the total number of bacteria isolated from the midline approach (n = 50) was found to be higher than in the flank approach (n = 20). The most frequently isolated bacteria were *Staphylococcus intermedius* group (SIG) (38.5%), followed by *Enterococcus faecium* (10%) and *Staphylococcus lentus* (8.5%). Of the isolated Staphylococcus spp. strains were positive 64% for beta-lactam resistance while 52% of that strains were also methicillin-resistant. Multidrug resistance to methicillin, beta-lactamase, and clindamycin was determined in two *Staphylococcus* spp. isolates. A high level of streptomycin resistance was observed in three *Enterococcus faecium* isolates. Overall, this study revealed that choosing the surgical site for ovariohysterectomy affected the bacterial profile and more than half of the isolates were resistant to antimicrobials. Thus, the lateral flank approach might be better than the ventral midline approach to prevent possible complications such as a surgical site infection in queens and bitches undergoing ovariohysterectomy.

Key words: Bacterial isolation, antimicrobial resistance, flank, midline, ovariohysterectomy

1. Introduction

Several methods, as well as current techniques, have been described for sterilizing small animals. Sterilization is reported to be beneficial for controlling animal populations and diseases, eliminating reproductive tract problems such as pyometra, mammary and ovarian tumors, and preventing inappropriate estrus behaviors [1]. In practice, ovariohysterectomy (OHE) is one of the most commonly recommended elective sterilization techniques and it is performed through two different surgical sites such as the traditional ventral midline or lateral flank approach [2–4]. The flank approach is preferred in the UK, whereas the ventral midline approach is favored in the USA. Therefore, national differences can be observed in the choice of surgical approach to OHE [3, 5]. Especially in queens, the lateral flank approach for OHE is preferred by many veterinarians. A ventral midline approach is recommended for serious genital tract problems such as tumors, genital lesions, cesarean, and pyometra [6]. Generally, the flank approach is preferred for OHE in conditions of the enlarged mammary gland in lactation or mammary gland hyperplasia [2]. In addition, the lateral flank approach provides an advantage for the visual assessment of surgical wounds from a distance without handling stray or aggressive animals. Also, some studies stated that the lateral flank approach reduced the potential risk of evisceration at the surgical site [4]. Roberts et al. [7] suggested that the ventral midline approach increased the risk of wound complication compared with a lateral flank approach in cats up to 12 weeks of age. Although numerous studies focused on determining a less painful and uncomplicated approach, there has been no consensus on a better approach for OHE [3, 8, 9].

Surgical site infection is one of the most common postoperative complications and the possibility of encounter-

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ing such disorders has become a growing concern in veterinary medicine [10, 11]. All surgical sites are at risk of contamination due to the breakage of the skin's protective barrier, however, not all surgical sites develop a postoperative infection. The ability of bacteria to infect a site depends on the level of contamination of the bacteria. In addition, all bacteria have different virulence factors that play a role in the pathogenesis of infections. Although prophylactic administration of antibiotics before and/or after surgery reduces the incidence of surgical site infection, choosing the appropriate antibiotic is essential in preventing unnecessary antibiotic use and antibiotic resistance.

Thus, the aims of this study were to evaluate the impact of the ventral midline and lateral flank approach on bacteria species in the surgical sites and their resistance to antibiotics in queens and bitches undergoing ovariohysterectomy.

2. Materials and methods

2.1. Animals

This study was performed on healthy animals brought to the Department of Obstetrics and Gynecology in the Faculty of Veterinary Medicine at Siirt University, for routine ovariohysterectomy requests. Twenty-two queens were aged between 1 to 3 years with a mean weight of 2.7 ± 0.5 kg. Twenty-one bitches were with an age range of approximately 4 months to 3 years and body weight between 13 to 36 kg. A general clinical examination was carried out to determine the health status of all animals. All animals enrolled in the study were not pregnant or lactating. After that, they were randomly attended to either a midline (11 queens and 10 bitches) or lateral flank approach (11 queens and 11 bitches) of OHE.

2.2. Surgical procedures and collection of swap samples

Food and water restriction were routinely performed for 8 h prior to surgery in all animals. A venous catheter was applied in the cephalic vein in preoperative preparation for an infusion of isotonic saline solution during the OHE. The surgical field was clipped from the xiphoid to the os pubis for the ventral midline approach and from just the last rib to the iliac crest for the lateral flank approach. When using the lateral flank approach, bitches were placed in right lateral recumbency and queens were placed in left lateral recumbency depending on the operator's preference. Following the preparation of surgical aseptic principles, ventral midline and lateral flank OHE were performed by the same operator who has good expertise in both surgical procedures. The flank approach of ovariohysterectomy was conducted with respect to the suggested by McGrath et al. [2] and the midline approach was performed as the technique described by Fingland [1]. However, abdominal muscles were not surgically incised for flank approach in queens and bitches in this study. After the skin incision, the abdominal muscles and peritoneum were stabbed by Halstead 'mosquito' by applying blunt trauma to pass into the abdominal cavity to prevent bleeding due to dissection of the abdominal muscles [12]. Subcutaneous meloxicam was injected (Demelox©, Denova, Turkey, 0.2 mg/kg SC) before surgery in all animals. Animals were sedated with xylazine (Xylazinbio 2%©, Bioveta, Turkey, 2 mg/kg IM); followed ketamine was performed for anesthesia induction (Ketasol 10%©, İnterhas, Türkiye, 8–10 mg/kg IM). Anesthesia was evaluated at 5 min intervals by the same person. No complications were observed in any animal during the surgical time. Samples of the surgical site were obtained just before aseptic preparation and following three days after the surgery. The samples were kept at –20 °C before to sent to the Department of Microbiology under a cold chain with Stuart transport medium (BD, Heidelberg, Germany). Postoperative wound abnormalities were recorded during the healing time.

2.3. Identification of bacteria and antimicrobial susceptibility

Swabs were inoculated to 5% sheep blood agar (GBL, Istanbul, Turkey) and incubated at 37 °C for 24–48 h in aerobic conditions. Then, colonies obtained from pure culture were examined by Gram staining. The Phoenix 100 automated system (BD Diagnostics, Oxford, United Kingdom) was used for the identification of all isolates using the Gram-positive combo panel (PMIC/ID-87) and the Gram-negative combo panel (NMIC/ID-450) in accordance with the manufacturer's instructions. Since we used an automated system and could not perform biochemical tests or molecular methods to distinguish SIG members properly, thus, *S. intermedius, S. pseudintermedius, and S.delphini* will be referred to as an *S. intermedius* group (SIG).

Antimicrobial susceptibility testing was performed concurrently with the identification using the Phoenix 100 automated systems according to EUCAST criteria. Gram-positive bacteria were evaluated for 21 antimicrobials in 9 different classes. The antimicrobials included in Gram positive combo panel were; Amikacin (AN, 4–16 µg/ml), Amoxicillin/Clavulanate (AMC, 2/1–8/4 µg/mL), Ampicillin (AM, 2–16 µg/mL), Cefoxitin (FOX, 2–16 µg/mL), Ciprofloxacin (CIP, 1–4 µg/mL), Clindamycin (CC, 0.25–1 µg/mL), Daptomycin (DAP, 1–4 µg/mL), Erythromycin (E, 0.25–4 µg/mL), Fosfomycin (FF, 8–32 µg/mL), Fusidic Acid (FA, 1–8 µg/mL), Gentamicin (GM, 1–4 µg/mL), Levofloxacin (LVX, 1–8 µg/mL), Linezolid (LZD, 2–8 µg /mL), Moxifloxacin (MXF, 0.25–1 µg/mL), Oxacillin (OX, 0.25–4 µg/mL), Penicillin (P, 0.125–0.5 µg/mL), Rifampicin (RA, 0.25–1 µg/mL), Teicoplanin (TEC, 1–8 µg/mL), Tetracycline (TE, 0.5–2 µg/mL), Trimethoprim/Sulfametoxazole (SXT, 2/38–8/152 µg/mL) and Vancomycin (VA, 1–16 µg/mL). The beta-lactamase

activity of the isolates was determined by the nitrocefin-based test included in the panels and the minimal inhibitory concentration (MIC) value of penicillin. To determine methicillin resistance, MICs of cefoxitin and/or oxacillin in *Staphylococcus (S.) aureus* and MIC of oxacillin in coagulase-negative staphylococci (CNS), SIG and *S. schleiferi* were evaluated. To investigate the high-level streptomycin resistance (HLSR) and high-level gentamicin resistance (HLGR) in *Enterococcus* spp. were evaluated by determining the MIC of streptomycin and the MIC of gentamicin, respectively.

2.4. Statistical analysis

All statistical procedures were analysed using SPSS 26 (Chicago, Illinois, USA, SPSS Inc.) statistical software. Independent sample t-test was used for paired comparisons of incision size and operation time between the ventral midline and lateral flank approaches in queens and bitches. Results are given as mean ± SEM (standard error of mean). Strains and antimicrobial susceptibility of isolates were evaluated as percentages. The chi-square test was used to compare the effect of vental midline or lateral flank approach on the percentage of positive bacteria culture. Differences were considered significant when $p < .05$ for all analyses.

3. Results

3.1. Surgical data

The average duration of surgery was recorded as 15.5 ± 1.5 min in the ventral midline approach and 14 ± 1.4 min in the lateral flank approach in queens ($p > 0.5$). The average duration of the surgery in the ventral midline approach was 15.1 ± 1.2 min and 17.2 ± 2.0 for the flank approach in bitches ($p > 0.5$). In queens, the length of surgical incisions was recorded which was 1.75 ± 0.3 cm and 1.3 ± 0.2 cm through the ventral midline and lateral flank approach, respectively ($p < 0.55$). In bitches, the ventral midline approach incision length was 2.80 ± 0.3 cm and the lateral flank approach was 3.10 ± 0.5 cm ($p > 0.5$). While postsurgical complications were observed in three queens (13.6%) subjected to a ventral midline approach, no postsurgical complication was observed at the surgical site in bitches.

3.2. Bacteriological results and antibiotic susceptibility

Overall, 70 strains were isolated from 50 of 172 (29.0%) samples collected from 43 animals. In queens, the percentage of positive bacteria culture was higher ($p < .01$) in the ventral midline approach (50%, 22/44) than that in the lateral flank approach (4.5%, 2/44). In bitches, a similar significant reduction ($p < .01$) in the percentage of positive bacteria culture was observed in the lateral flank approach (40.9%, 18/44) compared to the ventral midline approach (70%, 28/40). While pure culture was obtained from 36 samples, 2 different bacteria species were isolated from 13 samples, 3 species from 2 samples, and 4 species from 1 sample.

A total of 50 *Staphylococcus* spp. strains were isolated from the samples. Among them, 40% CNS and 60% coagulase-positive (CPS) staphylococci were identified. *S. intermedius* group (38.5%) was the most prevalent isolate, followed by *Enterococcus* (*E.*) *faecium* (10%) and *S. lentus* (8.5%). The distribution of isolated and identified bacteria according to the surgical site and animal species are presented in Tables 1 and 2.

Postoperative complications occurred in the surgical site in 3 queens who underwent midline approaches of the OHE. However, the causative agent was not isolated from the postoperative samples in one of the three queens. *E. faecium* and *E. faecalis* were detected on the 2nd and 3rd days from the surgical site in the other cat that had postsurgical complications. *Aerococcus* (*A.*) *viridans*, SIG, *Enterobacter* (*E.*) *cloacae*, and *S. aureus* were isolated on the 1st, 2nd, and 3rd days after the surgery from the surgical site infection in another cat.

When the antimicrobial susceptibilities of the isolates were examined, thirty-two (64%) of *Staphylococcus* spp. isolates were found to be beta-lactamase producers. Twenty-six (52%) of *Staphylococcus* spp. isolates were methicillin resistance. Methicillin and beta-lactamase resistance were determined in 40% (20/50) of the isolates. While 2 isolates had methicillin and inducible clindamycin resistance, another 2 of the isolates had methicillin, beta-lactamase, and inducible clindamycin resistance. In addition, both of beta-lactamase and inducible clindamycin resistance were observed in 6% (3/50) of *Staphylococcus* spp. isolates. Among *Enterococcu*s spp., 3 isolates of *E. faecium* had HLSR. HLSR and HLGR were also observed in one *E. faeciu*m isolate. *Streptococcus* spp. isolates were susceptible to all antimicrobials used in the study (Table 3).

4. Discussion

Contraceptive methods such as surgical or nonsurgical are reported around the world to control small animal overpopulation. Ovariohysterectomy is a surgical procedure and is mostly preferred by veterinarians as it reduces the risk of ovarian, uterine, and mammary pathology. Although there has been no standard surgical approach to applying the ovariohysterectomy, the lateral flank approach is preferred especially for neuter-return programs for stray animals in many countries [4]. On the other hand, the various advantages and disadvantages of the ventral midline and lateral flank approaches have been reported. Comparing the two approaches has been evaluated based on the surgical time, pain score, and postoperative complication in most studies [7, 13, 14]. However, there has been no study to evaluate the types of bacteria in the surgical site and their antibiotic resistance in small animals undergoing OHE with the ventral midline and lateral flank approaches. Previous

Table 1. Frequency of bacteria species isolated from the surgical area of the ovariohysterectomy in queens (n = 22).

Microorganism species	Gram (- / +)	Pre-op	Postop d 1	Postop d 2	Postop d 3
Ventral Midline OHE					
Aerococcus viridans	+	-	1/11	-	-
Enterobacter cloacae	-	-	1/11	1/11	1/11
Enterococcus faecalis	+	-	-	1/11	1/11
Enterococcus faecium	+	-	-	1/11	1/11
Enterococcus hirae	+	-	1/11	1/11	1/11
Staphylococcus intermedius	+	-	1/11	1/11	1/11
Staphylococcus aureus	+	-	1/11	1/11	1/11
Staphylococcus epidermidis	+	-	-	-	1/11
Staphylococcus haemolyticus	+	-	-	1/11	1/11
Streptococcus group C/G	+	-	-	1/11	1/11
Lateral Flank OHE					
Aerococcus viridans	+	-	-	-	-
Enterobacter cloacae	-	-	-	-	-
Enterococcus faecalis	+	-	-	-	-
Enterococcus faecium	+	-	-	-	-
Enterococcus hirae	+	1/11	-	-	-
Staphylococcus intermedius	+	-	-	-	-
Staphylococcus aureus	+	-	-	-	-
Staphylococcus epidermidis	+	-	-	-	-
Staphylococcus haemolyticus	+	-	1/11	-	-
Streptococcus group C/G	+	-	-	-	-

*22 isolates were obtained from 44 samples in the Ventral Midline OHE, 2 isolates obtained from 44 samples in the Lateral Flank OHE ($p < .01$).

studies indicated that the longer time of the surgery and anesthesia increased the risk of postoperative surgical site infection [15]. The postoperative surgical site infection rate is twice as high in animals with an operative time of 90 min than in animals with 60 min, and each hour passed doubles the infection rate [14]. In this study, all the surgical procedures were performed by the same surgeon who had equally familiar surgical experience with both approaches. Therefore, our findings were not influenced by different surgeon experiences. However, 13.6% (3/22) postoperative surgical site infection was recorded in queens after the midline approach in the current study. Our findings were similar to previous studies in which the overall incidence of postoperative surgical site complication is low following the OHE in healthy animals [7, 16]. In this study, any difference in the total duration of the OHE and incision length between the two approaches was found in queens and bitches and the results were within a similar range of other studies [3]. It could be considered that a low percentage of complications was related to similar findings following the OHE in this study.

Roberts et al. [7] reported that a midline approach was associated with a 2.95-fold increased risk of surgical site complication compared with a flank approach. In contrast, another study reported that discharge from the surgical site was more often in the lateral flank than midline approach. Additionally, the authors indicated that the greater visibility of the flank approach may have increased the probability of observing the problems of the surgical site of the patient owner [3]. On the other hand, swelling and discharge from the surgical site were reported in three queens after the midline approach in this study. The hunched posture of queens after abdominal surgery might have offered a suitable environment for the growth of bacteria in the ventral midline approach even if pain killer was administered in queens.

It was reported that the major contamination sources of surgical sites were the patient's skin and the operating

Table 2. Frequency of bacteria species isolated from the surgical area of the ovariohysterectomy in bitches (n = 21).

Microorganism species	Gram (-/+)	Pre-op	Postop d 1	Postop d 2	Postop d 3
Ventral Midline OHE					
Enterococcus faecium	+	1/10	2/10	1/10	1/10
Staphylococcus epidermidis	+	-	-	-	1/10
Staphylococcus haemolyticus	+	1/10	-	-	-
Staphylococcus hominis	+	-	-	1/10	1/10
Staphylococcus intermedius	+	3/10	2/10	6/10	4/10
Staphylococcus lentus	+	1/10	-	2/10	1/10
Staphylococcus schleiferi	+	-	-	-	-
Streptococcus equinus	+	-	-	-	-
Lateral Flank OHE		-			
Enterococcus faecium	+	-	-	-	-
Staphylococcus epidermidis	+	-	-	-	2/11
Staphylococcus haemolyticus	+	-	-	-	-
Staphylococcus hominis	+	-	-	-	1/11
Staphylococcus intermedius	+	1/11	1/11	3/11	4/11
Staphylococcus lentus	+	1/11	-	-	1/11
Staphylococcus schleiferi	+	-	1/11	1/11	1/11
Streptococcus equinus	+	-	-	1/11	-

*28 isolates were obtained from 40 samples in the Ventral Midline OHE, 18 isolates obtained from 44 samples in the Lateral Flank OHE ($p < .01$).

team [17]. On the other hand, surgical site infections have occurred as a relevant threat due to multidrug-resistant bacterial strains in human and veterinary medicine [18–20]. Choosing antibiotics as therapeutic and prophylactic the bacterial flora in the region and bacterial resistance patterns should be known.

In a recent large-scale study, Gómez-Beltrán et al. [21] isolated 771 *Staphylococcus* spp. from 1316 samples. The isolates were identified as *S. pseudintermedius* (52.6%), *S. intermedius* (20.7%), *S. aureus* (13.4%), and CNS (13.0%). Similar to our findings, *Staphylococcus* spp. is the most abundant (71.4%) bacterial species, isolated from surgical site samples, which are members of the normal skin microbiota causing various infections as opportunistic pathogens in healthy animals [20–22]. *S. intermedius* was the most identified (54%) strain among the *Staphylococcus* spp. in this study. *Staphylococcus intermedius* group may have a coagulase activity, methicillin resistance, and multiple antimicrobial resistance genes which can increase the pathogenicity and lead to treatment challenges. In addition to being an opportunistic pathogen in animals, it was also reported as a potential zoonotic pathogen of human wounds [23]. *S. lentus,* another mostly isolated staphylococci strain, is a coagulase-negative agent and opportunistic pathogen, also it can carry considerable resistance to antimicrobials [24–25]. *S. lentus* were isolated from both approaches in bitches, however, surgical site complication was not seen in any bitches. *E. faecium* is a naturally occurring intestinal commensal and has remained a problem since the 1980s as a leading cause of multidrug-resistant nosocomial infections [26]. A total of 7 *E. faecium*, 5 from bitches and 2 from queens, were isolated from the midline approach of OHE.

In addition to the differences in the numbers of identified bacteria from the midline and flank approaches, increasing levels of antimicrobial resistance were also remarkable in this study. *Staphylococcus* spp. are classified into two main groups based on the production of coagulase which has an important role to determine their pathogenicity. In this study, CPS (60%) *S. intermedius* group*, S. aureus* and CNS (40%) *S. lentus, S. haemolyticus, S. epidermidis, S. hominis, S. schleiferi* strains were obtained. While 23 (85.1%) of 27 SIG isolates were resistant to beta-lactamase, 11 of these isolates were also found to be resistant to methicillin. In addition, 2 of them were found to have both beta-lactamase and inducible clindamycin resistance. Similar

Table 3. Distribution of antimicrobial susceptibility.

	***Staphylococcus* spp. (n = 50)**		***Enterococcus* spp. (n = 13)**		***Streptococcus* spp. (n = 3)**		***Enterobacter cloacae* (n = 3)**		***Aerococcus viridans* (n = 1)**	
Antibiotics*	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Amikacin	0	47	-	13	-	1	-	-	-	-
Gentamicin	16	31	-	13	-	1	-	-	-	-
Cefoxitin	6	-	-	-	-	-	-	-	-	-
Ampicillin	-	-	3	9	-	3	-	-	1	0
Penicillin	3	-	-	-	-	3	-	-	1	0
Oxacillin	26	19	-	-	-	2	-	-	-	-
Amoxicillin-clavulanate	3	1	3	10	-	2	-	-	-	-
Daptomycin	0	46	-	-	-	2	-	-	-	-
Trimethoprim-sulfamethoxazole	16	31	-	-	-	2	-	-	-	-
Teicoplanin	0	50	-	13	-	3	-	-	-	-
Vancomycin	0	50	-	13	-	3	-	-	0	1
Clindamycin	20	23	-	-	-	3	-	-	-	-
Erythromycin	26	21	-	-	-	2	-	-	-	-
Fusidic acid	3	44	-	-	-	-	-	-	-	-
Linezolid	0	47	-	13	-	2	-	-	-	-
Fosfomycin	0	47	-	-	-	-	-	-	-	-
Ciprofloxacin	19	28	4	9	-	-	-	-	0	1
Levofloxacin	19	28	4	9	-	2	-	-	0	1
Moxifloxacin	19	28	-	-	-	2	-	-	-	-
Rifampin	19	28	-	-	-	2	-	-	1	0
Tetracycline	31	13	-	-	-	2	-	-	-	-

* The antibiotics were selected according to the isolates. Hence, some of the data missing for some isolates. R: Resistant, S: Susceptible

to our results, Cosgun et al. [27] reported that methicillin resistance was highly observed (76.1%) in CPS isolated from swab samples taken from the nasal cavity of bitches.

Even though CNS is less or nonpathogenic commensals, some studies indicated that CNS had a role in dog's and cat's wound infection, pyoderma, and human nosocomial infections [28–30]. It has been reported that the rate of methicillin-resistant CNS isolation varies between 3%–25% in various samples taken from small animals in recent studies [25,31]. Gülaydın et al. [25] found that methicillin resistance was determined in 19.1% of CNS isolates obtained from dogs and Göçmen et al. [24] reported that this rate was 20.5% in small animals. However, in our study, methicillin resistance in CNS was detected higher (45%) than that in other studies.

In the present study, 13 enterococci were obtained from 70 strains. One dog isolate and 2 cat isolates were identified as HLSR E. *faecium*. In addition, E. *faecium* which had HLGR was identified from a dog. Our results on the antimicrobial susceptibility of enterococci were consistent with the previous studies' findings [32, 33]. It is worth noting that the interspecies transmission of multidrug-resistant bacteria can cause treatment failure of bacterial-mediated diseases and it is important for global public health [18].

The major conclusion in this study is that the midline approach results in a more positive culture and the frequency of the isolation of the antimicrobial-resistant bacteria was higher in the midline approach than in the lateral flank approach. These findings revealed some useful epidemiological information to choose the

appropriate surgical site in small animals. Thus the lateral flank approach could be an appropriate surgical site for ovariohysterectomy in small animals, especially in queens. Furthermore, a greater emphasis should be devoted to the requirement to prevent the continuing increase of antimicrobial resistance by raising concerns about the improper use of antibiotics.

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Conflict of interest
The authors declared that they have no conflict of interest.

Ethical approval
This study was approved by the Siirt University Animal Research Local Ethics Committee (reference number: 2019/10), prior to the onset of the clinical investigations.

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