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ORIGINAL ARTICLE

TAXA PROFILING AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIAL PATHOGENS ASSOCIATED WITH CANINE SURGICAL WOUNDS AND OPERATING THEATRE SURFACES

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Surgical site infections are a major cause of morbidity and mortality in small animal surgical patients. This study evaluated the microbial profile and antibiotic susceptibility pattern of bacteria isolated from canine surgical wounds and operating room surfaces. Sterile swab samples (n = 41) were obtained from surgical wounds, operating tables, surgical instruments, and the recovery room. Aerobic and anaerobic bacteria were isolated and characterized using standard phenotypic methods. Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method and profiled for resistance pattern. Eleven genera of bacteria were isolated from surgical wounds, with *Staphylococcus aureus* being the most frequently isolated. Seven genera of bacteria were each isolated from surgical instruments, and kennels, respectively, while four genera of bacteria were isolated from surgical tables, with *Escherichia coli* being the most frequently isolated. The median number of isolated aerobic bacteria was significantly higher ($P \leq 0.05$) than anaerobic bacteria. Bacteria isolated showed resistance to amoxicillin + clavulanic acid, cefuroxime, ampicillin + cloxacillin, and ceftriaxone. *Staphylococcus aureus, Bacillus mycoides, Escherichia coli,* and *Flavobacterium* species showed lower than 10% antibiotic resistance rates. The results provide insight into possible nosocomial bacteria transmission with high-level multidrug resistance. Improvement in decontamination practices and regulation of antibiotic usage is recommended to prevent the emergence of theatre-related multidrug-resistant bacteria.

Keywords: antibiotics; bacteria; dogs; incision; surgery

INTRODUCTION

Surgical incision results in breakage of the integrity of the skin and underlying connective tissues. This process allows for entry, contamination, multiplication, and proliferation of infecting microorganisms within deeper tissue with an accompanied risk of surgical site infection [1]. Surgical site infections (SSI) are the most common nosocomial infections in human patient populations, accounting for 16% of all human infections and 38% of nosocomial infections among surgical patients [2].

In veterinary medicine, SSI is a complication of small animal surgical procedures with significant variations based on surgery type [2, 3]. Risk factors associated with SSI in dogs include age, breed, physical and nutritional status, type of surgical procedure, type and duration of anaesthesia, and skin antisepsis [2, 4, 5]. Bacterial contamination of wounds is a major cause of complications of surgical sites with resultant delay in the wound healing process. Despite the progress made in the field of veterinary surgery, surgical site infection remains a problem, accounting for significant morbidity and mortality. This may be associated with poor infection prevention and control programs in veterinary practices, inadequate guidelines on antibiotic stewardship, and the emergence of antibiotic-resistant bacteria [6].

In spite of the prevalence of surgical site infections and antimicrobial-resistance in small animals, clinical microbiological data on the associated bacteria are not available in most African countries [7]. With growing concerns for antimicrobial resistant bacteria, critical appraisal on the appropriate use of antibiotics in veterinary medicine is important. An understanding of the type of bacteria associated with surgical wounds and the antibiotics susceptibility pattern is also critical.

Currently, the prevalence rate of SSI and associated antimicrobial resistant bacterial strains in canine surgical patients in sub-Saharan Africa is unknown. Hence, this study evaluated the microbial profile and the antibiotic susceptibility patterns of bacteria associated with canine surgical wounds, surgical instruments, and various surfaces of the operating room.

MATERIALS AND METHODS

Informed consent was obtained from the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, prior to commencement of the study. In addition, ethical approval (FUNAAB/COLVET/CREC/2024/03/05) was received from the COLVET Research and Ethics Committee (CREC), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Sampling

Sterile swabs were used to obtain samples from the operating tables (n = 6), sterile surgical instrument packs (n = 6), admission kennels (n = 5), and surgically created wounds from dogs immediately before surgical closure of the wounds (n = 12) and at 48 hours post-surgery (n = 12) and preserved in transport broth (Bactec, Becton Dickinson & Company, Laoghaire, Ireland). The biodata distribution of sampled dogs consisted of males (n = 3) and females (n = 9) (Fig. 1A), which comprised three breeds, namely Boerboel, Alsatian, and local dogs (Fig. 1B), with ages ranging between 6 months and four years (Fig. 1C). Only dogs undergoing clean routine surgical procedure in any part of the body, such as castration, ovariohysterectomy, and splenectomy, were included, while dogs undergoing a clean-contaminated or contaminated surgery such as gastrotomy, cystotomy, intestinal resection, and anastomosis, were excluded.

Routine medical records of each dog were retrieved from the hospital database prior to surgery, while physical examinations of the dogs were performed to adjudge the dogs to be apparently healthy. All the dogs were subjected to at least six hours of routine fasting prior to surgery while routine aseptic preparation of the surgical site was performed. Steam (autoclave, Astell, UK) at temperatures 121°C–134°C was used for instruments sterilization, while surgical site disinfection was done using either povidone-iodine (5%) or chlorhexidine solution (0.05%). Each dog was fitted with an Elizabethan collar post-surgery to prevent contamination of their surgical wounds.

Bacterial biotyping and abundance estimation

A total of forty-one swab samples were collected and inoculated on MacConkey agar without salt (Oxoid CM 516, UK) and 7% blood agar (Oxoid, UK). All the inoculated plates were incubated at 37 °C for 18 to 24 hours

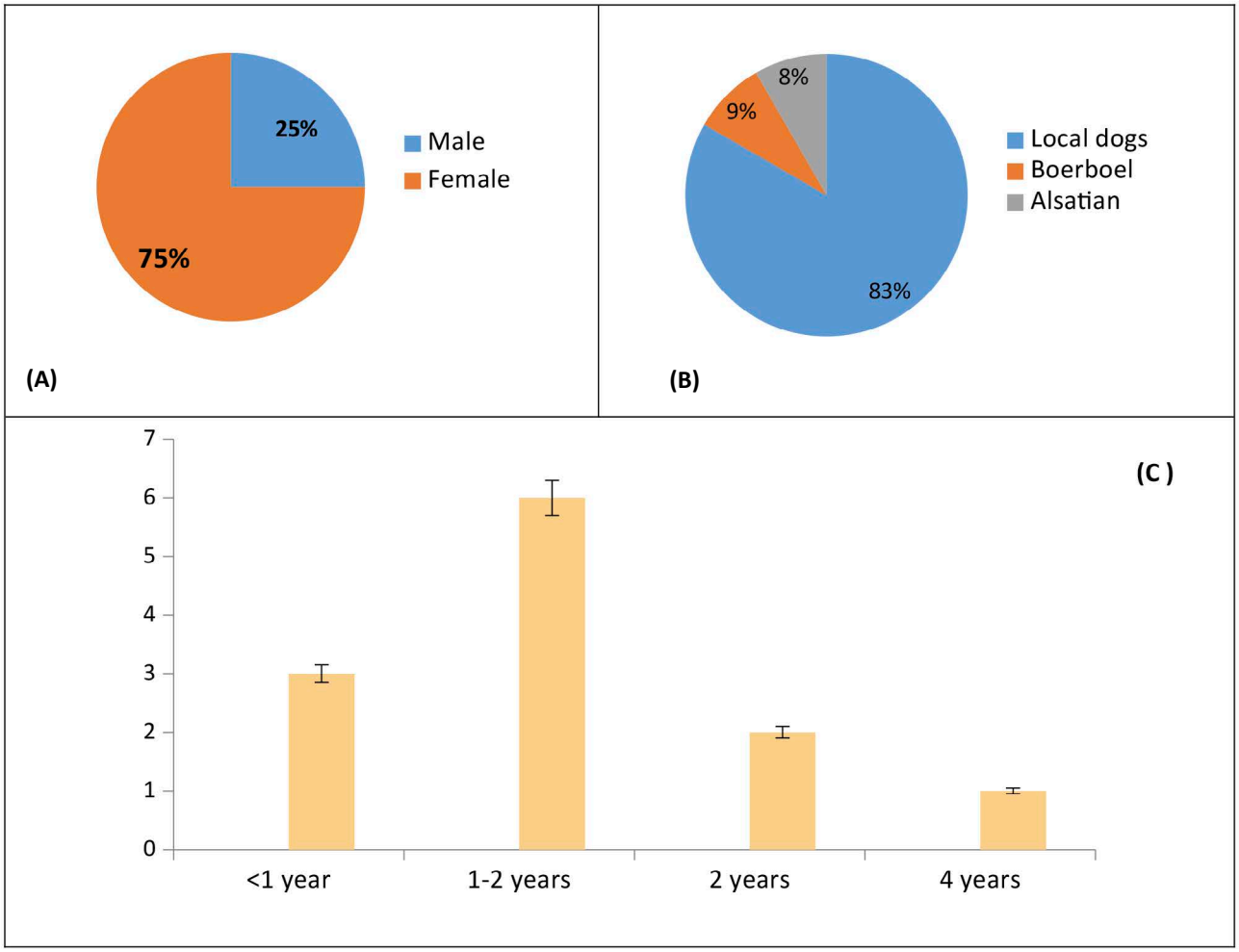

Fig. 1. Biodata distribution of selected dogs based on (A) gender, (B) dog breed and (C) age

and examined for cultural and cellular morphology by the Gram staining method [8]. Biochemical tests were performed using the Analytical Profile Index (API 20E and S36). The proportion of each strain of bacteria obtained from each source was estimated [9].

Antibiotic susceptibility testing

Susceptibility of each recovered bacteria isolate was analysed using the Kirby-Bauer disk diffusion antibiotic testing method [10]. Briefly, 3 to 5 pure colonies of the test organism were emulsified in 3–4 ml of sterile normal saline, and the suspension was adjusted to 0.5 McFarland (1.5×10^8 CFU/mL) turbidity. A sterile swab was dipped into the tube containing the bacteria suspension and spread uniformly on the Mueller Hinton agar surface. After a few minutes, antibiotic discs of cefuroxime (CXM), levofloxacin (LBC), gentamycin (GN), ofloxacin (OFX), iminepem (IMP), cefotaxime (CTX), ampicillin + cloxacillin (ACX), cefixime (ZEM), amoxicillin + clavulanic acid (AMC), and ceftriaxone (CRO) were gently placed on the inoculated agar surface and incubated at 37°C for 24 hours. After incubation, the zones of inhibition for each disc were measured and interpreted based on the criteria and breakpoints set by the Clinical and Laboratory Standards Institute [11].

Data analysis

Continuous variables, such as age and weight of the dogs, were assessed for normality. Data that were not normally distributed were presented as medians (ranges), while prevalence of SSI was presented as a percentage. Data on microbial distribution was presented using measures of central tendency. A p-value less or equal to 0.05 was considered significant. All statistical analysis was performed using STATA 15 [12].

RESULTS

High abundance rates of aerobic bacteria were isolated from operating tables, sterile surgical instruments, admission kennels, and surgical sites of the dogs (Fig. 2). High rates of *Escherichia coli, Staphylococcus saprophyticus, Staphylococcus aureus, ß-Streptococcus* species *Flavobacterium* species, *Proteus vulgaris,* and *Bacillus subtilis* were predominantly found in surgical wounds and surgical table samples (Tables 1 and 2). Samples from the surgical instruments and kennel floors yielded lower rate of *Bacillus subtilis, Staphylococcus saprophyticus, Proteus vulgaris,* and *Enterobacter* species (Tables 3 and 4). The bacteria isolated from the surgical site samples were mostly *Staphylococcus aureus, α-Streptococcus spp., ß-Streptococcus* spp., *Escherichia coli, Staphylococcus saprophyticus, Bacillus subtilis, Proteus vulgaris, Pseudomonas fluorescens, Klebsiella oxytoca, Flavobacterium* spp., and *Bacillus mycoides*.

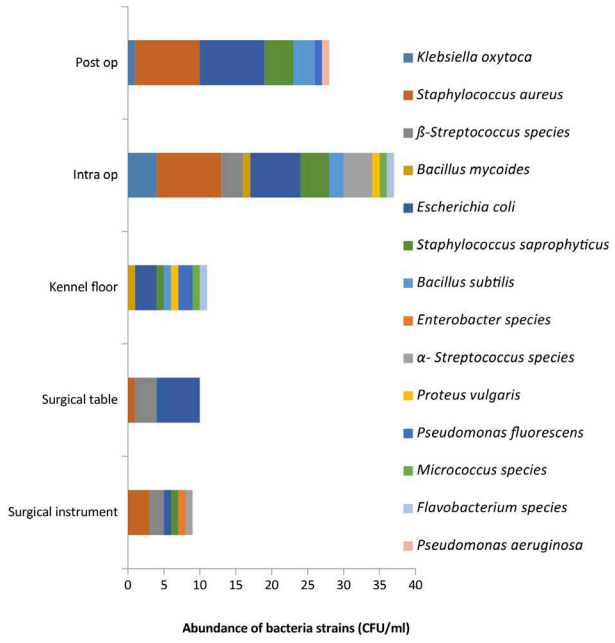

Fig 2. Bacterial populations from different operating room surfaces, surgical wounds, and kennel floors

Comparative analysis of microbial population from surgical incision prior to wound closure showed significant and higher abundance of *Staphylococcus aureus, α-Streptococcus* spp., *ß-Streptococcus* spp., *Escherichia coli, Staphylococcus saprophyticus, Bacillus subtilis, Proteus vulgaris, Klebsiella oxytoca, Flavobacterium* spp., *Bacillus mycoides* and *S. aureus* compared to the microbial population obtained from the surgical sites 48 hours post-surgery ($p = 0.002$; Table 5, Fig. 2).

Bacteria isolates recovered from surgical wound samples were resistant to augmentin, ceftriaxone, cefuroxime, ampicillin/cloxacillin, cefixime, levofloxacin, and gentamycin, while isolates from the kennel floor and surgical table showed resistance to ampicillin + cloxacillin, cefuroxime, and ceftriaxone, (Fig. 3A). Lower than 10% resistance rates were shown by *Klebsiella oxytoca*, *Staphylococcus aureus, Bacillus mycoides*, *Escherichia coli, Staphylococcus saprophyticus*, *Enterobacter* spp., *Streptococcus* spp., *Proteus vulgaris, Pseudomonas fluorescens*, *Micrococcus* spp., and *Flavobacterium* spp. (Fig. 3B).

DISCUSSION

The result of this study showed the presence of several bacteria genera from different operating room surfaces, as well as canine surgical wound prior to and 48 hours after wounds closure, suggesting nosocomial transmission of bacteria in canine surgical wounds. In addition, the antimicrobial susceptibility test performed on the isolated bacteria samples showed resistance to multiple antimicrobial agent indicating multidrug resistance.

The samples used for this study were obtained from dogs presented for clean surgeries such as orchidectomy, ovariohysterectomy, and splenectomy. This was done to prevent contamination of the surgical incision from bacteria within the gastrointestinal or urinary tract mucosa. In spite of sampling animals undergoing clean surgeries, the majority of the dogs still showed evidence of surgical site contamination. This supports the theory that there are several exogenous sources of wound contamination, such as the patient's skin, the operating room environment, surgical instruments, and the surgeons [13]. The small sample size of dogs in the study reflected the cultural practice of surgical procedures such as orchidectomy and ovariohysterectomy in sub- Saharan Africa where dog owners do not embrace neutering of their dogs because of financial gain [14].

Recycling of surgical instruments and linens may account for one of the sources of surgical site contamination in this study. Instruments are routinely cleaned after surgical procedures and then thermally disinfected using an au-

Table 1. Microbial population isolated from surgical wounds

Sample Identification	Bacteria Isolated	Number of Bacteria Genera
Intra Op 2	*Klebsiella oxytoca, S. aureus, ß-Streptococcus* spp., *E. coli, Bacillus mycoides*	5
Intra Op 3	*S. aureus., E. coli, S. saprophyticus, Flavobacterium* spp.	4
Intra Op 4	*Klebsiella oxytoca, S. aureus, E. coli*	3
Intra Op 5	*Klebsiella oxytoca, S. saprophyticus*	2
Intra Op 6	*Klebsiella oxytoca, S. aureus*	2
Intra Op 7	*S. aureus, α-Streptococcus* spp., *E. coli, S. saprophyticus*	4
Intra Op 8	*S. aureus, α-Streptococcus* spp., *E. coli, Proteus vulgaris*	4
Intra Op 9	*S. aureus, α-Streptococcus* spp., *E. coli, Bacillus subtilis*	4
Intra Op 10	*S. aureus, ß-Streptococcus* spp., *E. coli, S. saprophyticus, Bacillus subtilis*	5
Intra Op 11	*S. aureus, α-Streptococcus* spp., *ß-Streptococcus* spp.	3

Median number of bacteria genera isolated = 4

Table 2. Microbial population isolated from surgical tables

Sample Identification	Bacteria Isolated	Number of Bacteria Genera
T2	*E. coli*	1
T3	*E. coli, S. aureus, ß-Streptococcus* spp.	3
T4	*E. coli*	1
T5	*E. coli, ß-Streptococcus* spp.	2
T6	*Bacillus subtilis, E. coli, ß-Streptococcus* spp.	3
T7	*S. aureus, E. coli*	2

Median number of bacteria = 2

Table 3. Microbial population isolated from surgical instruments

Sample Identification	Bacteria Isolated	Number of Bacteria Genera
S2	*E. coli*	1
S3	No growth	0
S4	*S. saprophyticus, Enterobacter* spp.	2
S5	*S. aureus, ß-Streptococcus* spp., *Monococcus* spp.	3
S6	*S. aureus, ß-Streptococcus* spp., *Monococcus* spp.	3
S7	*S. aureus, ß-Streptococcus* spp.	2

Median number of bacteria = 3

Table 4. Microbial population isolated from kennel floors

Sample Identification	Bacteria Isolated	Number of Bacteria Genera
K2	*Bacillus subtilis, Proteus vulgaris*	2
K3	*E. coli, Pseudomonas fluorescens*	2
K4	*Pseudomonas fluorescens*	1
K5	*E. coli, Micrococcus* spp.	2
K6	*E. coli, Staphylococcus saprophyticus, Micrococcus* spp., *Flavobacterium* spp.	4

Median number of bacteria = 2

Table 5. Microbial population isolated from surgical site 48 hours after wound closure

Sample Identification	Bacteria Isolated	Number of Bacteria General
Post Op 2	*S. aureus, E. coli, S. saprophyticus*	3
Post Op 3	*S. aureus, E. coli, Pseudomonas fluorescens*	3
Post Op 4	*S. aureus, E. coli, Bacillus subtilis*	3
Post Op 5	*Klebsiella oxytoca, E. coli*	2
Post Op 6	*S. aureus, E. coli*	2
Post Op 7	*S. aureus, E. coli, S. saprophyticus, Bacillus subtilis*	4
Post Op 8	*S. aureus, E. coli, Micrococcus* spp., *Pseudomonas aeruginosa*	4
Post Op 9	*S. aureus, E. coli, S. saprophyticus*	3
Post Op 10	*S. aureus, E. coli, Bacillus subtilis*	3
Post Op 11	*S. aureus, E. coli, S. saprophyticus*	3

Median number of bacteria = 3

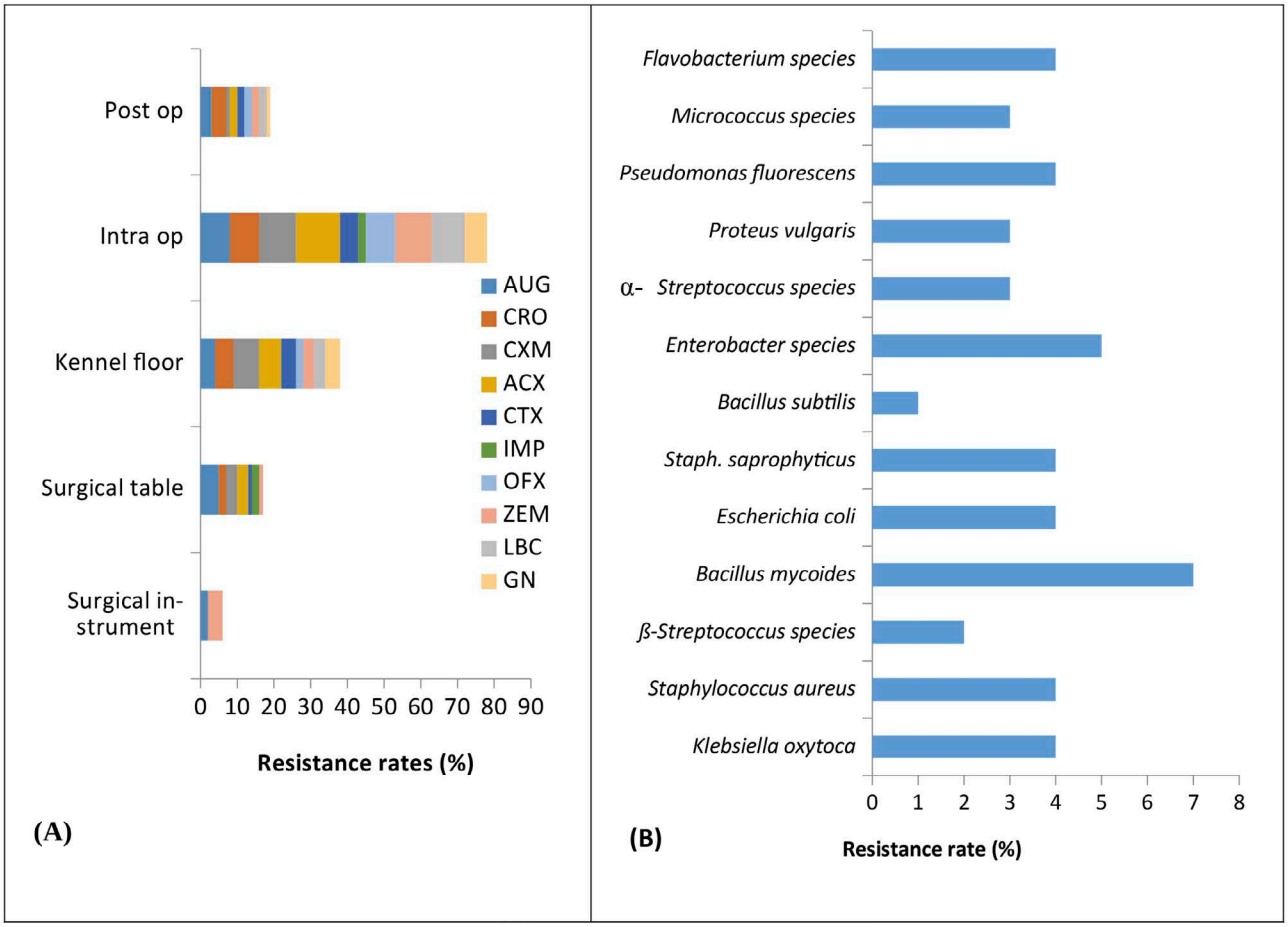

Fig. 3A. Antibiotic resistance pattern of bacteria isolated from surgical wounds and different theatre surfaces
Fig. 3B. Antibiotic resistance rates of different recovered bacteria isolates
Keys: CXM: cefuroxime, LBC: levofloxacin, GN: gentamycin, OFX: ofloxacin, IMP: iminepem, CTX: cefotaxime, ACX: ampicillin + cloxacillin, ZEM: cefixime, AUG: augmentin, CRO: ceftriaxone.

toclave, and stored in the theatre until the next operation. Contamination of the instruments can occur at any stage of the processing. For example, sterile surgical instruments could be contaminated in the protective area of the operating room when left open [15]. Autoclave malfunction due to failure of the sterilization cycle can also result in suboptimal steam sterilization. In this study, distribution pattern of the surgical linen (drapes) and instruments showed that surgical linen and instruments were reused in all the cases. This may account for bacteria being isolated from them despite the routine sterilization process. This finding is similar to that reported in the human operating room [16]. While the major bacteria isolated from surgical instruments and tables from this study was *E. coli,* coagulase-negative staphylococci were mostly isolated bacteria in a similar study in a human operating room [17]. It is thought that the air in the operating room might be the source of contamination of the surgical instruments, tables, and linens [18].

In this study, the bacteria isolated from the surgical instruments were *Escherichia coli, Staphylococcus saprophyticus, Enterobacter* spp., *Staphylococcus aureus, ß-Streptococcus* spp., and *Monococcus* spp., with *Staphylococcus aureus* being the most frequently isolated bacteria. This finding is similar to that reported in the surgery department of a resource-limited country [19]. This phenomenon may be associated with recycling of surgical instruments and sterilization failure. It is worrisome that despite the use of an autoclave for the sterilization of the instruments, multidrug-resistant bacteria were still isolated from the instruments. Sterilization failure has been reported to range between 12 and 17 percent in human dental facilities and has been associated with inadequate personnel training, poor equipment maintenance, and incorrect equipment maintenance [20]. These factors might be responsible for the presence of bacteria in sterilized instruments and linen despite sterilization with an autoclave.

Escherichia coli was the most frequently isolated bacteria on both the surgical table and the floor of the kennel. This is contrary to the report from a similar study in which *Rothia* spp., coagulase-negative *Staphylococcus* spp., *Staphylococcus aureus*, and *Enterococcus* spp. were the bacteria isolated from the operating room and admission kennels of a veterinary hospital [21]. In another study, *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Enterococcus* spp. were isolated [22]. The organisms isolated from the surgical tables and kennel floor may be associated with faecal contamination of these surfaces [21]. The presence of bacterial contamination of the surgical table despite sterilization with disinfectants such as chlorhexidine may be due to several factors, such as a lower concentration of the disinfectant used, the disinfectant not reaching the minimum contact time of the product with the surface, or taking very long cleaning intervals [22]. This will therefore necessitate strict disinfection protocol of all surfaces of the operating theatre to prevent the presence of contaminating bacteria.

The bacteria isolated from the surgical sites included *S. aureus, α-Streptococcus* spp., *ß-Streptococcus* spp., *E. coli, S. saprophyticus, Bacillus subtilis, Proteus vulgaris, Pseudomonas fluorescens, Klebsiella oxytoca, Flavobacterium* spp., and *Bacillus mycoides.* This finding is similar to that reported in previous studies, with the isolated bacteria probably being those present in the skin of the dogs rather than contamination from other sources [2, 23]. This observation underscores the importance of strict skin preparation, such as scrubbing and proper disinfection, prior to surgery. The use of a scalpel for shaving should also be discouraged, as it can help in the spread of normal bacterial flora of the skin to the incision site. Thus the use of clippers for hair removal is advocated.

It is important to note that the majority of the bacteria isolated from different surfaces of the operating room, kennel floor, and the surgical incision sites exhibited multidrug resistance pattern except *Bacillus subtilis* and β-*Streptococcus* spp. This finding is similar to reports from previous studies where multidrug-resistant bacteria have been isolated [2, 21, and 22]. This may not be unconnected with indiscriminate and inappropriate use of antibiotics in patients undergoing routine and elective surgical procedures in sub-Saharan Africa. Judicious use of antibiotics based on empirical data is therefore recommended.

Finally, there are few limitations in this study that will warrant that the present result be interpreted with caution. The samples studied were obtained from one hospital where it is expected that routine operating room practices such as disinfection and asepsis are similar. This makes it difficult to determine if a change in the operating room hygiene procedures will affect the pattern of bacterial contamination at the surgical site. The small number of the animals sampled is another limitation. However, small sample size reflects the cultural perception of dog owners regarding neuter surgery. It is important to note that in spite of the small size, a large number of bacteria were isolated from different surfaces of the operating room and the surgical site. The high number of bacteria isolated from different surfaces may be associated with the poor resource nature of the hospitals that resulted in the recycling of surgical instruments and linens.

CONCLUSION

In conclusion, aerobic bacteria populations from different surfaces of the operating room provide insight into possible nosocomial transmission in spite of routine disinfection protocol. The emergence of new strains with high-level multidrug resistance from contaminated surgical instrument and surgical incision sites during intraoperative and postoperative periods could serve as aetiological agent for postoperative systemic infection. From the study, it is evident that most bacterial contamination of the surgical site occurs intraoperatively rather than postoperative contamination of the wound at the kennel. Multi-centre bacteria profiling for comparative operating room practices, incorporating a larger sample size from veterinary surgical centres is recommended. The evidence of a high rate of sterilization failures suggested the need for improved personnel training, proper equipment maintenance, and adherence to proper equipment operation.

Data Availability Statement

Data sets for this study are available upon request from the corresponding author.

Ethical Statement

Ethical approval (FUNAAB/COLVET/CREC/2024/03/05) was received from the COLVET Research and Eth-

ics Committee (CREC), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

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Generative AI statement

No AI or AI-assisted technologies were used in the preparation of this manuscript.

Authors' Contributions

Conceptualization: ARA and TRA; Data Acquisition: TRA, SOL, OFK; Data Analysis: OFK, SOL, PAA; Manuscript Preparation: PAA, ARA. All the authors review and accept the final manuscript.

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