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# Contamination of Gloved Hands by Multidrug Resistant Bacteria during Small Animal Surgery Wet-labs and its Potential Implication for Occurrence of Surgical Site Infections

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**ABSTRACT**

This study was carried out to evaluate the level of asepsis at various stages of the surgical procedures during the undergraduate students' wet-lab sessions. Skin and/or wound swabs were collected from different wet lab groups, sessions and stages. The swabs were processed for bacteriological isolation using standard microbiological procedures. A total of 62 isolates of bacteria belonging to 8 genera: *Staphylococcus* (n=38), *Streptococcus* (n=1), *Corynebacterium* n=4), *Escherichia* (n=7), *Proteus* (n=8), *Klebsiella* (n=2), *Serratia* (n=1) and *Acinetobacter* (1), were isolated. The most commonly isolated species of bacteria were *Staphylococcus equorum* (n=31) and *Proteus* spp.(n=7), which were detected in swabs from ungloved and gloved hands of surgeon and his assistant, patient's surgical sites and surgical site infections. All the isolates (Gram-positive and negative) were resistant to at least one antibiotic with resistance to the β-lactam antibiotics: ampicillin (89.3% and 100% and amoxicillin (75% and 100%) most observed. The bacteria were more susceptible to doxycycline (75%) and imipenem (87.5%) respectively. Majority of the isolates (83.3%, n=30) were multidrug resistant, presenting in one of 24 different multidrug resistance patterns. The detection of these bacteria from the normally aseptic surgical procedure indicates a break in asepsis. Similarly, the danger of spreading multidrug resistant bacteria to the surgical wounds may result in wound infection, dehiscence, delayed healing and increased cost of post-surgical management. It is recommended that adherence to stringent pre-surgical and intra-surgical asepsis should be observed.

**Keywords**: Small animal; Wet-labs; Contamination; Aerobic bacteria; Multi-drug resistance

## INTRODUCTION

The last century witnessed the emergence of a number of practices in the field of surgery targeted at making surgical procedures safer and relatively free of the risk of transmission of micro-organisms between surgeon and patient and vice versa. However, surgical site infections (SSIs) remain a frequent complication in patients undergoing surgeries and reportedly accounts for complications in 0.8 % to 18.1 % of small animal surgical procedures (Walker *et al*., 2012; Andrade *et al*., 2016). Although the causes of SSI are reported to be complex and multi-factorial, the surgical team has a key role in the prevention of factors associated with the occurrence of SSI during the pre- and intra-operative periods (De Oliveira *et al*., 2016). Surgical hand antisepsis and the use of sterile surgical gloves are well known practices that contribute to the reduction/elimination of microorganisms that may cause SSI and therefore strongly recommended by organizations such as the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) (De Oliveira *et al*., 2016). The effectiveness of these practices is however dependent on the antiseptic agent use with their application method, and duration of surgical procedures.

Microorganisms particularly bacteria can gain access to a surgical wound either by direct contact of air borne dispersal or by contamination. Direct contact and poor hand washing techniques (asepsis) of health care practitioners during pre, intra and post-operative phases of patient care are considered to be major factors influencing the risk of developing a surgical wound infection. Bacteria species including *Staphylococcus, Enterococcus, Klebsiella, Salmon ella, Serratia, Clostridium difficile* and *Acinetobacter* have been identified as the major culprits for surgical site contamination (Harper *et al*., 2013).

SSIs are of increasing concern in veterinary hospitals as a result of the occurrence of multidrug-resistant (MDR) bacteria (Nelson, 2011; Meakins *et al*., 2016) which may result in delayed wound healing, prolonged hospital stay, increased trauma, high risk of surgical wound dehiscence, disarticulation, amputation, increased need for medical care,

increased treatment costs and failure and eventual death. This makes investigation and evaluation of sources of bacteria (in addition to their antibiotic susceptibility profile) for surgical wound infection a matter of concern. Currently, studies investigating the effectiveness of the surgical hand asepsis and gloving among surgical team members and their potential to serve as source of bacteria for surgical site infections during small animal surgery procedures in Nigeria are very scarce. Also, in spite of the relevance of these practices to achieving surgical asepsis, studies have shown insufficient adherence to this practice.

Knowledge of the potential sources of contamination during intra-operative period is critical in the search for the source of SSI, and allows for the development of evidence-based strategies to control the impact of pathogenic microbes in the surgical environment. The present study was therefore aimed at evaluating the effectiveness of the practice of surgical hand asepsis and gloving in the prevention of SSI by determining the presence of bacteria and their antimicrobial susceptibility profile using the wet-lab sessions in a Veterinary Teaching Hospital as a case study.

## MATERIALS AND METHODS

***Study design and population:*** A prospective study was carried out from June to August 2015 at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria targeting consecutive DVM student's small animal wet-lab surgical procedures during the period. The surgical procedures ranged from integumentary, abdomino-pelvic, ophthalmologic, aural, orthopaedic surgeries. Swabs of the various sites of importance were taken and labelled as follows: HSBS = hands of surgeon before surgery, HABS = hands of assistant-surgeon before surgery, HSAS = hands of surgeon after surgery, HAAS = hands of assistant-surgeon after surgery, GHS = gloved hands of surgeon, GHA = gloved hands of assistant-surgeon, PSSP = patient's scrubbed site pre-surgery, PDS = patient's draped site, SCS = skin closure, surgeon, SCA = skin closure, assistant-surgeon and SSI = surgical site infections (after 7 days). Swab samples were collected aseptically from the various surfaces indicated earlier. These samples were immediately returned into their packs into which 5 ml of sterile normal saline had been transferred each to prevent the swabs from drying up before processing. These swabs were immediately taken to the Diagnostic Bacteriology Laboratory of the Department of Veterinary Microbiology, A.B.U, Zaria; for processing. Swabs were also taken from the patient; scrubbed surgical site pre-surgery (PSP), draped site (PDS), surgical site after skin closure (SCP) and surgical site 7 days post-surgery (SSI) and evaluated for the presence of bacteria.

***Surgical Preparation:*** The same aseptic preparation protocol was used for all the patients and surgical team. Surgical sites were shaved and scrubbed with 0.05% Chlorhexidine gluconate solution. The patients after anaesthesia were moved from the induction room to the surgery room, and the surgical sites were scrubbed again using the Chlorhexidine gluconate solution just before draping. Preparation of the surgeons and assistants included scrubbing of both hands with 0.05% Chlorhexidine solution and wearing sterile surgical gowns, latex surgical gloves, masks, and caps. Other operating room personnel wore masks, caps, and scrub suites at all times.

***Bacterial isolation and identification*:** The swabs were processed for bacteriological isolation using standard procedures (Cheesbrough, 2006). Briefly, swabs were inoculated directly onto blood agar, MacConkey agar and mannitol salt agar plates (Oxoid, UK) each swab was inoculated per plate and incubated aerobically for 18-24 hours at 37 °C. The resulting isolates were presumptively identified using characteristic morphological appearances of colonies on media, Gram stains under the microscope and standard biochemical tests including catalase, coagulase, oxidase, Voges Proskauer, Hydrogen sulphide production, urease, indole, citrate, CAMP test and sugar utilization (Cheesbrough, 2006). Furthermore, the identity of the isolates was confirmed using the Microbact® 12E kit (Oxoid, UK).

***Antimicrobial susceptibility testing:*** All the bacterial isolates were tested for susceptibility to ampicillin (10 µg), amoxicillin (10 µg), cefepime 30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), doxycycline (30 µg), kanamycin (30 µg), nitrofurantoin (300 µg) lincomycin (30 µg) and vancomycin (30 µg) for Gram-positive isolates and imipenem (10 µg), enrofloxacin (5 µg) and ceftriaxone (30 µg) for Gram-negative isolates. The susceptibility testing was carried out using the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (Bauer, 1966; CLSI, 2016). Disc impregnated with the respective antibacterial (Oxoid, UK) were placed on the Mueller Hinton agar plates pre-inoculated (by spreading) with the isolates and incubated for 18-24 hours at 37 °C. The diameter of zone of inhibitions were measured and interpreted as susceptible or resistant based on the CLSI recommendation (CLSI, 2016). Multidrug resistance was defined as resistance of the isolate to three or more antimicrobial agents belonging to different classes (Basak *et al*., 2016).

## RESULTS

At the end of laboratory isolation and identification of aerobic bacteria from the various stages of the students' wet labs surgeries, a total number of 62 bacteria were isolated out of which 43 were Gram-positive giving 69.2% frequency of isolation. The total Gram-negative bacteria isolated were 19 with a frequency of isolation of 30.6%. Table 1 below shows details of the distribution of these bacteria where it could be seen that the most frequently isolated Gram-positive bacterium was *Staphylococcus equorum* 7 (11.3%); isolated from the patient's draped site (PDS).

The most frequently isolated Gram-negative bacterium was *Proteus* spp. which was also 7 (11.3%) but these were isolated from the surgical site infections (SSIs) where swabs were taken after seven days. The least frequently isolated Gram-positive bacteria were *Streptococcus* spp. and *Corynebacterium pseudodiphteriticum,* both at 1.6%; while the least frequently isolated Gram-negative bacteria were *Acinetobacter haemolyticus* at 1.6% and *Serratia liquefaciens* also at 1.6%.

**Table 1:** Distribution of Bacterial Species According to Gram's Staining

| Isolation site | Gram-positive Bacteria (n=43) | | Gram-negative Bacteria (n=19) | |
|---|---|---|---|---|
| | **Bacteria** | **Frequency (%)** | **Bacteria** | **Frequency (%)** |
| HSBS | *Staphylococcus equorum* | 5 (8.1) | *Acinetobacter haemolyticus* | |
| | *Staph. intermedius* | 2 (3.2) | | 1 (1.6) |
| | *Streptococcus* spp. | 1 (1.6) | | |
| HSAS | *Staph. equorum* | 1 (1.6) | No isolation | 0 (0) |
| HABS | *Staph. equorum* | 5 (8.1) | *Escherichia coli* inactive | 3 (4.8) |
| | *Staph. gallinarum* | 1 (1.6) | | |
| HAAS | *Staph. equorum* | 1 (1.6) | *E. coli* | 1 (1.6) |
| | *Staph. gallinarum* | 1 (1.6) | *E. coli* inactive | 1 (1.6) |
| GHS | *Staph. equorum* | 2 (3.2) | No isolation | 0 (0) |
| GHA | *Corynebacterium pseudodiphtheriticum* | | No isolation | 0 (0) |
| | | 1 (1.6) | | |
| PSSP | *Staph. equorum* | 3 (4.8) | No isolation | 0 (0) |
| PDS | *Staph. equorum* | 7 (11.3) | No isolation | 0 (0) |
| SCS | No isolation | 0 (0) | No isolation | 0 (0) |
| SCA | No isolation | 0 (0) | No isolation | 0 (0) |
| SCP | *Staph. equorum* | 5 (8.1) | No isolation | 0 (0) |
| SSIs | *Staph. intermedius* | 3 (4.8) | *Proteus mirabilis* | 1 (1.6) |
| | *Staph. equorum* | 2 (3.2) | *Proteus* spp. | 7 (11.3) |
| | *Corynebacterium* spp. | 3 (4.8) | *E. coli* | 2 (3.2) |
| | | | *Serratia liquefaciens* | 1 (1.6) |
| | | | *Klebsiella ozonae* | 1 (1.6) |
| | | | *Klebsiella pneumoniae* | 1 (1.6) |

**Key:** HSBS = hands of surgeon before scrubbing, HSAS = hands of surgeon after scrubbing, HABS = hands of assistant surgeon before scrubbing, HAAS = hands of assistant surgeon after scrubbing, GHS = gloved hands of surgeon, GHA = gloved hands of assistant surgeon, PSSP = patient's scrubbed site pre-surgery, PDS = patient's draped site, SCS = skin closure surgeon, SCA = skin closure assistant surgeon, SCP = skin closure patient, SSIs = surgical site infections (after 7 days).

Representative Gram-negative and Gram-positive isolates from Table 1 were screened for antimicrobial susceptibility as shown in Table 2. A high resistance to cefeprime (100%), ampicillin (89.3%) and amoxicillin (75%) was observed among Gram-positive bacteria which were also found to be most susceptible to ceftazidine (96.1%) followed by ciprofloxaxin at 71.4%. On the other hand, the Gam negative bacteria showed 100% resistance to ceftazidime, ampicillin and amoxicillin; but 87.5% resistance was shown to ceftriaxone. Meanwhile, they were most susceptible to doxycycline and imipenem both at 87.5%.

The frequency of occurrence of multidrug resistant phenotypes isolated was higher for AML-AMP-CAZ-CIP-FEP-K-VA and AML-AMP-CAZ-CRO-FEP-K with a frequency of occurrence of two each, while all other antimicrobial resistance patterns had frequency of occurrence of one each (Table 3).

**Table 2:** Antimicrobial Susceptibility Profiles of Gram-positive and Gram-negative Bacteria Isolated During Students Wet Labs

| Antimicrobial | Gram-positive bacteria (n=28) | | | Gram-negative bacteria (n=8) | | |
|---|---|---|---|---|---|---|
| | Susceptible | Intermediate | Resistant | Susceptible | Intermediate | Resistant |
| Ampicillin | 1(3.6) | 2(7.1) | 25(89.3) | 0(0) | 0(0) | 8(100) |
| Amoxycillin | 4(14.3) | 3(10.7) | 21(75) | 0(0) | 0(0) | 8(100) |
| Ciprofloxacin | 20(71.4) | 3(10.7) | 5(17.9) | 4(50) | 2(25) | 2(25) |
| Cefepime | 0(0) | 0(0) | 28(100) | 0(0) | 1(12.5) | 7(87.5) |
| Ceftazidime | 27(96.4) | 0(0) | 1(3.6) | 0(0) | 0(0) | 8(100) |
| Ceftriaxone | - | - | - | 1(12.5) | 2(25) | 7(87.5) |
| Doxycycline | 21(75) | 5(17.9) | 1(3.6) | 7(87.5) | 0(0) | 1(12.5) |
| Enrofloxacin | - | - | - | 4(50) | 2(25) | 2(25) |
| Imipenem | - | - | - | 7(87.5) | 0(0) | 1(12.5) |
| Kanamycin | 12(14.9) | 5(17.9) | 10(35.7) | 1(12.5) | 2(25) | 5(62.5) |
| Lincomycin | 19(67.9) | 1(3.6) | 6(21.4) | - | - | - |
| Nitrofurantoin | 7(25) | 13(46.4) | 6(21.4) | - | - | - |
| Vancomycin | 2(7.1) | 9(32.1) | 15(53.6) | - | - | - |

## DISCUSSION

In this study a total of 8 gloved hands, 8 ungloved hands, 6 patient surgical sites and 7 postsurgical wounds were sampled and after culture bacteria were phenotypically identified in majority (86.2 %) of the swabs collected with the exception of the gloved hands of the surgeon and assistant surgeon after skin closure. A total of 62 bacteria distributed into 8 genera were isolated in this study with the most frequently isolated being *Staphylococcus* (Gram-positive) and *Proteus* spp. (Gram-negative). In general, staphylococci particularly *S. equorum* was the most common bacterium isolated in this study. Although, the sources of these bacteria remain unclear, a possible reason for its high frequency of isolation may be due to the fact that the organisms are common skin inhabitants. It may also be connected to the hardy nature of these pathogens resulting in their ability to survive the actions of disinfectants (Davis *et al*., 2012). Consistent with the findings of this study, the CDC and other studies around the world have reported that, staphylococci and *E. Coli* were the most prevalent organisms associated with surgical wound infections (Spagnolo *et al*., 2013; CDC, 2014; Mundhada and Tenpe, 2015; Chaudhary *et al*., 2017). Also, most species of *Staphylococcus* are normal skin microflora, therefore they can potentially contaminate surgical materials and wounds causing infections.

In spite of the routine practices of pre-surgical hand asepsis, surgical site preparation, and strict aseptic technique, 100% of gloved hands of both surgeons and assistant surgeons in this study were contaminated with bacteria. This is a relatively high contamination rate for surgical procedure, thus suggesting that contamination of surgical wounds is likely a common occurrence with gloves being one source. This assertion is further supported by similarity of bacteria species on the gloved hands with those detected on the surgical site after closure (SCP) and 7 days post-surgery (SSI). This high level of bacterial contamination is also associated with a high risk for developing post-operative surgical site infection which may constitute a major source of morbidity and mortality which are negative indicators for surgery outcomes.

Similar to studies from other parts of the world (Weese, 2008; Godebo *et al*., 2013; Pirvanescu *et al*., 2014) this study detected a high rate of resistance among the Gram-positive and Gram-negative bacteria to the antimicrobials tested. All the isolates were resistant to at least one antibiotic. Resistance to the β-lactam antibiotics was by far the most observed among the isolates. This perhaps reflects the widespread use of this class of antibiotics in both human and Veterinary medicine. These antibiotics are relatively cheap and readily available across the counter without prescription.

**Table 3:** Multidrug Resistance Phenotypes Among Gram-positive and Gram-negative Bacteria Isolated During Students' Wet Labs

| Antimicrobial Resistance Pattern | Frequency of Occurrence |
|---|---|
| CAZ-F-FEP-VA | 1 |
| AMP-CAZ-FEP-VA | 1 |
| AML-CAZ-F-FEP | 1 |
| AML-AMP-CAZ-FEP-F-MY-VA | 1 |
| AMP-CAZ-FEP-VA | 1 |
| AML-AMP-CAZ-K-FEP | 1 |
| AML-AMP-CAZ-FEP-VA | 1 |
| AML-AMP-CAZ-FEP-VA | 1 |
| AML-AMP-CAZ-CIP-FEP | 1 |
| AMP-CAZ-FEP-MY-VA | 1 |
| AML-AMP-CAZ-FEP-VA | 1 |
| AMP-CAZ-CIP-FEP-K | 1 |
| AML-AMP-CAZ-FEP-K | 1 |
| AML-AMP-CAZ-CIP-FEP-VA | 1 |
| AML-AMP-CAZ-FEP-MY-VA | 1 |
| AML-AMP-CAZ-FEP-F-VA | 1 |
| AML, AMP,CAZ, FEP, K, VA | 1 |
| AML-AMP-CAZ-CIP-FEP-K | 1 |
| AML-AMP-CAZ-FEP-K-VA | 1 |
| AML-AMP-CAZ-CIP-FEP-K-VA | 2 |
| AML-AMP-CAZ-DOX-FEP-K-MY | 1 |
| AML-AMP-CAZ-F-FEP-K-VA | 1 |
| AML-AMP-CAZ-K* | 1 |
| AML-AMP-CAZ-CRO-FEP-K* | 2 |
| AML-AMP-CAZ-CRO-ENR-FEP* | 1 |
| AML-AMP-CAZ-CIP-DOX-ENR-FEP* | 1 |
| AML-AMP-CAZ-CRO-ENR-FEP-K* | 1 |
| AML-AMP-CAZ-CIP-CRO-ENR-FEP-IPM* | 1 |
| **Total** | **30** |

*Gram-negative isolates, AMP: ampicillin, AML: amoxicillin, FEP: cefepime, CAZ: ceftazidime, CIP: ciprofloxacin, DOX: doxycycline, K: kanamycin, F: nitrofurantoin, MY: lincomycin, VA: vancomycin, IPM: imipenem, ENR: enrofloxacin, CRO: ceftriaxone

These, together with the lack of regulation of antibiotics use and accessibility, might have caused the irrational overuse of these drugs which might have led to bacterial resistance.

Another important aspect to highlight in this study is the detection of multidrug resistance (defined as resistance to ≥ 3 antibiotics belonging to different classes) among majority of the isolates (Basak *et al*., 2016). This finding suggests that multidrug resistant bacteria may be widespread in the veterinary settings in Nigeria. Majority of the isolates (83.3 %, n=30) were multidrug resistant presenting in one of 24 different multidrug resistance patterns. Resistance to antibiotics that act by inhibiting the cell wall synthesis particularly ampicillin, amoxicillin and the cephalosporins was more common among the multidrug resistance phenotypes. The detection of a high rate of resistance to these agents is extremely worrisome, especially because these agents are usually the drugs of choice in the treatment of infections caused by these pathogens and this poses a substantial threat to the treatment and management of SSI (Weese, 2008). Similarly, the detection of a high number (53 %, n=15) of the Gram-positive bacteria resistant to vancomycin among which is the drug of last resort in the treatment of infections caused by multidrug drug resistant Gram-positive bacteria particularly *Staphylococcus* species is also of great concern as it narrows the spectrum of drugs available for choice by the clinician in the event of the occurrence of multidrug resistant SSI.

## Conclusion

The findings of this study revealed a relatively high level of wound bacterial contamination from surgeon and patient following a variety of small animal surgical procedures in spite of the aseptic measures carried out suggesting a break in asepsis or inefficient pre-surgical asepsis. It also brings to light the risk posed by break in asepsis in the spread of multidrug resistant bacteria to surgical wounds that may result in wound contamination, dehiscence, delayed healing and increased cost of post-surgical management.

## Conflict of interest

The authors declare that they do not have any conflict of interest.

## Authors Contributions

CAA designed and supervised the project. PHM collected and processed the samples in the laboratory and developed the draft manuscript. STM, AM and BAA performed the Surgical

procedure and assisted in sample collection. All authors read and approved the final manuscript.

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