UNIVERSIDADE LUSÓFONA

CENTRO UNIVERSITÁRIO DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

Efficacy of antisepsis and prophylactic administration of cefazolin in preventing contamination of the operating field in clean orthopaedical surgery

Dissertação apresentada a provas públicas para a obtenção do grau de mestre em Medicina Veterinária, orientada por Professor Doutor João Martins e Professora Doutora Adriana Belas

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Margarida Pinto Nogueira

VERSÃO FINAL

Dissertação defendida por Margarida Pinto Nogueira em provas públicas na Universidade Lusófona, Centro Universitário de Lisboa no dia 30/07 de 2025, perante o júri, nomeado pelo Despacho de Nomeação n.º: 412/2025, de 30 de julho, com a seguinte composição:

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Este trabalho também foi orientado por Professora Doutora Adriana Bela

Margarida Pinto Nogueira

2025

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Resumo

O principal objetivo deste estudo foi avaliar se a realização de antissepsia com uma solução de clorexidina a 2% e álcool isopropílico a 70%, assim como a administração profilática de cefazolina (22mg/kg, IV), seriam eficazes na prevenção da contaminação do campo cirúrgico durante a cirurgia ortopédica limpa.

Na realização deste estudo, foram recolhidas zaragatoas fecais e de pele de 63 animais submetidos a cirurgia ortopédica limpa, antes do protocolo de antissepsia e administração profilática de cefazolina. Adicionalmente, em todos os animais, após a realização da antissepsia e administração profilática de cefazolina foram também recolhidas zaragatoas da pele e do campo cirúrgico em vários momentos da cirurgia e de acordo com a duração da mesma: período de incisão (T0min), a 45 minutos de cirurgia (T45min), entre 45 a 90 minutos de cirurgia (T>45-90min) e após 90 minutos de cirurgia (T>90min). Todo o material recolhido foi inoculado e avaliado de forma a ser possível determinar a presença ou ausência de crescimento bacteriano, incluindo enterobactérias resistentes a cefalosporinas de 3º geração e carbapenemos, assim como *Staphylococcus* spp. resistentes à meticilina. O teste de Wilcoxon foi utilizado para comparar as diferenças entre a contaminação da pele antes e depois do protocolo de antissepsia. A comparação entre a contaminação e duração da cirurgia foi analisada com base no teste de Kruskal-Wallis.

Os resultados do estudo permitiram observar uma redução significativa do crescimento bacteriano na pele após a realização do protocolo de antissepsia e da administração de cefazolina (p <0.001). A contaminação intraoperatória apresentou diferenças significativas entre os tempos cirúrgicos (p = 0.024). Os resultados sugerem também que um protocolo de antissepsia com clorexidina a 2%e álcool isopropílico a 70%, juntamente com a administração profilática de cefazolina (22mg/kg, IV) são eficazes na prevenção da contaminação do campo cirúrgico em cirurgia ortopédica limpa por bactérias Gram-positivas. No entanto, não são eficazes na prevenção da contaminação por bactérias Gram-negativas. O estudo também indica que a escolha e o uso de antimicrobianos pós-cirúrgicos devem ser baseados nos resultados de culturas bacterianas positivas e nos testes de suscetibilidade, realizados com o material colhido antes do encerramento da ferida cirúrgica.

Palavras-chave: cirurgia, ortopedia, contaminação, antimicrobianos, antissepsia.

Abstract

The main objective of this study was to evaluate whether performing antisepsis with a solution of 2% chlorhexidine and 70% isopropyl alcohol and prophylactic administration of cefazolin (22mg/kg, IV) are effective in preventing contamination of the surgical field during clean orthopedic surgery.

In this study, fecal and skin swabs were collected from 63 animals submitted to clean orthopedic surgery, before the antisepsis protocol and prophylactic administration of cefazolin. In addition, in all animals, after antisepsis and prophylactic administration of cefazolin, swabs were also collected from the skin and the surgical field at various times of the surgery and according to the duration of the surgery: incision period (T0min), 45 minutes after surgery (T45min), between 45 and 90 minutes after surgery (T>45-90min) and after 90 minutes of surgery (T>90min). All collected material was inoculated and evaluated to determine the presence or absence of bacterial growth, including enterobacteria resistant to 3rd generation cephalosporins and methicillin-resistant carbapenems and *Staphylococcus* spp.

The Wilcoxon test was used to compare the differences between skin contamination before and after the antisepsis protocol. The comparison between contamination and duration of surgery was analyzed based on the Kruskal-Wallis test.

The results of the study showed a significant reduction in bacterial growth on the skin after the antisepsis protocol and cefazolin administration ($p < 0.001$). Intraoperative contamination showed significant differences between surgical times ($p = 0.024$). The results also suggest that an antisepsis protocol with 2% chlorhexidine and 70% isopropyl alcohol, together with the prophylactic administration of cefazolin (22mg/kg, IV) are effective in preventing contamination of the surgical field in clean orthopedic surgery by Gram-positive bacteria. However, they fail to prevent contamination by Gram-negative bacteria. The study also suggests that the use and selection of post-surgical antimicrobials should be based on the results of positive bacterial cultures and susceptibility tests of material collected before surgical wound closure.

Keywords: surgery, orthopedics, contamination, antimicrobials, antisepsis, wound

List of Abbreviations

ABHR – Alcohol-based hand rub

AF – Absolute Frequency

AMP – Antimicrobial Prophylaxis

AMR – Antimicrobial Resistance

AMROs – Antimicrobial resistant organisms

ASA – American Society of Anesthesia

ASP – Antimicrobial Stewardship Program

BLs – Beta-lactamases

CDC – Center for Disease Control and Prevention

CHX – Chlorexidine

COS – Clean orthopedic surgery

CRAA – Arrábida Animal Rehabilitation Center

ESBLs – Extended spectrum Beta-lactamases

HAS – Hydroalcoholic solutions

HVA – Arrábida Veterinary Hospital

RF – Relative Frequency

MRS – Methicillin-resitant *Staphylococcus*

MRSA – Methicillin-resistant *Staphylococcus aureus*

MRSP – Methicillin-resistant *Staphylococcus pseudintermedius*

OR – Operating room

PI – Povidone-iodine

SCIP – Surgical Care Improvement Project

SSI – Surgical Site Infection

TPLO – Tibial Plateau Leveling Osteotomy

WHO – World Health Organization

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I – Internship Report

The curricular internship to complete the Integrated master's degree in veterinary medicine occurred in two locations. The first part of the internship took place at the Arrábida Veterinary Hospital (HVA), in Vila Nogueira de Azeitão, from October 11, 2023, to December 8, 2023, under the supervision of Dr. Ângela Martins. The second part of the internship took place at the Pontovet Veterinary Clinic, in Amadora, between December 11, 2023, and March 8, 2024, under the supervision of Dr. Cláudio Cabral.

This internship report covers the activities carried out in both places, contributing to the consolidation and practical application of the knowledge acquired during the course. The species that took part in the internship were dogs and cats. During the internship, it was possible to attend prophylactic medicine and medical pathology appointments, especially in functional rehabilitation and neurology, carry out complementary diagnostic tests, and communicate with owners. In addition, the author was able to participate actively in emergency medicine and internal medicine protocols, as well as assist in day and night hospital stays. In the surgery department, the author drew up anesthetic protocols and participated as a surgeon's assistant or circulator in elective, orthopedic, and emergency surgeries. All the cases were discussed daily among the team, which also allowed the author to develop her counseling skills. All these activities were carried out under the supervision of a veterinarian.

The statistical data presented is in the form of absolute frequency (AF), representing the total number of cases observed, and relative frequency (RF), presented as a percentage (%), which represents the number of cases observed in the total number of cases. Thus, RF = (AF of each area / total AF) x 100.

1. Arrábida Veterinary Hospital

1.1 Distribution of cases by types of animals

Regarding types of animals, only two animals were seen during the internship period: dogs and cats. Figure 1 shows that 59% (721/1228) of the cases were of the canine species and 41%(507/1228) were of the feline species.

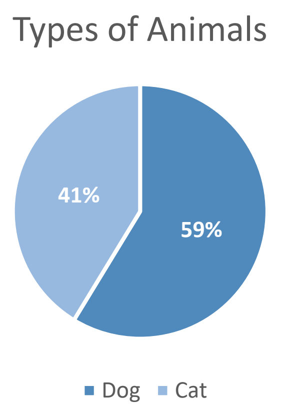

Figure 1 - Distribution of cases observed concerning type of animals

1.2 Distribution of cases by clinical area

During my internship at the HVA, several clinical areas were covered, such as internal medicine, emergency medicine, preventive medicine, surgery, physical medicine and functional rehabilitation. The area with the largest caseload was internal medicine, with 52.8% (649/1228) of cases (Table 1).

Table 1 - Number of cases seen in the various clinical areas, considering the species and respective AF and RF (n=1228)

Clinical Area	Dog	Cat	AF	RF
Preventive Medicine	12	9	21	1.71%
Internal Medicine	383	266	649	52.80%
Surgical Medicine	79	59	138	11.23%
Emergency Medicine	206	145	351	28.58%
Physical Medicine and Functional Rehabilitation	41	28	69	5.61%
Total	721	507	1228	100%

1.2.1 Internal Medicine

The areas of internal medicine with the largest caseloads observed during the internship are shown in Figure 2: gastroenterology, respiratory, neurology, and orthopedics/musculoskeletal system. Throughout the internship, it was possible to monitor clinical cases in all areas, including administering medication, carrying out therapeutic procedures, and monitoring them.

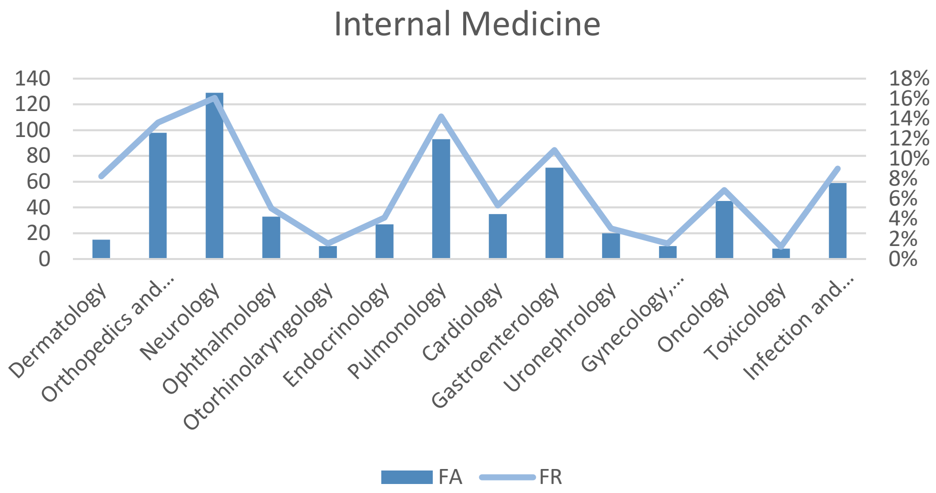

Figure 2 - Distribution of cases seen in the internal medicine area.

1.2.2 Emergency Medicine

Emergency medicine is one of the most important areas at the HVA. Table 2 shows the various areas covered by emergency medicine, with respiratory emergencies being the most prevalent.

Table 2. Emergency area distribution table (n=351)

Emergency Medicine	AF	RF
Respiratory	113	32%
Polytrauma	89	25%
Neurological	103	29%
Intoxication	46	13%
Total	351	100%

1.2.3 Preventive Medicine

Preventive medicine has the lowest percentage of cases, as the HVA's caseload is dominated by more complex and emergency cases. However, this area is fundamental to animal health, both in terms of disease prevention and public health. The main procedures carried out were vaccination, deworming, electronic identification, and general clinical examination.

1.2.4 Physical Medicine and Functional Rehabilitation

Physical medicine and functional rehabilitation are some of the HVA's most important areas since it is associated with the Arrábida Animal Rehabilitation Center (CRAA) and there is a casuistry common to both places.

The most common orthopedic alterations were hip dysplasia, osteoarthritis, and torn ligaments. The most common neurological disorders were herniated discs (Hansen's type I and II) and vestibular syndrome. In this area, it was possible to monitor the consultations for the various cases and draw up treatment protocols for each one.

1.2.5 Surgery

The clinical area of surgery is divided into two main types, soft tissue surgery, and orthopedic surgery, the former being the most prevalent (Figure 3). Soft tissue surgery includes orchiectomies, ovariohysterectomies, enterotomies, mastectomies, and nodulectomies.

In this area, it was possible to prepare the operating room, classify the animal's anesthetic risk, carry out the anesthetic protocol, and anesthetic monitoring, as well as take part in the surgery as a surgeon's assistant or circulator. It was also possible to take part in some emergency surgeries such as cesarean sections, gastric torsion, and exploratory laparotomies.

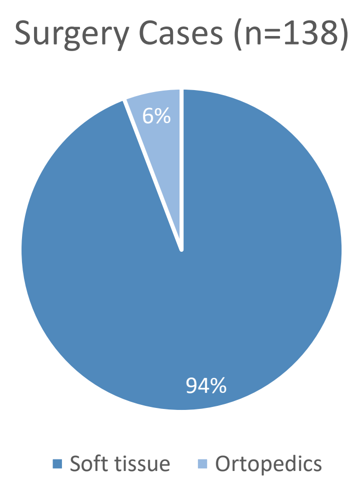

Figure 3 – Distribution of Surgeries observed

1.2.6 Complementary Diagnostic Exams

Complementary diagnostic tests (CDT) are essential procedures in the diagnostic approach and monitoring of animals. During the internship, it was possible to carry out blood tests, especially blood counts and biochemical tests, as well electrocardiograms, radiological tests, and POCUS (Point of Care Ultrasound), among others (Table 3).

Table 3 – Distribution of Complementary Diagnostic Exams

Complementary Diagnostic Exams	AF	RF
Hemogram	102	16%
Biochemical	91	15%
Ionogram	45	7%
Endocrine analysis	51	8%
Coagulation profile	38	6%
Ultrasound	74	12%
Echocardiography	13	2%
Radiography	55	9%
Eletrocardiography	123	20%
Urinalysis	22	4%
FNA	13	2%
Total	627	100%

2. PontoVet Clinic

2.1 Distribution of cases by types of animals

In the second internship period, only two types of animals were observed, the dog and the cat, which accounted for 51% (592/1168) and 49% (576/1168) of the sample, respectively.

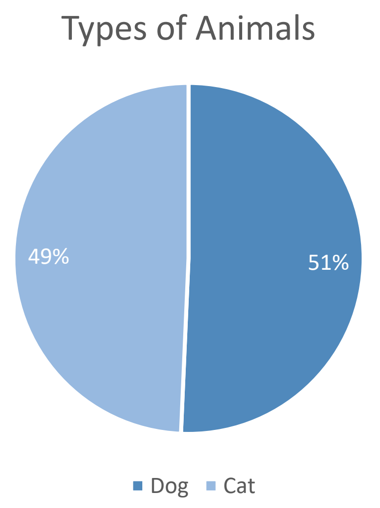

Figure 4 - Distribution of cases by types of animals

2.2 Distribution of cases by clinical area

During my internship at the Pontovet clinic, the author had contact with various clinical areas, such as preventive medicine, surgery, internal medicine, and emergency medicine. The areas with the largest caseloads were internal medicine, with 36.39% of cases, and surgery, with 35.27%.

Table 4 – Distribution of cases by clinical area

Clinical Area	Dog	Cat	AF	RF
Preventive Medicine	166	157	323	27.65%
Internal Medicine	208	217	425	36.39%
Surgical Medicine	213	199	412	35.27%
Emergency Medicine	5	3	8	0.68%
Total	592	576	1168	100%

2.2.1 Internal Medicine

The areas of internal medicine with the largest caseloads observed during the internship are shown in Table 5, namely gastroenterology, oncology, and endocrinology.

Table 5 – Distribution of Internal Medicine areas

Internal Medicine	AF	RF
Dermatology	38	9%
Orthopedics and Musculoskeletal	30	7%
Neurology	18	4%
Ophtalmology	23	5%
Otorhinolaryngology	25	6%
Endocrinology	42	10%
Respiratory	35	8%
Cardiology	32	8%
Gastroenterology	68	16%
Uronephrology	15	4%
Gynecology, obstetrics and andrology	4	1%
Oncology	57	13%
Infection and Parasitology	38	9%
Total	425	100%

2.2.2 Surgical Medicine

At Clínica PontoVet, surgery is divided into soft tissue surgery (51%;210/412), orthopedic surgery (35%;144/412), and dental scaling/extraction (14%; 58/412). Soft tissue surgery includes orchiectomies, ovariohysterectomies, enterotomies, mastectomies, and nodulectomies. In this area, they were allowed to prepare the operating room, classify the animal's anesthetic risk, carry out the anesthetic protocol, and anesthetic monitoring, as well as participate in the surgery as a surgeon's assistant or circulator.

2.2.3 Preventive Medicine

Preventive medicine is one of the most prevalent areas at the PontoVet clinic. The main procedures carried out were vaccinations (62%; 201/323), deworming (28%; 90/323), electronic identification (10%; 32/323), and general clinical examinations. In this area, general medical examinations and the preparation and administration of vaccines were allowed.

2.2.4 Emergency Medicine

Emergency medicine is not very prevalent at the clinic, as it doesn't have the same capacity as a veterinary hospital, and all the more complex cases were referred to this type of service. The clinical cases assisted were respiratory (62.5%; 5/8), neurological (25%; 2/8) and polytraumatic (12.5%; 1/8) emergencies.

2.2.5 Complementary Diagnostic Exams

At the Pontovet clinic, during the internship period, it was possible to carry out blood tests, especially blood count and biochemistry, radiography, and ultrasound (Table 6).

Table 6 – Distribution of Complementary Diagnostic Exams

Complementary Exams	AF	RF
Hemogram	106	25%
Biochemicals	98	23%
Ultrassound	102	24%
Radiography	89	21%
Urianalysis	15	4%
FNA	18	4%
Total	428	100%

II – Introduction

Surgical site infections (SSI) are a natural complication of surgery, directly associated with extended hospitalization, increased costs for the owner, and suffering for the patient (Belos *et* al., 2018; Paeckel *et al.*, 2024). SSI is a potentially serious problem in orthopedic surgery, with this kind of surgery tending to have a higher incidence, as it increases postoperative morbidity, cost of treatment, and duration of hospitalization, being one of the most common complications of extremity surgery, especially when implants are involved and when SSI occurs, surgery for implant removal is often required (Aiken, *et al.*, 2015; Coskun *et al.*, 2022; Sanders, *et al.*, 2019). Infection is a serious and dangerous complication of canine total hip replacement (THR) that can result in septic loosening, decreased hip function, and eventual removal of the hip prosthesis for resolution (Ireifej *et al.*, 2011). The single most common major complication following tibial tuberosity advancement (TTA) and tibial plateau leveling osteotomy (TPLO) surgery was SSI, with TPLO-associated infections reportedly ranging from 2,5% to 15,8% and the incidence of implant removal because of such infections is 3,5% to 7,4% (Hans *et al.*, 2017; Lopez *et al.*, 2018).

SSI can result from surgical bacterial contamination, with the animal's immune system and the bacteria virulence potential playing important roles in SSI development and severity. In a surgical context, skin antisepsis using appropriate products represents one of the most important preventive measures of SSI, targeting the elimination or a major reduction of the skin commensal microbiota, preventing its multiplication in the short term (Belos *et* al,2018; Marchionatti *et al.*, 2021). In recent systematic reviews in human medicine, chlorhexidine appears to be the superior antiseptic in preventing SSI, with up to 30% reduction in incidence (Marchionatti *et al.*, 2021).

In veterinary and human medicine, one of the main goals is to minimize the rate of SSI and the prophylactic administration of antimicrobials is one way of reducing this (Paeckel *et al.*, 2024; Välkki *et al.*, 2020; Whittem *et al.*, 1999). The use of antimicrobial prophylaxis (AMP) is routine in the treatment of contaminated and infected surgical wounds, however, AMP in clean and clean-contaminated surgeries is a decision of the surgeon (Nelson, 2011; Paeckel *et al.*, 2024). This decision is made based on a variety of risk factors, such as the expected duration of the procedure and anesthesia or the use of implants (Paeckel *et al.*, 2024; Whittem *et al.*, 1999). The use of postoperative antimicrobials is a common practice in veterinary medicine (Nelson, 2011; Paeckel *et* al., 2024; Välkii *et al.*, 2020).

III - Bibliographic Review

1. Surgical Site Infection

1.1 Definition

SSI are infections of the incision, organ, or space that emerge after surgery (Berríos-Torres *et al.,* 2017) and are an inherent risk of any surgical procedure since it is inevitable not to compromise the animal's protective barrier. This complication can lead to increased patient morbidity, mortality, prolonged hospitalization, increased treatment cost, and emotional strain on the pet owner and medical caregiver alike (Turk *et al*., 2015; Välkki *et al*., 2020; Weese, 2008). Besides the fact that SSIs have been present since the beginning of veterinary and human surgery, they are receiving more attention nowadays because of increases in antimicrobial resistance (AMR). The threat of SSI will always be present in veterinary medicine, so measures to control them and reduce their incidence and impact are extremely critical (Turk *et al*., 2015). Key strategies include maintaining aseptic conditions during surgery, using AMP wisely, identifying at-risk patients, and protecting the surgical wound postoperatively. If an SSI occurs, it is crucial to promptly identify the infection, assess its extent, administer culture-based antibiotics, manage the wound properly, and follow infection control protocols (Nelson, 2011).

Although the comprehensive health and economic burden of SSI in dogs and cats has not been quantified, considering the large volume of surgeries performed, the associated health and economic costs are likely to be substantial (Turk, *et al*., 2015). SSI has been described as a complication of 0,8% to 18,1% of small animal surgical procedures, with significant variation associated with surgery type (Nelson, 2011). Risk factors for developing SSI and their rates have been studied, however, these studies are often limited by sample size, reliance on retrospective design, the use of passive surveillance, failure to differentiate infection from inflammation, and failure to use standard definitions for SSI (Turk, *et al*., 2015). To properly discuss or evaluate a SSI, it is essential to understand what defines an SSI. In this case, standard definitions must be established to enable a reasonable comparison of data from different locations and to facilitate the accurate identification of SSIs (Weese, 2008).

The United States Centers for Disease Control and Prevention (CDC) has established standard guidelines for defining SSIs in human medicine, including both clinical and timeframe criteria. SSIs are categorized as superficial incisional (skin and subcutaneous tissues), deep incisional (fascial and muscle layers), and organ/space (areas beyond the skin affected during surgery) (Weese, 2008). Additionally, SSIs can be categorized as clean (non-traumatic, uninfected), clean-contaminated (controlled entrance of hollow viscus), contaminated (open traumatic wound, inflammation), or dirty (perforated viscus, pus) (Burgess, 2018). These criteria can also be applied to veterinary medicine, as it is critical to use an objective definition when evaluating SSIs (Burgess, 2018). However, these criteria exclude important presentations of veterinary orthopedic SSI, such as non-discharging joint infections (Aiken, *et al.*, 2015). SSI is an infection associated with a particular surgery procedure and the facility in which that procedure was performed (Verwilghen, 2015).

Surgery involves making at least one incision through the skin or mucous membrane or reoperating via a previous incision. Primary incisional closure is excluded from this definition, so infections in wounds not closed by primary intention should be included in SSI surveillance (Verwilghen, 2015). The duration of surgery is the time from the initial incision to when the surgeon completes all surgery-related procedures on the animal (Verwilghen, 2015).

1.2 Epidemiology of Surgical Site Infections

When analyzing the presence of a disease in a population it is important to consider the central point of epidemiology: disease does not occur randomly in populations (Burgess, 2018). Then, when managing risks associated with SSI, 3 key factors need to be considered in the disease development: the agent, the host, and the environment (Burgess, 2018). In human and veterinary medicine, SSIs and other hospital-acquired infections are increasingly complicated by multidrug-resistant pathogens. Without proper isolation and barrier protocols, these can spread within the hospital to other patients (Nelson, 2011). The risk of SSI for a given surgery involves the interaction between the size of the bacterial inoculum, the virulence of bacteria, and the host's resistance and each of these factors should be considered when assessing risk and developing prevention protocols for SSI (Nelson, 2011).

In SSIs, agents can be physical, chemical, or biological (Burgess, 2018). Key concerns include the types of surgical procedures and the presence of potential pathogens, whether endogenous (from the host) or exogenous (from the environment). Endogenous microorganisms may come from the host's gastrointestinal tract or infection sites. Exogenous microorganisms originate outside the patient. SSI events connected to specific agents have an etiology associated with those agents (Burgess, 2018). Despite this, 82% of American Veterinary Medical Association-accredited veterinary teaching hospitals reported infectious disease outbreaks in their facilities within a 5-year period, with *Salmonella* and methicillin-resistant *Staphylococcus* (MRS) the most identified bacteria (Burgess, 2018).

SSI can be originated in a variety of pathogens. However, normal skin bacteria are the main source, and due to their commensal and opportunistic nature, *Staphylococci* are the most cultured from canine SSI (Belos *et* al., 2018; Coskun *et al*., 2022; Verwilghen, 2015). *Staphylococcus pseudintermedius* is commensal in dogs, whereas *Staphylococcus aureus* is commensal in humans, which means the presence of this bacteria in dogs has a human origin (Belos *et al*, 2018). Over the past decade, *S. pseudintermedius* infections have swiftly become the most prevalent cause of SSI globally. A particularly concerning development is the notable increase in methicillin and multidrug resistance observed in these infections, affecting both dogs and cats (Belos *et al.*, 2018; Singh *et al.*, 2013; Verwilghen, 2015).

S. pseudintermedius is a coagulase-positive staphylococcus commonly found on the skin and mucous membranes of pets, often without causing symptoms. It is frequently isolated from veterinary patients with infections in wounds, surgical sites, external ear canals, and respiratory or urogenital tracts. In cats, it can be found in cases of respiratory tract infections, conjunctivitis, urogenital infections, dermatitis, otitis, and wounds (Bierowiec *et al.*, 2021). Factors that can increase the risk of *S. pseudintermedius* infection have been described for dogs and cats and infection by pathogens has been related to animals with previous hospitalization or frequent visits to veterinary settings, along with the administration of glucocorticosteroids and antibacterial chemotherapeutics (Bierowiec *et al.*, 2021). *S. aureus* is a leading cause of human SSIs and can also infect dogs, though less commonly than *S. pseudintermedius* (Verwilghen, 2015). The incidence of methicillin-resistant *S. pseudintermedius* (MRSP) SSI is increasing, presenting a major concern due to its multidrug resistance. Additionally, *S. pseudintermedius* has strong biofilm-forming abilities, complicating treatments, particularly in implant-associated SSIs (Verwilghen, 2015).

Host factors include intrinsic elements like genetics and immune status, as well as extrinsic elements such as population structure and exposure likelihood (Burgess, 2018). Despite the big association of wound contamination with SSI incidence, host factors are also critically important in SSI development (Nelson, 2011). The Centers for Disease Control and Prevention National Nosocomial Infections Surveillance identifies three categories of variables that predict the risk of SSI: 1) the intrinsic degree of microbial contamination at the surgical site, 2) the duration of the surgery, and 3) indicators of the patient's susceptibility. The American Society of Anesthesiology (ASA) classifies patients according to physical status score, which has served as a useful indicator of host susceptibility (Nelson, 2011). The ASA status of a patient at the time of surgery is a significant predictor of SSI risk in humans. In veterinary medicine, the classification of SSI risk based on patient status or comorbidity has not been thoroughly evaluated (Nelson, 2011).

Environmental factors, classified as extrinsic, include both animate and inanimate surroundings as well as therapeutic interventions. Studies on SSI indicate an elevated risk associated with the length of surgery, timing of perioperative clipping, and the number of individuals present in the operating room (Burgess, 2018). In veterinary medicine, factors associated with SSI include prolonged anesthesia and surgery time, the involvement of student surgeons, lack of appropriate AMP, hair clipping before induction, the number of people present in the operating room, the duration of hospitalization, drain placement, and early suture removal (Nelson, 2011).

1.3 Risk Factors of SSI

There are several procedures- and patient-related factors that can contribute to the incidence of SSI in dogs, making the risk of developing an SSI likely to be influenced by the characteristics of patients, such as concurrent diseases, surgery itself, like prolonged duration of anesthesia and surgery, and postoperative care (Cheng *et* al., 2017; Gibbons, 2012; Lopez *et al*., 2018). Studies focused specifically on the TPLO surgery and SSIs in dogs have revealed additional risk factors for SSI, including the type of surgical implant used, high *versus* low body weight, preoperative colonization with MRSP in the nares or rectum (Lopez *et al*., 2018).

Human medicine identifies risk factors for surgical procedures, such as patient health and bacterial carriage potential. Factors like the type of surgery, environment, expertise, and postoperative care also affect SSI risk (Verwilghen, 2015). These factors are also suitable for Veterinary Medicine and like in human medicine, the duration of the surgery and anesthesia time is an expected finding in risk analysis, though many risk factors can be attributed to our behavior, and the prime and the most threatening factor for development of SSI is probably ourselves (Verwilghen, 2015). Bacterial contamination in a surgical wound can be endogenous, from the animal's own microbiota, or exogenous, from the surgical team, environment, and materials used (Verwilghen, 2015). According to data collected in human medicine, approximately half of the risk factors for SSI are endogenous which means that they are difficult or impossible to change during and after the surgical period (Weese, 2008). In cases where a dog requiring TPLO exhibits active dermatitis at or near the surgical site, it is generally advised by surgeons that the dermatitis be treated and the surgery postponed until the dermatitis is resolved (Lopez *et al*, 2018).

When addressing the risks of SSI, it is important to consider four main categories: device-related events, procedures, compliance, and etiologic agents. Each category may play a role in the occurrence of SSIs within a facility (Burgess, 2018).

Procedure-associated events, frequently reported for SSI, may stem from factors related to surgical preparation or specific aspects of the surgical procedure, including the duration of the surgery, tissue handling techniques, or the experience level of the surgeon (Verwilghen, 2015). Surgery duration is recognized as an independent, modifiable risk factor for SSI. Studies indicate that longer surgeries, whether clean or clean-contaminated, elevate infection risk in dogs undergoing orthopedic, minimally invasive, or open procedures (Burgess, 2018; Cheng *et al.*, 2017). A study by Cheng *et al.,* 2017 demonstrated that most studies (87%) reported a statistically significant association between longer surgery time and SSI and a study by Ireifej *et al.*, 2011 showed that surgical and anesthetic duration were significantly associated with positive coxofemoral closing cultures results, in THR surgeries in dogs. The same study showed that, across a wide range of procedures, patients who developed an SSI had an average operative time that was 30 minutes longer than those without an SSI. The likelihood of SSI increased by 5% every 10 minutes, 13% every 15 minutes, 17% every 30 minutes, and 37% every 60 minutes, with infection rates doubling at 90 minutes (Cheng *et al.*, 2017; Ireifej, *et al.*, 2011). The mechanisms by which prolonged surgery time increases SSI incidence are not fully understood. However, several studies provide logical explanations: extended wound exposure times and longer tissue manipulation offer more opportunities for pathogens to enter the wound, resulting in a higher likelihood of SSI. These factors also increase the chance of tissue desiccation or other forms of damage, which further elevates the probability of contamination (Cheng *et al.*, 2017; Verwilghen, 2015).

Surgery time can be a compliance-associated risk factor, because a longer surgery time may also mean increased surgical team fatigue and an increased room for technical errors. Factors such as animals' characteristics, surgeons' skills, and the efficiency of support staff during procedures may influence surgery time (Cheng *et* al., 2017; Li *et al.*, 2013). Another frequently observed compliance-related issue involves hand hygiene, cleaning and disinfection protocols, and improper use of barrier precautions, potentially leading to significant animal morbidity and mortality (Burgess, 2018). An observational study in companion animal clinics reported variations and often low adherence to established surgical preparation practices. For example, the recommended duration for surgeon hand preparation and antiseptic cleansing of the animal surgical site is at least 2 minutes. However, this study observed that these times can be as short as seven to ten seconds, respectively. Additionally, the study noted that nonsterile contact with aseptically prepared surgical sites occurred in at least 36% of cases (Verwilghen, 2015). In addition, transmission of microbial pathogens by the hands of healthcare workers, outside the closed environment of the operating room, during patient care, plays a crucial role in the occurrence of SSIs. Although simple and cheap, the practice of washing hands is an efficient method of SSI prevention however overall compliance can be quite low among small-animal practitioners which promotes the transmission of infectious agents to animals, personnel, and the environment (Burgess, 2018; Verwilghen, 2015). Animal health care workers face zoonotic disease risks, making low hand hygiene compliance rates puzzling.

A human study found that more extensive hygienic measures did not significantly impact SSI, but failing to follow basic asepsis increased SSI risk by 3.5 times (Verwilghen, 2015). Several obstacles have been identified concerning hand hygiene compliance. These include forgetfulness or being "too busy," skin damage from frequent hand washing, and lack of available hand hygiene supplies. It is crucial to acknowledge that while personnel generally adhere to protocols during periods of increased observation, they tend to deviate from these protocols under normal circumstances (Burgess, 2018).

1.4 Prevention of SSI

SSIs are highly preventable healthcare-associated infections (Leaper & Ousey, 2015). Their prevention is crucial due to the related morbidity and expense (Nelson, 2011). Over a third of postoperative deaths can be associated with SSIs, ranging from a relatively trivial, short-lived, wound discharge to being life-threatening (Leaper & Ousey, 2015). A study developed by Gawande suggested that 50% of surgical complications could be avoided by improving perioperative routine. A study conducted by the World Health Organization (WHO) showed that the use of a surgical checklist reduced postoperative morbidity and mortality in people by 36% and 48%, respectively (Bergström, *et al.,* 2016).

In human medicine, three preventive measures have strong evidence: surgical hand preparation, appropriate AMP, and postponing elective surgeries during active infections. The veterinary field lacks such studies, so we rely on human literature (Verwilghen, 2015). About half of the identified SSI risk factors are endogenous and hard to change preoperatively and perioperatively. The other half are exogenous and easily modifiable. Studies suggest that simple, low-cost interventions are most effective at reducing healthcare-associated infections (Verwilghen, 2015). Burgess, 2019 identifies four key areas for managing SSI risks: identifying high-risk patients, adhering to aseptic principles, using antimicrobial drugs judiciously, and conducting SSI surveillance. Hospitals, where comprehensive integrated infection control and surveillance programs were used had significantly lower infection rates, especially those with four key components: intensive surveillance activities; intensive infection control activities; program for providing routine feedback to hospital personnel on infection rates, and use of trained personnel to implement and supervise infection control programs (Morley, 2004). A study by Bergström, *et al*., 2016 found that the rate of postoperative complications was reduced by 10% with the use of a surgical checklist in a small animal hospital, results that are comparable to several human studies, which shows that.the introduction of a checklist could improve the safety of veterinary surgery.

Surgical asepsis prevents wound contamination from microorganisms from the patient, surgeon, team, and environment (Verwilghen, 2015). Prevention requires multiple actions, including preparing the surgical facility, site, team, and equipment adequately. Everyone in healthcare is responsible for achieving aseptic procedures and ensuring successful surgeries (Verwilghen, 2015). Antisepsis is a relatively recent concept introduced into surgical practice by Joseph Lister in 1867 and includes all practices employed to prevent contamination with microorganisms, including the surgical facilities and environment, surgical site, surgical team, and surgical equipment and even though antisepsis will not eliminate all microorganisms from the surgical site, when properly done, it may reduce the number of microorganisms to a level that is of limited consequence, making the preoperative skin preparation with a topical antiseptic applied to the surgical site one of the most important measures in minimizing potential infections (Burgess, 2018; Coskun *et al*., 2022).

Patients should be evaluated for factors contributing to SSI development, including overall health, comorbidities, wound classification, and implanted medical devices. The presence of *Staphylococcus* spp. is a significant concern, as it is linked to SSIs in both humans and dogs carrying MRSP (Burgess, 2018). According to CDC guidelines, this matter is considered to have high evidential support and is corroborated by numerous retrospective studies. These studies found that remote infections are significant risk factors in the development of SSI, with the most common origins of remote site infections being the lungs, gastrointestinal tract, and urinary tract (Verwilghen, 2015). In human surgery, the patient's preoperative systemic inflammatory status has been associated with an increased risk, alongside various factors such as obesity, smoking, diabetes, and nutritional status. In veterinary surgery, wound and skin infections are both highly prevalent and pose significant risks. It is advisable to postpone elective surgeries in patients presenting with these conditions (Verwilghen, 2015).

Another important factor, according to surgeons, is the judgment and technique of the surgeon and surgical team. However, randomized trials are not feasible in this area. It is believed that a good surgical technique with adherence to Halsted principles reduces SSI. Learning to achieve hemostasis, maintain blood supply, handle tissues gently, remove devitalized tissue, eliminate dead space, and manage postoperative incisions can improve performance and reduce complications (Verwilghen, 2015). This affirmation is supported by several human and veterinary reports, in which the experience of the surgeon was associated with lower SSI, wound complications, or even patient survival.

Surgical and anesthesia time is often reported as a significant risk factor in the development of complications, which can be associated with longer surgery durations due to inexperience (Verwilghen, 2015). Surgery time is an independent SSI risk factor that may be partially modifiable, unlike certain patient intrinsic risk factors. Surgery time can be influenced by many factors, such as pre-operative planning, surgeon experience, and access to equipment (Cheng *et al*., 2017). While surgeon experience can extend operation time, the entire surgical team influences the duration. Effective pre-surgical planning can reduce decision-making time, ensure availability of instruments, efficiently deliver disposables, design implants or instruments, and coordinate with diagnostic imaging, all contributing to reduced surgical time and lower SSI incidence (Cheng *et* al., 2017; Verwilghen, 2015). Surgical team familiarity with the procedure and equipment should be addressed before the surgery, as it was shown that hospitals where the surgical anesthesia and nursing teams are unfamiliar with their role and communications function have been shown to prolong teamwork intervals, resulting in higher than usual SSI rates (Cheng *et* al., 2017; Verwilghen, 2015).

Investigating surgical techniques can reveal both detrimental and protective surgical-related techniques that affect SSI development in dogs after TPLO. Meniscal tears, often occurring with cranial cruciate ligament tears, typically require meniscectomy. Meniscectomy may increase the risk of SSI since the surgery involves more instruments and more manipulation of the joint than TPLO alone, which can result in more opportunities for breaks in asepsis (Lopez, 2018).

Verwilghen, 2015 notes that the importance of proper OR etiquette is often overlooked in the veterinary community. Hand hygiene is one of the most important infection control measures for the prevention of infectious disease transmission (Burgess, 2018). Modern hospitals require clean surgical clothing, including face masks, scrubs, and caps, to reduce infection rates in surgery rooms (Verwilghen, 2015). The surgery team must be alert to situations in the surgery room that may predispose to infections, such factors being the volume of traffic in the room, the behavior of the surgical team, the adequacy of environmental cleaning, and if there are no breaches in hand hygiene and aseptic technique (Gifford, *et al*., 2011). Noise levels in the surgical room are significantly correlated with higher SSI rates, suggesting that noise reduces concentration and increases surgical errors. Noise may justify more or louder talking, increasing aerosolization of pathogens in the surgery theater

(Verwilghen, 2015). A study on intraoperative behavior found that lapses in discipline by the surgical team, such as increased movement, team member changes, noise, and visitors in the OR, are independent risk factors for SSIs. Seeing that the implementation of checklists into surgical routine and improving OR discipline was shown to decrease surgical complications (Verwilghen, 2015).

Proposed mechanisms of reduction in SSI are via modulation of the inflammatory response, vasodilation leading to improved tissue oxygenation, and/or improved postoperative analgesia, particularly with epidural techniques, however, prospective evidence has yet to be collected in this area (Gifford, *et al*., 2011). Another anesthetic factor to take into consideration is the inspired gas composition, as increasing the partial pressure of oxygen in the blood and tissues, beyond what is required to fully saturate hemoglobin, has been postulated to improve the oxidative bactericidal activity of neutrophils (Gifford, *et al*., 2011). An analysis of neutrophil function revealed that elevated oxygen tension induces the production of reactive oxygen intermediates essential for intracellular cell death. This leads to an earlier peak and a more rapid decrease in tumor necrosis factor levels. Consequently, current guidelines for preventing SSI recommend administering only the necessary inspired oxygen concentration to maintain oxygen saturation above 95% (Gifford, *et al*., 2011). Additionally, another factor that must be considered is opioid-induced immunosuppression, as most opioids in current surgery practice have the potential to suppress the immune system. Drugs such as fentanyl, remifentanil, and to a lesser extent methadone have been shown to possess significant immunosuppressive properties. Buprenorphine does not significantly affect the immune system, while tramadol has immuno-enhancing properties (Gifford et al., 2011).

2. <u>Antisepsis in Surgery</u>

Antisepsis is a modern concept that has been incorporated into surgical practice. It encompasses all measures taken to prevent contamination by microorganisms, including those related to the surgical facilities and environment, the surgical site, the surgical team, and the surgical equipment (Burgess, 2018). Preoperative skin antisepsis aims to reduce the risk of SSI, as the skin isn't a sterile surface and is colonized by various bacterium and even though antisepsis will not eliminate all microorganisms from the surgical site when properly done, it may reduce the number of microorganisms to a level that is of limited consequence (Burgess, 2018; Dumville *et al*., 2015). Joseph Lister introduced antisepsis to clinical surgery in 1867. However, antiseptics have been empirically used for centuries. Hippocratic writings from the 4th and 5th centuries B.C. show that wine was used on wounds, as did the Good Samaritan in biblical times. Before Lister's work, at least 20 British publications discussed wound antiseptics

(Alexander, 1985). Louis Pasteur is a key figure in surgical infection control. His 1857 studies on fermentation revealed "organic corpuscles" in the air, leading to his 1863 putrefaction studies and supporting germ theory. Although his major contributions were yet to come, these early investigations caught Lister's attention, who observed higher post-amputation death rates in hospital-treated patients compared to those treated at home (Alexander, 1985). Lister assessed that septicemia was associated with venous invasion of the putrefaction process. Once convinced that invisible living particles were the cause of the suppuration, Lister experimented with carbolic acid as an antiseptic, since it was known to prevent putrefaction. Antiseptic principles were quickly developed and soon applied to operations, with the mortality from amputation falling from 45% to 15% (Alexander, 1985).

After anesthesia was introduced, surgeries became more frequent, though not necessarily more successful. By 1880, germ theory was accepted, and antisepsis was commonly used. Antisepsis proved crucial by preventing microorganisms from infecting wounds, highlighting the significance of contributions by Pasteur, Koch, and others. Surgical cleanliness evolved into asepsis, which ultimately surpassed antisepsis in importance (Alexander, 1985).

2.1 Antiseptics

Most SSI are caused by commensal and pathogenic agents from animal's microbiota, including antimicrobial resistant strains. Pre-surgical antisepsis of the skin using antiseptics is performed as a preventive measure that can be used to reduce the risk of SSI incidence and antibiotic resistance dissemination, by removing soil and transient organisms from the skin where a surgical incision will be made, however, in veterinary medicine there is no agreement on which biocide is most effective (Belo *et* al., 2018; Dumville *et al.*, 2015). The application of antiseptic products to the skin is crucial in preventing various healthcare-associated infections. Antiseptics work by both mechanically removing and chemically killing or inhibiting contaminating and colonizing flora. It is standard practice to use these products on the skin for multiple purposes in healthcare, including preoperative bathing, surgical site preparation, and surgical hand hygiene (Hadiati *et* al., 2020; Leaper & Ousey, 2015; Boyce, 2019). The ideal antiseptic agent would rapidly kill all bacteria, fungi, viruses, protozoa, tubercle bacilli and spores, be nontoxic, hypoallergenic and safe to use in all body regions, have residual activity, even in the presence of organic material and be safe for more than one use (Boucher *et* al., 2018; Dumville *et* al., 2015; Hadiati *et* al., 2020). Antiseptics are selected based on their effectiveness in killing bacteria and other microorganisms, speed of action, safety, ease of use, and duration of effect, among other criteria (Hadiati, *et* al.2020; Nye, *et al.*, 2024). With the objective of standardizing pre-, intra- and post-surgical procedures, some guidelines were recently developed (Belo *et al*., 2018; Berrios-Torres *et al*., 2017). WHO and the CDC recommend the use of alcohol-based agents for skin antisepsis, making the most used antiseptics for surgical preparation of patient's chlorhexidine, iodine compounds, and alcohols all of which have been shown to have an equivalent impact on bacterial growth on the skin (Belo *et* al., 2018; Berrios-Torres *et al*., 2017; Coskun *et al*., 2022; Nye, *et al*., 2024). In addition, povidone-iodine and chlorhexidine solutions reportedly decrease the occurrence of SSI among dogs undergoing orthopedic or soft tissue surgery, with chlorhexidine gluconate, when compared with povidone-iodine, showing, with moderate-certainty evidence, a slightly better reduction in the incidence of SSI (Burgess, 2018; Hadiati *et al*., 2020). Available antiseptic products are unable to eliminate all microorganisms present in the skin. Approximately 20% of the skin microbiota of small animals remains protected in deeper layers and follicles, regardless of the antisepsis protocol used (Belo *et al*., 2018).

Chlorhexidine (CHX) is a disinfectant and antiseptic that binds to and disrupts bacterial cell membranes. CHX has a broad spectrum of antimicrobial activity, being most effective against Gram-positive bacteria. It also shows activity against Gram-negative bacteria, anaerobes, fungi, and some enveloped viruses. However, it is ineffective against bacterial spores and has a mycobacteristatic effect (Nye *et al*., 2024). CHX's ability to bind to protein in the stratum corneum of epidermis allows for persistence of action which has been shown to have a cumulative effect with repeated use (Nye *et al.,* 2024). CHX requires a three-minute contact time to maximize the benefits of the product (Holzman & Kleist, 2015). The activity of CHX varies with concentration: at lower concentrations it has a bacteriostatic effect and at higher concentrations has a bactericidal (Tobias & Johnston, 2012). The residual activity of CHX has been well described and shown to last at least 24 h on skin and this is extended to 48h when combined with alcohol in a tincture, however bacterial resistance to CHX has also been described for several bacteria and bacterial strains (Boucher *et al*., 2018; Nye *et al.,* 2024). CHX is available as gluconate or diacetate salts, with CHX gluconate scrub being the preferred antiseptic for patient preparation in veterinary clinics. Studies have shown no significant difference between mechanical and non-mechanical aseptic preparations, or between different mechanical application techniques (linear scrubbing vs concentric circles) (Boucher *et* al., 2018; Nye, *et al*., 2024).

Iodine compounds function as disinfectants and antiseptics, capable of penetrating the cell wall of microorganisms and oxidizing proteins, nucleotides, and fatty acids in the cytoplasm and cell membrane, leading to cell death. They are effective against numerous Gram-positive and Gram-negative bacteria, tubercle bacillus, fungi, spores, and viruses. The effectiveness against spores depends on contact time (Dumville *et* al., 2015; Nye *et* al., 2024; Tobias & Johnson, 2012). Organic materials have been found to neutralize the effects of iodophors, such as whole blood because hemoglobin can bind a large amount of iodine and this also occurs in the face of purulent material and fat homogenate (Nye *et al*., 2024; Tobias & Johnson, 2012). Povidone-iodine (PI) is widely used in veterinary medicine for preparing patients, but it should only be applied to mucous membranes and external areas, avoiding open wounds (Nye *et al*., 2024; Tobias & Johnson, 2012). Human guidelines, however, suggest using diluted iodophor solutions to irrigate deeper layers of incisional wounds during surgery to prevent infections. This recommendation was recently evaluated in total hip arthroplasty in veterinary patients and was shown that postoperative SSI rates were lower when it was performed a pre-closure lavage of 0.035% PI solution (Nye, *et al*., 2024).

Alcohols, such as ethyl and isopropyl alcohol, act as disinfectants and antiseptics by likely denaturing cell wall proteins, which disrupts metabolism and causes cell lysis (Dumville *et al*., 2015; Nye *et al*., 2024; Tobias & Johnson, 2012). They are effective against Gram-positive and Gram-negative bacteria, viruses, and fungi, but not spores. The concentration, rather than the type of alcohol, determines their effectiveness (Dumville *et al*., 2015; Nye *et al*., 2024). Water is essential to increase microbicidal efficiency, with formulations of 60-90% alcohol being the most effective, and isopropyl alcohol has greater bactericidal action than ethanol, but less virucidal activity (Nye *et al*., 2024; Tobias & Johnson, 2012). Alcohol quickly and effectively reduces bacteria on the skin, but its effect ends once it dries. Adding emollients slows evaporation, enhancing efficiency, as seen in waterless hand sanitizers. Including other antiseptics like CHX or iodophors boosts alcohol's microbicidal activity and residual action (Nye, *et al*., 2024). Various studies indicate that alcohol and CHX preparations have improved efficacy and thereby, alcohol may be used as part of a combination product with CHX or iodophors or used as part of an initial preparation to remove excess antiseptic (Nye, *et al*., 2024; Tobias & Johnson, 2012).

The Society for Healthcare Epidemiology of America (SHEA) 2014 practice recommendation and the CDC guideline for preventing SSIs both suggest using an alcohol-containing preparation if there are no contraindications. The 2016 World Health Organization (WHO) guideline on the prevention of SSI advises preoperative surgical site preparation with an alcohol-based antiseptic solution containing CHX (Boyce, 2019). A study by Darouiche *et al*., 2010, demonstrated that preoperative cleansing of the patient's skin with chlorhexidine-alcohol is superior to cleansing with povidone-iodine for preventing SSI after clean-contaminated surgery. However, despite CHX having a wider range of activity in comparison to PI, a certain caution is being required as methicillin-resistant *Staphylococcus aureus*

(MRSA) and enterococci may be developing acquired resistance to it (Marchionatti *et al.*, 2021).

2.2 Preoperative Antisepsis Measures

2.2.1 Patient Preparation

Patient preparation before surgery includes examining for bacterial infections, such as evaluating the skin and urinary tract and if an infection is found, surgery should be postponed until it is resolved (Nelson, 2011). Issues like uncontrolled diseases, including diabetes mellitus, hyperadrenocorticism, obesity, malnutrition, or anemia, should be addressed to the extent feasible before surgery (Nelson, 2011). Dog skin can be a greater challenge when compared to humans since dogs have a thicker coating, low hygiene frequency, and contact with a more contaminated environment, which makes it understandable that the techniques that are effective in humans may be less efficient in animals (Belo *et* al., 2018). Presurgical bathing of surgical patients with an antiseptic agent 24 hours before surgery is suggested, though its effects in veterinary medicine are uncertain. If the coat has significant dirt or ectoparasites, a preoperative bath or insecticide treatment is advisable (Nelson, 2011). Most animals have fur and hair that needs to be removed from the surgical site (Coskun *et* al., 2022). Hair removal should be done with clean, well-maintained clippers after anesthetic induction and just before surgery to lower the risk of SSI in dogs (Coskun *et al.*, 2022; Nelson, 2011). After removing the hair, wash the incision site to eliminate contamination. Then apply an appropriate antiseptic in concentric circles moving outward from the incision area for final aseptic skin preparation (Nelson, 2011).

Preoperative surgical site antisepsis is based on the knowledge that pathogens leading to SSI are often acquired from the patient´s endogenous flora and involves the use of antiseptics to prevent wound contamination that may lead to the development of infections (Marchionatti *et* al., 2021; Nye *et al.*, 2024). Surgical asepsis refers to the absence of microorganisms in invasive procedures. Up to 20% of skin flora remains unaffected by antiseptics, thus preparation reduces resident flora and eliminates transient and pathogenic organisms (Nye *et al.*, 2024). Preoperative skin preparation is crucial for surgical asepsis (Burgess, 2018). In veterinary medicine, these protocols follow human guidelines and typically use aqueous or alcohol-based CHX or PI preparations (March*ionat*ti et al., 2021).

2.2.2 Surgical Team Preparation

According to WHO, hand hygiene is crucial for preventing infectious diseases. Perioperative hand hygiene significantly affects SSI risk and medical staff safety. However, both human and veterinary surgeons have limited knowledge of proper surgical hand preparation (Tsai *et* al., 2016; Verwilghen, 2015).

The impact of disinfection on hands-on infection rates was first demonstrated by Semmelweis in the 1840s and the requirement for the surgical scrub is a well-established principle for surgeons entering the operating theater (Gifford,*et al*., 2011). For many years, surgeons routinely scrubbed their hands with antimicrobial soap and water for 10 minutes before wearing sterile gloves. However, in 2002, CDC guidelines for hand hygiene in health care settings recommended that surgical hand antisepsis could be performed with either antimicrobial soap and water or an alcohol-based hand rub (ABHR) (Boyce, 2019). According to the WHO guideline on the prevention of SSIs, it is recommended that surgical hand antisepsis be conducted by either scrubbing with an appropriate antimicrobial soap and water or using a suitable ABHR prior to donning sterile gloves (Boyce, 2019).

Alcohol-based waterless formulations are as effective as scrubbing. Hydroalcoholic solutions (HAS) offer several advantages, such as rapid antimicrobial action, a wider spectrum of activity, fewer side effects, lower potential for resistance development, and no risk of recontamination with rinsing water. Therefore, this method is recommended over traditional hand scrubbing in both human and veterinary medicine (Verwilghen, 2015). Regardless of the surgeon, using an alcohol-based hand rub is as effective as a scrub with chlorhexidine gluconate or povidone-iodine in reducing bacterial counts on hands before surgery. However, it is important to note that veterinarians in training may not be as proficient in performing pre-surgical hand antisepsis, potentially making alcohol rubs less effective for this group of veterinary personnel (Burgess, 2018).

A survey conducted by the American and European College of Veterinary Surgeons indicates that specialists do not adhere to hand hygiene recommendations (Verwilghen, 2015). This finding supports the notion that surgeons' behavior in the operating room does not align with their scientific knowledge, and that compliance with established protocols is low and consequently, this highlights the role of healthcare professionals as a primary risk factor in the occurrence of SSI (Verwilghen, 2015). A lack of compliance can be addressed through hand hygiene education and awareness campaigns. Since frequent washing might harm skin integrity, providing lotions and moisturizers is crucial to maintain healthy skin and ensure compliance. Dedicated personnel should ensure hand hygiene supplies are always available (Burgess, 2018). Introducing HAS and improving material accessibility improve compliance rates (Verwilghen, 2015). Although biocides effectively reduce bacterial contamination, they must be used correctly (Burgess, 2018).

Hand hygiene is considered one of the most effective measures to control SSIs, especially superficial SSIs (Verwilghen, 2015). Numerous reports have demonstrated a temporal relationship between improved hand hygiene interventions, increased compliance rates, and reduced infection rates (Verwilghen, 2015). The objective of surgical hand preparation is to minimize the release of skin bacteria from the hands of the surgical team in the event of glove perforation. Surgical gloves are punctured in up to 35% of cases after 2 hours of surgery, with 80% of these punctures going unnoticed by the surgeon. The incidence of surgical glove perforation varies among surgeons, and such breaches significantly increase the risk of SSI, especially in patients who do not receive AMP (Lopez, 2018; Nelson, 2011). The presence of multidrug-resistant bacteria on the hands of medical professionals, including veterinary surgeons, underscores the importance of preventing the transmission of skin bacteria to surgical wounds. MRSA outbreaks in veterinary patients have been linked to colonized surgeons and staff (Nelson, 2011). Members of the surgical team should keep nails short, and avoid the use of artificial nails, and jewelry should also be removed. Before the aseptic preparation, hands and arms must be washed with soap and water to remove gross debris (Nelson, 2011).

3. Antimicrobial Prophylaxis

Antimicrobial prophylaxis in surgery consists of antimicrobial administration in the immediate proximity of the surgical procedure (Vällki, *et al*., 2020). In every surgical wound bacterial contamination occurs and can progress to an SSI if the host defense mechanisms are overwhelmed (Verwilghen, 2015). While the proper use of aseptic techniques during surgery is crucial for reducing infection, AMP is commonly used in human and veterinary medicine to lower bac*terial* contamination to a level where host defense mechanisms can prevent SSI development (Aiken, et al., 2015; Verwilghen, 2015). It is important to bear in mind that antimicrobials can help reduce the risk of SSI, but not reduce the risk factors (Paeckel *et al*., 2024). Multiple randomized controlled trials and systematic reviews have consistently shown antimicrobial prophylaxis to be of benefit in preventing infections after many types of surgery, however for animals undergoing most clean procedures, prophylactic use of antimicrobials is thought unnecessary and there is contradictory evidence regarding its protective benefit against SSI in clean orthopedic surgery in veterinary patients (Gifford, *et al*., 2011; Aiken, *et al.,* 2015). According to Nordic recommendations, in veterinary medicine, antimicrobial prophylaxis should be reduced, and strict surgical asepsis should be emphasized (Välkki, *et al*., 2020). According to Verwilghen, 2015 AMP serves as a complementary measure to meticulous aseptic surgical techniques and appropriate infection control strategies, and should not be considered a justification for compromising these areas.

Although AMP should be used when required, they should be used judiciously, as their misusage can induce the development of antimicrobial-resistant bacteria, which is a serious and increasing problem (Burgess, 2018; Aiken, *et al.,* 2015). This includes administering fully effective doses at appropriate intervals for relevant clinical indications, utilizing narrow-spectrum antimicrobial peptides specific to the isolated organisms based on susceptibility testing, selecting narrow-spectrum antimicrobial peptides against which the isolated organisms are not likely to develop resistance according to current scientific and clinical guidelines, altering narrow-spectrum antimicrobial peptides only after an adequate treatment period, limiting the indiscriminate use of antimicrobial drugs, and opting for topical or local therapy rather than systemic therapy whenever feasible (Burgess, 2018). The optimal timing, frequency, and dosage of antimicrobial administration necessary to achieve the best clinical outcomes are not well-defined, however, it is recommended that antimicrobials be administered intravenously in such a manner that bactericidal concentrations are present in serum and tissues at the time of incision and maintained at therapeutic levels throughout the surgical procedure, concluding within a few hours after wound closure (Aiken, *et al*., 2015; Nelson, 2011).

The UK National Institute of Clinical Excellence (NICE) issued guidelines in 2008 recommending a single dose of prophylactic antibiotics on starting anesthesia and recommending the use of AMP for contaminated surgery, clean-contaminated surgery, and clean surgery involving the placement of an implant or prosthesis (Gifford, *et al*., 2011). Clean surgery consists of not breaking the aseptic technique and the respiratory, gastrointestinal, or genitourinary tracts not being breached (Gifford, *et al*., 2011). Clean-contaminated surgery involves the oropharynx, sterile genitourinary or biliary tract, the gastrointestinal or respiratory tracts, or where there has been a minor breach in aseptic technique (Gifford, *et al*., 2011). Contaminated surgery is defined as the presence of acute inflammation, infected bilious secretions, urine, or gross contamination from the gastrointestinal tract (Gifford, *et al*., 2011).

AMP is recommended for surgical procedures involving contaminated or clean-contaminated wounds due to the higher risk of SSI. In both veterinary and human medicine, the use of AMP in clean wounds is debated because this type of wound carries a lower risk of SSI (Verwilghen, 2015). In veterinary orthopedic surgery, SSIs can have severe consequences despite being classified as clean wounds. Therefore, AMP is standard practice for most elective orthopedic surgeries, procedures with implants, or situations where SSIs would be disastrous (Verwilghen, 2015). A recent randomized clinical trial demonstrated that administering postoperative oral cephalexin or amoxicillin for one week significantly reduced the infection risk in dogs undergoing clean orthopedic surgery involving plate fixation (Burgess, 2018). Despite ongoing debate regarding prophylactic use, these findings indicate that this practice should be evaluated on a case-by-case basis, particularly if the patient presents additional risk factors for developing a SSI (Burgess, 2018). According to Danish Antibiotic Use Guidelines for Companion Animal Practice, animals with 1-2 ASA classification undergoing clean procedures or even apyretic ASA 3 animals undergoing clean or clean-contaminated procedures, don´t require antimicrobial prophylaxis (Välkki, *et al.*, 2020). Also, according to Swedish guidelines antimicrobial prophylaxis is not recommended in clean orthopedic procedures in low-risk animals, even when a surgical implant other than a joint prosthesis is placed and Finnish guidelines recommendations are similar: antimicrobial prophylaxis is not recommended in clean and clean-contaminated soft tissue surgeries that are under 60 minutes, however, according to these guidelines, in clean orthopedic surgery, the protocol consists of a first-generation cephalosporin given 30-60min before the surgical incision, being this administration continued until the end of surgery (Välkki, *et al.*, 2020).

The CDC and Centers for Medicare and Medicaid Services created the Surgical Care Improvement Project (SCIP) to reduce SSIs in humans. SCIP includes three key measures: selecting antimicrobials based on expected pathogens, timing antimicrobial administration to peak at incision, and discontinuing prophylactic antimicrobials within 24 hours post-op. These guidelines have decreased SSI-related morbidity and mortality in humans. Similar guidelines do not exist in veterinary medicine, though studies show AMP can reduce SSI based on surgical wound classification (Verwilghen, 2015). Veterinary practices can ensure judicious use by developing an antimicrobial stewardship program (ASP) aimed at improving patient outcomes while minimizing unintended consequences. These programs focus on the appropriate selection, dosage, route, and duration of antimicrobials (Burgess, 2018). ASP should be managed by a multidisciplinary team and incorporate a comprehensive approach, including core strategies such as prospective surveillance with feedback and educational initiatives to ensure foundational knowledge and enhance program compliance (Burgess, 2018).

3.1 Selection of Antimicrobials

Anticipating the pathogens to be encountered, depending on the surgical procedure is something the surgery team must do. The recommendation is to select a narrow spectrum of activity antimicrobials, intending to preserve the patient´s normal microbiota and reduce the development of antimicrobial resistance (Verwilghen, 2015). This means that susceptibility patterns of potential bacteria at the surgical site are one of the considerations for selecting perioperative antimicrobials (Chou, *et al*., 2020).

β-lactam drugs are crucial and widely used antimicrobials known for inhibiting cell wall synthesis, causing bacterial cell lysis, and thus being bactericidal. Named after their β-lactam ring structure, they include six major groups: penicillins, cephalosporins, cephamycins, carbapenems, monobactams, and beta-lactamase inhibitors (Rahman *et al.,* 2018). These antimicrobials represent about 50% of all prescribed antimicrobials globally, leading to increased resistance. Resistance mainly arises from beta-lactamases (BLs) that hydrolyze and inactivate these drugs. Over 900 BL types *are ide*ntified, with extended-spectrum beta-lactamases (ESBLs) posing significant concern due to their broad-spectrum activity against extended-spectrum cephalosporins (Rahman et al., 2018).

The β-lactam antibacterial activity involves inhibiting bacterial cell wall synthesis by preventing the proper functioning of penicillin-binding proteins, also known as transpeptidases. Peptidoglycan is an essential structural component of the bacterial cell and the periplasmic space, which provides rigidity, protection from high internal osmotic pressure, and defines the shape of the bacterial cell (Rahman et al., 2018). By blocking transpeptidases and activating autolysin, β-lactam disrupts the cell wall, resulting in cell lysis and the elimination of the microorganism (Rahman *et al.,* 2018). First-generation cephalosporins are the preferred prophylactic agents for human and small animal patients undergoing clean orthopedic procedures, because of their broad-spectrum effect on methicillin-sensitive staphylococci and streptococci and relatively low costs (Whittem,*et al*.,1999; Sanders, *et al*., 2019). Cefazolin (CFZ) is the most used perioperative antibiotic medication in human and veterinary orthopedic surgery because it presents low toxicity, has a rapid equilibrium between plasma and surgical wound fluid, favorable antimicrobial spectrum, sustained tissue concentrations, and high bone and joint tissue concentrations and its administration is associated with decreased rates of SSI. (Chou, *et al*., 2020; Välkki, *et al*., 2020; Budsberg, *et al*., 2001). Other second- and third-generation cephalosporins are also used as perioperative antimicrobials, which tend to be effective against β-lactamase-producing *Staphylococcus* spp., whose prevalence is increasing (Chou, *et al*., 2020).

3.2. Timing of administration

Prophylactic antimicrobial administration reduces the bacterial inoculum at the time of surgery and significantly decreases the rate of bacterial contamination of the surgical site. However, for effective prophylaxis, evidence has shown that the minimum inhibitory concentration of the antibiotic agent at the tissue level must be exceeded for at least the period from incision to wound closure, thus CDC guidelines for SSI prevention recommend that the time of administration of the surgery prophylactic antimicrobial allows the chosen drug to reach sufficient tissue concentrations at the surgical site before the incision is made, showing how much the timing of the prophylactic antibiotics is crucial (Gifford, *et al*., 2011; Välkki, *et al*., 2020). The timing of antimicrobial prophylaxis administration has garnered significant attention in human medicine, as it may be a critical aspect of AMP. According to the CDC, antimicrobial prophylaxis is not recommended beyond 24 hours post-surgery in humans. The guidelines for human medicine, as part of the SCIP, recommend administering an appropriately selected antimicrobial within 60 minutes of the surgical incision, providing doses every two half-lives to maintain therapeutic concentrations, and discontinuing antimicrobial use within 24 hours postoperatively. Additionally, the Swedish Council on Health and Technology Assessment supports limiting antimicrobial administration to prophylaxis only in cases involving closed fractures and elective prosthetic surgery (Paeckel et al., 2024; Välki et al., 2020; Verwilghen, 2015). WHO initiative *Safe Surgery Saves Lives* surgical safety checklist emphasizes the inclusion of antibiotic prophylaxis given within 60 minutes before skin incision (Gifford, *et al*., 2011). Although there is evidence suggesting that adherence to SCIP guidelines for the timing of AMP administration reduces morbidity and mortality, compliance remains low in human medicine. In veterinary medicine, where such guidelines are not available, principles based on human medicine recommendations are also applied (Paeckel *et al*., 2024; Verwilghen, 2015).

A 2014 retrospective study by Nazarali on 226 TPLO cases found that only 42.5% of dogs met SCIP guidelines for AMP timing and dosage. Although not statistically linked to SSI, a longer duration from antimicrobial administration to incision showed a trend toward higher SSI risk (Verwilghen, 2015).

3.3 Postoperative administration

Studies regarding the optimal use of antimicrobials in preventing SSI are inconclusive, as many of them suggest that postoperative antimicrobial administration after clean orthopedic surgeries is protective against SSI in dogs, but many others show evidence that antimicrobial administration in the postoperative period has no protective effect on the SSI incidence (Välkki, *et al*., 2020). A review of AMP in human surgery recommended discontinuing AMP within 24 hours postoperatively. This recommendation is based on substantial evidence indicating that extending AMP beyond this period does not reduce SSI and may contribute to antimicrobial resistance. In veterinary medicine, there are no established guidelines for postoperative AMP use, leading to frequent extrapolation from human medical practices. However, there remains ongoing debate within human literature regarding *the* optimal prophylactic antimicrobial regimen (Aiken et al., 2015; Verwilghen, 2015). Antimicrobials have risks and adverse effects, such as gastrointestinal symptoms and minor allergic reactions (skin rashes myalgias, and arthralgias), hence the routine antimicrobial prophylaxis not being recommended for clean, non-prosthetic, uncomplicated surgery (Gifford, *et al.*, 2011).

As mentioned before, there is little evidence in the veterinary literature, which means most published recommendations are based on studies on people. Although the use of postoperative antibiotics for clean surgery is not supported by the human medical literature, its use in veterinary medicine, particularly following orthopedic surgery, is common (Aiken, *et al.*, 2015; Nelson, 2011). In veterinary surgery, some reports suggest that extending the administration period beyond the 24 hours of surgery may offer improved protection in cases where implants are placed (Aiken, *et al.*, 2015).

Reported SSI rates in canine clean orthopedic surgery range from 0,6% to 7,1%. However, TPLO may have a higher infection rate than other clean canina orthopedic surgeries, with rates of 2.9% to 25.9% of infection (Fitzpatrick, *et al.*, 2010; Gallagher, *et al.*, 2012; Gatineau, *et al.*, 2011; Hans, *et al.*, 2017; Nazarali, *et al.*, 2014; Solano, *et al.*, 2015; Spencer, *et al.*, 2018; Välkki, *et al.*, 2020;) because of the limited soft tissue coverage of the bone plate, thermal bone necrosis caused by the saw blade, use of non-locking plates and presence of an implant (Aiken, *et al.*, 2015; Gallagher, *et al.*, 2012; Kim, *et al.*, 2008; Välkki, *et al.*, 2020; Verwilghen, 2015). For this type of surgery, only a few days of postoperative antimicrobial treatment is necessary. Extending antimicrobial therapy beyond this period does not provide additional benefits if SSI pathogens persist in the wound. Further research is needed to determine the optimal duration of postoperative antimicrobial administration to reduce the risk of TPLO SSI, limit the development of antimicrobial resistance, and minimize patient morbidity (Verwilghen, 2015). However, in a study by Aiken, *et al.*, 2015, there was no statistically significant difference between SSI rates in dogs administered antimicrobials over 3 hours and those who were administered a further 5 days of antimicrobials, in either TPLO or other clean orthopedic surgical procedures, a conclusion like other reports on similar surgical procedures.

The lack of clear guidelines AMP in veterinary surgery leads to the following recommendations based on current practice and human medical literature: 1) For clean procedures, AMP should be considered where the bone is incised, a prosthesis inserted, where infection would be considered catastrophic, high-risk animals or when surgery is expected to last more than 90 minutes. Antibiotic therapy should be discontinued no more than 12 to 24 hours postoperatively; 2) For clean-contaminated procedures, AMP is indicated with the selection of antimicrobials based on the expected flora at the site of contamination. The same guidelines for dosing onset and treatment duration for clean procedures apply; 3) For contaminated procedures, the general guidelines for clean-contaminated surgery apply (Nelson, 2011). If there is an infection or purulent exudate at the time of surgery, the procedure should be classified as dirty, and therapeutic rather than prophylactic antimicrobial therapy should be used. For dirty procedures, broad-spectrum antibiotic therapy appropriate for the most common pathogens should be administered before surgery and continued into the postoperative period until culture and sensitivity results allow narrowing of the antimicrobial spectrum (Nelson, 2011).

Additionally, it is essential to consider managing diseases without the use of AMP, as not all infections are bacterial in nature and systemic antimicrobial therapy is not required for all bacterial infections (Burgess, 2018). In certain cases, local treatment may be appropriate, while in other instances, resolving the underlying condition may eliminate a secondary bacterial infection without resorting to AMP. Moreover, antimicrobial therapy should not be used as a primary prevention strategy, as microorganisms in veterinary hospitals will inevitably be exposed to AMP, thereby exposing patients to resistant bacteria (Burgess, 2018).

4. Antimicrobial Resistance

Antimicrobials are antibacterial drugs with the capacity to eliminate or restrict the growth of target microorganisms (Rahman *et al.*, 2018). AMR, a natural phenomenon accelerated by the abuse and misuse of antimicrobial drugs is currently considered the most important emerging health-threat issue in both human and veterinary medicine worldwide (Cocca *et al.,* 2021; Palma, *et al.*, 2020; Scarpellini, *et al.*, 2024). Bacterial capability to face antimicrobial compounds is an ancient feature, enabling bacterial survival over time, as bacteria have evolutionary machinery that enables them to adapt to the selective pressure exerted by antimicrobial treatments, resulting in reduced efficacy of the therapeutic intervention against human and animal infections. Although AMR can be seen as a “modern” feature, resulting from unsuccessful and prolonged exposure to antimicrobial treatment, the identification of these resistance traits can be found in ancient permafrost, isolated caves, and mummies. Currently is employed a distinction between innate and acquired AMR: innate resistance is the result of a slow and long evolutionary process that microorganisms have performed to adapt to the changing environment; while acquired resistance is the result of a quick adaptation to a sudden selective pressure represented by the antimicrobial treatments (Palma, *et* al., 2020).

In industrialized countries, the companion animal population has dramatically increased and still expects steadily increasing growth, with the role of companion animals changing accordingly. On one hand, the increased attention level towards animal health results in the overall improvement of animal welfare. On the other hand, the increased contact between animals and humans leads to a higher risk of infections and cross-transmission of AMR traits. Additionally, the potential of reverse zoonosis along with the creation of animal reservoirs that maintain the loop of infection and AMR diffusion is gaining a steadily increasing concern. Hospitals are considered one of the major sources of AMR onset, due to the massive use of antimicrobials and the presence of high-risk patients the same issue is being experienced in veterinary hospitals, considering that some antimicrobials used are shared and so the emerging antimicrobial-resistant organisms (AMROs) (Palma, *et al*., 2020; Scarpellini, *et al*., 2024). In Veterinary Medicine, AMR represents an emerging problem that involves both commensal and pathogenic bacteria, as several multidrug-resistant (MDR) bacteria have emerged in dog and cat populations, constituting a serious threat due to possible therapeutic failure, longer hospitalization periods, increased treatment costs and morbidity (Cocca, *et al*., 2021).

From a global One Health perspective, some AMROs are considered particularly relevant, as for example methicillin-resistant Staphylococci (MRS), a well-recognized and important infectious agent, often showing multi-resistance patterns (Scarpellini, *et al*., 2024). In human medicine, the major pathogen MRS is MRSA, while in small animal practice is MRSP, an organism usually associated with skin, surgical sites, or urinary tract infections (Scarpellini, *et al*., 2024). According to a study by Cocca, *et al*., 2021, *S.pseudintermedius* was the most frequently observed isolate, which is consistent with previous veterinary literature. In this study, regardless of the risk category, *Staphylococcus spp*. Isolates showed high rates of AMR with resistance to at least one antimicrobial and MDR was found in a big percentage of the isolates. MRSP and MRSA were both encountered in animals and environmental surfaces in this study. Methicillin resistance is conferred by the *mecA gene* which codes for a new "penicillin-binding protein" which allows resistance to all β-lactam antimicrobials, making beta-lactam antimicrobials, such as cephazolin, ineffective against MRSP (Välkki, *et al*., 2020; van Duijkeren, *et al*., 2011). Reports on the prevalence of MRSP and MRSA colonization in small animals showed rates ranging from 0% to 4,6%, however, higher rates of MRSP and MRSA carriage are reported in hospitalized animals and hospital environments. Animals with MRSP often have a history of numerous or prolonged antimicrobial treatments or underlying skin or ear problems, as MRSP is often isolated from these areas. MRSP infections have associated risk factors, such as hospitalization, topical ear medications, glucocorticoid therapy, antimicrobial therapy, and the presence of skin and ear lesions (Beck, *et al.*, 2012; Cocca, *et al.*, 2021; Grönthal, *et al.,* 2014; Välkki, *et al.*, 2020).

The emergence of Gram-negative bacteria resistant to third-generation cephalosporins, including Enterobacterales able to produce ESBL is another health concern, as ESBL production is associated with resistance to penicillins, cephalosporins, and in some cases beta-lactamase inhibitors (Scarpellini, *et al.*, 2024). Small animal veterinary hospitals should be monitored with attention, as still little is known about their role in the onset and dissemination of AMRO's, besides the fact that they are an epidemiological point of risk along with the risk of zoonotic transmission (Scarpellini, *et al.*, 2024).

In recent decades, drug design and discovery of new targets have been a major focus in medicine, with significant investments in creating synthetic drugs to replace antibiotics (Rahman *et al.*, 2018). Based on the diversity of the origin of antimicrobials, there are three different bases of antimicrobial classification: 1) chemical structure, 2) target site of the drugs, and 3) impact of the outcome on the target such as bactericidal or bacteriostatic (Rahman *et al.*, 2018). Classification of antimicrobials can be ambiguous since some are bactericidal for certain bacteria but bacteriostatic for others. Categorizing based on chemical structure is also inadequate due to the diversity among agents (Rahman *et al.*, 2018).

The classification based on the target site is suitable as it aids in c*omprehe*nding the molecular basis and mode of action of antibacterial agents. Antimicrobials can act through one of five mechanisms: 1) inhibition of cell wall synthesis (penicillins, monobactams, carbapenems, and bacitracin), 2) inhibition of the cytoplasmic membrane (polymyxins), 3) blocking bacterial protein synthesis (chloramphenicol, lincosamides, macrolides, aminoglycosides, tetracycline), 4) blocking nucleic acid synthesis (quinolones, nitroimidazoles, and rifampicin), and 5) inhibiting folic acid synthesis (sulphonamides, trimethoprim) (Rahman et al., 2018). Blind and persistent use of antimicrobials is thought to be one of the important triggers to provoke and spread antimicrobial resistance (Rahman *et al.*, 2018).

5.1 The β-Lactam Antimicrobials

β-lactam drugs are essential and widely used antimicrobials that inhibit bacterial cell wall synthesis, leading to cell lysis. They are classified as bactericidal. Named for their β-lactam ring structure, they include penicillins, cephalosporins, cephamycins, carbapenems, monobactams, and beta-lactamase inhibitors (Rahman *et al.,* 2018). These drugs account for about 50% of all prescribed antimicrobials worldwide, but resistance to them is increasingly concerning. Resistance to β-Lactam is mainly due to the production of beta-lactamases (BLs) that hydrolyze and thereby inactivate beta-lactam antimicrobials (Rahman *et al.,* 2018). BLs exhibit a wide range of complexity and diversity, with over 900 types identified from various bacteria. ESBLs are notable for their broad activity against extended-spectrum ce*phalosp*orins (Rahman et al., 2018).

The β-lactam antibacterial activity functions by inhibiting bacterial cell wall synthesis. This is achieved through the prevention of penicillin-binding proteins (also known as transpeptidases) from functioning correctly. By simultaneously blocking transpeptidases and activating autolysin, β-lactam disrupts the cell wall, initiating its destruction and ultimately resulting in cell lysis and the elimination of the microorganism (Rahman et al., 2018).

5.1.1 Mechanism of Bacterial Resistance to β-Lactam Antimicrobials

Antimicrobial resistance occurs when effective antimicrobials can no longer eradicate the pathogen, making the treatment ineffective. A bacterium is considered resistant when it can survive even at higher doses of permissible drugs. When an organism becomes resistant to multiple types of antimicrobials, it is termed multidrug-resistant (Rahman *et al*., 2018). Bacteria develop resistance mechanisms over time. For β-lactams, the main methods include: 1) Producing β-lactamase to deactivate the antibiotic befo*re it* reaches its target; 2) Altering PBPs; 3) Reducing or lacking outer membrane proteins in Gram-negative bacteria; 4) Overexpressing efflux pumps to expel the antibiotic from the cell (Rahman et al., 2018).

β-lactamases are enzymes produced by microorganisms that can break β-lactam molecules, making them inactive, and contributing to β-lactam resistance and, until today, more than 500 β-lactamases have been reported, produced by diverse bacteria, making them one of the most common resistance mechanisms that leads the widespread resistance among Gram-negative microorganisms, where the βlactamase-mediated resistance is either plasmid-mediated or expressed chromosomally (Rahman *et al*., 2018).

6. **Objectives**

The principal objectives of the present study were to assess if the protocols of antisepsis with chlorhexidine and prophylactic administration of cephazolin were able to minimize the contamination of the surgery field. The other focus aims to lead to better surgery practice and contribute to the fight against antimicrobial resistance.

IV. Materials and Methods

1. Study Population

Data was collected from dogs and cats classified with I, II, III and IV categories by the American Society of Anesthesiologists (ASA) and submitted to clean orthopedic surgery (COS) with and without implants in three veterinary hospitals in the Lisbon area. Ethical approval was obtained from the Comissão de Ética e Bem-Estar Animal (CEBEA) from the Faculty of Veterinary Medicine of Lusófona University (16-2022).

A priori power calculation was performed using the G*Power 3.1.9.7 statistical software, which that a sample size of 63 animals would 80% power and a 0.4 size effect with a significance criterion of alfa=0.05 error level to detect differences in contamination of the surgical field during the intraoperative period.

After blood tests and anesthetic evaluation, patients were submitted to different anesthetic protocols and monitoring methods based on the anesthetist’s preferences. Venous access placement, trichotomy, and antisepsis were performed using a single solution of 2% chlorhexidine and 70% isopropyl alcohol (BBraun ®). All animals were submitted to different COS with or without implants and different anesthetic protocols and received IV cefazolin (22 mg/Kg), 30-60 minutes before surgical incision and repeated cefazolin in surgeries longer than 3 hours (Gonzalez *et al*., 2017). Swabs were collected from the surgical field skin in all animals before and after antisepsis and PA administration. Intraoperative swabs were taken at various surgical times based on the duration of the surgery: at skin incision (T 0min), after 45 minutes (T 45min), during the interval from 45 to 90 minutes (T >45-90 min) and after 90 minutes (T 90min). Additionally, fecal swabs were obtained from all animals before the PA administration.

Risk factors for SSI (CDC 2022; Lyssens *et al*., 2019; such as age, gender, body score, breed, ASA status, physical condition, anesthesia and surgery duration, hypoalbuminemia, use of surgical implants, hypotension, hypothermia, total time hospitalized, endocrine disease were obtained through individual questionnaires for each animal. SSI diagnosis was based on the following criteria: a) purulent/seropurulent drainage from the incision, b) spontaneous dehiscence or abscess formation in the incision line; c) microbial organisms identified on an aseptically obtained specimen from the incision; d) the incision deliberately opened in combination with at least one of the following signs or symptoms: localized swelling, localized pain, erythema, heat or fever (>39ºC). All collected swabs were inoculated and evaluated to determine the presence or absence of bacterial growth and antimicrobial resistance.

1.1 Microbiology Laboratory Sample Processing

Skin and intraoperative swabs were cultured on Columbia agar with 5% blood sheep (Oxoid, Hampshire, UK), MacConkey agar (Biokar Diagnostics, France), and MRSA (Oxoid, Hampshire, UK). Moreover, skin, intraoperative and fecal swab samples were cultured on MacConkey agar plates (Biokar Diagnostics, France) supplemented with 1.0 µg/mL of cefotaxime (CTX) (Sigma-Aldrich, St.Louis, MO, USA) and 1.5 µg/mL of meropenem (MEM) (Tcichemicals, Germany) and incubated overnight at 37º C for 24h to 48h. One isolate of each unique morphology was selected from each positive sample for further study. The bacterial species were identified using species-specific PCR by the Laboratory of Microbiology at the Faculty of Veterinary Medicine, Lusófona University.

Antimicrobial susceptibility testing and interpretation were performed using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2024). The antimicrobial agents (Oxoid, Basingstoke, Hampshire, UK) tested were: ampicillin (AMP) 10 µg, Amoxicillin/Clavulanate acid (AMC) 30 µg, cefotaxime (CTX) 30 µg, ceftazidime (CAZ) 30 µg, cefoxitin (FOX), cefepime (FEP) 30 µg, imipenem (IMP) 10 µg, meropenem (MEM) 10 µg, ertapenem (ETP), gentamicin (CN) 10 µg, amikacin (AK) 30 µg, ciprofloxacin (CIP) 5 µg, enrofloxacin (ENR) 5 µg, nalidixic acid (NA) 30 µg, nitrofurantoin (F) 300 µg, fosfomycin (FOS) 200 µg, tetracycline (TE) 30 µg, chloramphenicol (C) 30 µg and trimethoprim/sulfamethoxazole (SXT) 25 µg. *Escherichia coli* ATCC (American Type Culture Collection) 25922 was used as a reference strain for antimicrobial susceptibility quality control. Furthermore, the ESBL production was evaluated by using the double-disk synergy test (CLSI, 2024). The plates followed incubation at 35±2ºC for 24 hours, and then, the inhibition zone around disks was measured in millimeters, and results were interpreted according to the guidelines provided by the Clinical Laboratory Standards Institute (CLSI, 2024).

1.2. Statistical Analysis

Statistical analysis was performed using Jamovi version 2.6.13 computer software (https://www.jamovi.org). Results are presented as mean ± standard deviation or median (range) for continuous variables and as frequencies for ordinal variables. Wilcoxon signed-rank test was used to compare differences in skin contamination before and after skin antisepsis. Comparisons between the surgical times (T 0min, T 45min, T >45-90min, and T 90 min) contamination were analyzed using Kruskal-Wallis, followed by *post hoc* Pairwise comparisons. The significance level was set at $p < 0.05$.

V. Results

Sixty-three dogs and cats were included in the study. Data regarding the main demographic and surgical features is shown in Table 7.

Table 7. Main demographic and surgical features of the 63 animals with negative and positive intraoperative contamination.

		Intraoperative contamination	
		Negative	Positive
Sex, n (%)	Male	20/63 (31.7)	3/63 (4.8)
	Female	37/63 (58.7)	3/63 (4.8)
Age (mean ± SD)		4.26 ± 4.13	4.66 ± 3.62
Body score (median)		6	5
Species, n (%)	Canine	53/63 (84.1)	5/63 (7.9)
	Feline	4/63 (6.4)	1/63 (1.6)
Breed, n(%)	Crossbreed	18/63 (28.6)	3/63 (4.8)
	pure breed	40/63 (63.4)	2/63 (3.2)
Practice, n(%)	Private	54/63 (85.7)	5/63 (7.9)
	Teaching	3/63(4.8)	1 (1.6)
Antimicrobials last 12 months n (%)	Yes	13/63 (20.6)	4/63 (6.4)
	No	44/63(69.8)	2 (3.2)
ASA score, n (%)	I	29/63 (46)	0
	II	24/63 (38)	4/63 (6.3)
	III	2/63 (3.2)	2/63 (3.2)
	IV	2/63 (3.2)	0
	V	0	0
Surgical drapes n (%)	Adhesive	23/63 (36.5)	3/63 (4.8)
	non-adhesive	19/63 (30)	3/63 (4.8)
	Both	16/63 (25.4)	0
Type surgery n (%)	TPLO	23/63 (36.5)	3/63 (4.8)
	Arthroscopy	0	1/63 (1.6)
	Fracture	5/63 (7.9)	2/63 (3.2)
	Femoral head/ neck arthroplasty	5/63 (7.9)	0
	Hip prosthesis	7/63 (11.1)	0
	Bicipital tenotomy	1/63 (1.6)	0
	Arthrotomy	1/63 (1.6)	0
	Patellar luxation	9/63 (14.3)	0
	Tail amputation	1/63 (1.6)	0
	Hemilaminectomy	1/63 (1.6)	0
	Osteotomy	1/63 (1.6)	0
Hypothermia n (%)	Yes	43/63 (68.3)	4/63 (6.3)
	No	14/63 (22.2)	2/63 (3.2)
Hypotension n (%)	Yes	8/63 (12.7)	2/63 (3.2)
	No	49/63 (77.8)	4/63 (6.3)

Legend: ASA - American Society of Anesthesiologists; TLPO - Tibial Plateau Leveling Osteotomy

The duration of surgery and anesthesia was 81.3 ± 39.3 and 127 ± 49.6 minutes, respectively. Before antisepsis protocol and cefazolin administration, 45 out of 63 (71.4%) animals showed bacterial growth from the skin. After antisepsis of the surgical site only 6 out of 63 (9.5%) displayed bacterial growth. The use of antisepsis and cefazolin significantly reduced skin bacterial growth ($p < 0.001$). Following antisepsis and cefazolin administration bacterial contamination of the surgical field wound occurred in six animals and was essentially by Gram-negative bacteria. Notably, no common endogenous skin bacteria such as *Staphylococcus* spp. was detected during intraoperative time (Table 8).

Table 8. Bacteria responsible for intraoperative contamination at different surgical times

Surgical time	Bacteria identification
T_{0min}	*Enterococcus* spp. *Pseudomonas aeruginosa.*
T_{45min}	*Enterococcus* spp. *Pseudomonas aeruginosa.* *E. coli*
$T_{>45\text{-}90min}$	*Enterococcus* spp. *Pseudomonas aeruginosa.* *Proteus mirabilis.*
$T_{>90min}$	*Enterococcus* spp. *Pseudomonas aeruginosa.* *Proteus mirabilis.*

Regarding intraoperative contamination, significant differences ($p = 0.024$) were observed in bacterial contamination between the surgical times T 0min 2/63 (3.2%), T 45min 2/63 (3.18%), T40-90min 1/42 (2.3%) and T90min 3/16 (18.8%).

The results of our study also showed that 13 out of 63 (20.6%) of the companion animals were colonized with 3GC-resistant Enterobacterales. About 84.6% (n=11/13) were identified as *E. coli*, 7.7% (n=1/13) as *Klebsiella pneumoniae* and 7.7% as *Enterobacter cloacae*. 3GC-resistant *E. coli* was also resistant to carbapenems (18.2%, n=2/11), fluoroquinolones (45.5%, n=5/11), tetracyclines (54.5%, n=6/11), trimethoprim/sulfamethoxazole (27.3%, n=3/11), chloramphenicol (18.2%, n=2/11), nitrofurantoin (9.1%, n=1/11). Overall, 54.4% (n=6/11) of these isolates were MDR. Regarding, *K. pneumoniae* and *Enterobacter cloacae* isolates had resistance to fluoroquinolones, aminoglycosides, tetracyclines, trimethoprim/sulfamethoxazole, and chloramphenicol. *K. pneumonia* also showed resistance to nitrofurantoin (Table 9). However, the bacteria that contaminated the operative field did not coincide with those that were antimicrobial resistant at the fecal level.

Table 9: Antimicrobial resistance in 3 GC-resistant Enterobacterales isolated from fecal samples of companion animals.

Antimicrobials	***Escherichia coli* % R *(n)***	***Klebsiella pneumoniae* % R *(n)***	****Enterobacter cloacae* % R *(n)***
Ampicillin	100%, (n= 11)	100%, (n=1)	100%, (n=1)
Amoxicillin/clavulanate	100%, (n=11)	100%, (n=1)	100%, (n=1)
Cefoxitin	45.5%, (n=5)	100%, (n=1)	100%, (n=1)
Cefotaxime	100%, (n=11)	100%, (n=1)	100%, (n=1)
Ceftazidime	72.7%, (n=8)	100%, (n=1)	100%, (n=1)
Cefepime	63.6%, (n=7)	100%, (n=1)	100%, (n=1)
Imipenem	18.2%, (n=2)	0%, (n=0)	0%, (n=0)
Meropenem	0%, (n=0)	0%, (n=0)	0%, (n=0)
Ertapenem	18.2% (n=2)	100%, (n=1)	100%, (n=1)
Nalidixic acid	45.5%, (n=5)	100%, (n=1)	100%, (n=1)
Ciprofloxacin	36.4, (n=4)	100%, (n=1)	100%, (n=1)
Enrofloxacin	45.5% (n=5)	100%, (n=1)	100%, (n=1)
Levofloxacin	45.5%, (n=5)	100%, (n=1)	0.0%, (n=0)
Nitrofurantoin	9.1%, (n=1)	100%, (n=1)	0.0%, (n=0)
Fosfomycin	0.0%, (n=0)	0.0%, (n=0)	0.0%, (n=0)
Gentamicin	0.0%, (n=0)	100%, (n=1)	100%, (n=1)
Amikacin	9.1%, (n=1)	100%, (n=1)	0.0%, (n=0)
Tetracycline	54.5%, (n=1)	100%, (n=1)	100%, (n=1)
Chloramphenicol	18.2% (n=2)	100%, (n=1)	100%, (n=1)
Trimethoprim/sulfamethoxazole	27.3% (n=3)	100%, (n=1)	100%, (n=1)
MDR	54.4% (n=6)	100%, (n=1)	100%, (n=1)

Legend: %R, percentage of resistant isolates; MDR- Multidrug resistant bacteria; * K. *pneumoniae* is intrinsic resistant to AMP; ** E. cloacae is intrinsic resistant to AMP, AMC, first-generation cephalosporins, and FOX (CLSI,2024)

No animals in the study developed SSI after surgery; however, different antimicrobial protocols were used postoperatively.

VI. Discussion

The main findings of our study suggest that antisepsis with an alcohol-based combination of 2% chlorhexidine and prophylactic administration of cefazolin (22mg/Kg, IV) are effective in preventing contamination of the operative field in COS since it decreases the skin bacterial growth from 71.4% to 9.5% after antisepsis. It was also possible to verify that despite maintaining strict rules of surgical antisepsis and administration of PA, intraoperative contamination of the operative field occurred. Significant differences in surgical field contamination were observed, with an increase of contamination after 90 minutes of surgery. Although there were no cases of SSI in the postoperative period, it was not possible to evaluate the impact of surgical field contamination on SSI due to the use of different postoperative antimicrobial protocols by the surgeons involved in the study.

To the author's knowledge, this is the first study conducted in veterinary medicine, namely in orthopaedical surgery, that investigated the effect of antisepsis, PA administration, and the duration of surgery on intraoperative contamination. This study suggests that the implemented protocol effectively prevented and eliminated the growth of expected endogenous skin bacteria, particularly *Staphylococcus* spp. However, our study raises a concern, given that all the bacteria that grew from samples collected from the operative field were *Enterococcus* spp. and Gram-negative bacteria. This indicates that these bacteria managed to escape antiseptic measures and the prophylactic effect of cefazolin. This concern becomes more important given the fact that in this study 20.6% of the companion animals presented 3GC and carbapenems-resistant Enterobacterales before surgery. This raises significant worries about the potential for fecal contamination of the skin, which could increase the risk of serious SSI and even the zoonotic risk, as these animals carry fecal antimicrobial-resistant bacteria. These results agreed with the study by Belas *et al.,* 2021.

According to Gonzales *et al.,* 2017, administering a single intravenous injection of cefazolin (22mg/Kg, IV) 30 to 60 minutes before surgery for surgical procedures lasting 4 hours or less provides antimicrobial activity against the most common commensal organisms found on the skin of dogs, such as *Staphylococcus pseudintermedius* and *Streptococcus* spp. This is particularly beneficial for COS, as interstitial fluid concentrations of cefazolin are maintained above 4 mcg/mL for 4 hours. However, despite the minimal concentration of cefazolin necessary to inhibit the growth of 90% of the isolates (MIC_{90}) for *Staphylococcus* *pseudintermedius* and *Streptococcus* spp. is under 4 µg/mL (Rosin *et al.,* 1993; Stegemann *et al.,* 2006; Westermeyer *et al.,* 2010). In another study, *E. coli* MIC_{90} was 128 mcg/mL (Thungrath *et al.,* 2015). So, when *Enterococcus* spp., *E.coli* or other Gram-negative bacteria are suspected, another antimicrobial and dosing regimen should be considered (Gonzales *et al*., 2017). This fact may explain why, in our study, surgical field contamination was associated with *Enterococcus* spp. and with Gram-negative bacteria. Another possible explanation for the presence of these bacteria in the surgical field could be that CHX is less effective against Gram-negative than Gram-positive bacteria (Baines *et al.,* 2012).

In this study, no growth was detected in 18 animals in skin samples before antisepsis. This fact may be related to inadequate sample acquisition or the fastidious nature of some bacteria that grow poorly with conventional culture methods (Garcia-Fonticoba *et al.,* 2020; Stewart, 2012).

Although there is some controversy regarding the ideal concentrations of chlorhexidine to be used for operative field antisepsis, in a study in cats by Duque *et al*., 2024, the authors demonstrated that concentrations of 1 and 2% of aqueous or alcohol-based combinations of CHX are effective in the surgical field antisepsis. The cefazolin dose and administration intervals used in this study were based on a study by Gonzales *et al*., 2017, which demonstrated that tissue concentrations remain stable in tissues for *Staphylococcus pseudintermedius* and *Streptococcus* spp. for 4 hours. Taking into account the recommendations in human medicine and veterinary medicine, after the formation of a fibrin clot in the surgical wound, approximately 3-6 hours after closing the surgical wound, there is no need to administer further doses of PA, as there is no longer a risk of contamination of the surgical wound, therefore the continued administration of antimicrobials is contraindicated as it is associated with the development of bacterial resistance (Baines *et al.,* 2012). This aspect assumes greater importance given that these results show that adequate antisepsis and the use of cefazolin eliminated Gram-positive bacteria but allowed the growth of *Enterococcus* spp. and Gram-negative bacteria. Moreover, *Enterococcus* spp. is one of the most common causes of nosocomial infections and can be responsible for contamination of various hospital devices, contributing to wounds and skin/soft tissue infections. The clinical relevance of *Enterococcus* spp. is emphasized by their intrinsic resistance to several antimicrobial drugs, including aminoglycosides, cephalosporins, and sulfonamides (CLSI, 2024). Additionally, it is concerning that in this study, 20,6% of the Enterobacterales isolates from the fecal samples were 3GC and carbapenems, as well as being MDR. These bacteria, typically found in fecal or urinary sources, can contaminate the skin in the surgical area, thereby affecting the surgical wound.

The administration of postoperative antimicrobials after COS is controversial in veterinary medicine and is normally contraindicated in human medicine. Generally, this selection of postoperative antimicrobials is empirical and based on the expected bacterial agent, usually *Staphylococcus* spp. According to the findings of this study, the authors suggest that the decision to use postoperative antimicrobials should be based on whether a positive culture is obtained before closing the wound, as well as the antimicrobial susceptibility test of the isolated bacteria. This approach will avoid the inappropriate use of antimicrobials, allowing for more effective management of bacteria that may contaminate the surgical field and have the potential for SSIs. Through this strategy, it will be possible to reduce the incidence of SSI and prevent the development of antimicrobial-resistant bacteria due to their inappropriate use. On the other hand, given the concerns that orthopedic surgeons have regarding the development of catastrophic infections in animals with implants, this strategy allows for an adequate selection of antimicrobials. Moreover, these results could be helpful for infection management and prevention. It is a priority for healthcare providers to prevent the transmission and widespread do MDR bacteria by implementing a variety of safety measures such as active surveillance by rapid diagnosis, using standard precautions (hand hygiene, protective equipment), and proper decontamination methods.

Regarding the effect of the duration of surgery on contamination of the operating field, we found that there were significant differences between surgical times, with an increase in contamination after 90 minutes of surgery. Future studies should focus on the relationship between intraoperative contamination and the incidence of SSI, particularly in cases where postoperative antimicrobials are not administered. In this study, we were unable to obtain this conclusion, because the animals received postoperative antimicrobials.

The limitations of this study stem from the use of postoperative antimicrobials, making it impossible to establish a clear relationship between the contamination of the operative field and the incidence of SSI. This may have contributed to the observed absence of SSI cases during the postoperative period. Additionally, including two different species and the presence of different risk factors may have influenced our results. Another limitation of this study is that we were unable to demonstrate how the most common risk factor for SSI impacted the contamination of the operative field.

VII. Conclusion

These results suggest that antisepsis with 2% alcohol-based chlorhexidine solution and preoperative administration of cefazolin is effective in preventing contamination of the operating field in COS by Gram-positive bacteria. However, they fail to avoid contamination by *Enterococcus* spp. and Gram-negative bacteria. Moreover, this study also highlights the need for the use and selection of postoperative antimicrobials in COS to be based on the presence of positive bacterial cultures and their antimicrobial susceptibility tests obtained from samples before the closure of the surgical wound.

The development of resistance to antimicrobials is expected; however, the current increase in resistance to essential antibiotics and its transmission to commensal bacteria is particularly concerning. The genetic elements that enable bacteria to counteract the toxic effects of otherwise effective drugs are either encoded within the chromosome, resulting in vertical propagation, or acquired horizontally from the environment. Resistance elements that inactivate extended spectrum B-lactam drugs are often found in transferable elements like plasmids. These can spread through the gut, environment, and food animals, turning harmless bacteria into pathogens. Understanding the structural and genetic background of these elements is crucial for preparing against resistant bacterial epidemics. Additionally, much further research must be made, as the antibiotic treatment should be improved with the help of better diagnostic tools, that allow a better, faster and more accurate diagnoses, avoiding empirical treatments.

VIII. References

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