UNIVERSIDADE LUSÓFONA

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SURGICAL FIELD ANTISEPSIS: A COMPARATIVE STUDY IN FELINE SURGERY

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Presidente: Prof. Doutora Margarida Alves , por delegação da Profª. Doutora Laurentina Pedroso;

Arguente: Prof. Doutor Lénio Ribeiro

Orientador: Prof. Doutor João Martins

Universidade Lusófona

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All we have to decide is what to do with the time that is given us

J. R. R. Tolkien

To my family.

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Resumo

Após a introdução da antissepsia por Joseph Lister no século XIX, houve um decréscimo súbito da mortalidade associada a amputação para menos de 10% e os casos de septicemia diminuíram (Alexander, 1985). Estes resultados pós-cirúrgicos, anteriores e posteriores à introdução do método de Lister, indicam que existia de facto um problema de infeção associado a mortes após os principais procedimentos cirúrgicos (Alexander, 1985).

Em qualquer cirurgia invasiva existe um local de incisão que originará a chamada ferida cirúrgica. Uma possível infeção da mesma pode causar dor e desconforto, retardar a cicatrização e até mesmo a propagação sistémica da mesma, ameaçando assim a vida do paciente. Por conseguinte, a antissepsia do local cirúrgico é fundamental, pois permite a remoção de agentes microbianos que podem causar infeção da incisão cirúrgica (Yool, 2012).

Comparativamente à medicina humana, a preparação do campo cirúrgico em animais pode ser mais difícil devido à sua pelagem, banhos pouco frequentes, e ambiente geralmente mais contaminado (Gibson *et al.*, 1997). Em medicina veterinária, alguns fatores essenciais na prevenção das infeções das feridas cirúrgicas estão ligados ao procedimento realizado (duração, manipulação do tecido e experiência do cirurgião), à assepsia do cirurgião e desinfeção do bloco operatório (limpeza das mãos, uso de luvas, touca e bata cirúrgica, limpeza e desinfeção regulares do bloco operatório), ao uso de antimicrobianos pré-operatórios (o combate de qualquer possível infeção pós-cirúrgica, deve ser realizado com cautela e considerado caso a caso), e à antissepsia do campo cirúrgico (Burgess, 2019). Esta última é de particular importância, pois previne não só as infeções pós-cirúrgicas através da eliminação de bactérias no local da incisão, mas também na propagação da resistência antimicrobiana, uma vez que a sua eficácia na eliminação de bactérias pode diminuir a utilização de antimicrobianos profiláticos (Belo *et al.*, 2018; Burgess, 2019).

Apesar da iodopovidona ainda ser utilizada em alguns centros veterinários, atualmente a clorexidina e o álcool são os mais utilizados regularmente na antissepsia do campo cirúrgico. A clorexidina é um antisséptico de largo espectro, tendo sido introduzida no século XX no Reino Unido, é eficaz contra bactérias gram-negativas e gram-positivas e alguns fungos e vírus. Tem um tempo de ação mais rápido do que a maioria dos antissépticos e não é afetada pela presença de fluidos corporais como o sangue, contrariamente à iodopovidona (Lim & Kam, 2008; Silla *et al.*, 2008).

O álcool é utilizado como antisséptico desde o século XIV, estando provado ser um excelente bactericida e bacteriostático, eficaz contra alguns vírus (especialmente vírus envelopados) e alguns fungos (McDonnell & Hansen, 2020). Tem demonstrado eficácia contra bactérias gram-positivas e gram-negativas, incluindo bactérias multirresistentes tais como *Staphylococcus aureus* meticilina-resistente (MRSA) (McDonnell & Hansen, 2020).

Quando comparados os antissépticos, existem relatos que indicam que o uso de clorexidina a 2% associada a álcool na antissepsia reduz a probabilidade de infeção local em comparação com o uso da iodopovidona, estando esta última mais associada ao desenvolvimento de reação alérgica local (Dumville et al., 2015; Medeiros et al., 2018; Guenezan et al., 2021). Apesar disto, a maioria dos estudos afirma que ambos os antissépticos (clorexidina e iodopovidona) são excelentes antimicrobianos, e ambos previnem o aparecimento de infeções de feridas cirúrgicas em animais, sem diferença estatística entre os dois (Belo *et al.*, 2018; Medeiros *et al.*, 2018; Guenezan *et al.*, 2021).

A escolha e aplicação de um antisséptico apropriado é significativa, mas também a técnica empregue para aplicar a solução antisséptica tem o mesmo nível de importância, no entanto, constitui um tema menos abordado em medicina veterinária (Kerrigan, 2018).

Existem dois métodos de preparação da pele do campo cirúrgico: o método linear, que começa no local de incisão da pele, onde a pressão/fricção é mantida na compressa com o antisséptico e o movimento é feito para a periferia na mesma direção para a frente e para trás até o local da incisão cirúrgica parecer limpo e livre de sujidade; e o método circular, que começa no centro, trabalhando para o exterior com movimentos em círculos concêntricos em direção aos bordos do campo cirúrgico (Swales & Cogan, 2017; Kerrigan, 2018).

Comparando os dois métodos, vários estudos realizados em medicina humana e veterinária, relatam não existir qualquer diferença entre os dois métodos de antissepsia do campo cirúrgico (circular e linear) (Swales & Cogan, 2017; Mann, 2018; Carre et al., 2020; Tan et al., 2021). No entanto, num estudo em medicina humana, o método linear mostra-se mais eficaz, embora o circular seja ainda o mais comummente utilizado nos hospitais veterinários (Monstrey, 2022).

Relativamente à microbiota da pele, em medicina veterinária, ainda não existem estudos suficientes sobre a mesma, especialmente em gatos, onde a informação existente parece ser ainda mais limitada (Older *et al.*, 2017; Bierwiec *et al.*, 2021). Num estudo de Older et al. sobre a microbiota da pele felina, realizado em gatos de exterior e de interior, mostrou que o principal filo identificado foi Proteobacteria (44,3%), seguindo Firmicutes (21,04%), Bacteroidetes (16,65%) e Actinobacteria (10,38%) (Older et al., 2019).

Nos casos de infeções do local cirúrgico, tanto em cães como em gatos, as bactérias mais frequentemente encontradas eram organismos comensais da pele (cocos gram-positivos) e da flora normal do trato gastrointestinal e outras vias (especialmente bactérias gram-negativas) (Gonzalez et al., 2017). Assim, os antimicrobianos com eficácia contra estes organismos, especialmente *Staphylococcus spp.*, *Streptococcus spp.* e *Escherichia coli*, devem ser os recomendados profilaticamente (Gonzalez *et al.*, 2017).

O conceito de "*One Health"* traduz-se em "uma só saúde" ou "saúde única" e descreve-se como o esforço colaborativo de múltiplas profissões das ciências da saúde para atingir uma saúde ótima para as pessoas, animais domésticos, vida selvagem, plantas e o nosso ambiente (McEwen & Collignon, 2018).

Os antimicrobianos são utilizados para eliminar ou restringir o crescimento de microrganismos (Rahman *et al*., 2018). Em medicina veterinária, é comum a utilização de antimicrobianos profiláticos em cirurgias, incluindo cirurgias eletivas como ovariohisterectomias, como prevenção de possíveis infeções pós-cirúrgicas graves. No entanto, devido ao processo de seleção natural de Darwin, com o uso prolongado e pouco razoável dos mesmos antimicrobianos, começaram a surgir resistências em vários microrganismos que os levou a perseverar e a propagar (McEwen & Collignon, 2018).

Uma vez que a maioria das classes de antimicrobianos utilizados em medicina veterinária são também utilizados na medicina humana (por exemplo, cefalosporinas, fluoroquinolonas e tetraciclinas), existe um maior risco de surgirem novas resistências e estas serem transmitidas pelos animais aos seres humanos e vice-versa (McEwen & Collignon, 2018). Assim, uma vez que as medicinas humana e veterinária estão interligadas em termos da emergência e propagação da resistência antimicrobiana, parece lógico adotar uma abordagem "*One Health"* para este importante problema (McEwen & Collignon, 2018).

Uma das resistências anteriormente referidas mais relevantes são *Staphlyococcus* meticilina-resistentes, que são bactérias que contêm uma proteína adicional que se liga à penicilina, conferindo resistência aos antibióticos beta-lactâmicos (Walther *et al.*, 2016). Podem ser *Staphylococcus aureus* meticilina-resistente (MRSA), que são frequentemente encontrados em humanos e animais, e *Staphylococcus pseudintermedius* meticilina-resistente (MRSP), que parecem ter uma maior prevalência e importância clínica em cães e gatos comparativamente com humanos (Feßler *et al.*, 2018).

Uma das classes de antimicrobianos mais utilizada em medicina veterinária em todo o mundo são os beta-lactâmicos (Belas *et al*., 2021). Contudo, ao longo do tempo, a sua utilização frequente, levou ao aparecimento de resistência aos mesmos, nomeadamente as Enterobacteriaceae produtoras de beta-lactamases de espectro alargado (ESBLs), que são enzimas que têm resistência à maioria dos antibióticos beta-lactâmicos. As bactérias mais frequentes que produzem ESBLs são *Escherichia coli* e *Klebsiella pneumoniae,* que são as principais fontes de infeções hospitalares adquiridas em medicina humana e veterinária (Belas *et al.*, 2021).

O objetivo deste estudo é determinar se existe uma diferença entre a utilização de diferentes concentrações de clorexidina e entre os dois métodos de limpeza utilizados na antissepsia pré-cirúrgica: linear e circular, em gatos. Para atingir este objetivo, foram obtidas e cultivadas amostras do campo operatório para identificação bacteriana e foram contabilizadas as unidades formadoras de colónias (UFCs) para comparação.

Para este estudo, foram selecionadas 51 gatas fêmeas com indicação cirúrgica para ovariectomia eletiva, não tendo sido utilizados critérios de raça ou peso. Todos os animais pertenciam a colónias (gatos de exterior). Após a sedação dos animais, foi realizada tricotomia do abdómen entre o processo xifoide e púbis com um pente previamente esterilizado (máquina de tosquia *Aesculap*®).

Em seguida, o campo cirúrgico foi lavado com compressas humedecidas em solução salina (NaCl 0,9%) até que a sujidade, pelo e os detritos fossem completamente removidos. Uma vez dentro do bloco operatório, as duas primeiras amostras foram recolhidas por aposição com zaragatoas estéreis, uma do hemiabdomén esquerdo e a outra do hemiabdomén direito. A antissepsia do campo cirúrgico foi feita com divisão aleatória dos animais em três grupos: grupo antisséptico 1 (A1), que incluiu amostras G1 (1º felino do qual foram recolhidas amostras) a

G17, e no qual foi utilizada uma solução única de clorexidina 2% e álcool isopropílico 70%; grupo antisséptico 2 (A2), que incluiu amostras G18 a G34, e no qual foi utilizada uma solução de clorexidina 2% e álcool etílico 70%; e finalmente grupo antisséptico 3 (A3), que incluiu amostras G35 a G51, e no qual foi utilizada uma solução de clorexidina 1% e álcool etílico 70%. Cinco passagens com compressas estéreis foram realizadas no grupo A1 e cinco passagens alternadas entre a clorexidina e o álcool, terminando a limpeza com a primeira, sempre com compressas estéreis, foram realizadas nos grupos A2 e A3. Em todos os animais, a antissepsia foi realizada utilizando o método circular no hemiabdomén esquerdo, onde a limpeza começou no local da incisão trabalhando em direção à periferia em movimentos circulares; e o método linear no hemiabdomén direito, utilizando um movimento de vaivém na área da incisão trabalhando em direção à periferia em movimento ascendente e descendente. Após 3 minutos para os antissépticos atuarem e a pele secar, foram recolhidas mais duas amostras: uma do hemiabdomén esquerdo do animal e outra do hemiabdomén direito e devidamente identificadas e preservadas em métodos de cultura microbiana. Assim, foram recolhidas quatro amostras por animal, resultando num total de 204 amostras.

As amostras recolhidas da pele foram inoculadas em 1 ml de NaCl 0,85% e depois 100 µl da mesma foram retirados e inoculados em várias placas de cultura [agar *Tryptic soy* (TSA) (*Biokar Diagnostics*, França), Agar de Manitol (MSA) (*Biokar Diagnostics*, França) e Agar *MacConkey* (MCK) (*Biokar Diagnostics*, França)] para observação do crescimento bacteriano. Além disso, foram utilizadas placas *BrillianceTM MRSA 2* (Oxoid, Espanha) para detectar *Staphylococcus spp.* resistente à meticilina, e placas *Brilliance™ ESBL Agar* (Oxoid, Espanha), para detectar ESBLs.

As colónias foram contadas e registadas como "unidades formadoras de colónias" (UFCs) nas placas de TSA. As placas de MSA, MCK, MRSA e ESBL foram observadas para detetar se havia crescimento, facilitando assim a identificação das bactérias. Finalmente, as placas com crescimento bacteriano foram repicadas para placas *UriSelect 4* (URI) (*Bio-Rad Laboratories Ltd, Hemel Hempstead*, Reino Unido) para chegar a uma identificação final presumível da bactéria.

A diferença e variação de UFCs entre pré e pós-antissepsia em ambos os métodos em cada grupo de desinfetantes foram calculadas e registadas. A diferença é o resultado da

subtração de UFCs na pré-antissepsia com a pós-antissepsia. A variação corresponde à percentagem da diferença.

A análise estatística foi realizada utilizando o software SPSS (v.28.0). Todos os dados quantitativos foram testados para uma distribuição normal utilizando o teste *Shapiro-Wilk*. Uma vez que os dados não apresentavam distribuição normal ($p<0.05$), foram aplicados testes não paramétricos. O Teste *Wilcoxon* foi realizado para estudar se nas UFCs existiam quaisquer diferenças estatisticamente significativas entre pré e pós-antissepsia nos três grupos de antissépticos e nas duas técnicas de limpeza do campo cirúrgico. O teste *Mann-Whitney* U foi realizado para determinar se havia qualquer diferença entre as variações das UFC nas duas técnicas de limpeza antisséptica (circular e linear) em cada grupo de antisséptico. Finalmente, realizou-se o teste *Kruskal-Wallis* para determinar se havia qualquer diferença entre as variações das UFCs dentro de cada técnica de limpeza antisséptica para os três grupos de antissépticos. Os resultados foram considerados estatisticamente significativos quando $p\leq0.05$.

No grupo A1 no método circular foi observada uma diferença de $1{,}0x10^5$ UFCs e uma variação de 80%, enquanto que no método linear foi observada uma diferença de $4{,}7x10^5$ UFCs e uma variação de 50%. Em ambos os métodos houve uma diminuição significativa do número de UFCs da pré-antissepsia para o pós-antissepsia (***p*=0.021** e ***p*=0.005**, respetivamente).

No grupo A2, a diferença e variação foram $2{,}1x10^4$ e 87% no método circular e $2{,}8x10^3$ e 100% no método linear. Em ambos os métodos, houve diferença significativa no pré e pós-antissépsia (***p*=0.021** e ***p*=0.014**, respetivamente)

No grupo A3, a diferença e variação foram 123 e 89% no método circular e 108 e 68% no método linear. Houve uma diferença significativa na variação do método circular (***p*=0.005**), contudo no linear não houve diferença estatisticamente relevante entre pré e pós antissepsia (***p*=0.064**).

Dentro do grupo A1 houve um total de 25 amostras com crescimento bacteriano e, portanto, com variação, com 11 observadas no método circular e 14 no linear. Ao comparar os dois métodos dentro deste grupo não parece haver diferença entre os dois (***p*=0.774**). No grupo A2 houve 22 amostras com variação, com 9 no método circular e 13 no linear. Ao comparar os dois métodos não parece haver diferença significativa (***p*=0.164**). Finalmente, no grupo A3 houve um total de 25 amostras com variação, 14 das quais no método circular e 11 no linear. Apesar disto, não há diferença significativa entre os dois métodos dentro deste grupo (***p*=0.570**)

Relativamente aos dois métodos, no método circular houve 11 amostras com variação no grupo A1, 9 no grupo A2 e 14 no A3; resultando num total de 34 amostras com variação neste método. Ao comparar os três grupos dentro deste método não parece haver diferença significativa (***p*=0.902**). No método linear houve 14 amostras com variação no grupo A1, 13 no grupo A2 e 11 no grupo A3; o que resulta num total de 38 amostras com variação. Ao comparar os três grupos neste método parece não haver diferença significativa (***p*=0.117**).

Quanto ao crescimento bacteriano, foram observadas 118 amostras e 5 com a presença de fungos. Das bactérias identificadas, 88% eram gram-positivas e 12% gram-negativas. Dentro das bactérias gram-positivas, 70% foram observadas no pré e 30% no pós-antissepsia; nas bactérias gram-negativas, 86% foram identificadas no pré e 14% no pós-antissepsia.

No total, foram observados 123 microrganismos em todas as amostras, dos quais foram identificados *Staphylococcus spp.* (46%), *Enterococcus spp.* (11%), *Pseudomonas spp.* (5%), *Micrococcus spp.* (3%), Fungos não patogénicos (4%), *Proteus spp.* (2%), MRSA (2%), *Klebsiella spp.* (1%), *Escherichia coli* (1%), MRSP (1%). Os restantes 24% pertenciam à categoria de bactérias não identificadas, em que 87% eram gram-positivas e 13% gram-negativas.

Os três grupos de antissépticos e ambos os métodos de limpeza do campo cirúrgico foram eficazes na eliminação de UFCs, com a exclusão do grupo A3, apenas nas amostras em que o método linear foi utilizado, provando ser o menos eficaz em termos de resultados pré e pós-antissepsia. Contudo, comparando os diferentes grupos de antissépticos dentro de cada método, considerando a variação (subtração do número de UFCs no pré e pós-antissepsia) não houve diferença na utilização de cada um ($p>0.05$).

Atualmente, em termos de antissepsia de campo cirúrgico, existem ainda dúvidas sobre quais os antissépticos, concentrações, ou métodos de preparação mais eficazes para prevenir infeções do local cirúrgico, com estudos que recomendam a clorexidina (Medeiros *et al.*, 2018), outros iodopovidona (Osuna *et al.*, 1990), e outros que afirmam não haver diferença entre ambos (Marchionatti *et al.*, 2022). No entanto, em medicina humana, as recomendações geralmente indicam uma eficácia superior no uso de clorexidina 2% com álcool (Guenezan *et al.*, 2021). Assim, este estudo visou avaliar a eficácia de diferentes concentrações de clorexidina e dois métodos diferentes de antissepsia do campo cirúrgico, a fim de detetar se existem diferenças entre eles.

Alguns estudos referem que quanto maior a concentração de clorexidina, maior a sua ação antisséptica (Sharma *et al.*, 2021). Isto explicaria o facto de que a única concentração de clorexidina que não teve impacto na eliminação de microrganismos neste estudo foi a mais baixa 1%. Seria relevante no futuro utilizar uma concentração mais elevada (4%, por exemplo) para comparação com os outros antissépticos.

Embora o método circular ainda seja o mais rotineiramente utilizado nos hospitais veterinários, vários estudos comparando ambos os métodos (linear e circular) concluíram que não existe vantagem em utilizar um ou outro (Swales & Cogan, 2017; Monstrey, 2022). Este estudo apoia o que foi descrito até agora em medicina veterinária, uma vez que não houve diferença significativa na utilização do método linear ou circular em cada grupo de antisséptico.

No total foram identificadas mais bactérias gram-positivas do que gram-negativas nas amostras. Entre as bactérias gram-positivas, as bactérias com maior incidência foram *Staphylococcus spp.* seguidas por *Enterococcus spp.*; entre as bactérias gram-negativas foram a *Pseudomonas spp.* seguidas de *Proteus spp.* Na literatura está descrito que os principais filos identificados foram Proteobacteria e Firmicutes, por essa ordem; sendo que neste estudo o principal filo identificado foi Firmicutes (às quais pertencem *Staphylococcus spp.* e *Enterococcus spp*.), seguido de Proteobacteria (onde pertencem *Pseudomonas spp.* e *Proteus spp.*), diferindo neste sentido (Older et al., 2019).

Atualmente, ainda há informação limitada sobre a microbiota cutânea dos gatos, pelo que este estudo contribui para a caracterização da microbiota cutânea, especialmente em gatos no exterior. Algumas das bactérias observadas não puderam ser identificadas e foram preservadas juntamente com as restantes para futura identificação.

Os agentes resistentes a antimicrobianos constituem um problema crescente em ambientes hospitalares veterinários, pelo que intervenções ativas para prevenir a sua transmissão e emergência, especialmente por alguns serem agentes zoonóticos (MRSA), contribuem para o conceito de "*One Health*". O aparecimento e a propagação destes agentes em animais de companhia, como os gatos, estão relacionados com o contacto próximo com os seres humanos. Isto é particularmente verdade quando se fala de MRSA, já que se trata de um agente facilmente transmissível entre humanos e animais (Boucher 2017).

Apesar de a amostra ser composta por gatos errantes, MRSA (2%) e MRSP (1%) também foram encontrados neste estudo, mas estes representaram uma percentagem muito

baixa em comparação com o que é normalmente encontrado em gatos de interior (Elmoslemany *et al*., 2021). Não foram detetadas ESBLs em nenhuma amostra.

Assim, são necessárias diretrizes sobre o uso de antimicrobianos, bem como programas de controlo de infeções relativas ao pessoal, pacientes e ambiente (exemplo: saneamento pessoal e ambiental regular) em pequenas clínicas e hospitais de animais de companhia para melhorar a gestão antimicrobiana e prevenir o aparecimento e transmissão de resistências antimicrobianas (Boucher 2017).

Dado o constante aumento de resistências aos antimicrobianos e a utilização regular de antimicrobianos de forma profilática, mesmo em cirurgias regulares como ovariohisterectomias, seria desejável diminuir a sua utilização, uma vez que este estudo mostra que essencialmente todos os grupos de antissépticos e ambos os métodos utilizados (exceto o grupo A3 no método linear) foram eficazes na eliminação da maior parte da carga bacteriana presente na pele dos gatos, o que poderia causar possíveis infeções do local cirúrgico.

Relativamente às limitações deste estudo, considera-se o tamanho da amostra (baixo número de animais), e como eram gatos errantes, não ter sido possível acompanhar os casos após a cirurgia, para saber se desenvolveram reações cutâneas ou mesmo infeções do local cirúrgico.

Atualmente existe pouca informação disponível sobre antissepsia em medicina veterinária, sendo a mesma por vezes contraditória, especialmente em gatos. Assim, este estudo é pioneiro na medida em que compara três tipos diferentes de soluções antissépticas, bem como os dois métodos (linear e circular) descritos na limpeza pré-cirúrgica. Além disso, como não há muita informação relativa à caracterização da microbiota cutânea do gato, este estudo apresenta resultados relativos a este tópico que também podem ser inovadores em medicina veterinária.

Concluindo, todos os antissépticos utilizados (solução única de clorexidina 2% e álcool isopropílico 70%; clorexidina 2% e álcool etílico 70%; clorexidina 1% e álcool etílico 70%) no método circular mostraram eficácia na eliminação de microrganismos presentes na pele durante a antissepsia pré-cirúrgica. No método linear, apenas o grupo A3 (1% clorexidina e 70% álcool etílico) não foi eficaz na eliminação de microrganismos. Contudo, não houve diferença estatística no uso dos três antissépticos, nem no uso do método circular ou linear. As bactérias com maior incidência foram *Staphylococcus spp.*, *Enterococcus spp.* e *Pseudomonas spp.*. Três

amostras com MRSA e uma com MRSP foram identificadas e eliminadas pelos respetivos antissépticos utilizados.

Outros estudos sobre esta temática deverão ser realizados com a finalidade de eleger os antissépticos e métodos de limpeza mais eficazes, contribuindo assim para a diminuição do aparecimento de resistências antimicrobianas.

Palavras-chave: cirurgia; felino; antissepsia; clorexidina; infeção

Abstract

Rigorous technique of antisepsis in the surgical field is of great importance for surgical site infections (SSIs) prevention and should be based on the application of efficacious antiseptic solutions and antisepsis scrubbing techniques. The present study aimed to compare the effectiveness of three antiseptics with different concentrations of chlorhexidine, also compares two different surgical field scrub techniques. This way, samples were collected before and after surgical field antisepsis of cats from colonies (n=51) undergoing elective ovariectomy. Samples were cultured and colony forming units (CFUs) were counted for comparison. A total of 123 microorganisms were observed: *Staphylococcus spp.* (46%), *Enterococcus spp.* (11%), *Pseudomonas spp.* (5%), *Micrococcus spp.* (3%), Non-pathogenic fungi (4%), *Proteus spp.* (2%), methicillin-resistant *Staphylococcus aureus* (MRSA) (2%), *Klebsiella spp.* (1%), *Escherichia coli* (1%), methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) (1%) and 24% belonged to the category of unidentified bacteria. There was a statistically significant difference between pre and post antisepsis samples for all antiseptic solutions ($p<0.05$), except for the linear method in the 1% chlorhexidine and 70% ethyl alcohol antiseptic group ($p>0.05$). In this work, there was no statistical evidence demonstrating the superiority of any of the three antiseptic solutions or the two scrub techniques used for surgical field antisepsis. In the future, further studies should be carried out in order to choose the most effective antiseptic solutions and antisepsis scrub techniques, contributing to SSIs reduction, and in this way to reduce antimicrobials use and bacterial resistance emergence.

Keywords: surgery; feline; antisepsis; chlorhexidine; infection

List of Abbreviations

A1 – Antiseptic one

A2 – Antiseptic two

A3 – Antiseptic three

CDC – Centers for Disease Control and Prevention

CFU – Colony Forming Unit

CFUs – Colony Forming Units

CM – Circular method

CT – Computed tomography

DPO – Double Pelvic Osteotomy

ESBLs – Extended Spectrum Beta-Lactamases

Et al. – Latin term "et alii", meaning "and others"

Kg – Kilogram

LM – Linear method

MCK – MacConkey Agar

MDR – Multi-drug resistance

Mg – Milligram

MRS – Methicillin-resistant *Staphylococci*

MRSA – Methicillin-Resistant *Staphylococcus aureus*

MRSP – Methicillin-Resistant *Staphylococcus pseudintermedius*

MSA – Mannitol Salt Agar

MSSA – Methicillin-susceptible *Staphylococcus aureus*

OR – Operating Room

SSIs – Surgical Site Infections

THR – Total Hip Replacement

TPLO – Tibial Plateu Leveling Osteotomy

TSA – Tryptic Soy Agar

UFCs – Unidades Formadoras de Colónias

URI – UriSelect 4

Vs - Versus

µg – Microgram

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Internship report

The author started her curricular internship on the 6th of September 2021 and ended on the 31st of December 2021. It was performed at VetOeiras, central veterinary hospital of linha de Cascais, under the supervision of Dr. Diogo Santos.

The internship was carried out on a rotating schedule, with the author spending 140 hours in hospitalization, 80 hours in consultations, 140 hours in surgery and anaesthesia, 210 hours on night shifts and 98 hours on weekends and holidays (Figure 1). All this adds up to a total of 668 hours of internship. It focused on small companion animals, having been observed 75 animals in surgery and anaesthesia and 69 animals in consultations, of this total of 144 animals, 65 were females and 79 males, and 91 were dogs and 53 cats (Figure 2).

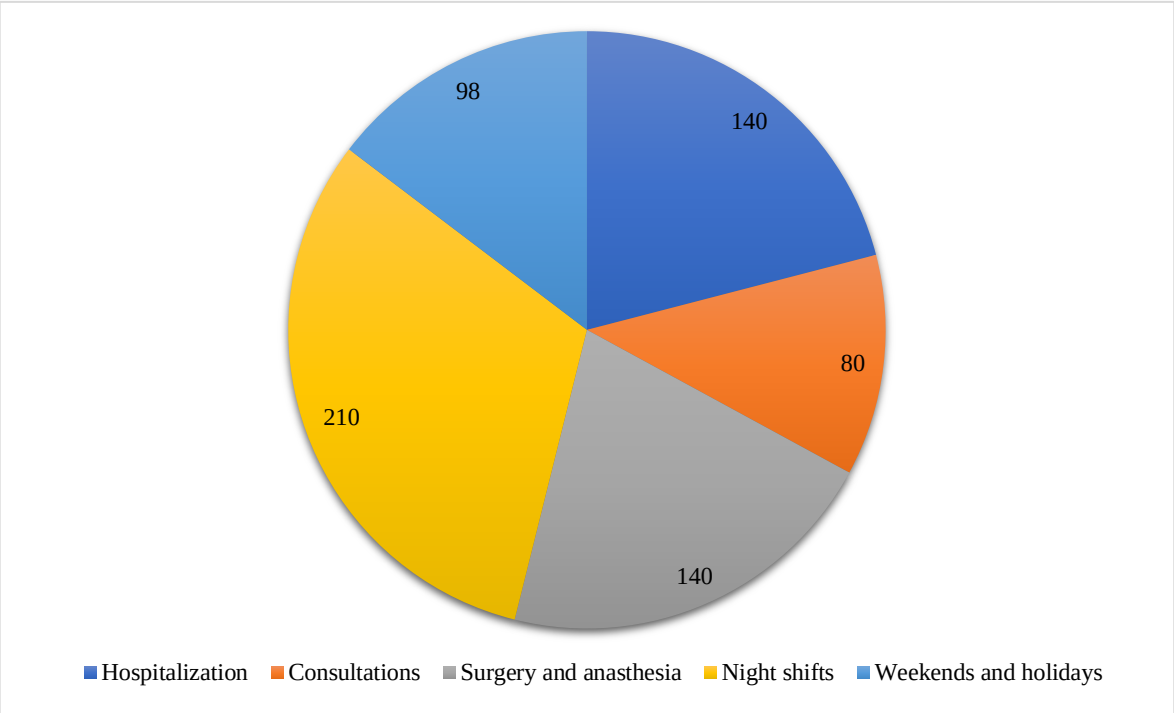

Figure 1. *Number of hours spent on the internship divided into categories.*

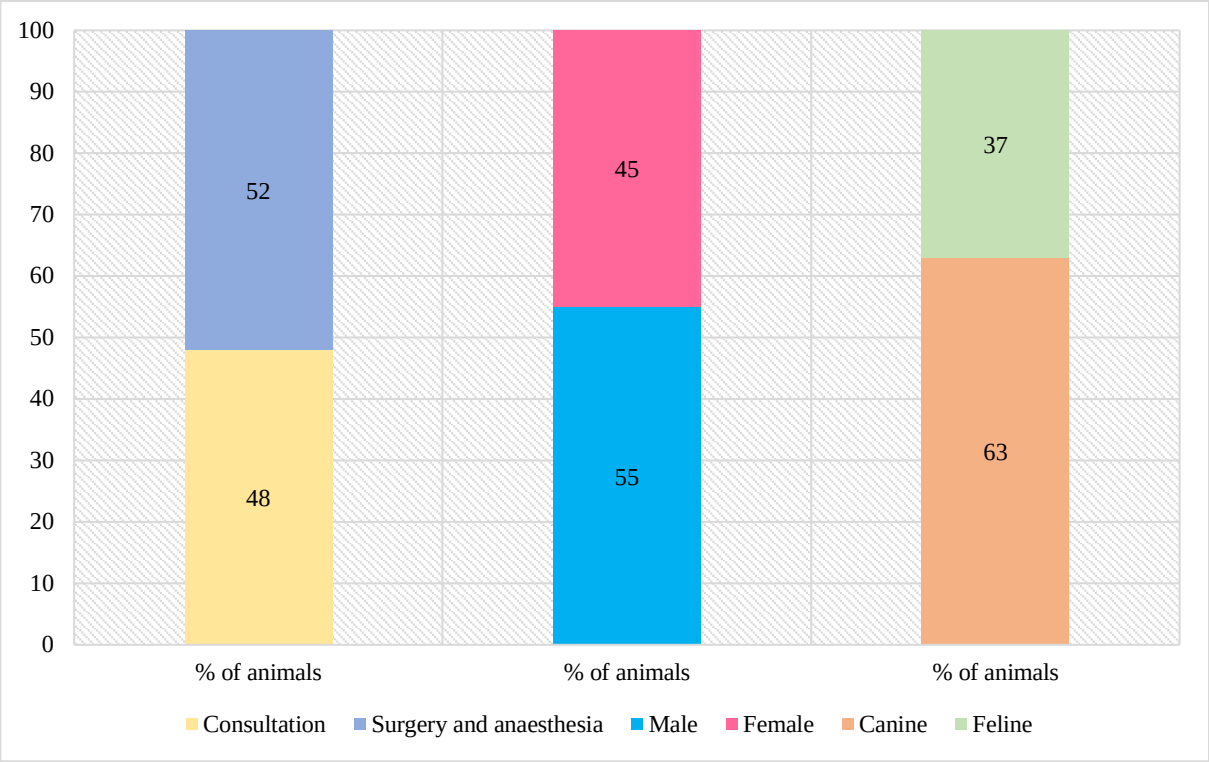

Figure 2. *Percentage of animals divided in three categories (consultation/surgery and anesthesia, male/female, canine/feline).*

Throughout the 4 months, the author had the opportunity to perform several medical-veterinary acts such as drug administration, x-rays and ultrasounds, blood sampling and catheterization, intubations, clinical analyses and cytology, assisting in surgeries and animal restraint, etc. There was also the possibility to attend the daily passages of cases at the hospitalization and improve communication skills with the tutors during consultations. During night shifts, weekends and holidays, the author was able to help and assist in emergencies and intensive care.

The author had the opportunity to observe 75 surgeries, mostly in orthopedics (52% of all surgeries attended). Thus, 14 Tibial Plateau Leveling Osteotomies (TPLO), 11 orchiectomies and arthroscopies, 8 fracture resolutions, 7 ovariohysterectomy, 5 nodular removals, 4 ophthalmologic surgeries, 3 gastric torsion resolutions, 2 foreign body removals, hemilaminectomies, mastectomies, splenectomies, Total Hip Replacements (THR) and Double Pelvic Osteotomies (DPO) were observed (Figure 3).

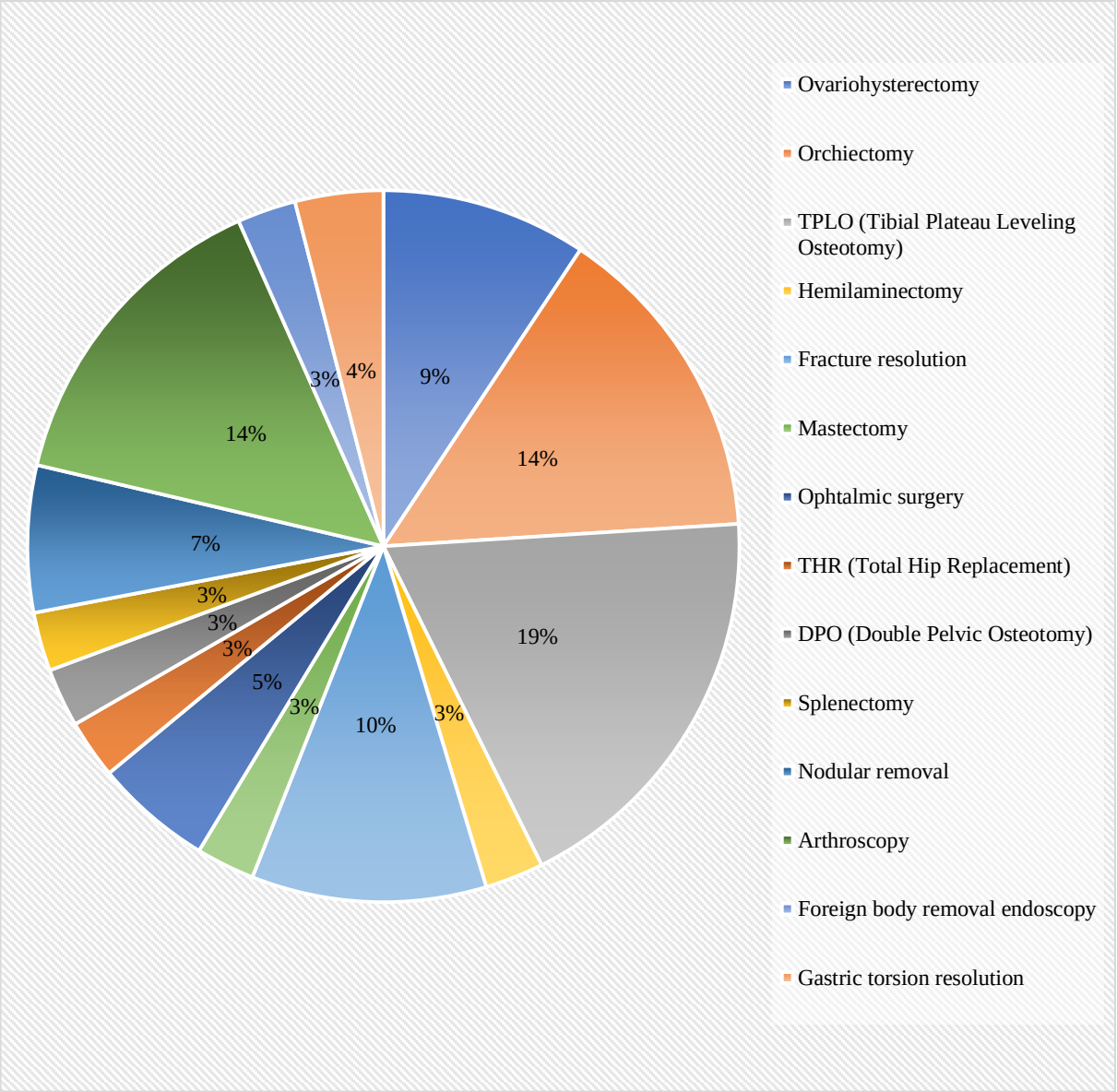

Figure 3. *Percentage of surgeries.*

It was possible to attend 69 consultations of various specialties of veterinary medicine, including: 11 general consultations (vaccinations, deworming and/or microchipping), 10 imaging consultations (ultrasounds, echocardiograms and CT scans), 8 gastroenterology, 7 cardiology and endocrinology, 6 orthopedics and urinary system, 5 neurology, 4 dermatology, 3 oncology and 2 respiratory system (Figure 4).

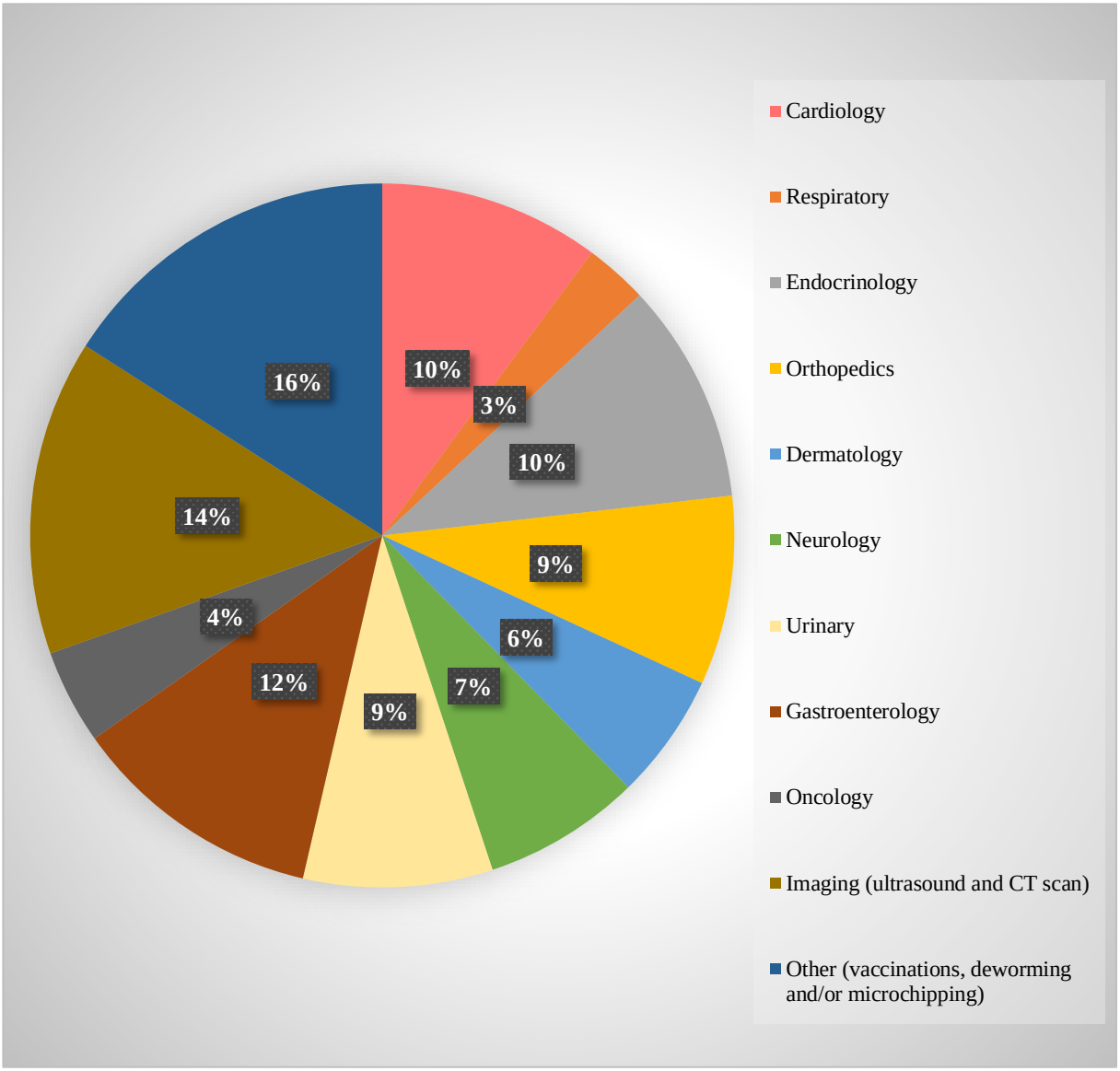

Figure 4. *Percentage of consultations by specialty.*

I. Introduction

1. Historical background of antisepsis

Before the concept of antisepsis, anaesthesia was introduced in 1846 by Morton. This revolutionary step in medicine allowed more freedom for surgeons to operate and the patients were no longer in pain during some procedures (Alexander, 1985; Newsom, 2008). However, most patients were still reluctant to the idea of surgery, especially elective surgeries, due to the high probability of ending in shock, pyaemia, or hospital gangrene (at the time, 80% of post operations resulted in gangrene), and since almost half of all the patients died after such procedures. At the time, the death rate of amputations performed by surgeons in hospitals was from 30 to 50%. It was also shown that the successful rate was superior when surgeries were made at home instead of in the hospital (Alexander, 1985; Newsom, 2008).

With the invention of the microscope in 1693, Leeuwenhoek demonstrated the presence of organisms in infections, however, these were observed both in infected wounds and in uninjured areas of the human body, such as the mouth. Thus, at the time, it was interpreted that these organisms could not be pathogenic (Alexander, 1985).

Known as the father of modern medicine, Joseph Lister was the first person that published about antisepsis in the late 19th century. He used carbolic acid as an antiseptic due to its putrefaction prevention properties (Alexander, 1985; Worboys, 2013).

Another great scientist who ended up having an impact on antisepsis was Louis Pasteur who, in the middle of the 19th century, showed that germs causing fermentation and putrefaction could be eliminated through heat. All his studies and experiments concerning the germ theory caused the theory of spontaneous regeneration, which was the current one at the time, to be refuted (Alexander, 1985).

With the introduction of Lister's antiseptic method, it became easy to see the results as mortality from amputation would suddenly drop to less than 10% and cases of sepsis would also be lower. These post-surgical results before and after Lister's method indicate that there was indeed an infection problem in these deaths after major surgical procedures. It is curious that Lister, despite being considered one of the pioneers in preventive medicine and antisepsis, never used a mask, gown or gloves (Alexander, 1985). Still in the same century, Professor John Dewar, a Scottish veterinarian, affirms that Lister's work *"led to a better and more accurate understanding of the causes of failure, and conversely to a clearer and more correct*

apprehension of the principles which should guide the surgeon in his endeavour after better results" (Dewar, 1891).

Lister characterized bacteria as particles only, and in 1877, Koch went further and proved that there were several types of bacteria through studies on animals. Then, in 1881, this scientist perfected the "plate technique" that allowed us to begin to identify bacteria by their appearance (Newsom, 2008).

2. The importance of antisepsis in surgery

In any invasive surgery there is an incision site that will originate the so-called surgical wound. This can be contaminated in various ways, through surgical instruments, skin and hair of the animal, the surgeon's hands and clothing, and even the environment where the surgery is performed. A possible infection of the surgical wound can cause pain and discomfort, delay healing and even spread systemically, threatening the patient`s life. Therefore, surgical site antisepsis is extremely necessary, as it allows removal of microbial agents that may cause infection in the surgical incision area. However, it is impossible to eliminate all bacteria present on the skin surface and the skin quickly recolonizes (Yool, 2012).

Compared to human medicine, surgical field preparation in animals may be more challenging due to their thick fur coat, infrequent bathing, and generally more contaminated environment; this may indicate that some antiseptic techniques used successfully in humans may not be as effective in animals (Gibson *et al.*, 1997).

3. Surgical site infections

Medicine has evolved a lot over the years, there was a time when the treatment was worse than the disease and most surgical procedures ended in serious infections. Nowadays, there are numerous protocols to prevent the so-called surgical site infections (SSIs) (Verwilghen, 2015). SSIs are infections that occur at the surgical incision site and are among the most common surgical complications. They increase the length of post-surgical hospital stay, associated costs, morbidity and mortality (Berríos-Torres *et al.*, 2017; Espinel-Rupérez *et al.*, 2019).

According to data collected in human medicine, approximately half of the risk factors for SSIs are endogenous (for example patient age and presence of concomitant diseases), which

means that they are difficult or impossible to change during and after the surgical period. The other half refers to exogenous factors such as the use of surgical gown and gloves, and entry and exit to and from the operating room (OR) during surgery, which can easily be protocolled and adapted to each situation (Verwilghen, 2015).

In veterinary medicine, some essential factors in the prevention of SSIs are linked to the procedure performed, the asepsis of the surgeon and operating room, the use of preoperative antimicrobials, and antisepsis of the surgical field (Burgess, 2019). Regarding the procedure, the risks may be associated with its duration (the longer, the higher the risk) and with the handling of the tissues, both factors usually depending on the surgeon's experience (Espinel-Rupérez *et al*., 2019; Burgess, 2019). Regarding the asepsis of the surgeon and the OR, pre-surgical hand cleaning, use of gloves, surgical cap and gown, and regular cleaning and disinfection of the entire OR should be carried out to prevent the accumulation of biofilms (Burgess, 2019). The use of preoperative antimicrobials to prevent SSIs should be undertaken with caution and considered on a case-by-case basis, for example if the patient is at risk of developing SSIs, to avoid the emergence of resistant organisms (Burgess, 2019). Finally, antiseptic preparation of the surgical field is an important component not only in preventing SSIs by eliminating bacteria at the incision site, but also the spread of antimicrobial resistance, as its effectiveness in eliminating bacteria may decrease the use of prophylactic antimicrobials (Belo *et al.*, 2018; Burgess, 2019).

3.1. Clipping

A study in humans reported that clipping performed 24 hours before surgery increased the risk of SSIs. This may be due to aggression to the skin that could cause cuts leading to bacterial contamination, so, in veterinary medicine, clipping of the surgical field should be performed immediately prior to surgery (Reynolds & Nichols, 2019).

Hair clipping is believed to cause less trauma and thus less incidence of skin infections than shaving with a razor. In dogs, as in humans, some of the bacteria that represent the flora present in the hair follicles are *Staphylococcus spp*. and *Micrococcus spp.* (Messiaen *et al*., 2019).

In a 2020 study, it was found that in short hair dogs pre-surgically washed and not clipped prior to arthrocentesis had no increase in skin bacteria counts. This indicates that pre-surgical clipping may not have a great antiseptic impact in some cases (Lavallée *et al.*, 2020).

4. Types of antiseptics

Lister is considered the founder of antiseptic medicine and a pioneer in preventive medicine, nevertheless, other people before him helped in his discovery. In the 5th century B.C., Hippocrates described the use of wine to heal wounds, which shows that some antiseptics were already being used empirically centuries before Lister. Also, before Lister, silver nitrate, mercuric chloride and creosote were used to decrease infections. In 1363 turpentine began being used for its styptic properties, along with myrrh, benzoin, frankincense and balsam. Even though it was made popular by Lister, carbolic acid was already used for local infection reduction by Demaux and Wolfe in 1859 and 1865 respectively (Alexander, 1985).

Clark (1907) describes other antiseptic agents and compares them to the carbolic acid, such as iodoform, that seems to not have any advantage over the first, and stearoptenes (thymol and eucalyptol). The use of peroxide of hydrogen starts to get attention and is considered the prime rival to the carbolic acid. The article also refers that all surgical instruments should be sterilized before and after the surgery, most commonly with boiling water. The hands of the surgeon such as the surgical instruments and the surgical region should be disinfected with some type of antiseptic before any procedure to avoid the "*danger of introducing morbid germs into open wounds*". It also refers that the OR should be clean and removed of all unnecessary objects (Clark, 1907).

In the late 20th century, another article was published comparing two antiseptics: an iodophor solution and chlorhexidine gluconate in one hundred animals that underwent elective ovariohysterectomy. In this study, cultures of the skin before and after the antisepsis seem to demonstrate no difference in terms of efficacy (Gibson *et al.*, 1997).

4.1. Chlorhexidine

Chlorhexidine is a broad-spectrum antiseptic that was introduced in 1954 in the United Kingdom (Silla *et al.*, 2008). It is effective against gram-negative and gram-positive bacteria and some fungi and viruses. It has faster action time than other antiseptics and is not affected

by the presence of body fluids such as blood, unlike the povidone-iodine (McDonnell & Denver, 1999; Lim & Kam, 2008). Depending on its concentration, it can have bacteriostatic (inhibits bacterial growth) or bactericidal (eliminates bacteria) effects (Genuit *et al.*, 2001). It is a positively charged molecule (cation) that binds to negatively charged sites (anions) on the cell wall, damaging it. Once damaged, chlorhexidine penetrates the wall and attacks the cytoplasmatic membrane, which promotes an escape of intracellular components and leads to apoptosis (McDonnell & Denver, 1999; Silla *et al.*, 2008).

When comparing various concentrations of chlorhexidine solutions and their efficacy, Sharma *et al.* states that there is no significant difference between 1% and 2% chlorhexidine in skin antisepsis in neonate humans (Sharma *et al.*, 2021). However, Stinner (2011) concludes that the dilution of chlorhexidine is directly correlated with its bactericidal activity, meaning that the higher the concentration, the greater its antiseptic ability. This study also recommends the use of a 4% chlorhexidine solution when preparing the surgical field and a waiting time of 2 minutes to let the antiseptic act before the skin incision (Stinner *et al.*, 2011). Yool (2012), however, recommends 5 minutes of action time for maximum antimicrobial effect and suggests that chlorhexidine has a prolonged antimicrobial effect which prevents recolonization of the skin for several hours post contact (Yool, 2012). There are reports that the antimicrobial mechanisms of chlorhexidine can last up to 48 hours on human skin (Hibbard, 2005).

A downside is that chlorhexidine also seems to have a cytotoxic effect on some human cells *in vitro*, stimulating apoptosis. However, *in vivo* effect is still poorly known and should be studied in the future (Karpiński & Szkaradkiewicz1, 2015). Regarding bacterial resistance, to date there are no reports of bacteria or fungi resistant to chlorhexidine. However, Buxser (2021) in a systematic review published in 2021 on the subject, in human medicine, reports that some bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* have shown a slight increase in resistance to chlorhexidine over the years. Microorganisms such as *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Candida albicans* showed no increased resistance to chlorhexidine and even seem to have increased susceptibility to it. A difference in susceptibility was also detected between methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *Staphylococcus aureus* strains (Buxser, 2021).

The low magnitude of emergence of chlorhexidine resistance over the years may indicate frequent changes or mutations of the cell surface and metabolic processes. These changes may be due to the use of chlorhexidine as an antiseptic in a hospital setting over a long period of time that has led to the creation of a low level of resistance. The main concern is whether these changes may also lead to cross-resistance with clinically relevant antimicrobials. However, although there is scientific evidence that the use of biocides leads to the appearance of resistance to them, so far there is no evidence that this could also increase antibiotic resistance to some extent (Oggioni *et al.*, 2013; Buxser, 2021).

4.2. Alcohol

Alcohol has been used as an antiseptic since the 14th century and is now proven to be an excellent bactericide and bacteriostatic, effective in killing some viruses (especially enveloped viruses) and some fungi. It has shown efficacy against gram-positive and gram-negative bacteria, including multi-resistant bacteria such as MRSA [Centers for Disease Control and Prevention (CDC), 2016; McDonnell & Hansen, 2020].

Like most chemical germicides, alcohol acts at multiple sites in the cell and its antimicrobial activity is related to coagulation/denaturation of cellular proteins. However, the percentage of water included in an alcohol solution is directly related to its efficacy, because increased concentrations of alcohol lead to water deprivation in the bacteria cells, inducing an impermeable cell membrane and hindering coagulation/denaturation. Thus, the ideal concentration for good antisepsis should be between 60 and 90% (CDC, 2016; McDonnell & Hansen, 2020). The recommended action time for best antibacterial effect is usually about 3 minutes and typically these alcohol-based products are generally those that cause the least contact dermatitis (Yool, 2012).

Alcohol has been shown to have no activity against spores and little activity on some non-enveloped viruses, and since it is a flammable element, it should be used with care (CDC, 2016; McDonnell & Hansen, 2020). Another downside is that alcohol has no residual effect which means that the bacteria will recolonize the skin surface faster (Yool, 2012).

4.3. Antiseptics comparison

There are reports that conclude that the use of 2% chlorhexidine plus alcohol for antisepsis reduces the likelihood of local infection compared to the use of povidone-iodine, which is more likely to cause allergic site reaction (Dumville *et al.*, 2015; Medeiros *et al.*, 2018; Guenezan *et al.*, 2021). However, most studies claim that both antiseptics (chlorhexidine and povidone-iodine) are excellent antimicrobials, and both prevent the onset of surgical wound infections in animals, with no statistical difference between the two (Belo *et al.*, 2018; Medeiros *et al.*, 2018; Guenezan *et al.*, 2021).

A study in human medicine favours the use of alcohol-based formulations when cleaning the surgical field, in deterioration of chlorhexidine and povidone-iodine and further warns of a possible emergence of resistance to these antiseptics that are frequently used in hospital settings (Boyce, 2019).

5. Antisepsis scrub techniques

Since whenever the skin is punctured there is an entry point for microorganisms, its aseptic care before any incision is of utmost importance to reduce the incidence of surgical wound infection. The application of an appropriate antiseptic is significant, but so is the technique employed to apply the solution itself, even if this is still given undoubtedly less importance (Kerrigan, 2018).

There are two methods of preparing the skin of the surgical field: the linear method (LM), with a "back and forth" motion, and the circular method (CM), which starts in the center, working outwards towards the edges of the surgical site (Swales & Cogan, 2017).

5.1. Circular method

The circular or concentric circle method is probably the most widely used in small animal veterinary medicine today for antiseptic preparation of the surgical field. This technique involves applying a pad with the selected antiseptic first to the incision site, rubbing outwards from the surgical field always in concentric circles until the end of the outer margins is reached.

Figure 5. *Illustration of the CM, starting at the orange point and ending at the blue one (Adapted from Worldwide Veterinary Service Academy, 2022).*

The procedure is usually repeated three to five times or until the skin appears clean and there is no more dirt on the pads. Applying pressure/friction to the skin during this technique is not mentioned and should not be used (Kerrigan, 2018).

5.2. Linear method

Friction increases the antimicrobial effect of the antiseptics used and so, in the LM, antisepsis also begins at the skin incision site where pressure/friction is maintained on the pad with the antiseptic and movement is made to the periphery in the same direction back and forth.

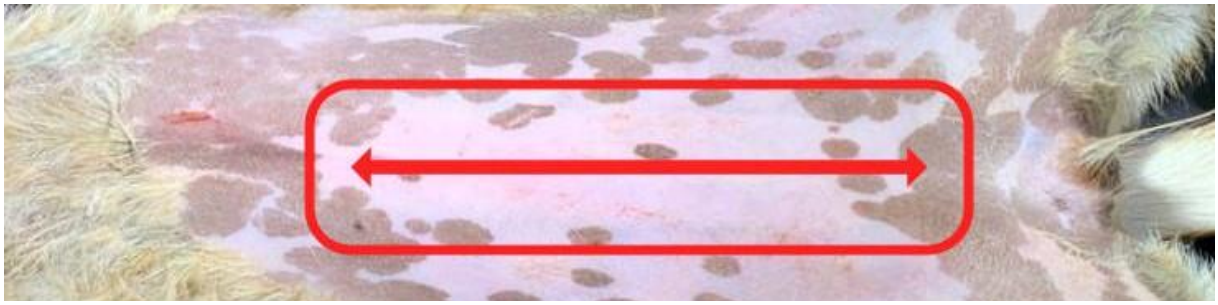

Figure 6. *Illustration of the start of the LM, starting with back-and-forth movements in the incision area (Adapted from Worldwide Veterinary Service Academy, 2022).*

The process is repeated until the surgical incision site appears clean and free of dirt (Kerrigan, 2018).

5.3. Comparison of the two antisepsis methods

An effective way to distinguish the two methods is from the anatomy of the skin, which is composed of 25 layers, and several studies show that about 80% of all skin flora resides in the first five outer layers. A back-and-forth motion, in the LM, covers the same area several times, while creating friction and pressure. This is possibly more effective at penetrating cracks and crevices in the skin and reaching deeper cell layers, then the CM (Casey *et al.*, 2017; Kerrigan, 2018).

The CM ensures that the entire surgical field is painted with an antiseptic solution and therefore the skin is assumed to be clean. However, this does not guarantee that the same path is maintained during each application, so some areas of the skin may not have been effectively cleaned. The incision site is a more likely access for bacterial contamination of the wound than the periphery, however, when preparing the skin using the CM, most of the time used during preparation is spent away from the incision line rather than on it (Kerrigan, 2018).

Several studies have been performed comparing the two scrub techniques in both human and veterinary medicine, and it appears that in most no difference was observed between the two methods of antisepsis (circular and linear) (Swales & Cogan, 2017; Mann, 2018; Carre *et al.*, 2020; Tan *et al.*, 2021). However, in a study in human medicine, the LM is shown to be more effective, although the CM is still the most commonly used scrub method in hospitals (Monstrey, 2022).

Another study conducted in veterinary medicine in the UK, indicates that the friction provided during the LM offers no advantage over the CM, and further suggests that the technique used may be that of personal preference, and that there is no observable benefit to selecting either method (Swales & Cogan, 2017).

6. Skin Microbiota

The body of all mammals is colonized by a diversity of microorganisms, that can be beneficial or pathogenic to the host (Older *et al.*, 2017; Bierwiec *et al.*, 2021).

Different parts of the body have different populations of microorganisms, just as various animals can have different microorganisms present. This variation can be due to intrinsic factors, such as the autoimmune system, or extrinsic factors, such as their environment. To understand how opportunistic skin microorganisms can cause or contribute to infections or other pathologies, there first needs to be an understanding of the skin microbiota in healthy animals (Older *et al.*, 2017).

In veterinary medicine, there are still not enough studies on the microbiota in the skin of animals, especially in cats, which seem to be even more limited (Older *et al.*, 2017; Bierwiec *et al.*, 2021).

In a study by Older *et al.* about the feline skin microbiota, made in outdoor and indoor only cats, showed that the main phyla identified was Proteobacteria (44.3%), following Firmicutes (21.04%), Bacteroidetes (16.65%) and Actinobacteria (10.38%), by that order. In this study, no significant differences were observed between the outdoor and indoor only cats, mainly because the indoor only cats came into contact regularly with microbes associated with the environment carried in the air or on their tutors. It was also shown many differences between different breeds of cats, likely due to features such as haircoat which may allow growth off different communities of microbes, concluding that the breed, more than the environment, plays a major role in shaping the cutaneous microbiota of the cat (Older *et al.*, 2019).

In cases of SSIs, both in dogs and cats, the most commonly found bacteria were commensal organisms of the skin (gram-positive cocci) and normal flora of the gastrointestinal and other tracts (especially gram-negative rods). Thus, antimicrobials with efficacy against these organisms, especially *Staphylococcus* spp., *Streptococcus* spp. and *Escherichia coli*, should be the recommended prophylactically (Gonzalez *et al.*, 2017).

7. Antimicrobial resistance

The concept of One Health describes itself as *"the collaborative effort of multiple health science professions to attain optimal health for people, domestic animals, wildlife, plants, and our environment"* (McEwen & Collignon, 2018).

Antimicrobials are used for eliminating or restricting growth of microorganisms (Rahman *et al*., 2018). In veterinary medicine, it is common to use prophylactic antimicrobials in surgeries, including ovariohysterectomies, as a prevention of SSIs and possible serious post-surgical infections. However, following Darwin's process of natural selection, with prolonged and unreasonable use of the same antimicrobials, resistance begins to emerge in various microorganisms that causes them to persevere and spread (McEwen & Collignon, 2018).

Since most classes of antimicrobials used in veterinary medicine are also used in human medicine (such as cephalosporins, fluoroquinolones, and tetracyclines), there is a greater risk of new resistances emerging and being transmitted from animals to humans (McEwen & Collignon, 2018). Thus, since human and veterinary medicine are interconnected in terms of the emergence and spread of antimicrobial resistance, it seems logical to have a One Health approach to this important problem (McEwen & Collignon, 2018).

As previously mentioned, the use of antimicrobials may decrease the incidence of SSIs. However, for routine clean surgical procedures some researchers believe that there is no effect on the use of antimicrobials, while others argue that it decreases the incidence of postoperative SSIs (Gonzalez *et al.*, 2017).

With the increased use of antimicrobials in human medicine and veterinary, resistances to these by the pathogenic organisms has become a problem with costs on the treatment of infectious diseases (Shaikh *et al.*, 2015). This resistance to antimicrobials, which includes multi-drugs resistance (MDR), also comes from an inappropriate and indiscriminate use of them, and so some researchers believe that antimicrobials should only be recommended in procedures associated with high risk of local infection, or when the emergence of postoperative infection can have serious consequences in the outcome (Gonzalez *et al.*, 2017). In veterinary medicine, the most commonly associated with SSIs opportunistic bacteria are methicillin-resistant *Staphylococci* (MRS), including MRSA and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), and extended-spectrum beta-lactamases (ESBLs) - producing Enterobacteriaceae (Walther *et al.*, 2016).

When using prophylactic antimicrobials, it should be considered the degree of contamination of the surgery to be performed, the choice of a specific antimicrobial, which targets the pathogenic bacteria, the history of antimicrobial use of the specific animal, the tissue concentration, the cost and side effects. (Chou *et al.*, 2020).

The most used and indicated antimicrobial at pre-surgical level has been cefazolin, due to its efficacy, spectrum, for being well tolerated, having a low rate of adverse effects and being low cost (Gonzalez *et al.*, 2017).

7.1. Methicillin-Resistant *Staphylococci*

Methicillin-resistant Staphylococci (MRS) are an ongoing and increasing problem in veterinary and human medicine. The MRS contain an additional protein that binds to penicillin, conferring resistance to beta-lactam antibiotics (Walther *et al.*, 2016). MRSA are commonly found in humans and animals, whereas MRSP seem to have a higher prevalence and clinical importance in dogs and cats than in humans (Feßler *et al.*, 2018).

7.1.1. Methicillin-Resistant *Staphylococcus aureus*

MRSA is a leading pathogen with antimicrobial resistance. It is responsible for causing a variety of infections worldwide, ranging from mild to severe life-threatening infections (Aires-de-Sousa, 2016).

The first report of MRSA infection in animals occurred in Belgium in the 1970s, in a case of bovine mastitis. In relation to human contact with companion animals, an outbreak of feline-borne MRSA infections in humans occurred in 1988 in a geriatric home in the United Kingdom due to a colonised resident feline. Close contact between pets and their tutors creates optimal conditions for transmission of MRSA, such that the same tutors are more frequently colonized with MRSA than the general population. This suggests that companion animals also act as reservoirs for MRSA, and that it is an important pathogen in veterinary medicine, especially at the zoonotic level (Aires-de-Sousa, 2016).

Reports from ten years ago tell that the prevalence of MRSA in cats (n=540) in the London area were 1.8% (Loeffler *et al.*, 2011). In a more recent study, a total of 179 cats were

used to isolate MRSA and MRSP, of which were 8.4% and 1.1%, respectively (Elmoslemany *et al.*, 2021).

7.1.2. Methicillin-Resistant *Staphylococcus pseudintermedius*

Although transmission of MRSP from animals to humans is uncommon, even though there are described cases of it, transmission from one animal to another is a common concern in veterinary medicine (Walther *et al.*, 2016; Feßler *et al.*, 2018). Hospitalisation is considered an important risk factor for MRSP infections, showing the impact of veterinary hospitals as a source of MRSP transmission in hospitalised animals. Severe clinical cases presenting with MRSP isolates often reveal limited or no therapeutic options, possibly resulting in long-term suffering of these patients, or euthanasia, based on animal welfare (Walther *et al.*, 2016).

A study made in 2021 in healthy (n=595) and sick (n=81) cats reports that the prevalence of *Staphylococcus pseudintermedius* was 2.49% and 7.61%, respectively. When comparing with MRSP the results were 0.12% in healthy and 2.98% in sick cats (Bierowiec *et al.*, 2021).

Despite the existence of numerous reports on the incidence and spread of MRSP, data on the investigation of epidemiological outbreaks are relatively limited (Walther *et al.*, 2016).

7.2. Extended Spectrum Beta-Lactamases - producing Enterobacteriacea

One of the most used antimicrobials in veterinary medicine worldwide are β-lactams. However, over time and due to their frequent use, resistance to them has been created, namely the Extended-spectrum β-lactamases (ESBLs) which are enzymes that have resistance to most beta lactam antibiotics, including penicillin, cephalosporins, and the monobactam aztreonam (Belas *et al.*, 2021). The most frequent bacteria that produce ESBLs are *Escherichia coli* and *Klebsiella pneumoniae*, which are major sources of hospital-acquired infections in human and veterinary medicine (Belas *et al.*, 2021).

In a study by Belas *et al.* (2021), concludes that the appearance of ESBL-producing Enterobacteriaceae increased significantly with prophylactic use of antimicrobials. It also indicates that 20% of dogs were already colonised with ESBL-producing Enterobacteriaceae prior to surgery and before they were admitted to hospital (Belas *et al.*, 2021).

ESBL infections are associated with increased morbidity and mortality. Sometimes, they are difficult to identify, and their prevalence rates continue to rise, pointing to the need for action at hospital level, by restricting the use of broad-spectrum cephalosporins, for example, through the strict implementation of antimicrobial policies, and effective cleaning of the environment (Ghafourian *et al.*, 2015).

8. Objectives

The aim of this study is to determine whether there is a difference between the use of different concentrations of chlorhexidine and between the two scrub methods used in pre-surgical antisepsis: linear and circular, in cats. To achieve this objective, samples were cultured for bacterial identification and colony forming units (CFUs) were counted for comparison.

II. Material and Methods

1. Sampling procedure and collection of data

For this project, 51 female cats with surgical indication for elective ovariectomy were selected, and no breed or weight criteria were used. All animals belonged to colonies (outdoor cats) and were rescued by the municipal kennel of Vila Franca de Xira, thus any previous or recurrent pathology was unknown. Animals with visible dermatological pathologies in the sample collection area were excluded. Sample collection for the project was carried out in February 2022 and the bacteriological inoculation and identification methods were carried out during February and June 2022, both took place at the veterinary hospital in the University *Lusófona de Humanidades e Tecnologias*, in Lisbon, Portugal. Institutional Ethics and Animal Welfare Commission approval was obtained (119-2022) for the study, and an approved informed client consent form was signed.

All cats were submitted to pre-medication with dexmedetomidine (20 μg kg^{-1}) and methadone (0.2 mg kg^{-1}), via an intramuscular injection, anaesthetic induction with an intravenous injection of propofol to effect (usually 2-4 mg kg^{-1}) and maintenance with isoflurane were monitored with a multiparametric monitor. After sedation of the animals, the ventral abdomen was clipped between the xiphoid process and the pubis with a previously sterilised clipper blade (Aesculap® shearing machine).

Then, the surgical field was first washed with compresses dampened in saline solution (NaCl 0.9%) until dirt, hair, and debris were completely removed. Once inside the OR, the first two samples were collected by apposition with sterile swabs, one from the left hemiabdomen and the other from the right hemiabdomen. The surgical field antisepsis was made with random division of the animals into three groups: Antiseptic group 1 (A1), which included samples G1 (1st feline from which samples were collected) to G17, and in which a single solution of 2% chlorhexidine and 70% isopropyl alcohol was used; antiseptic group 2 (A2), included samples G18 to G34, and in which a 2% chlorhexidine solution and 70% ethyl alcohol was used; and finally antiseptic group 3 (A3), which included samples G35 to G51, and in which a 1% chlorhexidine solution and 70% ethyl alcohol was used. Five passages with sterile compresses were performed in the A1 group and five alternating passages between the chlorhexidine and alcohol, ending the cleaning with the first one, always with sterile compresses, were performed in the groups A2 and A3. In all animals, antisepsis was performed using the CM on the left

hemiabdomen, where the cleaning started at the incision site working towards the periphery in circular movements; and the LM on the right hemiabdomen, using a back-and-forth motion in the incision area working towards the periphery in an up-and-down motion (Figure 7). After 3 minutes for the antiseptics to act and the skin to dry, two more samples were collected: one from the animal's left hemiabdomen and another from the right hemiabdomen and properly identified and preserved in microbial culture methods. Thus, four samples were collected per animal, resulting in a total of 204 samples (Figure 8).

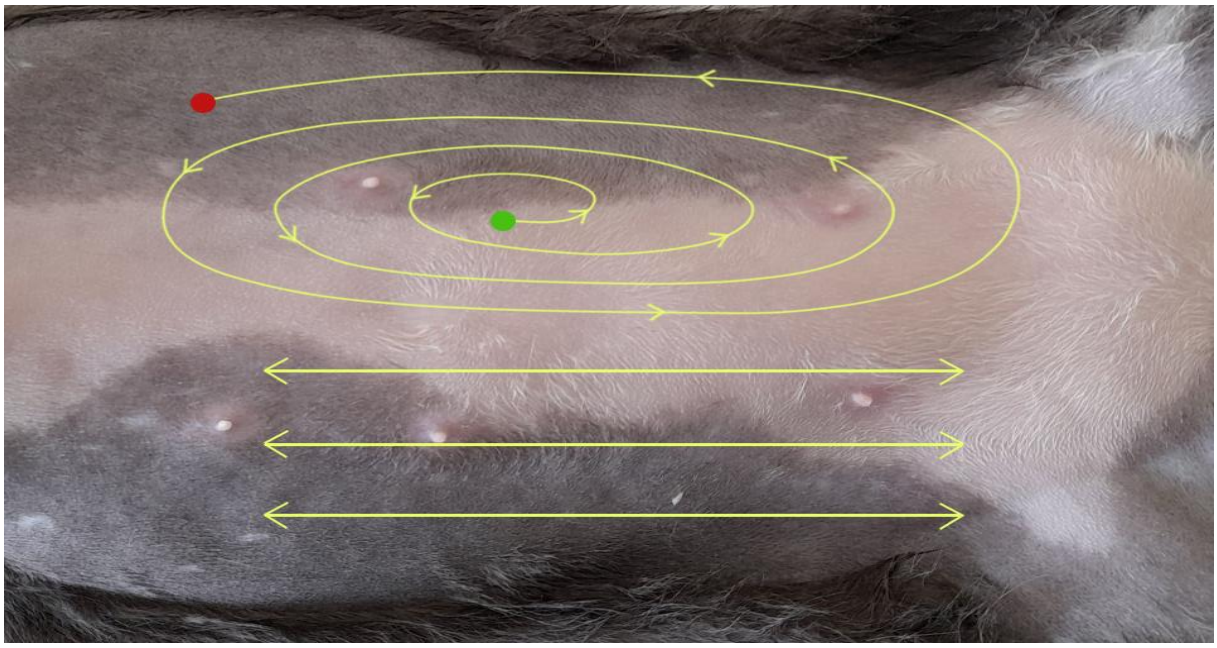

Figure 7. *Illustration of how the circular and linear methods were performed on all animals. The green circle marks the beginning and the red circle the end of the cleaning. The arrows indicate the direction of the movement. (Original picture from the author).*

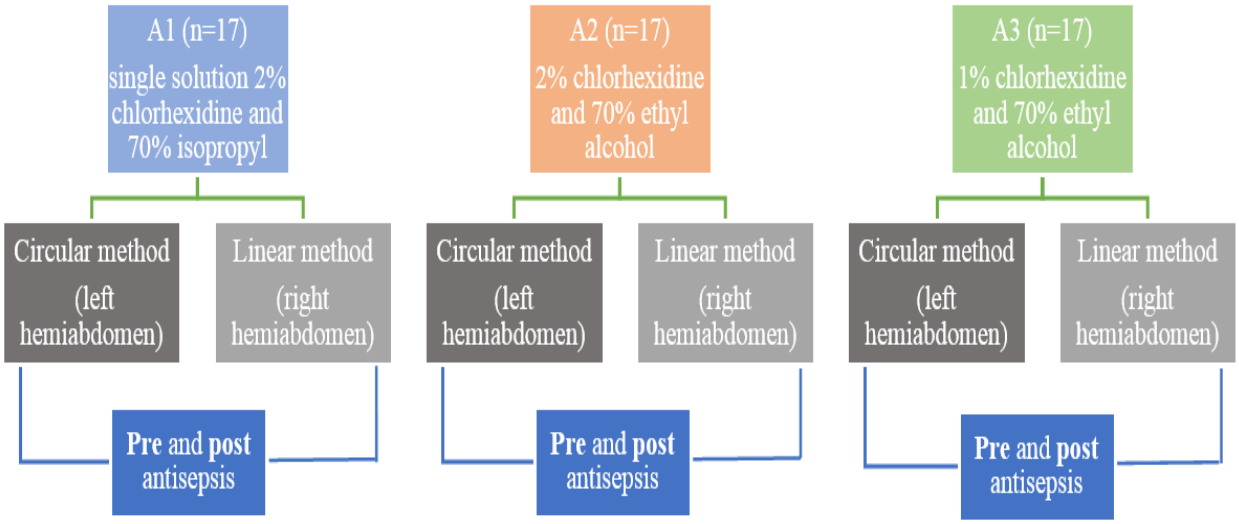

Figure 8. *Sample collection diagram. (Original picture from the author).*

To avoid contamination of the surgical field, washed and disinfected surgical pyjamas were used, as well as surgical cap and disposable gloves that were changed between each patient.

2. Bacteriological Methods

Swabs taken from the skin were inoculated in 1 ml of NaCl 0.85% and then 100 µl of this sample was taken and inoculated into various culture plates [Tryptic soy agar (TSA) (Biokar Diagnostics, France), Mannitol Salt Agar (MSA) (Biokar Diagnostics, France) and MacConkey agar (MCK) (Biokar Diagnostics, France)] using the quadrant streak method (Figure 9) for observation of bacterial growth.

Furthermore, Brilliance™ MRSA 2 plates (Oxoid, Spain) were used to detect methicillin-resistant *Staphylococcus* spp., and Brilliance™ ESBL Agar plates (Oxoid, Spain) were used to detect ESBLs.

All plates were properly labelled with the sample number, whether it was collected pre or post antisepsis (control or antiseptic group number), the method used (linear or circular) and date, and then incubated at 37°C for 48 hours. After this time, colonies were counted and registered as "colony forming units" (CFUs) in the TSA plates. The MSA, MCK, MRSA and ESBL plates were observed to see if there was any growth, thus facilitating identification of the bacteria. Finally, the plates with bacterial growth were repotted onto UriSelect 4 (URI) (Bio-

Rad Laboratories Ltd, Hemel Hempstead, UK) plates to arrive at a final presumptive identification of the bacteria.

The isolated strains were preserved in BHIB (brain heart infusion brith) with 20% glycerol at -80ºC.

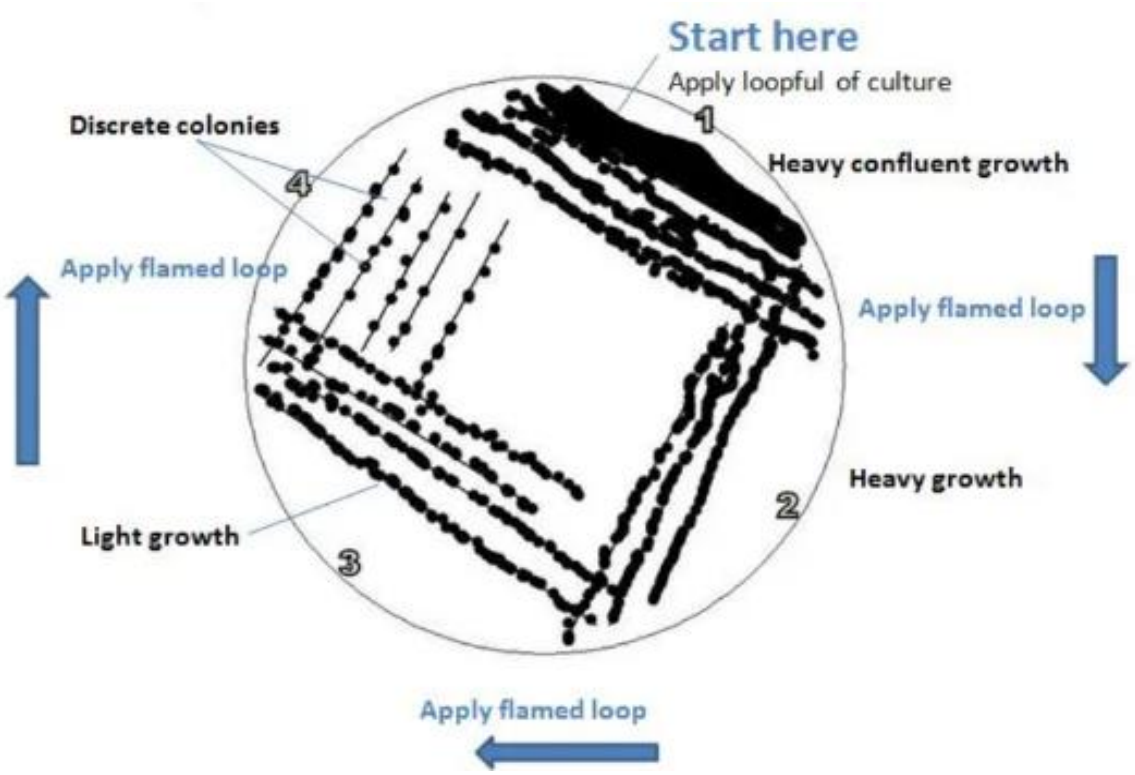

Figure 9. *How to perform the quadrant streak method (Adapted from "Streak plate method: Principle, procedure, uses. In Microbe Online. Retrieved July 26, 2022, from https://microbeonline.com/streak-plate-method-principle-purpose-procedure-results/).*

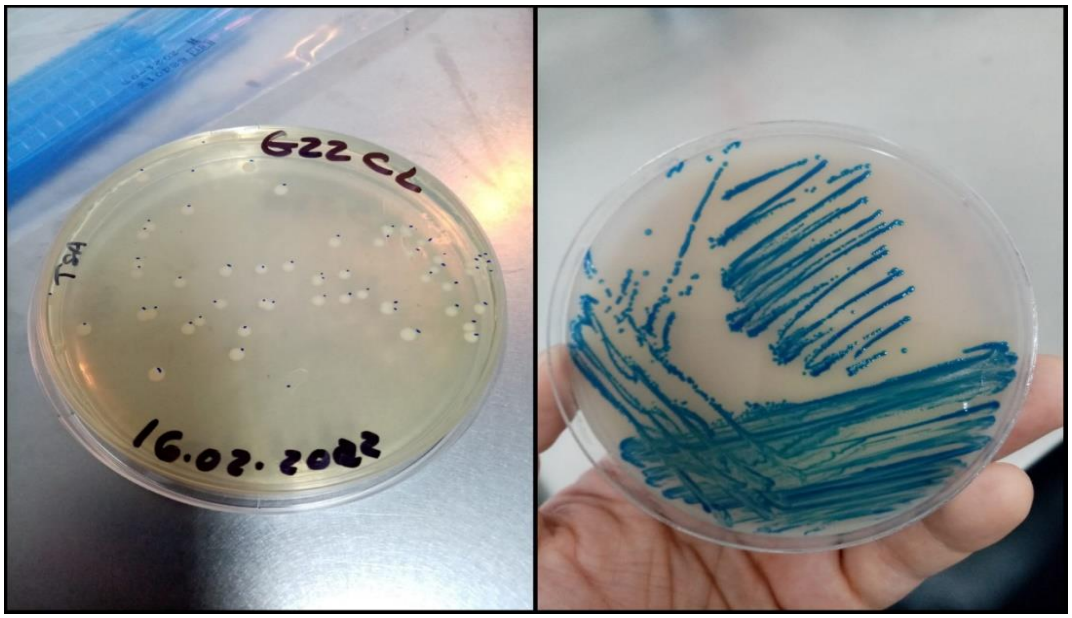

Figure 10. *Examples of plaque identification and bacterial growth. (Original picture from the author).*

Figure 11. *Types of chlorhexidine used, in order: 1% chlorhexidine solution; 2% chlorhexidine and 70% isopropyl alcohol single solution; 2% chlorhexidine solution. (Original picture from the author).*

3. Statistical analysis

Statistical analysis was performed using SPSS software (v.28.0). All quantitative data were tested for normal distribution using the Shapiro-Wilk test. Since data were not normally distributed (p <0.05), non-parametric tests were selected. Wilcoxon signed rank tests were performed on CFUs count to identify any statistically significant differences between pre and

post antisepsis in the three antiseptic solutions and the two antisepsis scrub techniques. Mann-Whitney U Test was performed to determine if there was any difference between variation of CFUs in the two antisepsis scrub techniques in each antiseptic group. Finally, Kruskal-Wallis test was performed to determine if there was any difference on CFUs variation inside each antisepsis scrub technique for the three antiseptics. The results were considered statistically significant when $p \leq 0.05$.

4. Future perspectives

The bacterial conservation carried out makes it possible to conduct phenotypic (identification of all bacteria) and genotypic (search for resistance) characterization in a future study.

III. Results

In total, the number of samples with pre antisepsis growth was 72 and post antisepsis 32, indicating that 56% of the microorganisms initially identified were eliminated in all groups.

Table 1. *Comparison of the number of samples with growth in pre and post antisepsis in all antiseptic groups.*

	Antisepsis	**A1**		**A2**		**A3**		Total
		CM	**LM**	**CM**	**LM**	**CM**	**LM**	
Hemiabdomen*(n)*		17	17	17	17	17	17	102
Number of samples with growth *(n)*	**Pre**	11	14	9	13	14	11	72
	Post	8	5	3	4	7	5	32

A1 – 2% chlorhexidine + 70% isopropyl alcohol; A2 – 2% chlorexidine + 70% ethyl alcohol; A3 – 1% chlorhexidine + 70% ethyl alcohol; CM – Circular method; LM – Linear method; n – number of samples

1. Pre *vs*. post antisepsis

The mean CFU count observed on the pre antisepsis plates in the CM on A1 was $1.0x10^5 \pm 4.1x10^5$, on A2 $2.1x10^4 \pm 8.7x10^4$ and on A3 135±169. In the LM in A1 the total number of CFUs observed was $4.7x10^5 \pm 1.3x10^6$, $2.8x10^3 \pm 8.6x10^3$ in A2 and 122±362 in A3. In contrast, the mean number of CFUs in the post antisepsis in the CM in A1 was 24±54, in A2 2±4 and in A3 12±28. In the LM the means were 16±35 in A1, 44±169 in A2 and 15±48 in A3.

The difference and variation in the number of CFUs between pre and post antisepsis in both methods in each antiseptic group were calculated and registered. The difference being the result of subtracting the number of CFUs in the pre antisepsis with the post. The variation corresponds to the percentage of the difference.

In group A1 in the CM a difference of $1.0x10^5$ CFU and a variation of 80% was observed, while in the LM a difference of $4.7x10^5$ CFU and a variation of 50% was found. In both methods there was a significant decrease in the number of CFUs from pre antisepsis to post antisepsis (***p*=0.021** and ***p*=0.005**, respectively) (Table 2).

Table 2. *CFU values of pre and post antisepsis in the circular and linear method in group A1.*

Scrub technique		Mean ± SD	Min.	Max.	Mean 95% CI		Dif.	Var.	*p*
					LB	UB			
CM	Pre	$1.0x10^5$±$4.1x10^5$	0	$1.0x10^6$	$-1.1x10^5$	$3.1x10^5$	$1.0x10^5$	80%	0.021
	Post	24±54	0	200	-4	51			
LM	Pre	$4.7x10^5$±$1.3x10^6$	0	$4.0x10^6$	$-2.0x10^5$	$1.1x10^6$	$4.7x10^5$	50%	0.005
	Post	16±35	0	120	-2	35			

CI – Confidence interval; CM – Circular Method; LM – Linear Method; Dif. – Difference; LB – Lower boundary; UB – Upper boundary; SD – Standard deviation; Min – Minimum; Max – Maximum; p – Significance; Var. – Variation.

In the group A2, the difference and variation were $2.1x10^4$ and 87% in the CM and $2.8x10^3$ and 100% in the LM. In both methods there was significant difference in the pre and post antisepsis (***p*=0.021** and ***p*=0.014**, respectively) (Table 3).

Table 3. *CFU values of pre and post antisepsis in the circular and linear method in group A2.*

Scrub technique		Mean ± SD	Min.	Max.	Mean 95% CI		Dif.	Var.	*p*
					LB	UB			
CM	Pre	$2.1x10^4$±$8.7x10^4$	0	$3.6x10^5$	$-2.3x10^4$	$6.6x10^4$	$2.1x10^4$	87%	0.021
	Post	2±4	0	10	0	4			
LM	Pré	$2.8x10^3$±$8.6x10^3$	0	$3.6x10^4$	$-1.6x10^3$	$7.3x10^3$	$2.8x10^3$	100%	0.014
	Post	44±169	0	700	-43	131			

CI – Confidence interval; CM – Circular Method; LM – Linear Method; Dif. – Difference; LB – Lower boundary; UB – Upper boundary; SD – Standard deviation; Min – Minimum; Max – Maximum; p – Significance; Var. – Variation.

In the group A3, the difference and variation were 123 and 89% in the CM and 108 and 68% in the LM. There was a significant difference in the pre and post anitsepsis in the CM (***p*=0.005**), however in the LM there was no statistically relevant difference between pre and post antisepsis (***p*=0.064**) (Table 4).

Table 4. *CFU values of pre and post antisepsis in the circular and linear method in group A3.*

Scrub technique		**Mean ± SD**	**Min.**	**Max.**	**Mean 95% CI**		**Dif.**	**Var.**	***p***
					LB	**UB**			
CM	**Pre**	135±169	0	500	48	222	123	89%	0.005
	Post	12±28	0	100	-2	27			
LM	**Pre**	122±362	0	$1.5x10^3$	-64	309	108	68%	0.064
	Post	15±48	0	200	-10	39			

CI – Confidence interval; CM – Circular Method; LM – Linear Method; Dif. – Difference; LB – Lower boundary; UB – Upper boundary; SD – Standard deviation; Min – Minimum; Max – Maximum; p – Significance; Var. – Variation.

In the CM, in all groups of antiseptics, the variation in samples that presented microbial load was greater than 99% and in the LM the variation was 100%, which indicates that there was practically total elimination of microorganisms in both methods in general (Figure 12). There was one outlier in the CM (95% variation) and at least three in the LM (99%, 98% and 96%).

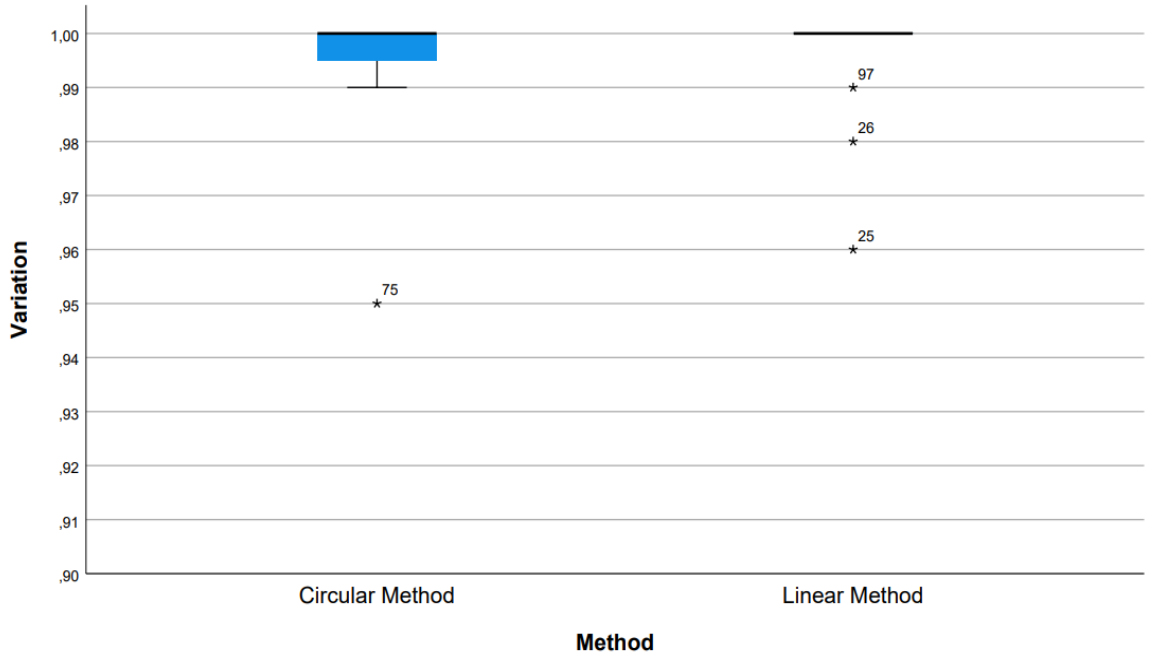

***Figure 12**. Simple boxplot of the variation by method. Variation scale in percentages: 1,00 means that 100% of CFUs were eliminated.*

Regarding the antiseptic groups in both methods, the variation was 100% in A1 and A2 with the presence of three outliers in A1 (99%, 98% and 96%) and one in A2 (99%). In group A3, 75% of the samples had a 95% variation and the remaining 25% had a variation between 88% and 95% (Figure 13).

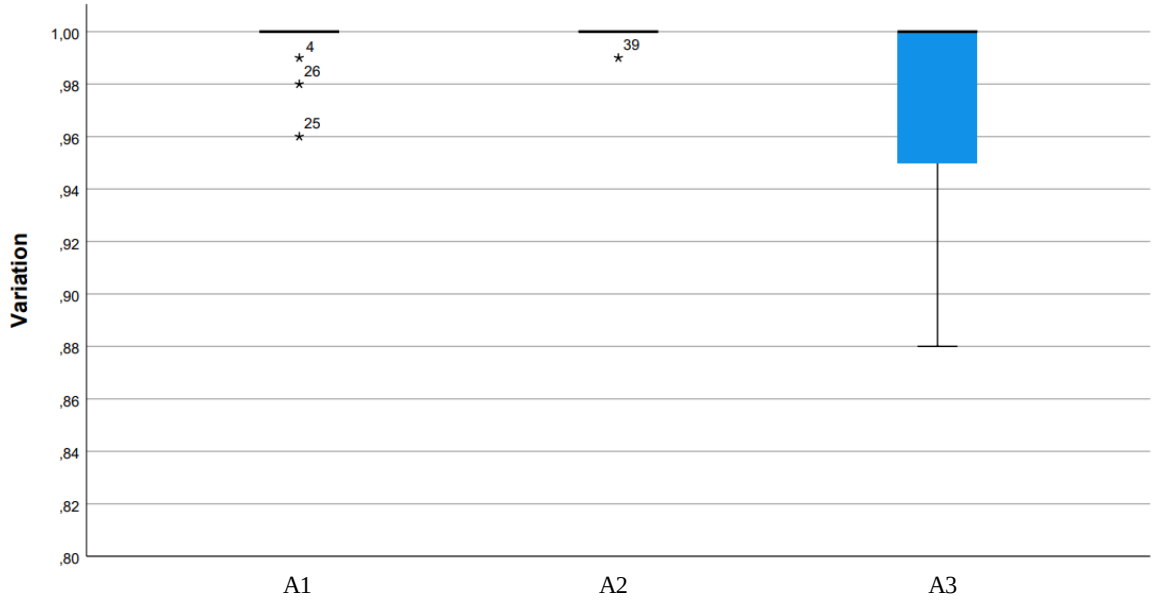

***Figure 13**. Simple boxplot of the variation by antiseptic group.*

2. Antiseptics

2.1. A1

Within the group A1 there were a total of 25 samples with bacterial growth and therefore with variation, with 11 observed in the CM and 14 in the LM. When comparing the two methods within this group there appears to be no difference between the two (***p*=0.774**). In the CM all samples presented 100% of variation, with only one outlier (99%), having, in average, eliminated all CFUs. In the LM, 75% of the samples had at least 99% of variation, being 98% the lowest value reached. There was only one outlier (96%) (Figure 14).

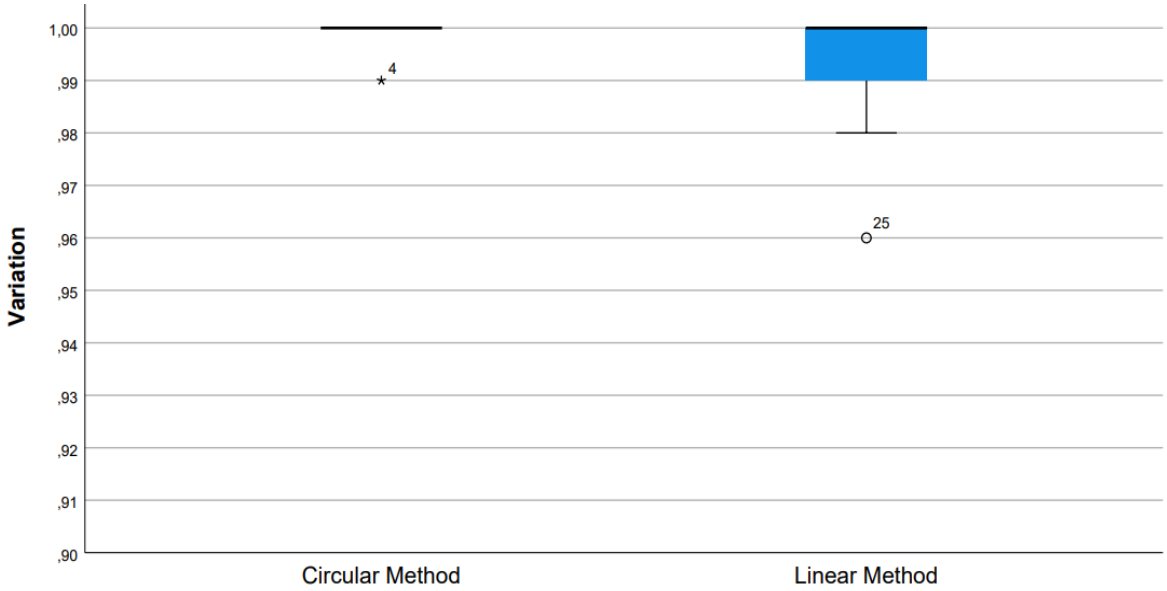

Figure 14. *Simple boxplot of the variation by method for the A1 group.*

2.2. A2

In group A2 there were 22 samples with variation, 9 in the CM and 13 in the LM. In the CM the lowest value reached was 99% and in the LM all samples presented 100% variation, meaning there was total elimination of CFUs (Figure 15). When comparing the two methods there appears to be no significant difference (***p*=0.164**).

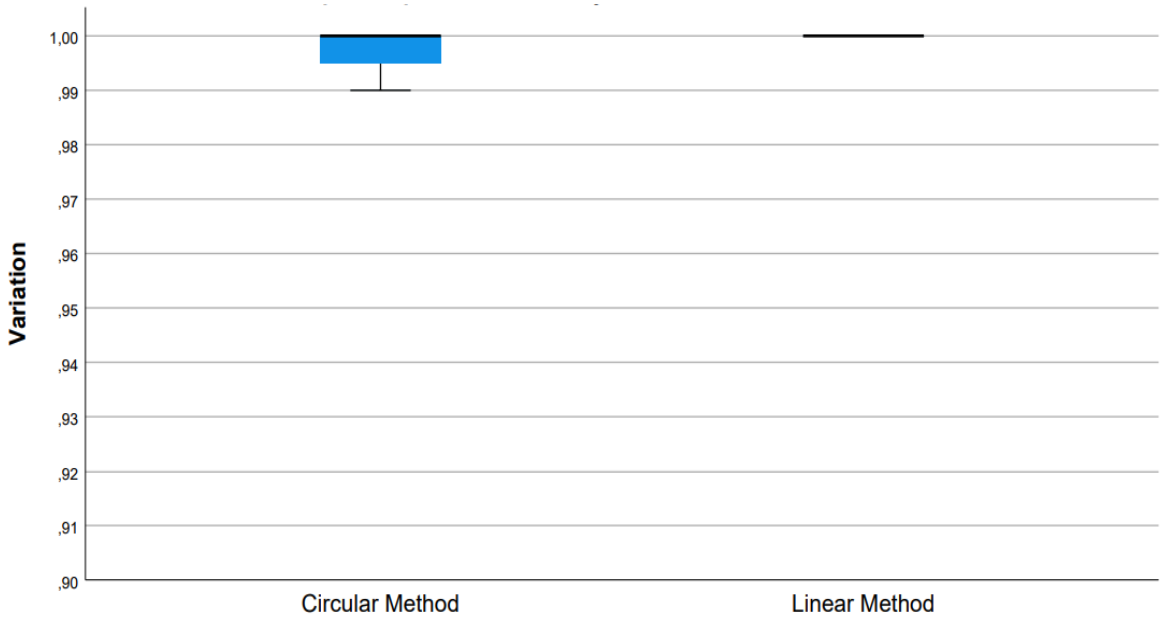

Figure 15. *Simple boxplot of the variation by method for the A2 group.*

2.3. A3

In group A3 there were a total of 25 samples with variation, 14 of them in the CM and 11 in the LM. In the CM 75% of the samples had a variation greater than 95%, with ≃87% variation as the lowest value. There was the presence of one outliner (≃67%). In the LM despite the median being 100% of variation, 75% of samples were above ≃74%, and 25% were between 50% (lowest value achieved across all antiseptic groups) and ≃74%. Despite this, there is no significant difference between the two methods within this group (***p*=0.570**) (Figure 16).

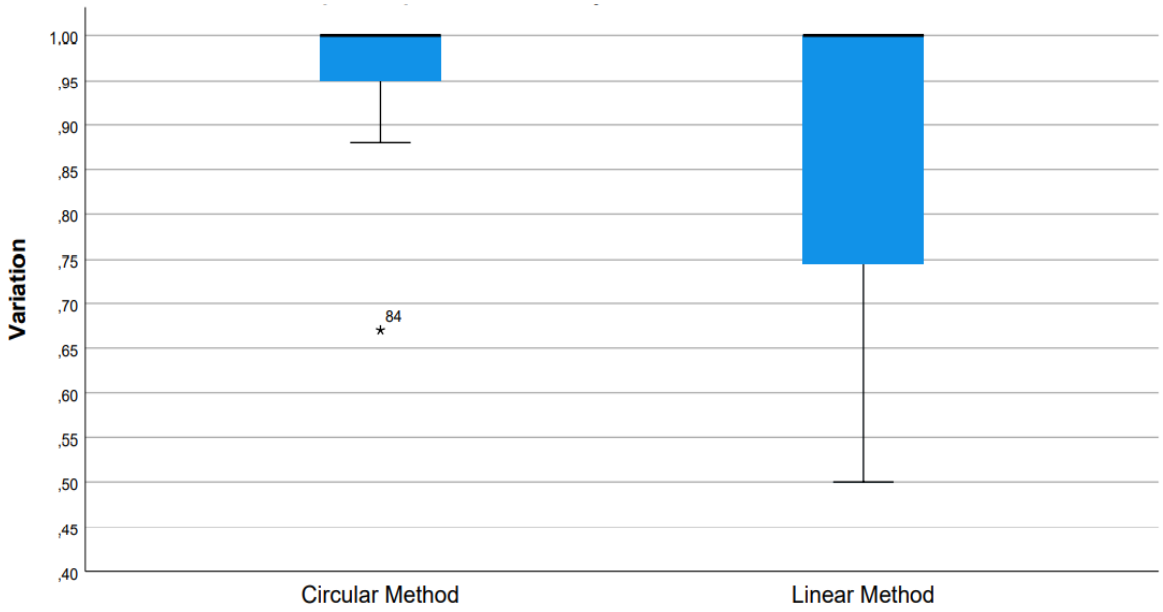

Figure 16. *Simple boxplot of the variation by method for the A3 group.*

3. Antisepsis scrub techniques

3.1. Circular method

In the circular method there were 11 samples with variation in group A1, 9 in group A2 and 14 in A3; resulting in a total of 34 samples with variation in this method. When comparing the three groups within this method there appears to be no significant difference (***p*=0.902**).

3.2. Linear method

In the linear method there were 14 samples with variation in group A1, 13 in A2 and 11 in the A3 group; this results in a total of 38 samples with variation. When comparing the three groups in this method it seems there's no significant difference (***p*=0.117**).

4. Skin colonization

Regarding bacterial growth, 118 samples were observed and 5 with the presence of fungi. Of the bacteria identified 88% were gram-positive and 12% gram-negative. Within the gram-positive bacteria 70% were observed in the pre and 30% in the post antisepsis; in the gram-negative bacteria 86% were identified in the pre and 14% in the post antisepsis. (Figure 17).

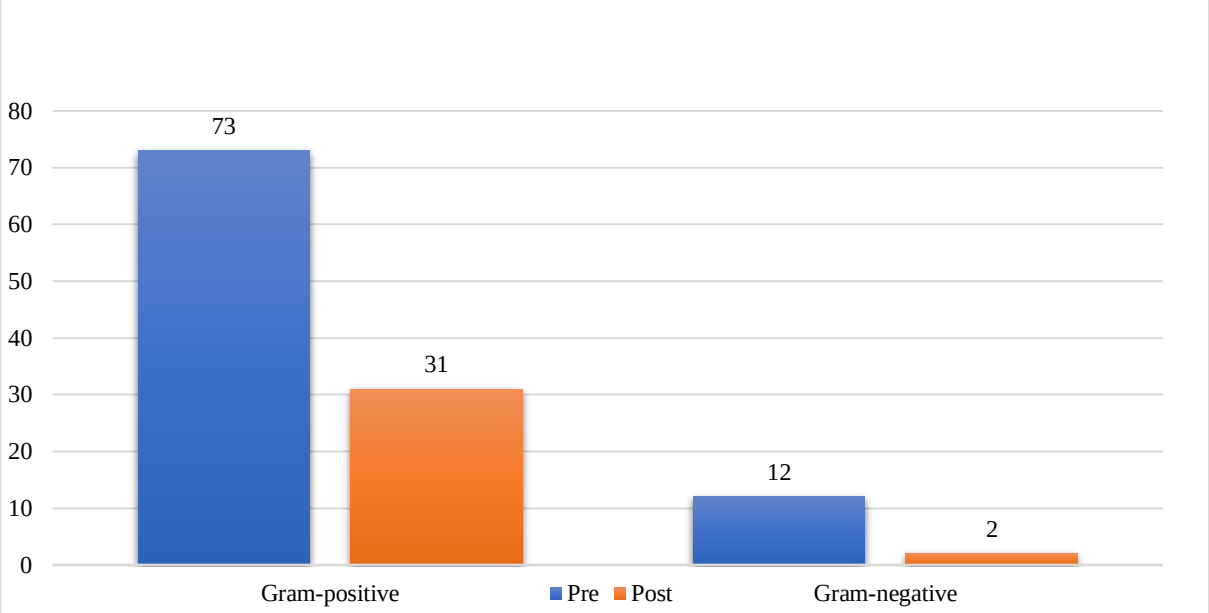

Figure 17. *Number of samples with bacterial growth (gram-positive and gram-negative).*

In total, 123 microorganisms were observed in all samples, of which *Staphylococcus spp.* (46%), *Enterococcus spp.* (11%), *Pseudomonas spp.* (5%), *Micrococcus spp.* (3%), Non-pathogenic fungi (4%), *Proteus spp.* (2%), MRSA (2%), *Klebsiella spp.* (1%), *Escherichia coli* (1%), MRSP (1%) were identified. The remaining 24% belonged to the category of unidentified bacteria, in which 87% were gram-positive and 13% gram-negative (Figure 18).

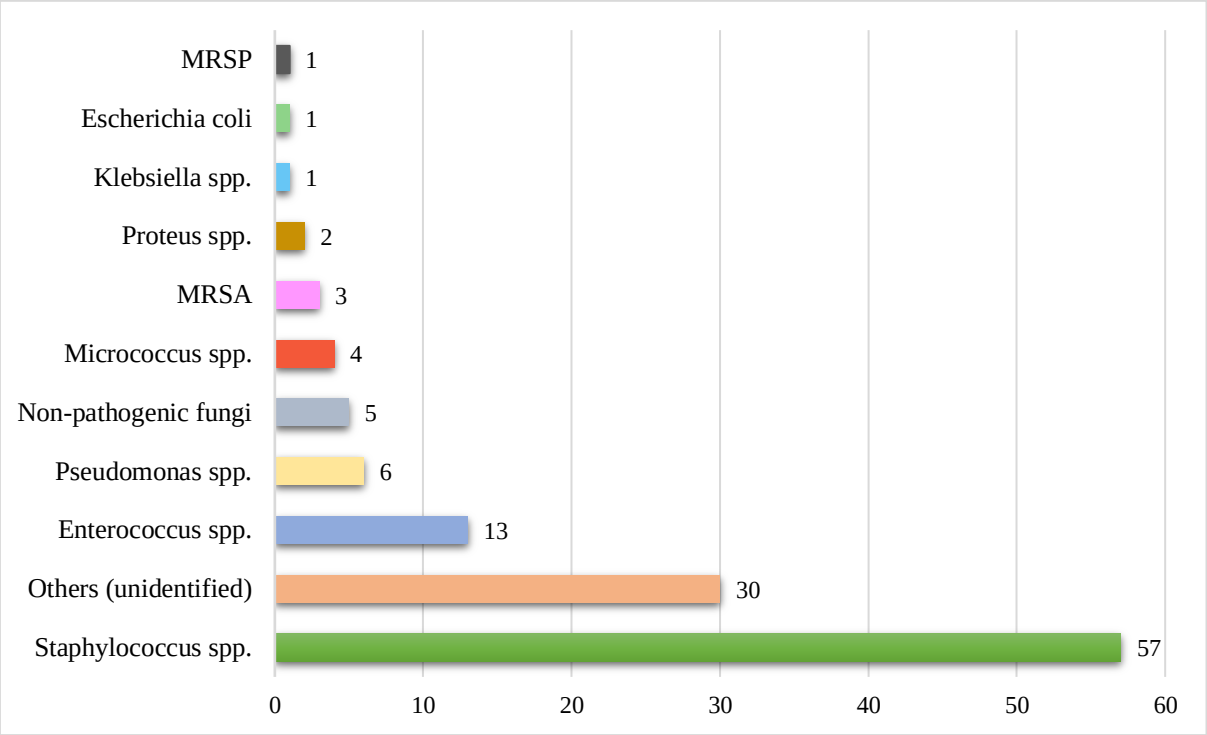

Figure 18. *Number of samples with microbial growth and identification.*

The three samples where MRSA was identified were part of the A2 CM, A3 LM and CM groups, all pre antisepsis. One MRSP was identified in the A1 LM group pre antisepsis. Both MRSA and MRSP found represented 3% of all discovered bacteria and were all eliminated by the respective antiseptics.

IV. Discussion

Currently, there are still doubts about which antiseptics, concentrations, or preparation methods are more effective for surgical field antisepsis to prevent SSIs, with studies recommending chlorhexidine (Medeiros *et al.*, 2018), others povidone-iodine (Osuna *et al.*, 1990), and others stating that there is no difference between antiseptics (Marchionatti *et al.*, 2022). However, in human medicine, recommendations generally indicate a superior efficacy in the prevention of SSIs associated with the use of 2% chlorhexidine with alcohol (Guenezan *et al.*, 2021). Therefore, this study aimed to assess the effectiveness of different concentrations of chlorhexidine and two different methods of antisepsis, in order to detect whether there are differences between them.

All antiseptic solutions and both scrub methods were effective in eliminating CFUs, with the exclusion of group A3 only in those samples where the linear method was used, proving to be the least effective in terms of pre and post antisepsis results (Tables 2, 3 and 4). However, comparing the different groups of antiseptics within each method considering the variation (subtraction of the number of CFUs in the pre with the post antisepsis) there was no difference in the use of each (p >0.05).

It is described that there is no significant difference in the use of 1% or 2% chlorhexidine in human neonates (Sharma *et al.*, 2021). However, other reports conclude that the higher the concentration of chlorhexidine the greater its antiseptic action (Stinner *et al.*, 2011). This would explain the fact that the only chlorhexidine concentration that had no impact on the elimination of microorganisms in this study was the lowest 1% (Table 4). It would be relevant in the future to use a higher concentration (4% for example) for comparison with the others.

Although the CM is still the most routinely used in veterinary hospitals, several studies comparing both methods (linear and circular) have concluded that there is no advantage to using one or the other (Swales & Cogan, 2017; Monstrey, 2022). This study supports what is described so far in veterinary medicine, as there was no significant difference in using the linear or circular method in each antiseptic group (Figures 14, 15 and 16).

In total there were more gram-positive than gram-negative bacteria in the samples (Figure 16). Among the gram-positive bacteria, the bacteria with the highest incidence were *Staphylococcus spp.* followed by *Enterococcus spp.*; among the gram-negative bacteria it was

Pseudomonas spp. followed by *Proteus spp.* (Figure 18). In the literature it is described that the main phyla identified were Proteobacteria and Firmicutes, in that order; in this study the main phyla identified was Firmicutes (to which belong *Staphylococcus spp.* and *Enterococcus spp*.), followed by Proteobacteria (where belong *Pseudomonas spp.* and *Proteus spp.*), differing in this sense (Older et al., 2019).

Currently, there is still limited information on the skin microbiota of cats, so this study contributes to the characterization of the skin microbiota especially in stray cats. Some of the bacteria observed could not be identified and were preserved together with the rest for future identification.

The patient's hair was clipped with a sterilised clipper blade which is thought to cause less trauma and therefore less incidence of SSIs than using a razor for shaving (Messiaen *et al*., 2019).

The surgery duration is directly related to the number of microorganisms that access the surgical wound, so the skills of the surgeon are important when it comes to keeping the surgical wound as clean as possible (Boucher 2017). In this study, all surgeries were performed by two senior surgeons with many years of practice and skill, which made the duration of it short.

Some authors suggest that the contact time for chlorhexidine and alcohol solutions could be only of 30 seconds (Genuit *et al.*, 2001), while others indicate a minimum of two minutes (Boucher 2017). For this study, the chlorhexidine action time in all animals was 3 minutes, a time considered ideal based on previous literature.

MDR agents continue to be epidemic in veterinary hospital settings, so active interventions to prevent their transmission and emergence, especially since some are zoonotic agents (MRSA), contribute to the concept of One Health. The appearance and spread of these agents in small companion animals such as cats are related to close contact with humans. This is particularly so when talking about MRSA, as it is an agent which is easily transmissible between humans and animals (Boucher 2017).

MRSP, on the other hand, is considered a recent serious problem that has been appearing more frequently in healthy animals mainly in environments with agglomerations of them (such as veterinary hospitals and kennels). It is a problem considered less severe than

MRSA transmission because it is less frequently transmitted from animals to their tutors, although reports already exist, however it is getting more common among companion animals (Couto *et al.*, 2016).

Despite being stray cats, MRSA (2%) and MRSP (1%) were also found in this study, but these were a very low percentage compared to what is usually found in indoor cats (Elmoslemany *et al.*, 2021). No ESBL were visualized in any samples.

Thus, guidelines on antimicrobial use as well as infection control programs regarding staff, patients and environment (such as regular personal and environmental sanitation) in small companion animal clinics and hospitals are needed to improve antimicrobial management and prevent the emergence and transmission of antimicrobial resistances (Boucher 2017).

Given the constant increase in antimicrobials resistance and the regular use of antimicrobials prophylactically even in regular surgeries such as ovariohysterectomies, it would be desirable to decrease their use since this study shows that essentially all groups of antiseptics and both methods used (except for the A3 group in the LM) were effective in eliminating most of the bacterial load present in the skin of cats, which could cause SSIs.

Regarding limitations, we can consider the sample size (low number of animals), and as they were stray cats, it was not possible to follow up the cases post-surgery, to know if they developed skin reactions or even SSIs.

There is still little information available on antisepsis in veterinary medicine, especially in cats. Thus, this study is pioneer in that it compares three different types of antiseptic solutions as well as the two methods (linear and circular) described in pre-surgical cleaning. Furthermore, because there is not much information regarding the characterization of the cat's skin microbiota, this study presents results regarding this topic that may also be innovative in veterinary medicine.

V. Conclusion

All antiseptics used (single solution of 2% chlorhexidine and 70% isopropyl alcohol; 2% chlorhexidine and 70% ethyl alcohol; 1% chlorhexidine and 70% ethyl alcohol) in the circular method showed efficacy in eliminating microorganisms present on the skin during pre-surgical antisepsis. In the linear method, only the A3 group (1% chlorhexidine and 70% ethyl alcohol) was not effective in eliminating microorganisms. However, there was no statistical difference in the use of the three antiseptics, nor in the use of the circular or linear method.

The bacteria with the highest incidence were *Staphylococcus spp.*, *Enterococcus spp.* and *Pseudomonas spp.*. Three samples with MRSA and one with MRSP were found and eliminated by the respective antiseptics used.

Further studies on the subject should be carried out in order to elect the most effective antiseptics and cleaning methods, contributing to the decrease in the appearance of antibiotic resistances.

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VII. Appendices

Appendix I. Surgical antisepsis protocol used.

Protocolo de Antissepsia cirúrgica em OVH / OVE

Data:

Identificação Animal (nome e nº doente)	**Identificação Proprietário**

Pré-medicação:

Indução:

Manutenção:

Método:

1. Tricotomia do abdómen ventral entre o processo xifóide e o púbis com uma lâmina previamente esterilizada (máquina tosquia Aesculap®);
2. Realizar uma primeira lavagem com compressas humedecidas em NaCl até remoção completa de sujidade, pêlos e detritos;
3. Recolha 1ª zaragatoa esterilizada, após a tricotomia e lavagem e antes da antissepsia campo. As amostras serão enviadas para cultura aeróbios para avaliar crescimento bacteriano e isolamento bacteriano.
4. No quadrante abdominal esquerdo aplicar mediante o método circular, cinco passagens (alternadas em 2 e 3, terminado com clorexidina) p de um dos 3 esquemas antissepsia (1- solução única clorexidina a 2% + álcool isopropílico a 70% //2- clorexidina 2% e álcool etílico a 70%// 3-solução clorexidina 1% + álcool etílico a 70%), com luvas e compressas estéreis.
5. No quadrante abdominal direito aplicar mediante o método linear, cinco passagens (alternadas em **2 e 3**, terminado com clorexidina) pde um dos 3 esquemas antissepsia (**1**- solução única clorexidina a 2% + álcool isopropílico a 70% //**2**- clorexidina 2% e álcool etílico a 70%// 3-solução clorexidina 1% + álcool etílico a 70%), com luvas e compressas estéreis
6. Aguardar 3 minutos após a última passagem do antisséptico e deixar secar pele;
7. Recolher 1 zaragatoa de cada quadrante com uma zaragatoa estéril após a evaporação dos antissépticos com posterior colocação no meio de cultura associado.

Responsável: _____

Appendix II. The content of this thesis was presented (Poster presentation) in the XVIII Congresso Internacional Veterinário Montenegro, 4th-5th November 2022, Portugal.

EFFECTIVENESS IN ANTISEPTIC PREPARATION OF THE SURGICAL FIELD

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Introduction

In any invasive surgery there is an incision site that will originate the so-called surgical wound, which can be contaminated in various ways (Yool, 2012). Therefore, antisepsis of the surgical site is extremely necessary, as it allows for a removal of microbial agents that may cause local infection, as well as the use of prophylactic antibiotics, eliminating or restricting growth of microorganisms (Yool, 2012; Rahman *et al.*, 2018). The use of antimicrobial agents in the prevention of surgical site infections (SSIs), together with the cleaning of the surgical field, contributes to the emergence of bacterial resistance (Shaikh *et al.*, 2015). There are two methods of preparing the skin of the surgical field: the linear method, with a "back and forth" motion, and the circular method, which starts at the centre, working outwards towards the edges of the surgical site (Swales & Cogan, 2017).

This study aims to test an antiseptic (single solution of 2% chlorhexidine and 70% isopropyl alcohol) and to compare two methods of antisepsis (linear and circular) of the surgical field.

Materials and Methods

Swabs from surgical field skin were collected from healthy rescues cats (n=17) that were admitted to elective ovariohysterectomy. The first two samples were collected by sterile swabs apposition, one from the left hemiabdomen and the other from the right hemiabdomen. Five passages with sterile compresses with a single solution of 2% chlorhexidine and 70% isopropyl alcohol were performed, using the circular method on the left hemiabdomen and the linear method on the right hemiabdomen (Figure 1). After 3 minutes, one sample per hemiabdomen was collected. Thus, a total of 68 swab skin samples were taken and sent to microbiology laboratory, where the bacteria were isolated accordingly with microbiology procedures. Furthermore, Brilliance TM MRSA 2 medium was used to detect methicillin-resistant *Staphylococcus spp.*, and Brilliance™ ESBL Agar medium was used to detect Extended-Spectrum-Beta-Lactamases (ESBLs)-Enterobacterales producing. Data were analysed by non-parametric methods using SPSS software (v.28.0). The results were considered statistically significant when $p \leq 0.05$.

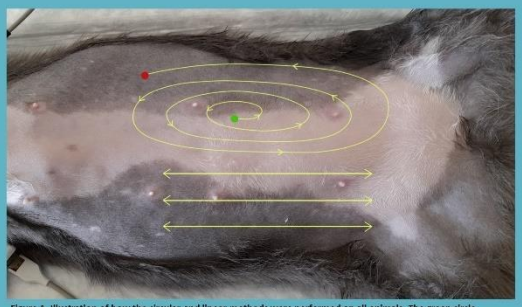

Figure 1. Illustration of how the circular and linear methods were performed on all animals. The green circle marks the beginning and the red circle the end of the cleaning. The arrows indicate the direction of the movement.

Figure 2. Pre and post-antiseptic bacterial quantification in the circular and linear method.

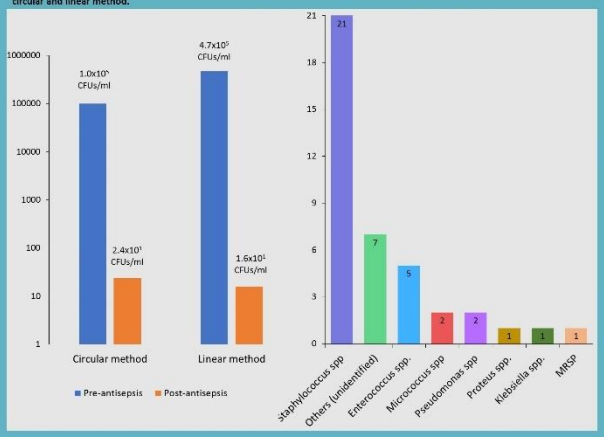

Figure 3. Number and identification of isolated bacteria.

Results

In the circular method, the bacteria load mean of pre-antisepsis and post-antisepsis were $1.0x10^5 \pm 4.1x10^5$ CFUs/ml and 24±54 CFUs/ml, respectively. In the linear method for pre-antisepsis and post-antisepsis were $4.7x10^5 \pm 1.3x10^6$ CFUs/ml and 16±35 CFUs/ml (Figure 2). Regarding pre- and post-antisepsis swab samples for the circular method and linear method there was statistical significance (**p=0.021** and **p=0.005**, respectively) regarding bacterial load. However, the mean variation in CFUs pre- and post-antisepsis for both methods showed no statistical evidence to differentiate the use of the two methods (**p=0.774**). Furthermore, the most frequent bacteria agent found was *Staphylococcus spp.* (53%, n=21/40), followed by unidentified bacteria (18%, n=7/40) and *Enterococcus spp.* (13%, n=5/40) (Figure 3). The presence of one MRSP was only observed in the pre-antiseptic linear method.

Conclusions

The use of a single solution of 2% chlorhexidine and 70% isopropyl alcohol with circular method or linear method was successful in eliminating most of the bacteria present on the cats skin. Currently, in small animal veterinary medicine, the circular method is the most used antisepsis method (Swales & Cogan, 2017). However, with this study no difference was observed in the use of both methods. Further studies on the subject should be carried out to choose the most effective antiseptics and cleaning methods, contributing to the reduction of antimicrobial bacteria resistance and SSIs.

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