BIOTEKNOLOGI
Volume 15, Number 1, May 2018
Pages: 13-25

ISSN: 0216-6887
E-ISSN: 2301-8658
DOI: 10.13057/biotek/c150102

Phenotypic and molecular characterization of Methicillin Resistant *Staphylococcus aureus* from surgical patients and normal dogs

CECILIA NJOROGE, JOHN DEMESI MANDE♥, SIMON ERIC MITEMA, JAFRED M.A. KITAA
Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi. P O BOX 29053, Nairobi, Kenya. Tel.: +254 733 725 212, +254 713 023 440, ♥email jdmande@uonbi.ac.ke, jdemesi@gmail.com

Manuscript received: 26 October 2017. Revision accepted: 15 April 2018.

Abstract. *Njoroge C, Mande JD, Mitema SE, Kitaa JMA. 2018. Phenotypic and molecular characterization of Methicillin Resistant Staphylococcus aureus from surgical patients and normal dogs. Bioteknologi 15: 13-25.* The objectives of this study were to determine bacterial ecology and their antimicrobial susceptibilities from a wound and ear swabs with emphasis on *Staphylococcus aureus* and to determine the prevalence of MRSA/MRSP in normal dogs and surgical patients using phenotypic and genotypic assays. The findings revealed that the most prevalent microbial isolates recovered from dogs diagnosed with wounds, surgical site infections, and otitis externa, were *S. aureus* 50% (133/267) and *Proteus* spp. 14% (38/267). Other frequently recovered isolates included *Pseudomonas* spp. 10% (28/267), other *Staphylococcus* spp. 8.2% (22/267), *Streptococcus* spp. 6.7% (18/267), and *E. coli* 5.6% (15/267). Resistance to antimicrobial drugs was observed in the majority of the isolates in the retrospective study, with 97% (262/267) of the isolates demonstrating antimicrobial resistance to at least one drug. Resistance to sulphonamides (96%), potentiated sulphonamides (89%), ampicillin (68%), amoxicillin (62%) and tetracycline (56%) was relatively high for all bacterial species examined. *S. aureus* isolates showed 95% resistance to sulfamethoxazole, 55% to ampicillin, 52% to tetracycline and 52% to amoxicillin/clavulanic acid. *Pseudomonas* spp. showed the highest multidrug resistance with all (100%) isolates showing resistance to amoxicillin, amoxicillin/clavulanic acid and sulfamethoxazole, the isolates also showed high resistance to cotrimoxazole (93%), ampicillin (93%) and tetracyclines (80%). Low resistance to gentamicin (9%), norfloxacin (24%) and chloramphenicol (33%) was observed in all bacterial isolates. Data from the prospective study revealed that presumptive *Staphylococcus* spp. were isolated from 34% (65/191) of the samples. Coagulase-positive *Staphylococcus* spp. (COPS) accounted for 43% (28/65) of the *Staphylococcus* spp. isolated. Phenotypic resistance to oxacillin was detected in 53.6% (15/28) of COPS. Further analysis of the resistant determinants by BLAST revealed that all the resistant *Staphylococcus* strains were *S. aureus* strains. This study confirms *S. aureus* as the most prevalent bacterial isolate from wounds, surgical site infections, and otitis externa. *Proteus* spp., *Pseudomonas* spp., other *Staphylococcus* spp., *Streptococcus* spp. and *Escherichia coli*, in descending order, were also frequently isolated. Gentamicin, norfloxacin, and chloramphenicol, in that order, were the most effective antimicrobial agents in the management of wounds, surgical site infections and otitis externa in the retrospective study. The study reports the first case of MRSA strains in dogs in Kenya, which were associated with mobile genetic elements (*SCCmec*) and had the potential to be transferred from dogs to humans. The MRSA resistant determinants observed are like some human-like isolates reported in several countries.

Keywords: Dog, methicillin resistant, *Staphylococcus*, surgical patient

INTRODUCTION

Staphylococcal species are commensal bacteria and leading causes of community and hospital-associated disease in humans and animals worldwide (Vengust et al. 2006). The most clinically relevant staphylococci in veterinary medicine are the coagulase positive *Staphylococcus aureus* and members of the *Staphylococcus intermedius* group, particularly *Staphylococcus pseudintermedius* (Weese and Duijkeren 2009). Although *S. aureus* can colonize and infect companion animal species, the most common commensal staphylococci of canines is *S. pseudintermedius* (formerly *S. intermedius)* with isolation rates of between 46-92% of healthy dogs compared to 10% *S. aureus* (Hanselman et al. 2009; Rubin and Chirino-trejo 2011; Paul et al. 2011). *S. pseudintermedius* can be isolated from the nares, mouth, pharynx, forehead, groin, and anus of healthy dogs and cats. It is an opportunistic pathogen and a leading cause of skin and ear infections, infections of other body tissues and cavities, and post-operative wound infections in dogs and cats (Guardabassi et al. 2004; van Duijkeren et al. 2011).

Staphylococcal infections are frequently treated with antibiotics and, consequently, antibiotic resistance and⁄or acquired resistance have developed (Normand et al. 2000). The increasing prevalence of antimicrobial resistance has made staphylococcal infections more dangerous and costly to treat. Of considerable concern are Methicillin-resistant *S. aureus* (MRSA) and the emergence of Methicillin-resistant *S. pseudintermedius* (MRSP) in dogs and cats. These resistant strains of bacteria pose a new threat to animal health due to the limitations of their management (EMA 2010).

MRSA was first identified in the United Kingdom and was then recognized as a nosocomial pathogen worldwide (HA-MRSA) (Petinaki and Spiliopoulou 2012). Subsequently, there have been reports of MRSA infections occurring in people with no exposure to a healthcare setting; these have been designated community-acquired (CA-MRSA). There are differences in the epidemiology of

HA-MRSA and CA-MRSA including resistance determinants, *SCCmec* types and clonal complexes. HA-MRSA has also been found to be resistant to more antimicrobials than CA-MRSA and to be responsible for more invasive infections (Cohn and Middleton 2010). In animals, methicillin resistance was first documented in the early 1970's, after isolation of MRSA from a dairy cow with mastitis. The emergence of MRSA in livestock and people in contact with the animal, have introduced a new epidemiological dimension to MRSA infections. These strains are designated LA-MRSA and are phenotypically and genotypically distinct from the HA-MRSA and CA-MRSA genotypes (Petinaki and Spiliopoulou 2012). Although pet animals, especially dogs and cats, may become contaminated, colonized, or infected with *S. aureus*, including MRSA, these species are not believed to be natural reservoir hosts for *S. aureus*. The MRSA strains found in companion animals are frequently identical to human epidemic strains of MRSA, making it more likely that MRSA originates from a person than a pet (Cohn and Middleton 2010). Majority of MRSA infections in dogs and cats appear to be in high-risk patients and are acquired by direct contact with human carriers (Duquette and Nutall 2004).

The MRSA isolates in dogs have been associated with clinical samples from surgical site infections, wound infections (Baptiste et al. 2005; Vincze et al. 2014), catheter site infections, urinary tract infections, pneumonia, and skin infections (Vengust et al. 2006). These observations demonstrate the clinical importance and therapeutic challenge of MRSA in the management of conditions of dogs and cats.

Methicillin-resistant *S. pseudintermedius* (MRSP) has recently emerged in small animals worldwide and represents a significant challenge for small animal practitioners due to its characteristic multidrug resistance phenotype (Paul et al. 2011) and its characteristics of a nosocomial pathogen (Frank and Loeffler 2012). It has been isolated from various conditions, including wound infections, otitis externa and canine pyoderma (Beck et al. 2012). An essential aspect of MRSA and MRSP control is the identification of potential sources of exposure.

There are limited reports of MRSA in humans in Kenya; Maina et al. (2013) found MRSA prevalence of 84.1% amongst *S. aureus* isolated from patients with skin and soft tissue conditions. A different study by Aiken et al. (2014) reported low carriage rate of *S. aureus* (85/950) in hospitalized patients, with only 7.0% of these isolates being MRSA. There is limited data in the literature on the prevalence, as well as phenotypic and molecular characteristics of microbial isolates from normal and surgical conditions in dogs in Kenya. Mande and Kitaa (2005) found *S. aureus* as the most common isolate from ear swabs of dogs suffering from otitis externa and reported multidrug resistance among bacterial strains. Available records in the University of Nariobi (UoN), Small Animal Clinic laboratory indicated frequent isolation of bacteria from dogs and cats. However, no systematic data or meta-analysis was available describing the full extent of the phenotypic and molecular characteristics of the different types of microbial isolates in dogs in Kenya.

This study was therefore designed with the aim of addressing the identified gap in the knowledge and skills to improve the therapeutic and clinical management of dogs undergoing surgical or medical procedures in Kenya.

The specific objectives of the study were: (i) To determine retrospectively the prevalence of common bacterial flora in isolates from wounds surgical site infections and otitis externa with emphasis on *Staphylococcus aureus*. (ii) To determine the antimicrobial susceptibility profiles of the various bacterial isolates to antibiotics used in the antimicrobial susceptibility tests. (iii) To assess the prevalence of MRSA/MRSP in normal dogs and surgical patients using phenotypic and genotypic tools. (iv) To sequence resistant PCR amplicons and validate the sequences.

MATERIALS AND METHODS

Study site

The study was undertaken at the UoN Small Animal Clinic, Upper Kabete. This facility receives patients mostly from the suburbs of Nairobi region and its environs. It also serves as a referral center for cases from other small animal clinics in Kenya.

Study design

This study involved both retrospective and prospective components. The retrospective study component involved a review of microbial isolates and antibiogram data from the bacteriology laboratory of samples submitted from surgical patients and dogs with otitis externa at the UoN Small Animal Clinic. The prospective component was a cross-sectional study that involved sampling of surgical patients and normal dogs presented at the UoN Small Animal Clinic and a Community veterinary clinic located in Nairobi County.

Retrospective study: Survey of common bacterial isolates from wounds and otitis externa and their respective antimicrobial susceptibility profiles

Animal patient biodata

The bacteriology laboratory records of clinical samples submitted between January 2004 and December 2013 were investigated. All the samples were from animals presented to the UoN's Small Animal Clinic during the study period. The records were examined to retrieve data on culture samples of dogs and cats presented with otitis externa and wounds. Animal biodata extracted from these records included: date of submission, sex, and the site where the sample was collected from (wound or ear swab).

Bacterial profile

For each clinical sample submitted, the number of microbial isolates and microorganisms isolated from either wounds or ear swab were recorded. The total number of various bacterial flora isolated were calculated and expressed as percentages. Bacteria of the Genus

Staphylococcus were recorded as *S. aureus* or broadly classified as other *Staphylococcus* spp. (for those that did not fit the characteristics of *S. aureus* in biochemical tests).

Antimicrobial susceptibility testing (AST)

Routine disk diffusion procedures were employed in AST by the laboratory. The bacterial isolates were tested against a panel of 8 antimicrobial agents namely, ampicillin (2µg), gentamicin (10µg), cotrimoxazole (25µg), chloramphenicol (10µg), tetracycline (10µg), potentiated amoxicillin (amoxicillin-clavulanic acid) (30µg), norfloxacin and sulfamethoxazole (25µg). Various bacteria in the AST were scored by the laboratory as either being susceptible or resistant to the respective antibiotic. If the zone of inhibition around the disk was found to be ≤14mm, the organism was scored as being resistant to that drug.

Wound characteristics

Patient case records, from which wound and abscess swabs were collected, were retrieved for further review. Information recorded for analysis included the cause and location (body region) of the wound or abscess swab.

Data analysis

All data were entered into a spreadsheet (Microsoft Excel 2010) and a pivot table generated. The frequency of the various parameters (species, breed, sex) over the study period was calculated and expressed as percentages. The total number of bacterial flora isolated was estimated and expressed as percentages. Antimicrobial susceptibility was shown as either susceptible or resistant. Overall resistance for each antimicrobial agent was calculated. Percentage resistance for each bacterium was calculated for each antimicrobial agent.

Prospective study: Prevalence of MRSA/MRSP in dogs

Study population

The following formula was used to calculate an appropriate sample size for the study

$$n = \frac{1.96^2\, p(1-p)}{d^2}$$

Where:
p = Estimate of the expected proportion (15%)
d = Desired level of absolute precision (0.05)

An estimated MRSA prevalence of 15% (Bond and Loeffler 2012) in the population was used at 95% confidence interval. From the formula, we estimated our sample size to be 196 samples.

A total of 191 dogs were enrolled in this cross-sectional study, which entailed convenience sampling at the UoN Small Animal Clinic and a Community Owned Clinic. Criteria for inclusion necessitated: dogs of any age, sex, breed and obtaining written consent from the owner or attending veterinarian to collect samples; preference was given to dogs presented for surgery, those with wounds and otitis externa. A brief questionnaire was filled by the owner or attending veterinarian to obtain information on the patient including biodata like breed, sex, age, presenting complaint, history of the condition (first time/recurrent) and prior treatment administered (antibiotic use) in the past three months preceding the study.

Sample collection

Sampling was carried out between March 2014 and June 2015. Samples were collected from four sites on the affected surgical patients and normal dogs, specifically, anterior nares, buccal mucosa, perianal area, a wound swab if the patient presented with a wound, and an ear swab in patients presenting with otitis externa. A sterile cotton-tipped swab moistened with sterile normal saline was used to collect samples by swabbing the sites above. A separate swab was used for each anatomic location and swabs from each dog were pooled in a bijou bottle containing 3 ml of transport medium (Stuart's medium) and transported to the laboratory where they were stored in a refrigerator at 4^{o}C awaiting processing.

Bacteriological examination

Recovery of isolates. Samples were removed from the refrigerator and kept at room temperature for 4 hours before being cultured onto a nutritive medium, tryptone soya broth supplemented with 6.5% NaCl for selective enrichment of *Staphylococcus.* After incubation at 37^{o}C for 24 hrs, a loopful of broth was taken and cultured to Mannitol Salt Agar (MSA), a selective medium and incubated at 35^{o}C for 24-48 hrs. The growth of yellow colonies on this medium and color change of the media to yellow was taken as positive fermentation of mannitol and presumptive *S. aureus* (Kateete et al. 2010). Pink colonies on mannitol salt agar were also sub-cultured and designated as presumptive *S. pseudintermedius.*

The presumptive *S. aureus* or *S. pseudintermedius* colonies were sub-cultured on 5% sheep blood agar (SBA) and incubated at 37^{o}C for 24 hours to isolate a pure culture. Those SBA plates that did not show any growth after 24 hours were incubated for a further 24 hours. Final identification of the presumptive coagulase positive *Staphylococcus* spp. characteristic colonies were by colonial morphology, gram stain reaction, and positive catalase and coagulase tests. The presumed staphylococcus colonies were subjected to a Gram stain and the slide examined under a light microscope to check for gram reaction, size, and shape of the colonies. Gram-positive cocci, that appeared as grapelike clusters in pairs and singles, were presumed to be *Staphylococcus* spp.

Biochemical tests for confirmation

Catalase test. A sterile loop was used to pick organisms from the plate and place them on a slide. A drop of 3% Hydrogen peroxide was added to the slide and mixed with the organisms. Visualization of bubbles was regarded as a positive reaction.

Tube coagulase test. This test was performed by transferring a single colony of inoculum to 1 ml of reconstituted rabbit plasma. The two were mixed by gently rotating the tubes. The tubes were then incubated at 37^{o}C

and evaluated after 24 hrs. Formation of a clot in the tube was taken as a positive reaction. Presumptive coagulase positive Staphylococcus colonies were sub-cultured on Tryptic soy agar, awaiting susceptibility testing.

Catalase test. A sterile loop was used to pick organisms from the plate and place them on a slide. A drop of 3% Hydrogen peroxide was added to the slide and mixed with the organisms. Visualization of bubbles was regarded as a positive reaction.

Tube coagulase test. This test was performed by transferring a single colony of inoculum to 1 ml of reconstituted rabbit plasma. The two were mixed by gently rotating the tubes. The tubes were then incubated at 37°C and evaluated after 24 hrs. Formation of a clot in the tube was taken as a positive reaction. Presumptive coagulase positive Staphylococcus colonies were sub-cultured on Tryptic soy agar, awaiting susceptibility testing.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed according to the Kirby-Bauer disc diffusion method. A sterile loop was used to pick organisms from the tryptone soy agar plate.

The organisms were added to a tube containing 4.5 ml of sterile physiological saline. The mixture was vortexed to create a smooth suspension. The turbidity of the suspension was adjusted to 0.5 McFarland standard. A sterile swab was dipped into the inoculum suspension. The Mueller Hinton (MH) plate was then inoculated by streaking across the agar surface ensuring that the entire plate was covered. The lid of the plate was left slightly open for 3-5 minutes for the agar surface to dry up.

Oxacillin was used as the surrogate antibiotic to methicillin (CLSI 2008). Oxacillin (1 µg) discs (HiMedia Laboratories Pvt. Ltd, Mumbai, India) were peeled from the cartridge using forceps. The lid of the MH agar was lifted to allow placement of the discs on the agar surface. Once the disc was placed, it was gently pressed with forceps to ensure total contact with the agar surface. Plates were incubated at 35-37°C for 24 hrs. The zone diameters of complete inhibition, including that of the discs, were measured to the nearest whole millimeter using a ruler. To measure the zones of inhibition, the ruler was held on the back of an inverted petri dish while keeping it a few inches from a black non-reflecting background illuminated with reflected light.

For each isolate, antimicrobial susceptibility testing was done in duplicate and the mean zone diameter of inhibition calculated. The resistance zone diameter of <17mm around a one µg oxacillin disc was used as an indicator for methicillin resistance as recommended by Bemis et al. (2009) and approved by the Clinical and Laboratory Standards Institute (CLSI) subcommittee on Veterinary Antimicrobial Susceptibility Testing (CLSI 2013).

Molecular identification and PCR detection of *mecA*

Isolates found to be resistant were amplified by polymerase chain reaction (PCR). *S. aureus* ATCC 25923 served as the reference quality control strain. Primer pairs, sequences and amplicon size of primers used in the PCR reactions are shown in Table 2.

DNA extraction

Extraction of DNA was performed as described by Diaz-Campos (2012). Two or three colonies were obtained from 18 -48 hours cultures inoculated on tryptic soy agar (4.1%) and suspended in 400 µL of sterile distilled water. The bacterial suspension was boiled at 95°C for 7 minutes and then centrifuged at 15,000 *g* for 1 min and the supernatant collected. The DNA supernatant extracts were stored at -20°C until used as a template for the PCR reactions.

Validation of isolates

Amplification of 16S rRNA gene of all strains was performed at first to confirm that they were *Staphylococcus* strains. This was accomplished in a protocol adapted from Kondo et al. (2007). PCR reaction was done in a total volume of 20 µL containing 5 µL of DNA template and 0.25 µL of primers Staph-F and Staph-R. Thermal cycling reactions consisted of initial denaturation at 94°C for 10 min; followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 50°C for 15 s, extension at 72°C for 1 min; and a final elongation at 72°C for 5 min. Amplification products were analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide. Gels were visualized under U.V light. Amplification of the 416bp PCR product indicated the strain to belong to the genus *Staphylococcus.*

Identification of coagulase positive staphylococci

Primers for species identification were designed to amplify a portion of the *nuc* gene. The procedure used was adapted from Asfour and Darwish (2014). The reaction was established in 25 µL reaction volume containing 10 µL of DNA as a template. The amplification cycles were carried out in a thermocycler. Reaction conditions were optimized to be 94°C for 5 min, as initial denaturation, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 60 seconds. A final extension step at 72°C for 10 min was followed. DNA isolated from *S. aureus* ATCC 25923 was used as positive control. Amplification of 295 bp and 381 bp indicated the isolate to be *S. aureus* and *S. pseudintermedius* respectively.

Detection of mecA

Detection of the *mec*A gene was performed as previously described by Kondo et al. (2007). PCR reaction was performed in a final reaction volume of 25 µL containing 5 µL of DNA template. Amplification was done in an MJ minicycler (MJ Research Inc., USA) under the following conditions: initial denaturation at 94°C for 2 minutes, followed by 30 cycles of 94°C for 2 minutes, annealing temperature at 57°C for 1 minute, extension temperature at 72°C for 2 minutes, and a final extension step of 72°C for 2 minutes. A 1.5% agarose gel was used for electrophoresis after staining with ethidium bromide. Gels were visualized under ultraviolet illumination. A 100 bp DNA ladder was run simultaneously as a DNA marker. Amplification of the 286 bp band indicated the strains to harbor the *mecA* gene.

Sequencing of resistant genes

The PCR products obtained using gene-specific primers for resistance were purified and submitted for sequencing. The PCR products were purified with QIAquick PCR Purification Kit (Qiagen, USA). This was done to remove excess primers, salts and Taq polymerase, which can interfere with the sequencing reaction. The purified products, together with the forward and reverse primers initially used for the PCR detection of resistance, were submitted to International Livestock Research Institute (ILRI), Segolip laboratory for sequencing which was done using the ABI PRISM 3770 genetic analyzer (Applied Biosystems, US).

Basic Local Alignment Sequence Tool (BLAST) analysis

The BLASTN tool of the NCBI Genbank database (http: /blast.ncbi.nlm.nih.gov/Blast.cgi) was used to analyze the sequenced DNAs. The nucleotides were first read using GeneRunner software for further analysis. Analysis of the BLAST output was used to determine the *Staphylococcus* spp. harboring the assayed resistance genes, their geographical distribution, and hosts from which these homologs had been previously isolated. The homologs to the sequences including their nucleotide and amino-acid identity were identified using the BLASTN output.

Submission to NCBI GenBank

The sequenced resistance gene, that was longer than 200 bp, was submitted to the NCBI GenBank database for validation and assignment of an accession number.

Ethical issues

Ethical approval for the study was granted by the UoN's Faculty of Veterinary Medicine's Biosecurity, Animal Use and Ethics Committee (Ref No. 15/10). Informed consent was sought from the owners before sample collection. Permission to collect samples from the UoN Small Animal Clinic was requested and granted by the Chairman of the Department of Clinical Studies. The dogs used were the sole responsibility of the owner. Hospitalized dogs were housed in kennels located at the clinic.

RESULTS AND DISCUSSION

Retrospective study: Survey of common bacterial isolates from wounds and otitis externa of dogs and their antimicrobial susceptibility patterns

During the period between January 2004 and December 2013, a total of 291 samples were recorded from 191 individual dogs. The swab samples were obtained from wounds 27% (n=80) and ear infections 73% (n=211) respectively. Of these samples, growth was observed in 267 (92%) of the samples with 24 (8%) of the samples showing no growth after culture.

Animal patient biodata

The samples (n=291) were submitted from 200 dogs; of which of which 145 were sampled once, 34 sampled twice, 15 sampled thrice, 3 sampled four times, 2 sampled 5 times and one animal sampled 12 times over the study period. Adult animals accounted for 89% (178/200) compared to 6% (12/200) young animals. Males accounted for 68% (136/200) compared to 27% (53/200) females, while the sex of 11 animals was not indicated. Of the 200 samples from dogs, 119 (59.5%) were German shepherd dogs, 29 (14.5%) dogs were cross breeds, 8 (4%) Japanese spitz, 7 (3.5%) rottweilers dogs. The rest were breeds with 4 or less dogs in each breed.

Microbial isolates

The predominant isolates were *S. aureus* 50% (133/267) and *Proteus* spp. 14% *(38/267)*. Other frequently isolated bacteria included *Pseudomonas* spp. 10% (28/267), *Staphylococcus* spp. 8.2% (22/267), *Streptococcus* spp. 6.7% (18/267), *Escherichia coli* 5.6% (15/267). The frequency and source of isolation of the different spp. is represented in Table 3**.**

Table 2. Primer pairs and sequences used in the PCR reactions for identification of the Genus *Staphylococcus*, species identification of *Staphylococcus aureus* and detection of *mec*A medicated resistance.

Primer name	Sequence 5'-3'	Gene	Amplicon size
MecA1	F-TGC TAT CCA CCC TCA AAC AGG R-AAC GTT GTA ACC ACC CCA AGA	mecA	286bp
MecA2	F-AGA AAT GAC TGA ACG TCC GAT TT R-CAC CTG TTT GAG GGT GGA TAG	mecA	887bp
Sau	F-CGA AAG GGC AAT ACG CAA AG R-GGA TGC TTT GTT TCA GGT GTA TC	Nuc	295bp
Staph	F-GTA GGT GGC AAG CGTTAT CC R-CGC ACA TCA GCG TCA G	16S rRNA	416bp

Note: F-Forward Primer, R-Reverse Primer, Sau-*Staphylococcus aureus*

Table 3. Prevalence of bacterial isolates from clinical samples of wounds and ear swabs in dogs

Isolate	Ear (Percent)	Wound (Percent)	Total	Percent
Staphylococcus aureus	103 (48.8%)	30 (37.5%)	133	49.8
Proteus spp.	35 (16.5%)	3 (3.75%)	38	14.2
Pseudomonas spp.	26 (12.3%)	2 (2.5%)	28	10
Staphylococcus spp.	19 (9%)	3 (3.75%)	22	8.2
Streptococcus spp.	10 (4.7%)	8 (10%)	18	6.7
Escherichia coli	3 (1%)	12 (16.3%)	15	5.6
Corynebacterium spp.	3 (1.4%)	2 (2.5%)	5	1.9
Actinomyces pyogenes	1 (0.5%)	1 (1.25%)	2	0.75
Diphtheroids	1 (0.5%)	-	1	0.4
Klebsiella spp.	-	1 (1.25%)	1	0.4
Norcardia spp.	-	1 (1.25%)	1	0.4
Pasteurella spp.	-	1 (1.25%)	1	0.4
Total	201	66	267	100

Table 4. Resistance (%) of six bacterial isolates from dogs to various antimicrobial agents (n=262).

Antimicrobial agent	*S. aureus* n=139	Proteus n=40	Pseud n=28	*E. coli* n=15	Staph n=24	Strep n=19	Total n=262
Amoxycillin	69%	100%	100%	N/A	67%	N/A	62%
Amoxicillin/ Clavulanic	51%	58%	87%	100%	75%	46%	58%
Ampicillin	55%	94%	93%	79%	68%	65%	68%
Chloramphenicol	24%	45%	64%	33%	33%	7%	32%
Gentamicin	12%	0%	4%	8%	0%	29%	9%
Norfloxacin	28%	14%	15%	33%	21%	8%	22%
Tetracycline	50%	69%	79%	36%	50%	71%	56%
Cotrimoxazole	87%	97%	93%	93%	89%	67%	89%
Sulfamethoxazole	95%	100%	100%	91%	92%	100%	96%

Note: *S. aureus*-*Staphylococcus aureus*; Proteus-*Proteus* spp.; Pseud-*Pseudomonas* spp.; Staph; Other *Staphylococcus* spp; Strep-*Streptococcus* spp.

Staphylococcus aureus remained the most common isolate, regardless of the source of the sample. *Proteus* spp. were more frequently isolated in ear swabs (16.5%), than from wounds (3.7%). *Pseudomonas* spp. were also recorded as important pathogens in ear infections (12.3%) but were found to be minor pathogens in wound infections with isolation rate of 2.5%. *E. coli* was a common cause of contamination in wounds (16.3%) but did not seem to be an important cause of ear infections (0.95%).

Antibiogram profile

Resistance to antimicrobial drugs was observed in most of the isolates in the study, with 97% (262/267) of the isolates demonstrating antimicrobial resistance to at least one drug. Four isolates were not resistant to any drug and one of the isolates was a fungal, thus antimicrobial susceptibility was not done. Resistance to sulphonamides (96%), potentiated sulphonamides (89%), ampicillin (68%), amoxicillin (62%) and tetracycline (56%) was relatively high for all bacterial species examined (Table 4).

Staphylococcus aureus isolates displayed high multidrug resistance to sulfamethoxazole (95%), cotrimoxazole (87%), ampicillin (55%) and amoxicillin/ clavulanic acid (51%). Resistance to sulfamethoxazole was a common finding, with more than (90%) of the isolates being resistant to this drug. *Proteus* spp. isolates were 100% resistant to amoxicillin and sulfamethoxazole and showed high level resistance to ampicillin (94%), cotrimoxazole (97%) and tetracyclines (69%). All *Pseudomonas* spp. isolates (100%) were resistant to sulfamethoxazole and amoxycillin. High level resistances to ampicillin (93%), amoxicillin/clavulanic acid (87%) tetracyclines (79%) and chloramphenicol (64%) were also observed among the *Pseudomonas* spp. isolates (Table 4).

Low resistance to gentamicin (9%), norfloxacin (22%) was observed in all bacterial isolates. The results of antimicrobial susceptibility testing are presented in Table 4. Multidrug resistance was also observed with most of the isolates displaying resistance to 2 or more drugs (Table 5).

Table 5. Phenotypic multidrug resistance profiles displayed by the bacterial isolates from dogs to various antimicrobial agents

Resistance profile	Number of isolates resistant
COT, SXT	7
AMP**, COT**	5
AMP, COT, TET	7
AMP, COT, AMC, SXT	6
AMP, COT, TET, SXT	5
AMP, TET, AMC, SXT	5
AMP, COT, TET, AMC, SXT	7
AMP, CEF, COT, TET, AMC	6
AMP, COT, CHP, TET, AMC, SXT	6

Note: AMP-Ampicillin; AMC-Amoxicillin/Clavulanic Acid; COT-Cotrimoxazole; TET-Tetracycline; CEF-Cefaclor; CHP-Chloramphenicol; SXT; Sulfamethoxazole.

Wound characteristics

Of the 80 samples collected from wounds in the retrospective study, only 58% (46/80) of records were retrievable from the medical records. Wounds commonly involved the limbs of the affected animals, with hindlimbs (32.6%) more affected than forelimbs (27.8%). The head region was also frequently presented with wounds 8 out of 46 (17.3%), Table 6.

Surgical site infections were a more frequent source of wound swabs than other causes, representing 23.9% of the sources. Bite wounds and traumatic wounds were also frequently sampled for culture and susceptibility testing (Table 7). The cause of 18 wounds tested (33%) was not specified.

Prevalence of MRSA/MRSP from normal dogs and surgical patients

Clinical history and animal biodata

Samples from the Community veterinary clinic accounted for 103 (54%) of the samples, while 88 samples (46%), were collected from the University of Nairobi Small Animal Clinic. It was seen in 72 (37.7%) of the 191 dogs sampled, that wound (s) were present on their body. Males were the predominant dogs sampled accounting for 56% (n=107) of the samples, with 44% (n=84) being females. Majority of the animals (60%) had received antimicrobial treatment in the past three months before sampling.

Prevalence of staphylococci

All the 191 samples successfully formed colonies in the enrichment media (Tryptone soya broth). The selective media, MSA, detected 65 (34%) presumptive staphylococci species, the other samples yielded gram -ve bacteria, which were not considered for further screening. The *Staphylococcus* spp. Were subjected to a tube coagulase test, and only 28 (14.7%) of the isolates tested positive and thus designated coagulase positive staphylococci.

Phenotypic characterization of resistance

Antimicrobial susceptibility testing was done on the 28 coagulase positive isolates of which, 13 isolates (46.4%) were susceptible to oxacillin. Phenotypic resistance to oxacillin was observed in 15 isolates (53.6%).

Validation of isolates

The control PCR was performed to exclude any false positive results. It was done using a control primer pair targeting 416 bp fragment of 16S rRNA gene of genus Staphylococcus. Eleven out of the 15 presumptive coagulase-positive staphylococci were confirmed to be staphylococci (Figure 2).

Identification of coagulase-positive staphylococci (COPS)

This PCR assay was done to differentiate the COPS by amplification of the 295 bp and 381bp specific PCR product for *S. aureus* and *S. pseudintermedius* respectively. Out of the 11 confirmed *Staphylococcus* species, this assay identified 7 (63.6%) *S. aureus* strains. No *S. pseudintermedius* strains were detected in this study.

MecA gene

Two *mecA*-positive MRSA strains were isolated from two dogs (Figure 3). One of the strains was from the wound of a dog with a post-operative infection that resulted after inguinal herniorrhaphy, while the other was from a normal healthy puppy presented for vaccination.

BLAST analysis

Identification of DNA sequences. Analysis of the sequenced resistant determinants from the two samples revealed the genes were harbored by *Staphylococcus* spp. strains. The nucleotide sequence of isolate 1 (Lab ID: CS 100), was 97% identical to GenBank accession number AB547235.1, which is a *Staphylococcus sciuri mecA* gene and 96% identical to GenBank accession number KF058902.1 which is a *S. aureus mecA* gene.

The nucleotide sequence of isolate 2 (Lab ID: CS 148) revealed 99% nucleotide identity to sequences in the NCBI databases belonging to different *Staphylococcus* spp. This isolate was 99% identical to GenBank accession numbers KR187111.1, KP265312.1 and HE984157.2 which were *mecA* genes from *S. aureus*, *Staphylococcus epidermidis* and *S. pseudintermedius* respectively (Table 8).

Geographical distribution and host diversity of the homolog genes. The homologs containing the *mecA* gene showed a varied global distribution with isolates from Brazil, Japan, China, Madagascar, Israel, and Ireland. These strains were isolated from diverse sources including human, dogs, rodents, and primates and with different conditions (Table 9).

Accession numbers. The sequenced resistance gene submitted to the GenBank database was validated and subsequently assigned the accession number KX689749.

Table 6. Number of dogs presented with injury to different regions of the body.

Region	Number of dogs	%
Abdomen	4	8.7
Cervical region	4	8.7
Forelimbs	13	28.3
Head	8	17.3
Hindlimb	15	32.6
Pelvic region	1	2.2
Thorax+abdomen	1	2.2
Total	46	100

Table 7. Causes of wounds sampled for culture and sensitivity in dogs presented to the clinic.

Cause	Number of dogs	%
Bite wound	10	21.7
Cellulitis	1	2.2
Fracture	1	2.2
Pododemodicosis	1	2.2
Surgical site infection	11	23.9
Traumatic	7	15.2
Unknown	15	32.6
Total	46	100

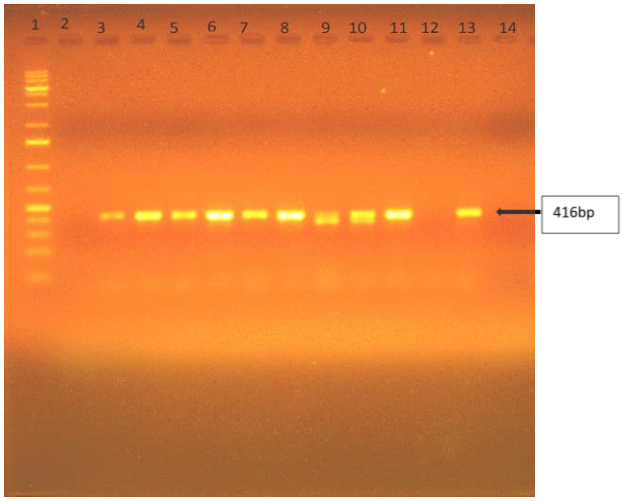

Figure 2. Agarose gel electrophoresis of PCR products of control PCR done for identification of *Staphylococcus* targeting 16S rRNA gene. Lane 1: 100bp ladder DNA marker, Lane 2: Negative Control; Lanes 3-11 Representative *Staphylococcus* (PCR Product 416 bp); Lane 13: *Staphylococcus aureus* ATCC 25923.

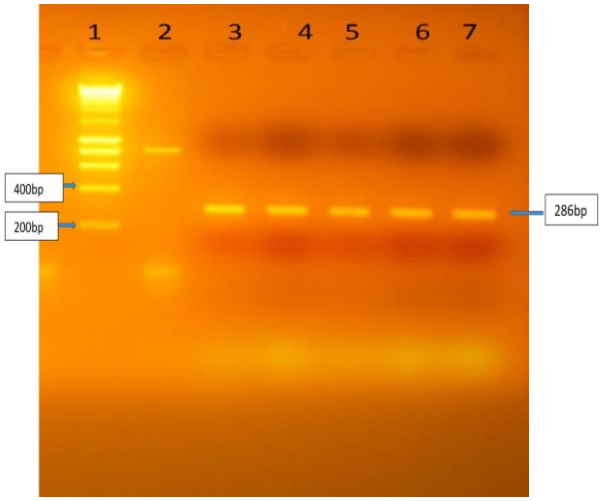

Figure 3. Agarose gel electrophoresis of PCR products of mecA positive strains. Lane 1: 1KB ladder DNA marker, Lane 3-6 mecA positive isolates (Positive PCR product 286 bp), Lane 7 Positive control.

Table 8. Resistant gene nucleotide homologues and their identities in expressed in percentages.

I.D	Homologue	%Identity	Accession number
CS 100	*Staphylococcus sciuri mecA* gene	97%	AB547235
	Staphylococcus sciuri mecA gene	96%	JX094435.1
	Staphylococcus aureus mecA gene	96%	KF058902.1
CS 148	*Staphylococcus aureus mecA* gene	99%	KR187111.1
	Staphylococcus epidermidis mecA gene	99%	KP265312.1
	Staphylococcus aureus mecA gene	99%	KF058908.1
	Staphylococcus pseudintermedius mecA gene	99%	HE984157

Table 9. Diversity of hosts and geographical distribution of resistant gene homologues.

Isolate I.D	Accession number	Host	Country
CS 100	AB547235	Rat	Japan
	JX094435.1	Primate (Sifaka)	Madagascar
	KF058902.1	Bovine (Mastitic milk)	Brazil
CS 148	KR187111.1	Bovine (Mastitic milk)	China
	KP265312.1	Canine (Fracture site)	Ireland
	KF058908.1	Human	Brazil
	HE984157	Canine (Rhinitis)	Israel

Discussion

In this study, *Staphylococcus* spp. were the most common isolates from samples submitted in the laboratory. The findings of the retrospective study confirmed the etiological and clinical importance of *Staphylococcus* organisms as colonizers of skin and important causes of infection in the skin of animals. The high percentage of staphylococci (59.1%) was expected since Staphylococcal species are present on or in clinically normal individuals as commensals (Weese 2010). However, they are opportunistic pathogens including *S. pseudintermedius* as well as *S. aureus* as leading cause of surgical site infections in animals (Vengust et al. 2006; Turk 2015). The observation in this study that *S. aureus* was the most prevalent isolate from wounds is like reports from Bangladesh (Rahman et al. 2003) In contrast, Vincze et al. (2014) recorded a low prevalence of *S. aureus* from wounds with isolation rates of 5.8% and 12.2% for dogs and cats, respectively, in Germany. *S. aureus* has been recognized as an important wound pathogen and a major cause of delayed wound healing and infection. The prevalence of *S. aureus* (37.5%) isolated from wounds of dogs in Kenya has previously not been reported.

The high prevalence of *S. aureus* in this study was surprising. Other authors (Meyers et al. 2007; Urumova et al. 2012; Padhy et al. 2014) have reported *S. intermedius* to be the primary isolate from wounds in dogs. While dogs and cats may become colonized, contaminated, and infected with *S. aureus*, they are not considered reservoir hosts of this organism (Cohn and Middleton 2010). The predominant *Staphylococcus* spp. in dogs has been reported to be *S. pseudintermedius* (Griffeth et al. 2008; Hanselmann et al. 2009). This finding may be because at the laboratory, all coagulase positive Staphylococci were designated as *S. aureus.*

In the present study, *E. coli*, *Streptococcus* spp., other *Staphylococcus* spp., and *Proteus* spp. were other microorganisms isolated from the wound swabs. This finding is like the study by Rahman et al. (2003) in Bangladesh, who isolated *E. coli*, *Klebsiella* spp. and *Proteus* spp. in wound swabs. The results of the present study are also in agreement with Urumova et al. (2012) who also found a high incidence of Enterobacteriaceae in particular *E.coli* in wounds. In this study, the polymicrobial growth was demonstrated, with 24% of the swabs yielding more than one organism was consistent with other reports of similar nature conducted elsewhere (Meyers 2007; Padhy et al. 2014). Colonization in wounds is mostly polymicrobial involving different potentially pathogenic microorganisms (Bowler et al. 2001). The number and diversity of microbes in any wound is influenced by several factors among them are wound type, depth, location, and quality, the level of tissue perfusion, and the antimicrobial efficacy as well as the host immune response.

In vitro antimicrobial agent susceptibility of the isolates showed a high frequency of resistant strains, with 97% of the isolates showing resistance to at least one drug. These observations are the cause for concern, as they are an indication of the existence of multidrug-resistant isolates among dogs that might pose a clinical as well as therapeutical challenges.

In the retrospective study, 58% of bacteria isolated from ear swabs belonged to the Genus *Staphylococcus*; this is comparable to other studies (Lilenbaum et al. 2000; Lyskova et al. 2007; Petrov et al. 2013). Malayeri et al. (2010) reported a high prevalence of 73.8% of *Staphylococcus* spp. in Iran. Other bacteria isolated in the present study included *Proteus* spp. 16.5%, *Pseudomonas* spp. 12.3% and *Streptococcus* spp. 4.7% . This was comparable to a previous study by Mande and Kitaa (2005) where *S. aureus* was found to be the most prevalent isolate (51.2%), and *Streptococcus* spp. (14%), *Pseudomonas* spp. (14%) and *Proteus* spp. (10%). This study demonstrated an increase in staphylococcal isolation from otitis externa to 58% vs. 51.2% compared to a previous Kenyan study. This study also shows that *Proteus* spp. is increasingly becoming an important pathogen with a prevalence of 16.5% up from 10% in the survey by Mande and Kitaa (2005).

Previous studies in dogs, reported the pathogens isolated from wounds to be most sensitive to potentiated sulphonamides and amoxicillin/clavulanic acid preparations (Meyers et al. 2007; Urumova et al. 2012). This observation is not in agreement with findings in this study, where comparatively higher resistance rates were observed to potentiated sulphonamides (89%) and amoxicillin/ clavulanic (58%). Interestingly, Pedersen et al. (2007) found no resistance to amoxicillin/clavulanic acid in their study which involved bacterial isolates from clinical submissions in Denmark. Earlier reports by Authier et al. (2006), suggested amoxicillin/clavulanic acid to be an appropriate antimicrobial for the treatment of skin infections by *Staphylococcus* spp. However, based on the results of this study, the use of these antimicrobials as the first line of treatment for empirical therapy might result in treatment failure if the observation made represents the general population in Kenya. The findings of this study demonstrated that gentamicin and norfloxacin were the most effective antimicrobial agents against many of the isolates. Gentamicin has been indicated for the treatment of Staphylococcal infections (Lilenbaum et al. 2000). However, Authier et al. (2006) suggest that its use should be limited to cases where initial treatment has failed.

In the present study, surgical site infections were found to be a frequent cause for wound swabbing representing 24% of the wound swabs. Seventy five percent of surgical site infections sampled resulted from fracture fixation using an implant. Turk et al. (2015) reported the use of implants increases the risk for surgical site infections. Gallagher et al. (2012) and Turk et al. (2015) further points out that, implants frequently become colonized with bacteria and may also act as substrates for bacterial biofilm formation. Majority of the wounds sampled were located on the extremities with the hindlimbs being more affected than the forelimbs. These results are comparable with the report by Shamir et al. (2002) where the extremities and the head were reported as the most frequent sites of bite wounds in dogs. Similarly, Meyers et al. (2007) also observed most wounds to involve the cranial half of the body, especially the head and thoracic limbs in dogs.

Concerning the susceptibility of coagulase positive *Staphylococcus* spp. to various antimicrobials the present study found 97% of the *Staphylococcus* isolates to be resistant to at least one drug. This finding agrees with a previous report report by Lilenbaum et al. (2000), who reported Staphylococcus isolates to display a high level of resistance in Brazil. They found 90.9% of the isolates in their study to show resistance to at least one drug. However, the findings of this study were in contrast with the findings reported by Junco and Barrasa (2002), who reported only 64.8% of COPS displaying resistance.

In the present study, the least effective antimicrobials against *S. aureus* were sulphonamides (sulfamethoxazole), potentiated sulphonamides (cotrimoxazole), ampicillin and tetracycline. The highest level of resistance noted was for potentiated sulphonamides with a resistance rate of 95%. Lilenbaum et al. (2000) also found many staphylococcal isolates in Brazil resistant to this drug though at a lower rate 72.7%. On the other hand, a study in Denmark by Pedersen et al. (2007) described very low resistance of *S.intermedius* ear isolates to this drug combination. The most effective agent against Staphylococci was gentamicin, chloramphenicol, and norfloxacin. Gentamicin

susceptibility rate was 88% which is like the one reported by Lilenbaum et al. (2000). Most of the isolates were also found to be susceptible to amoxicillin (62%), suggesting that this drug can be used as the first line of treatment before results of antimicrobial susceptibility testing.

Pseudomonas and *Proteus* isolates observed in this study displayed the highest resistance to most antimicrobial agents. *Pseudomonas* spp. are mostly isolated in chronic cases of canine otitis externa (Scott et al. 2001; Greene 2006). This organism has been reputed for its high level of resistance to most antimicrobials. The multidrug resistance was observed to be the case in this study, with 92% of *Pseudomonas* spp. isolates showing resistance to 4 or more drugs. Highest resistance was recorded to amoxicillin and sulfamethoxazole, with all the isolates tested against these drugs showing 100% resistance. These isolates also showed high resistance to ampicillin (93%) and amoxicillin/ clavulanic acid (87%). Significant resistance to chloramphenicol (64%) and tetracycline (79%) was also observed in the *Pseudomonas* spp. isolates in this study. Pedersen et al. (2007) in their study found that all the *Pseudomonas* spp. isolates were resistant to ampicillin, amoxicillin/clavulanic acid, and erythromycin. Malayeri et al. (2010) also concurred with these observations with all *Pseudomonas* spp. isolates in their study showing 100% resistance to ampicillin, amoxicillin/clavulanic, erythromycin, rifampin, and penicillin G. In another study, Hariharan et al. (2006) found that Pseudomonas isolates to be highly resistant to chloramphenicol (99%) and doxycycline (98%). The least antimicrobial resistance in this study was observed against gentamicin (4%) and norfloxacin (15%). This observation agrees with Petersen et al. (2002), who reported most *Pseudomonas aeruginosa* isolates to be 100% susceptible to the two drugs.

In the present study, *Proteus* spp. was the second most frequently isolated microorganism after *S. aureus*, accounting for 14.2% of all isolates; a finding that was like reports by other researchers (Pedersen et al. 2007; Lyskova et al. 2007; Petrov et al. 2013). The present study found all isolates to be resistant to amoxicillin and sulphonamides, but susceptible to gentamicin. High resistance was observed against ampicillin (94%), cotrimoxazole (97%) and moderate resistance to tetracyclines (69%), amoxicillin/clavulanic (58%) and chloramphenicol (45%). Similar results have previously been reported by Petrov et al. (2013), who found all isolates to be susceptible to gentamicin. Also, they observed resistance to tetracycline (81%) and chloramphenicol (74%) though at higher rates, and the isolates in their study were resistant to ampicillin. In contrast to this study, Pedersen et al. (2007), found all *Proteus* spp. isolates in their study to be resistant to tetracyclines and majority of the isolates susceptible to gentamicin and ciprofloxacin, which agrees with the findings in this study.

Prospective screening of dogs in this study showed a carriage rate of 34% (65/191) of *Staphylococcus* spp. This may be due to the prolonged storage time of some samples (up to 8 months for a few samples). In other studies, samples were cultured within 12 hrs (Gingrich et al. 2011) and 24 -36 hours of collection (Bergstrom et al. 2012; Walther et al. 2012). The recent use of antimicrobial agents in most of the study animals before sampling may have led to the suppression of the number of commensal bacteria, especially that resident on the skin.

Detection of the *mecA* gene by PCR revealed 2 out of the 15 (13%) phenotypically resistant isolates to be genotypically resistant to methicillin (oxacillin). These two isolates contained the *mecA* gene that encodes for resistance to β-lactam antibiotics. Ozturk et al. (2010) reported similar results in their study where all 5 *Staphylococcus* spp. isolates that were phenotypically resistant to oxacillin were *mecA* negative on PCR. This discrepancy between phenotypic and genotypic resistance in the isolates has been reported by Schmidt et al. 2014 and Elhassan et al. 2015. This discrepancy could be due to the existence of the so-called borderline (low-level resistant) strains. These *mecA* negative strains are thought to result from overproduction of β-lactamase (Chambers 1997). Other mechanisms associated with borderline resistance include the acquisition of modified PBPs (Elhassan et al. 2015). The existence of these borderline strains emphasize the need to screen *mecA* negative strains for other resistance mechanisms.

Two genes are known to encode for methicillin resistance in *Staphylococcus* spp. namely *mecA* and *mecC*. However, in this study, only *mecA* was investigated for genotypic characterization of methicillin resistance since reports of *mecC* positive MRSA isolates are low with the prevalence of 0-3% reported in European countries (Paterson et al. 2014). Previous studies have also shown *mecA* to be the most common gene encoding for methicillin resistance in *Staphylococcus* spp. (Weese 2010; van Duijkeren et al. 2011).

No MRSPs were observed in this study, which is like reports by Garbacz et al. (2011). Their research involved 39 *S. pseudintermedius* isolates from clinical submissions and found all the isolates to be susceptible to oxacillin. Several studies by different authors have also failed to isolate any MRSP isolates (Murphy et al. 2009; Rubin and Chirino-Trejo 2011; Schmidt et al. 2014). In most of these studies, the investigators collected samples from healthy animals. In the present study, samples were collected both from normal and clinically sick animals, some of which had received antibiotic treatment before sampling. This study found a prevalence rate of 7% (2/28) of MRSA among coagulase positive *Staphylococcus* spp. and an overall prevalence of 1% (2/191). In a Swedish animal hospital, no MRSA was isolated from surgical patients and healthy animals, although the prevalence of MRSA in the environment was found to be 5.3% (Bergstrom et al. 2012). The low prevalence of MRSA in this study is also like reports by Couto et al. (2011), who reported MRSA prevalence of 1% from 287 dogs and cats presented to a veterinary teaching hospital in Portugal and is consistent with findings of Quitoco et al. (2013). A higher prevalence of MRSA of 15.8% was observed in a study on surgical site infections (Turk et al. 2015). A recent report by Aiken et al. (2014), found a similar prevalence (7%) of MRSA among *S. aureus* strains isolated from patients admitted to a hospital in Kiambu County, Kenya. One of the

MRSA isolates in this study was recovered from a patient with a surgical site infection. The patient had been hospitalized for a week before the infection and was under treatment with an antimicrobial agent. This finding is like that by Middleton et al. (2005), whose sole postoperative MRSA isolate was from a canine patient with an orthopedic pin tract infection. MRSA has emerged as an important pathogen in post-operative infections in previous studies (Tomlin et al. 1999; Turk et al. 2015). Antimicrobial drug therapy, hospitalization, and surgery have been cited as factors predisposing to MRSA infection (Loeffler et al. 2005; Faires et al. 2010; Magalhaes et al. 2010; Davis et al. 2013). Multidrug-resistant Staphylococci isolated from dogs with post-operative infections and wounds should raise suspicion of MRSA infection and appropriate care taken in handling such patients.

Analysis of the sequenced resistant determinants showed that the resistant genes were harbored by *Staphylococcus* spp. strains and that the resistant determinant is geographically widespread across various regions of the globe having previously been isolated from countries such as Ireland (McManus et al. 2015), China (Wu et al. 2015), Brazil (Melo et al. 2014), Israel (Perreten et al. 2013). The strains have been isolated from different *Staphylococcus* spp. isolated from bovine (milk), canine (orthopedic implant and rhinitis) and human clinical submissions. The *mecA* genes contained in *SCCmec* have been reported to be almost identical regardless of the *Staphylococcus* species carrying it (Tsubakishita et al. 2010). This observation alludes to the fact that the *mecA* gene is transferable from different *Staphylococcus* spp. The *mecA* gene encodes for penicillin-binding (*PBP2*) protein. PBP 2a is a low-affinity penicillin-binding protein (PBP) that mediates methicillin resistance in *Staphylococcus* spp. (Weese 2010). This modified PBP2 has a low affinity for β -lactams and therefore cell wall construction is not prevented by these antimicrobials (van Duijkeren et al. 2011).

Haphazard use of antimicrobials before testing can lead to the selection of multidrug-resistant strains. This phenomenon may have been the case for one of the *S. aureus* strains isolated that showed resistance to the complete panel of the 8 drugs tested against. This strain was isolated from a dog suffering from recurrent otitis externa. Selective pressure exerted by previous antimicrobial treatment in recurrent cases may lead to the emergence of resistant strains (Guardabassi et al. 2004). The occurrence of antimicrobial resistance in companion animals is of significance to human health (Hawkey 2008). The close contact between household pets and humans offers favorable conditions for the transmission of bacteria by direct contact or indirectly through contamination of the environment. Transfer of mobile resistance determinants between companion animals and humans may also occur (Guardabassi et al. 2004).

In conclusion, the present study confirms that the most prevalent microorganisms associated with wounds and otitis externa were *S. aureus* (50.5%), *Proteus* spp. (14.04%), *Pseudomonas* spp. (9.82%), Other *Staphylococcus* spp. (8.42%), *Streptococcus* spp. (7.67%) and *E. coli* (5.62%). From the results of our study, gentamicin, an aminoglycoside, is the most effective antimicrobial agent against all the isolates from wounds, surgical site infections and otitis externa in dogs. Norfloxacin, a fluoroquinolone, is relatively effective against Gram-negative isolates (*Proteus* spp. and *Pseudomonas* spp.) and *Streptococcus* spp. *Pseudomonas* spp. and *E. coli* from otitis externa and wounds respectively, are the most challenging organisms to treat in dogs. The study findings report the first two cases of MRSA isolated from a healthy dog and a dog with a surgical site infection in Kenya. The study observed MRSA prevalence of 7% among coagulase positive *Staphylococcus* spp. The resistant determinant *mecA* in this study was like some MRSA strains from human patients in other parts of the world and therefore demonstrates the zoonotic importance of these resistant strains.

REFERENCES

Aiken AM, Mutuku IM, Sabat AJ, Akkerboom V, Mwangi J, Scott JAG, Morpeth SC, Friedrich AW, Grundmann H. 2014. Carriage of *Staphylococcus aureus* in Thika Level 5 Hospital, Kenya: a cross-sectional study. Antimicrob Resist Infect Control 3 (1): 22.

Asfour HAE, Darwish SF. 2014. Evaluation of phenotypic methods versus molecular methods for differentiation of coagulase positive Staphylococci causing bovine mastitis with a special reference to atypical *Staphylococcus aureus*. Intl J Curr Microbiol Appl Sci 3 (5): 543-558.

Authier S, Paquette D, Labrecque O, Messier S. 2006. Comparison of susceptibility to antimicrobials of bacterial isolates from companion animals in a veterinary diagnostic laboratory in Canada between 2 time points 10 yrs apart. Canadian Vet J 47: 774-778.

Baptiste K, Williams K, Williams N, Wattret A, Clegg P, Dawson S, Corkill J, O'Neill T, Hart C. 2005. Methicillin-resistant staphylococci in companion animals. Emerg Infect Dis 11: 1942-1944.

Beck KM, Waisglass SE, Dick HL, Weese JS. 2012. Prevalence of methicillin resistant *Staphylococcus pseudintermedius* (MRSP) from skin and carriage sites of dogs after treatment of their methicillin-resistant or methicillin-sensitive staphylococcal pyoderma. Vet Dermatol 23: 369-75.

Bemis DA, Jones RD, Frank LA, Kania SA. 2009. Evaluation of susceptibility test breakpoints used to predict *mecA*-mediated resistance in *Staphylococcus pseudintermedius* isolated from dogs. J Vet Diagnostic Invest 21: 53-58.

Bergström A, Gustafsson C, Leander M, Fredriksson M, Grönlund U, Trowald-Wigh G. 2012. Occurrence of methicillin-resistant Staphylococci in surgically treated dogs and the environment in a Swedish animal hospital. J Small Anim Pract 53 (7): 404-410.

Bond R, Loeffler A. 2012. What's happened to *Staphylococcus intermedius*? Taxonomic revision and emergence of multi-drug resistance. J Small Anim Pract 53: 147-154.

Bowler PG, Duerden BI, Armstrong DG. 2001. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 14: 244-269.

Chambers HF. 1997. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin *Microbiol Rev* 10: 781-791.

CLSI. 2008. Performance Standards for Antimicrobial Disk Dilution Susceptibility Test for Bacteria Isolated from Animals; Approved Standard, 3rd edition. CLSI document M31-A3. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI (ed.). 2013. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; Approved Standard, 4th edition. CLSI document VET01-A. Clinical and Laboratory Standards Institute, Wayne, PA.

Cohn LA, Middleton JR. 2010. A veterinary perspective on Methicillin Resistant Staphylococci. J Vet Emerg Crit Care 20: 31-45.

Couto N, Pomba C, Moodley A, Guardabassi L. 2011. Prevalence of methicillin-resistant staphylococci among dogs and cats at a veterinary teaching hospital in Portugal. Vet Rec 162: 72.

Davis MF, Peterson AE, Julian KG, Greene WH, Price LB, Nelson K, Whitener CJ, Silbergeld EK. 2013. Household risk factors for colonization with multidrug-resistant *Staphylococcus aureus* isolates 8. PLoS ONE 8 (1): e54733. DOI: 10.1371/journal.pone.0054733

Diaz-Campos DV. 2012. Molecular epidemiology and genetic analysis of *Staphylococcus* species in companion animal medicine [Dissertation]. Auburn University, Auburn, AL, USA.

Duquette RA, Nuttall TJ. 2004. Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? J Small Anim Pract 45: 591-597.

Elhassan MM, Hani AO, Hassan AH, Miskelyemen AE, Leila MA. 2015. Absence of the mec A Gene in Methicillin Resistant *Staphylococcus aureus* Isolated from Different Clinical Specimens in Shendi City, Sudan. BioMed Res Intl. DOI: 10.1155/2015/895860

EMA [European Medicines Agency]. 2010. Reflection paper on Methicillin-resistant *Staphylococcus pseudintermedius*. European Medicines Agency, London, UK.

Faires MC, Traverse M, Tater KC, Pearl DL, Weese JS. 2010. Methicillin-resistant and -susceptible *Staphylococcus aureus* infections in dogs. Emerg Infect Dis 16: 69-75.

Frank LA, Loeffler A. 2012. Methicillin resistant *Staphylococcus pseudointermedius*; Clinical challenge and treatment options. Vet Dermatol 23: 283-291.

Gallagher AD, Mertens WD. 2012. Implant removal rate from infection after tibial plateau leveling osteotomy in dogs. Vet Surg 41: 705-711.

Garbacz K, Piechowicz L, Żarnowska S, Haras K, Dąbrowska-Szponar M. 2011. Heterogeneity of methicillin-sensitive *Staphylococcus pseudintermedius* strains isolated from diseased dogs. Polish J Vet Scis 14 (2): 283-284.

Gingrich EN, Kurt T, Hyatt DR, Lappin MR, Ruch-Gallie R. 2011. Prevalence of methicillin-resistant staphylococci in northern Colorado shelter animals. J Vet Diagnostic Invest 23: 947-950.

Greene CE. 2006. Otitis Externa. In: Greene CE (ed) Infectious Diseases of the Dog and Cat, 3rd ed. Saunders, Missouri, USA.

Griffeth GC, Morris DO, Abraham JL, Shofer FS, Rankin SC. 2008. Screening for skin carriage of methicillin-resistant coagulase-positive staphylococci and *Staphylococcus schleiferi* in dogs with healthy and inflamed skin. Vet Dermatol 19: 142-149.

Guardabassi L, Loeber ME, Jacobson A. 2004. Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. Vet Microbiol 98: 23-27.

Hanselman BA, Kruth SA, Rousseau J, Weese JS. 2009. Coagulase positive staphylococcal colonization of humans and their household pets. Canadian Vet J 50: 954-958.

Hariharan H, Coles M, Poole D, Lund L, Page R. 2006. Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. Canadian Vet J 47: 253-255.

Hawkey PM. 2008. The growing burden of antimicrobial resistance. J Antimicrob Chemother 62 (Suppl. 1): i1-i9.

Junco TM T, Barrasa Martin JL. 2002. Identification and antimicrobial susceptibility of coagulase positive Staphylococci isolated from healthy dogs and dogs suffering from otitis externa. J Vet Med B 49: 419-423.

Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, Joloba ML, Najjuka FL. 2010. Identification of *Staphylococcus aureus*: Dnase and Mannitol salt agar improve the efficiency of the tube coagulase test. Ann Clin Microbiol Antimicrob 9: 23-30.

Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, Hiramatsu K. 2007. Combination of Multiplex PCRs for Staphylococcal Cassette Chromosome *mec* Type Assignment: Rapid Identification System for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob Agents Chemother 51 (1): 264-274.

Lilenbaum W, Veras M, Blum E, Souza GN. 2000. Antimicrobial susceptibility of Staphylococci isolated from otitis externa in dogs. Lett Appl Microbiol 31: 42-45.

Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, Dalsgaard A, Smith H, Stevens K B, Lloyd D H. 2005. Prevalence of methicillin-resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK. J Antimicrob Chemother 56 (4): 692-697.

Lyskova P, Vydrzalova M, Mazurova J. 2007. Identification and antimicrobial susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. J Vet Med A 54: 559-563.

Magalhaes SRJ, Loeffler A, Lindsay J, Rich M, Roberts L, Smith H, Lloyd DH, Pfeiffer DU. 2010. Risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) infection in dogs and cats: a case-control study. Vet Res 41: 55.

Maina EK, Kiiyukia C, Wamae CN, Waiyaki PG, Kariuki S. 2013. Characterization of methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections in patients in Nairobi, Kenya. Intl J Infect Dis 17 (2): e115-119. DOI: 10.1016/j.ijid.2012.09.006.

Malayeri HZ, Jamshidi S, Salehi TZ. 2010. Identification and antimicrobial susceptibility patterns of bacteria causing otitis externa in dogs. Vet Res Commun 34: 435-444.

Mande JD, Kitaa JMA. 2005. Microbial profile and antimicrobial susceptibility of isolates from dogs with otitis in Kenya. Kenya Vet 29: 40-44.

McManus BA, Coleman DC, Deasy EC, Brennan GI, O' Connell B, Monecke S, Ralf Ehricht R, Leggett B, Leonard N, Shore AC. 2015. Comparative Genotypes, Staphylococcal Cassette Chromosome mec (SCCmec) Genes and Antimicrobial Resistance amongst *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* isolates from infections in humans and companion animals. PLoS ONE 10 (9): e0138079. DOI: 10.1371/journal.pone.0138079.

Melo DA, Coelho IS, Motta CC. 2014. Impairments of mecA gene detection in bovine *Staphylococcus* spp. Braz J Microbiol 45 (3):1075-1082.

Meyers B, Schoeman JP, Goddard A, Picard J. 2007. The bacteriology and antimicrobial susceptibility of infected and non-infected dog bite wounds: fifty cases. Vet Microbiol 127 (3-4): 360-368.

Middleton J, Fales W, Luby C, Oaks J, Sanchez S, Kinyon J, Wu C, Maddox C, Welsh R, Hartmann F. 2005. Surveillance of *Staphylococcus aureus* in veterinary teaching hospitals. J Clin Microbiol 43: 2916-2919.

Murphy C, Reid-Smith RJ, Prescott JF, Bonnett BN, Poppe C, Boerlin P, Weese JS, Janecko N, Mcewen SA. 2009. Occurrence of antimicrobial resistant bacteria in healthy dogs and cats presented to private veterinary hospitals in southern Ontario: preliminary study. Canadian Vet J 50: 1047-53

Normand EH, Gibson NR, Reid SW, Carmichael S, Taylor DJ. 2000. Antimicrobial-resistance trends in bacterial isolates from companion animal community practice in the UK. Prevent Vet Med 46: 267-278.

Öztürk D, Avki S, Türütoğlu H, Yiğitarslan K, Sağnak S. 2010. Methicillin resistance among Coagulase-positive Staphylococci isolated from dogs with otitis externa, skin wounds and pyoderma. Kafkas Universitesi Veteriner Fakultesi Dergisi 16 (4): 651-656.

Padhy A, Mishra R, Behera SS, Sahu AR, Sahoo S. 2014. Microbial profile of canine persistent wound infections. Vet World 7 (4): 244-247.

Paterson GK, Harrison EM, Holmes MA. 2014. The emergence of *mecC* methicillin-resistant *Staphylococcus aureus*. Trends Microbiol 22: 42-47.

Paterson GK, Morgan FJE, Harrison EM, Peacock SJ, Parkhill J, Zadoks RN, Holme MA. 2014. Prevalence and properties of *mecC* methicillin resistant *Staphylococcus aureus* (MRSA) in bovine bulk tank milk in Great Britain. J Antimicrob Chemother 69: 598-602.

Paul NC, Moodley A, Ghibaudo G, Guardabassi L. 2011. Carriage of Methicillin-Resistant *Staphylococcus pseudointermedius* in small animal veterinarians; Indirect Evidence of Zoonotic transmission. Zoonoses Public Health 8: 533-539.

Pedersen K, Jensen H, Finster K, Jensen VF, Heuer OE. 2007. Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs. J Antimicrob Chemother 60: 775 -781.

Perreten V, Chanchaithong P, Prapasarakul N, Rossano A, Shlomo E, Blum SE, Elad D, Schwendener S. 2013. Novel Pseudo-Staphylococcal Cassette Chromosome *mec* Element (SCC*mec*57395) in Methicillin-Resistant *Staphylococcus pseudintermedius* CC45. Antimicrob Agents Chemother 57 (11): 5509-5515.

Petersen AD, Walker RD, Bowman MM, Schott HC, Rosser Jr, EJ. 2002. Frequency of isolation and antimicrobial susceptibility patterns of *Staphylococcus intermedius and Pseudomonas aeruginosa* isolates from canine skin and ear samples over a 6-year period (1992-1997. J Amer Anim Hospitals Assoc 38: 407-413.

Petersen AD, Walker RD, Bowman MM, Schott HC, Rosser EJ Jr. 2002. Frequency of isolation and antimicrobial susceptibility patterns of *Staphylococcus intermedius* and *Pseudomonas aeruginosa* isolates

from canine skin and ear samples over a 6-year period (1992-1997). J Amer Anim Hospitals Assoc 38: 407-413.

Petinaki E, Spiliopoulou I. 2012. Methicillin-resistant *Staphylococcus aureus* among companion and food-chain animals; Impact of human contact. Clin Microbiol Infect 18: 626-634.

Petrov V, Mihaylov G, Tsachev I, Zhelev G, Marutsov P, Koe K. 2013. Otitis externa in dogs: microbiology and antimicrobial susceptibility. Revue de Médecine Vétérinaire 164 (1): 18-22.

Quitoco IM, Raimundo MS, Silva-Carvalho MC, Souza RR, Beltrame CO, Oliveira TF, Araújo R, del Peloso PF, Coelho LR, Figueiredo AM. 2013. First report in South America of companion animal colonization by the USA1100 clone of community-acquired methicillin-resistant *Staphylococcus aureus* (ST30) and by the European clone of methicillin-resistant *Staphylococcus pseudintermedius* (ST71) 336. BMC Res Notes 6: 336. DOI: 10.1186/1756-0500- 6-336.

Rahman MM, Biswas DB, Islam MM, Islam MA. 2003. Cultural sensitivity of septic wounds in animals. Pakistan J Biol Sci 6: 741-744.

Rubin JE, Chirino-Trejo M. 2011. Prevalence, sites of colonization, and antimicrobial resistance among *Staphylococcus pseudintermedius* isolated from healthy dogs in Saskatoon, Canada. J Vet Diagnos Invest 23: 351-354.

Schmidt VM, Williams NJ, Pinchbeck G, Corless CE, Shaw S, McEwan N, Dawson S, Nuttall T. 2014. Antimicrobial resistance and characterisation of staphylococci isolated from healthy Labrador retrievers in the United Kingdom. BMC Vet Res 10: 17. DOI: 10.1186/1746-6148-10-17.

Scott DW, Miller WH, Griffin CE. 2001. Muller and Kirk's small animal dermatology. 6th ed. W.B. Saunders; Philadelphia.

Shamir MH, Leisner S, Klement E, Gonen E, Johnston De. 2002. Dog bite wounds in dogs and cats: a retrospective study of 196 cases. J Vet Sci A 49: 107-112.

Tomlin J, Pead M.J, Lloyd DH, Howell S, Hartmann F, Jackson HA, Muir P. 1999. Methicillin-resistant *Staphylococcus aureus* infections in 11 dogs. Vet Rec 144: 60-64.

Tsubakishita S, Kuwahara-Arai K, Sasaki T, and Hiramatsu K. 2010. Origin and molecular evolution of the determinant of Methicillin Resistance in Staphylococci. Antimicrob Agents Chemother 54 (10): 4352-4359.

Turk R, Singh A, Weese J.S. 2015. Prospective surgical site infection surveillance in dogs. Vet Surg 44 (1): 2-8.

Urumova V, Chaprazov TS, Lyutskanov M, Borisov I. 2012. Microbiological analyses of canine infected wounds. Revue Médecin Veterinaire 163 (4): 201-205.

Van Duijkeren E, Catry B., Greko C., Moreno MA, Pomba MC, Pyörälä S, Ruzauskas M, Sanders P, Threlfall EJ, Torren-Edo J, Törneke K. 2011. Review on methicillin-resistant *Staphylococcus pseudintermedius*. J Antimicrob Chemother 66 (12): 2705-2714.

Vengust M, Anderson MEC, Rousseau J, Weese JS. 2006. Methicillin-resistant staphylococcal colonization in clinically normal dogs and horses in the community. Lett Appl Microbiol 43: 602-606.

Vincze S, Stamm I, Kopp P A, Hermes J, Adlhoch C, Semmler T, Wieler L H, Lubke-Becker A, Walther B. 2014. Alarming proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) in wound samples from companion animals, Germany 2010-2012. PLoS ONE 9 (1): e85656. DOI: 10.1371/journal.pone.0085656.

Walther B, Hermes J, Cuny C, Wieler LH, Vincze S, Abou EY, Stamm I, Kopp PA, Kohn B, Witte W, Jansen A, Conraths FJ, Semmler T, Eckmanns T, Lübke-Becker A. 2012. Sharing more than friendship--nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. PLoS ONE 7 (4): e35197. DOI: 10.1371/journal.pone.0035197.

Weber S, Herwaldt LA, Mcnutt LA, Rhomberg P,Vaudaux P, Pfaller MA, Perl TM. 2002. An outbreak of *Staphylococcus aureus* in a pediatric cardiothoracic surgery unit. Infect Control Hospital Epidemiol 23: 77-81.

Weese JS. 2010. Methicillin resistant *Staphylococcus aureus* in animals. Inst Lab Anim Res J 51: 233-244.

Weese JS, Duijkeren VE. 2009. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microb 140: 418-429.

Windahl U, Reimega RD, Holst BS, Egenvall A, Fernstro¨ ML, Fre-Driksson M, Trowald-Wigh G, Andersson UG. 2012. Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in dogs—a longitudinal study. BMC Vet Res 8: 34-42.

Wu Z, Li F, Liu D, Xue H, Zhao X. 2015. Novel type XII of Staphylococcal cassette chromosome mec harboring a new cassette chromosome recombinase, CcrC2 allele. Antimicrob Agents Chemother 59 (12): 7597-7601.