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Occurrence of community-acquired Panton-Valentine leukocidin-producing and enterotoxin-producing methicillin-resistant staphylococci in companion dogs

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Abstract. In Nigeria, available data on drug-resistant bacterial infections that are caused by companion dogs are scarce. Hence the present study evaluated the occurrence of some community-acquired toxigenic methicillin-resistant staphylococci (MRS) on companion dogs harboured in Nigerian homes, as a pointer to the extent of exposure of humans to these pathogens. Samples were collected from 70 healthy companion dogs during dry and rainy season periods by swabbing a 125 cm^2 fur area on the lumbar and thoracic sites. Phenotypic tests, Kirby Bauer disc diffusion test and 16S rRNA gene analysis were used to identify presumptive colonies of staphylococci and MRS. Molecular methods were employed to detect Panton-Valentine leukocidin (PVL) and prototypic enterotoxin B in MRS isolates. The counts of staphylococci on fur of companion dogs during the rainy season exceeded usual limits of bacteria (≤ 2.54 log_{10} CFU cm^{-2}) on a healthy dog, thus, suggesting that companion dogs harboured in homes situated in Nigeria may be reservoirs of bacteria, especially during rainy season. The mean counts of staphylococci during the rainy season were estimated at 3.09 ± 2.78 log_{10} CFU cm^{-2} and 2.77 ± 2.43 log_{10} CFU cm^{-2} in Edo and Delta States, respectively. The main *Staphylococcus* species that were

carried on fur of companion dogs included *S. pseudintermedius*, *S. aureus*, *S. epidermidis*, *S. simulans* and *S. saprophyticus*. Amongst the staphylococci, expression of methicillin and multidrug resistance was mainly exhibited by *S. pseudintermedius* and *S. aureus*, while enterotoxigenicity was mainly expressed by methicillin-resistant *S. aureus*. Enterotoxigenic *S. aureus* was carried on the fur of companion dogs during the rainy season at estimated prevalence of 8.57% in both Edo and Delta States, respectively; while PVL-producing *S. aureus* was estimated at 5.71% and 2.86%, with PVL-producing *S. pseudintermedius* estimated at 25.71% and 34.29%, respectively. The high prevalence of toxigenic-producing isolates seen on the fur of companion dogs, especially during rainy season, could pose a risk for humans, particularly those that harbour pet dogs at their homes.

Keywords: Panton-Valentine toxin, Enterotoxin B, Methicillin resistance, *Staphylococcus aureus*, *Staphylococcus pseudintermedius.*

Introduction

There is a surge in companion animals harboured within households. In European countries, it is estimated that the population of dogs and cats within households exceeds 127 million (FEDIAF, 2012). Household companion animals refer to animals that are harboured within homes by people for company, psychological support or enjoyment (Damborg *et al.*, 2016). The skin of companion animals, such as dogs and cats, is covered by dense hair referred to as fur. The fur performs vital roles in dogs, some of which include protecting the skin of dogs against chemical, microbial and physical damage, as well as insulating the skin (Miller *et al*., 2013; Cuscó*et al*., 2017). The fur on the skin of these companion animals is often colonized or infected with myriads of pathogenic bacteria. Thus, the close contact between companion animals and humans may cause zoonotic transmission by direct human contact or indirectly by cross-contamination of food. Though, bacterial zoonoses directly associated with companion animals are relatively negligible when compared to foodborne zoonoses (Damborg *et al.*, 2016). Various studies (Chah *et al*., 2014; Rodrigues-Hoffmann *et al*., 2014; Bradley *et al*., 2016; Chermprapai *et al*., 2019; Suepaul *et al*., 2021) have reported that *Staphylococcus, Pseudomonas*, *Corynebacterium* and *Microbacterium* were the dominant bacterial genera that often colonize healthy companion dogs. Staphylococci, the dominant bacteria that colonize the fur and skin of dogs, have been categorized into two main groups, namely, coagulase-positive staphylococci and coagulase-negative staphylococci. Some

common coagulase-negative staphylococci that colonize the skin of dogs include *S. schleiferi*, *S. epidermidis*, *S. simulans*, *S. haemolyticus* and *S. sciuri* (Kloos and Bannerman, 1994; Chah *et al*., 2014), while the most dominant coagulase-positive staphylococci colonizer and infectious agent in dogs is *S. pseudintermedius* (Lynch and Helbig, 2021; Suepaul *et al*., 2021). Coagulase-positive *S. aureus*, the most clinically important coagulase-positive species in humans, is often found colonizing the skin of companion dogs (Wang *et al*., 2019; Suepaul *et al*., 2021). The coagulase-positive staphylococci are often associated with virulence because of their ability to express coagulase-mediated clotting of blood, thereby, evading the host's immune responses (Lamers *et al*., 2012). Coagulase-negative staphylococci have been widely associated with negligible virulence and are often regarded as contaminants (Kloos and Bannerman, 1994; Chah *et al*., 2014). Various studies (Lloyd, 2007; Findik *et al*., 2018; Li *et al*., 2021) have reported that staphylococci, particularly the coagulase-positive staphylococci, can develop resistance to antibiotics, such as the methicillin, in companion dogs due to their close contact with humans and the extensive use of broad-spectrum antibiotics to treat companion dogs. Methicillin-resistant *S. aureus* and methicillin-resistant *S. pseudintermedius* have been found to colonize both humans and dogs, though methicillin-resistant *S. aureus* may only be temporary colonizers of dogs (Gortel *et al.*, 1999; Gronthal *et al*., 2014; Ventrella *et al*., 2017). The carriage of these methicillin-resistant strains on the skin of companion dogs plays a fundamental role in the pathogenesis and epidemiology of community-associated infections. The infections caused by methicillin-resistant *S. aureus* and *S. pseudintermedius* could progress to become severe due to necrotizing processes mediated by the production of Panton-Valentine leukocidin (PVL) by these strains (Weese *et al*., 2009; Reyes-Robles *et al*., 2013; Maali *et al*., 2018). PVL-producing *S. aureus* and *S. pseudintermedius* are dominant causative agents of skin infections, such as furnicles, in canine and human subjects (Prevost *et al*., 1995; Weese *et al*., 2010). *S. aureus* carried by companion dogs has also been reported to produce enterotoxins that are the causative agents of staphylococcal food poisoning outbreaks in humans (Abdel-Moein and Samir, 2011). From both human and veterinary perspectives, understanding the prevalence/occurrence of toxigenic-producing drug-resistant staphylococci among companion dogs is imperative. However, available data on drug-resistant bacterial infections that are caused by companion dogs are scarce, particularly because cases of these pet-related infections are not often recorded and monitored. Hence the present study evaluated the occurrence of community-acquired Panton-Valentine leukocidin-producing and enterotoxin-producing methicillin-resistant staphylococci in companion dogs harboured in Nigerian homes, as a pointer to the extent of exposure of humans, particularly dog owners, to these pathogens.

Materials and methods

Recruitment of companion dogs

Healthy companion dogs with no clinical symptoms were enrolled for this study, while ill dogs with vivid infections were excluded. The breeds of companion dogs that were recruited include Caucasian Shepherd, German Shepherd, Doberman Pinscher, American Eskimo, Lhasa Apso and Alsatian. The registers in 12 veterinary clinics within the study localities were used to locate the homes of companion dog owners. Informed consent was obtained from owners of companion dogs before the participation of their dogs in the study. As an incentive, dog owners were promised that the results of the investigation on their dogs will be transmitted to their veterinarian who will implement any probable treatment regime.

Sample collection

The sample collection was performed in homes of dog owners and experimental analysis of the samples were done in the facility of Igbinedion University, Okada from January 2020 to October 2020 to cover dry and rainy seasons. A total of 35 companion dogs were recruited from 27 homes situated in Edo State (latitude: 6.5438^{o}N, 5.8987^{o}E). Other 35 companion dogs were recruited from 23 homes situated in Delta State (latitude: 5.7040^{o}N, 5.9339^{o}E). Overall, a total of 70 companion dogs were recruited from 50 homes situated in Edo (latitude: 6.5438^{o}N, 5.8987^{o}E) and Delta (latitude: 5.7040^{o}N, 5.9339^{o}E) states of Nigeria. Samples were initially collected from each of the companion dogs during the dry season period (January – March 2020) and then repeated during the rainy season period (July – October 2020). Sampling was done according to the previously prescribed techniques (Cuscó *et al.,* 2017). A 125 cm^2 fur area on the lumbar and thoracic sites of each dog was swabbed with 10 sterile swab sticks moistened with sterile phosphate-buffered saline. After swabbing each dog, the swab sticks were put into a sterile bottle containing 30 ml of sterile phosphate-buffered saline and it was stored on ice while being transported to the laboratory. The bacterial analysis took place in the laboratory within six hours of sample collection.

Isolation and enumeration of staphylococci and methicillin-resistant Staphylococci (MRS)

Presumptive isolation of staphylococci and MRS was performed with the spread plate method (Public Health England, 2014). Each sterile bottle containing swab sticks was thoroughly agitated to disperse the contents in the

fur into the saline diluents and serial dilutions ranging from 10^{-1} to 10^{-4} were made. Sterile mannitol salt agar plates, containing 75 g sodium chloride per litre of agar medium, were prepared and used for isolation of *Staphylococci*; while sterile mannitol salt agar plates containing 4 µg oxacillin per millilitre of the agar medium were used to isolate MRS. Twenty-five millilitre portion of the fur contents in each bottle was mixed with 225 ml of sterile phosphate-buffered saline to obtain the 10^{-1} dilution. Serial dilutions up to 10^{-4} were subsequently prepared from the fur contents of the 10^{-1} dilution. One hundred microlitres (100 µl) aliquots of each of the serial dilutions and the undiluted sample were spread on the duplicate agar medium (HiMedia Laboratories, Mumbai, India) and the inoculated Petri dishes were incubated at 35°C for 48 hours. After incubation, colonies on the Petri plates were presumptively identified as *Staphylococci* and were counted. The colony counts on the Petri plates were subsequently used to deduce the count of presumptive staphylococci and MRS on the fur of dog samples with the following equation and expressed as colony-forming units per square centimeter (CFU/cm^2).

$$CPS \text{ or } CPMS\ (CFU\ cm^{-2}) = \frac{CFU \times Df \times V}{v \times A} \quad (1)$$

CPS: count of presumptive staphylococci on the fur of dog; *CPMS*: count of presumptive MRS on the fur of dog; *CFU*: bacterial colony-forming units on the Petri plates; *Df*: reciprocal of the sample dilution selected for counting; *V*: total volume of diluents (30 ml); v: unit volume of diluents inoculated on each of the plates (0.1 ml); A: surface area of the dog's fur sampled (125 cm^2).

Genus-level identification of Staphylococci and MRS

Genus-level identification of presumptive staphylococci and MRS colonies were performed with standard methods (Krieg and Holt, 1984). The phenotypic tests included the bacterial colony examination, Gram-stain, tube coagulase, catalase, oxidase, haemolysis and mannitol fermentation, including maltose fermentation that was used to presumptively distinguish between *S. aureus* and *S. pseudintermedius*. Cefoxitin antibiotic susceptibility test was further performed for confirmation of MRS colonies using the cefoxitin disc (30 µg) diffusion method (CLSI, 2014). Colonies with zones of inhibition greater than or equal to 22 mm (≥ 22 mm) were suspected to be methicillin-sensitive or susceptible *S. aureus* while those that had zones of inhibition less than or equal to 21 mm (≤ 21 mm) were suspected to be methicillin-resistant *S. aureus*. However, for suspected *S. pseudintermedius* colonies, 1 µg oxacillin disc was

specifically used to carry out the disc diffusion test, and colonies with zones of inhibition greater than or equal to 20 mm (≥ 20 mm) were suspected to be methicillin-susceptible *S. pseudintermedius* while those that had zones of inhibition less than 20 mm (< 20 mm) were suspected to be methicillin-resistant *S.pseudintermedius*.

Species-level identification of staphylococci and MRS

The presence of methicillin resistance phenotype in the suspected MRS was confirmed by using PCR to detect the *MecA* gene in their DNA templates. The PCR was performed according to previously described methods (Adhikari *et al.*, 2017). *MecA* gene amplification was done with the specific primers:*mecA*F (5'-AAA ATC GAT GGT AAA GGT TGG C -3') and *mecA*R(5'-AGT TCT GGA GTA CCG GAT TTG C -3'), with amplicon size of 533 base pairs. The PCR protocol was performed with a 24 µL reaction mixture containing 1 µL of DNA template, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each of the deoxynucleoside triphosphates (dNTPs) (Fermentas Inc., Burlington, Canada), 0.1% Triton X-100, 2 U of GoTaq Hot Start DNA Polymerase (Promega Corporation, Madison, WI) and 20 µM of each primer. PCR was run on a GeneAmp PCR system 9700 (Applied Biosystems, Waltham, MA). The amplification program involved 30 cycles, with each cycle consisting of denaturation at 95°C for 1 minute, followed by annealing at 55°C for 1 minute and extension at 72°C for 1 minute. The PCR products were analyzed by gel electrophoresis on a 1.5% agarose gel (Sigma-Aldrich, Taufkirchen, Germany). DNA bands in the gel were then visualized and documented on the gel documentation system (Applied Biosystems).

Partial 16S rRNA gene analysis confirmed the presence of staphylococci and its methicillin-resistant strains on the fur of companion dogs. The gene analysis was done by polymerase chain reaction (PCR) and sequencing methods (Lane, 1991; Schuurman *et al*., 2004). Zymo-spin column (Zymo Research Corporation, Irvine, CA) was used to extract ultra-pure DNA templates from suspected staphylococci and MRS colonies at the Microbiology Laboratory of Igbinedion University according to prescriptions of the manufacturer. The DNA templates were subsequently used to perform PCR and sequencing of PCR amplicons. Universal 16S rRNA primers (27F [forward primer]: AGA GTT TGA TCM TGG CTC AG; 1492R [reverse primer]: GGT TAC CTT GTT ACG ACT T) were used for the taxonomic classification. PCR protocol was performed with a 50-µl reaction mixture containing 10mmol/l Tris–HCl (pH 8.3), 2mmol/l $MgCl_2$, 2µl of template DNA, 200mmol/l of each of the deoxynucleoside triphosphates (dNTPs) (Fermentas Inc., Burlington, Canada), 50mmol/l KCl,

2 U of GoTaq Hot Start Polymerase (Promega Corporation, Madison, WI) and 0.5 µmol/l of each primer. Amplification was done on a GeneAmp PCR system 9700 (Applied Biosystems, Waltham, MA) with the following cycling conditions: initial denaturation at 95°C for 2 minutes, followed by 40 cycles, with each cycle consisting of denaturation at 94°C for 45 seconds; annealing at 55°C for 1 minute; extension at 72°C for 2 minutes; and a final extension at 72°C for 5 minutes. DNA sequencing of PCR amplicons was carried out with the dideoxy chain termination method (Sanger *et al*., 1977). Query nucleotide sequence comparison with a database of reference nucleotide sequence to confirm the identity of suspected *Staphylococci* was done with the BLASTN 2.8.0+ program (National Center for Biotechnology Information [NCBI]).

Phylogenetic analysis

16S rRNA gene sequences of some staphylococci strains that colonized companion dogs harboured in Nigerian homes were compared with reference strains from other environmental sources to infer their ancestral lineages. Phylogenetic analysis was constructed with the neighbour-joining method using MEGA software, version 6 (Tamura *et al*., 2013), and the robustness of the groupings in the tree was assessed with 1000 bootstrap iterations.

Molecular detection of Panton-Valentine leukocidin (PVL) toxin in MRS isolates

PCR was used to detect the presence of PVL genes in the genomic DNA templates of MRS isolates according to previously described methods (Lina *et al*., 1999). Specific primers employed for amplification of the PVL genes were *luk-PV-1* (5'- ATCATTAGGTAAAATGTCTGGACATGATCCA-3') and *luk-PV-2* (5′-GCATCAASTGTATTGGATAGCAAAAGC-3′). The amplification program involved 30 cycles, consisting of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 1 minute. The PCR products were analyzed by gel electrophoresis on a 1.5% agarose gel (Sigma-Aldrich, Taufkirchen, Germany) and the bands were visualized as previously described. DNA sequencing of the PCR products was carried out with the dideoxy chain termination method (Sanger *et al*., 1977). Comparison of query nucleotide sequences against sequence database (non-redundant protein sequences) was done with BLASTX 2.8.0+ program (NCBI) to confirm the presence of PVL in the MRS isolates. All the databases of non-redundant GenBank CDS translations + PDB + SwissProt + PIR + PRF excluding environmental samples from WGS projects were searched for reference sequences that were homologous to the query translated nucleotide sequences of the MRS isolates.

Molecular detection of enterotoxin B in MRS isolates

The PCR was done with specific primers that targeted the prototypic enterotoxin B gene (Machado *et al*., 2020). Specific primers used for amplifying enterotoxin B gene were *SEB*-F (5'-ACATGTAATTTTGATATTCGCACTG-3') and *SEB*-R (5′-TGCAGGCATCATGTCATACCA-3′), with amplicon size of 667 base pairs. The DNA templates were amplified with the following cyclic conditions: initial denaturation for 5 minutes at 94°C followed by 30 cycles of denaturation, with each cycle consisting of denaturation at 94°C for 2 minutes; annealing at 50°C for 1 minute; extension at 72°C for 1 minute; and a final extension step at 72°Cfor 5 minutes. The amplified products were subsequently run on a 2% agarose gel and the amplicons were sequenced and compared using BLASTX 2.8.0+ program as previously described.

Antibiotic susceptibility testing of MRS isolates

Susceptibility of confirmed MRS isolates to other classes of antibiotics was performed with the Kirby Bauer disc diffusion test as earlier described (CLSI, 2014). The bacterial colony suspension adjusted to 0.5 McFarland turbidity standard was inoculated on a Petri dish containing sterile Mueller-Hinton agar medium (HiMedia Laboratories, Mumbai, India) and antibiotic discs attached to the agar surface. The Petri dish was incubated at 35°C for 16 – 18 hours. Inhibitory zone diameter was interpreted as sensitive (susceptible), intermediate or resistant based on zone diameter interpretive standards set by CLSI. *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as control. Ciprofloxacin (5 µg), pefloxacin (5 µg); cotrimoxazole (30 µg), erythromycin (15 µg) and gentamycin (10 µg) were the antibiotic discs that were used.

Calculation of the confirmed (actual) count of staphylococci, MRS, PVL-producing and enterotoxin-producing MRS

Each presumptive staphylococci or MRS colony that was subjected to phenotypic and molecular tests was confirmed to be staphylococci if it was shown to be Gram-positive coccus, coagulase-positive, catalase-positive, oxidase negative, beta-haemolytic and if its 16S rRNA gene sequence (query sequence) significantly matched with a reference *Staphylococci* 16S rRNA gene sequence in the NCBI GenBank database. Presumptive MRS colonies that were first confirmed as staphylococci and also produced cefoxitin zone diameter of less than or equal to 21 mm (≤ 21 mm) and whose DNA templates also contained the *MecA* gene were ultimately confirmed as MRS. The relative occurrence (*F*) of staphylococci and MRS colonies in each of the dog samples was deduced as follows:

$$F = \frac{\text{Number of bacterial colonies that were confirmed to be staphylococci or MRS}}{\text{Total number of presumptive staphylococci or MRS colonies examined}} \quad (2)$$

The counts of confirmed staphylococci (*CS*) or confirmed MRS (*CMS*) in each of the dog samples was deduced with equation 3.

$$CS/CMS = H \times CPS/CPMS \quad (3)$$

H is the relative occurrence of staphylococci/MRS in each of the dog samples. *CPS* is the count of presumptive staphylococci while *CPMS* is the count of presumptive MRS in each of the dog samples.

The count of PVL-producing MRS (*CLS*) in each of the dog samples was deduced as follows:

$$CLS = P \times CMS \quad (4)$$

CMS is the count of confirmed MRS. *P* is the ratio of PVL-producing MRS colonies to the total methicillin-resistant bacterial colonies examined.

The count of enterotoxigenic-producing MRS (*CES*) in each of the dog samples was deduced as follows:

$$CES = O \times CMS \quad (5)$$

CMS is the count of confirmed MRS. *O* is the ratio of enterotoxigenic-producing MRS colonies to the total methicillin-resistant bacterial colonies examined.

Probability of exposure

The probability of exposure of humans, especially dog owners, to staphylococci, MRS, PVL-producing MRS and enterotoxigenic-producing MRS isolated from the companion dogs was mathematically deduced from the prevalence of companion dogs that are colonized by these microbes (FDA/CFSAN/JIFSAN/RSI 2021).

Statistical analysis

Descriptive statistics of staphylococci counts and relative occurrence datasets was done with NCSS ver. 12 data analysis software. Levene test of homogeneity, Shapiro–Wilk test, Kruskal–Wallis nonparametric one-way ANOVA test, Fisher (F) one-way ANOVA test for normally distributed datasets with equal variances, Welch's one-way ANOVA test for normally distributed datasets with unequal variances and one-tailed Student's t-test were also performed with NCSS ver. 12 data analysis software. The test of the hypothesis was considered statistically significant if the achieved level of significance (p) was less than 0.05. The accuracy of the phylogenetic tree implemented with MEGA software was evaluated by Monte Carlo simulation of the original tree-building dataset using the bootstrap sampling technique.

Results

Identification of bacterial colonies on MSA plates

Tab. 1 and 2 present the phenotypic and molecular characterization of bacterial colonies on MSA Petri plates with oxacillin (4 μg ml^{-1}) and without oxacillin. Results of phenotypic and molecular analysis performed on the bacterial colonies in MSA Petri plates without oxacillin (Tab. 1) indicated that staphylococci and non-staphylococci were isolated from companion dogs harboured in homes situated in Edo State, Nigeria during the dry season. The isolated staphylococci were classified as *S. pseudintermedius*, *S. aureus*, *S. epidermidis*, *S. simulans* and *S. saprophyticus*; while the non-staphylococci were found to be mainly *Micrococcus* spp. and *Bacillus* spp. Representative *S. pseudintermedius* strain ADEOLAAKINNIBOSUN 200, *S. epidermidis* strain ADEOLAAKINNIBOSUN 202, *S. simulans* strain ADEOLAAKINNIBOSUN 203, *S. pseudintermedius* strain ADEOLAAKINNIBOSUN 201, *S. aureus* strain ADEOLAAKINNIBOSUN 204, *S. pseudintermedius* strain ADEOLAAKINNIBOSUN 215, *S. aureus* strain ADEOLAAKINNIBOSUN 231, *S. aureus* strain ADEOLAAKINNIBOSUN 232, *S. pseudintermedius* strain ADEOLAAKINNIBOSUN 230, *S. simulans* strain ADEOLAAKINNIBOSUN 240 and *S. saprophyticus* strain ADEOLAAKINNIBOSUN 219 isolated from the fur of companion dogs harboured in homes situated in Edo and Delta States, Nigeria were deposited in the GenBank (NCBI) database under accession numbers MW965474, MW965579, MW965587, MW965574, MW965613, MZ008355, MZ461457, MZ461460, MZ461942, MZ488580, and MZ021337.

All staphylococcal colonies on MSA plates without oxacillin that were isolated from companion dogs during the dry season were susceptible to cefoxitin, with *MecA* prevalence estimates of 0.00%. However, during the rainy season, some staphylococci isolates were resistant to cefoxitin. Cefoxitin/oxacillin resistance was observed only in staphylococci isolates that were identified as *S. pseudintermedius* and *S. aureus*, with *MecA* prevalence of 53.06% and 34.52% for *S. aureus* and *S. pseudintermedius* isolates obtained from dog samples situated in Edo State, Nigeria.

No bacterial growth was seen in all the dry season samples from Edo State that were cultured on MSA plates with oxacillin (Tab. 2), but bacterial growth was seen in the rainy season samples. The bacteria that were identified on MSA plates with oxacillin were similar to the bacteria that were found on MSA plates without oxacillin. Unlike the case with MSA plates without oxacillin, the staphylococci on MSA plates with oxacillin were all resistant to cefoxitin/oxacillin, with *MecA* prevalence estimates of 100%, thus, were all termed as methicillin-resistant staphylococci.

The bacterial species that were isolated from companion dogs in homes situated in Delta State, Nigeria during dry and rainy season samplings significantly conformed to those isolated from companion dogs harboured in homes situated in Edo State, Nigeria.

Table 1. Phenotypic and molecular characterization of bacterial colonies on MSA Petri plates without oxacillin

Sampling locations	Period of sampling	Representative bacterial colonies	Colonial and morphological characteristics		Biochemical characteristics of bacterial colonies										Molecular analysis				Identified bacteria
					CO	CA	OX	MA	HT	MR	VP	FOX			16S homology	16S identity	*Mec4* prevalence		
			Growth on MSA Petri plates	Gram staining								Z (mm)	I				(F)	(%)	
Edo State, Nigeria	January – March 2020 (Dry season)	1	Yellow colonies	Positive cocci	-	+	+	-	v	+	v	23 - 33	S		NP	NP	NP		*Micrococcus* spp.
		2	Dry/mucoid colonies	Positive bacilli	NP	+	v	-	v	-	+	23 - 36	S		NP	NP	NP		*Bacillus* spp.
		3	Mucoid colony	Positive cocci	-	-	-	-	-	-	+	28 - 35	S		98 - 100%	95 - 99%	0/5	0.00	*Staphylococcus simulans*
		4	White colonies	Positive cocci	+	+	-	+	+	-	+	25 - 33	S		99 - 100%	95 - 100%	0/23	0.00	*Staphylococcus pseudintermedius*
		5	Yellow colonies	Positive cocci	+	+	-	-	+	-	+	23 - 26	S		94 - 99%	92 - 99%	0/3	0.00	*Staphylococcus aureus*
		6	Mucoid colonies	Positive cocci	-	+	-	-	-	-	+	24 - 33	S		96 - 100%	96 - 100%	0/20	0.00	*Staphylococcus epidermidis*
	July – September 2020 (Rainy season)	1	Yellow colonies	Positive cocci	-	+	+	-	v	+	v	17 - 28	S/R		NP	NP	NP		*Micrococcus* spp.
		2	Dry/mucoid colonies	Positive bacilli	NP	+	v	-	v	-	+	23 - 33	S		NP	NP	NP		*Bacillus* spp.
		3	Yellow colonies	Positive cocci	+	+	-	-	+	-	+	12 - 33	S/R		93 - 100%	92 - 100%	26/49	53.06	*Staphylococcus aureus*
		4	Mucoid colonies	Positive cocci	-	+	-	-	-	-	+	23 - 29	S		97 - 100%	99 - 100%	0/7	0.00	*Staphylococcus epidermidis*
		5	Mucoid colonies	Positive cocci	-	+	-	-	-	-	+	30	S		99%	100%	0/1	0.00	*Staphylococcus saprophyticus*
		6	White colonies	Positive cocci	+	+	-	+	+	-	+	23 - 33	S/R		98 - 100%	97 - 99%	29/84	34.52	*Staphylococcus pseudintermedius*
		7	Mucoid colonies	Positive cocci	-	-	-	-	-	-	+	23 - 27	S		99 - 100%	100%	0/3	0.00	*Staphylococcus simulans*
Delta State, Nigeria	January – March 2020 (Dry season)	1	Yellow colonies	Positive cocci	-	+	+	-	v	+	v	22 - 35	S		NP	NP	NP		*Micrococcus* spp.
		2	Dry/mucoid colonies	Positive bacilli	NP	+	v	-	v	-	+	22 - 36	S		NP	NP	NP		*Bacillus* spp.
		3	White colonies	Positive cocci	+	+	-	+	+	-	+	24 - 30	S		95 - 100%	95 - 99%	0/22	0.00	*Staphylococcus pseudintermedius*
		4	Mucoid colony	Positive cocci	-	-	-	-	-	-	+	29 - 30	S		96 - 100%	98 - 99%	0/6	0.00	*Staphylococcus simulans*
		5	Mucoid colonies	Positive cocci	-	+	-	-	-	-	+	25 - 33	S		99 - 100%	100%	0/17	0.00	*Staphylococcus epidermidis*
		6	Mucoid colonies	Positive cocci	-	+	-	-	-	-	+	28 - 29	S		99 - 100%	99 - 100%	0/3	0.00	*Staphylococcus saprophyticus*
	July – October 2020 (Rainy season)	1	Yellow colonies	Positive cocci	-	+	+	-	v	+	v	12 - 28	S/R		NP	NP	NP		*Micrococcus* spp.
		2	Dry/mucoid colonies	Positive bacilli	NP	+	v	-	v	-	+	23 - 25	S		NP	NP	NP		*Bacillus* spp.
		3	Mucoid colonies	Positive cocci	-	+	-	-	-	-	+	23 - 25	S		97 - 100%	97 - 100%	0/11	0.00	*Staphylococcus epidermidis*
		4	White colonies	Positive cocci	+	+	-	+	+	-	+	12 - 25	S/R		99 - 100%	97 - 100%	36/72	50.00	*Staphylococcus pseudintermedius*
		5	Mucoid colonies	Positive cocci	-	-	-	-	-	-	+	23 - 27	S		95 - 99%	97 - 99%	0/4	0.00	*Staphylococcus simulans*
		6	Yellow colonies	Positive cocci	+	+	-	-	+	-	+	17 - 26	S/R		94 - 99%	92 - 99%	27/43	62.79	*Staphylococcus aureus*

Voges-Proskauer test. FOX: cefoxitin/oxacillin antibiotic test; Z: zone inhibition diameter; I: interpretive criteria; S: sensitive, R: resistant; F: fractional prevalence of *MecA* gene; NP: not performed; +: positive result; -: negative result; v: variable result.

Table 2. Phenotypic and molecular characterization of bacterial colonies on MSA Petri plates containing 4 µg oxacillin per millilitre

Sampling locations	Period of sampling	Representative bacterial colonies	Colonial and morphological characteristics		Biochemical characteristics of bacterial colonies										Molecular analysis				Identified bacteria
					CO	CA	OX	MA	HT	MR	VP	FOX			16S	16S	*Mec A*		
			Growth on the MSA Petri plates	Gram staining								Z (mm)		I	homology	identity	prevalence (F)	(%)	
Edo State, Nigeria	January – March 2020 (Dry season)		No bacterial growth in all MSA plates examined																
	July – September 2020 (Rainy season)	1	Yellow colonies	Positive cocci	-	+	+	-	v	+	v	8 - 13	R		NP	NP	NP		*Micrococcus* spp.
		2	Dry/mucoid colonies	Positive bacilli	NP	+	v	-	v	-	+	12	R		NP	NP	NP		*Bacillus* spp.
		3	Yellow colonies	Positive cocci	+	+	-	-	+	-	+	0 - 8	R		98 - 100%	99 - 100%	29/29	100.00	*Staphylococcus aureus*
		4	White colonies	Positive cocci	+	+	-	+	+	-	+	0 - 10	R		94 - 99%	95 - 99%	34/34	100.00	*Staphylococcus pseudintermedius*
		5	Mucoid colonies	Positive cocci	-	-	-	-	-	-	+	13	R		98%	100%	1/1	100.00	*Staphylococcus similans*
		6	Mucoid colonies	Positive cocci	-	+	-	-	-	-	+	12	R		100%	99%	1/1	100.00	*Staphylococcus epidermidis*
Delta State, Nigeria	January – March 2020 (Dry season)		No bacterial growth in all MSA plates examined																
	July – October 2020 (Rainy season)	1	Yellow colonies	Positive cocci	-	+	+	-	v	+	v	0 - 13	R		NP	NP	NP		*Micrococcus* spp.
		2	Dry/mucoid colonies	Positive bacilli	NP	+	v	-	v	-	+	12	R		NP	NP	NP		*Bacillus* spp.
		3	Mucoid colonies	Positive cocci	-	+	-	-	-	-	+	12 - 13	R		100%	100%	3/3	100.00	*Staphylococcus epidermidis*
		4	White colonies	Positive cocci	+	+	-	+	+	-	+	0 - 10	R		96 - 100%	97 - 99%	38/38	100.00	*Staphylococcus pseudintermedius*
		5	Mucoid colonies	Positive cocci	-	-	-	-	-	-	+	12 - 13	R		99 - 100%	99%	5/5	100.00	*Staphylococcus similans*
		6	Yellow colonies	Positive cocci	+	+	-	-	+	-	+	0 - 8	R		97 - 100%	99 - 100%	30/30	100.00	*Staphylococcus aureus*

Staphylococcal phylogeny

Phylogenetic tree highlighting the evolutionary relatedness of some staphylococci strains that colonized companion dogs harboured in Nigerian homes and reference strains that were isolated from other environmental sources in the world is shown in Fig. 1. *S. epidermidis* strain ADEOLAAKINNIBOSUN 223 (MZ021414), *S. epidermidis* strain ADEOLAAKINNIBOSUN 229 (MZ021508), *S. epidermidis* strain ADEOLAAKINNIBOSUN 208 (MZ971554) and *S. epidermidis* strain ADEOLAAKINNIBOSUN 207 (MW971519) isolated from the fur of the companion dogs in Nigeria were found to have shared a common ancestry with *S. epidermidis* strain 100911 (NR 113957) collected from Japan, with a 97% likelihood. There was 88% likelihood that *S. aureus* strain ADEOLAAKINNIBOSUN 209 (MW971572), *S. aureus* strain ADEOLAAKINNIBOSUN 210 (MW978696), *S. aureus* NBRC 100910 (NR 113956) and *S. aureus* strain S33 R (NR 037007) evolved from a common ancestor. A common ancestor was also inferred to be the origin from where *S. pseudintermedius* strain ADEOLAAKINNIBOSUN 221 (MZ021339), *S. pseudintermedius* strain ADEOLAAKINNIBOSUN 205 (MW970345), *S. pseudintermedius* strain ADEOLAAKINNIBOSUN 206 (MW971449), *S. pseudintermedius* strain ADEOLAAKINNIBOSUN 215 (MZ008355) and *S. intermedius* strain H11/68 (NR 036829) evolved, with a 99% likelihood.

Occurrence and counts of staphylococci and non-staphylococci on the companion dogs

The occurrence and counts of bacteria seen on MSA plates without oxacillin are presented in Tab. 3. *Bacillus* spp., a non-staphylococci, was found to be the most abundant bacteria that were seen on the companion dogs harboured in homes situated in Edo State, Nigeria during the dry season because they had a relative occurrence estimated at 65.96% and a mean count estimated at 0.79 ± 0.15 $\log_{10}$ CFU cm^{-2}.

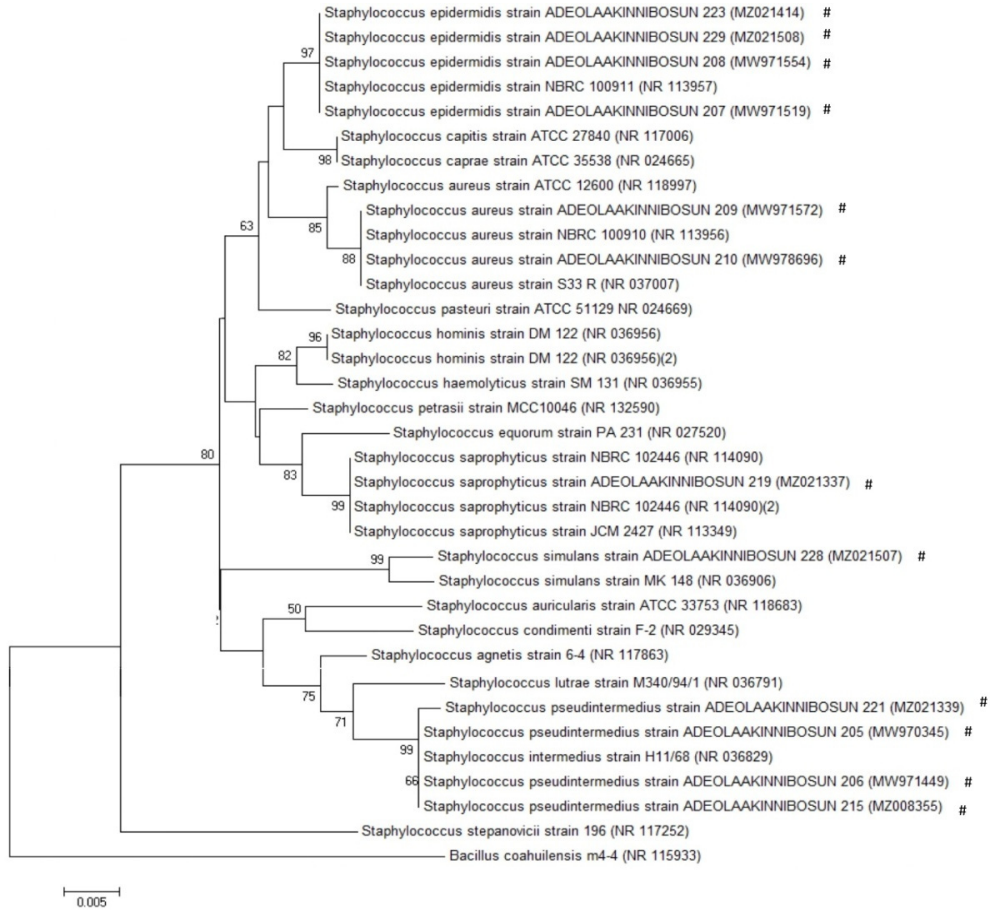

Figure 1. Phylogenetic tree constructed with the neighbour-joining method (# is used to indicate some novel *Staphylococcus* strains on the fur of the companion dogs examined in this study. GenBank accession numbers of all the strains used to implement the phylogenetic tree are indicated in parenthesis. The tree was rooted on midpoint and only bootstrap values that were above 50 % are displayed on branches).

Table 3. Occurrence and counts of bacteria seen on MSA plates without oxacillin

Sampling locations	Period of sampling	Counts of presumptive staphylococci (*CPS*)		Counts of identified bacteria					Counts of confirmed staphylococci (*CS*)	
		Mean ± SE (Log_{10} CFU cm^{-2}) (N = 35)	95% CI (Log_{10} CFU cm^{-2}) (N = 35)	Identified bacterial colonies	Relative occurrence of bacterial colonies		Relative counts		Mean ± SE (Log_{10} CFU cm^{-2}) (N = 35)	95% CI (Log_{10} CFU cm^{-2}) (N = 35)
					(*H*)	(%)	Mean ± SE (Log_{10} CFU cm^{-2}) (N = 35)	95% CI (Log_{10} CFU cm^{-2}) (N = 35)		
Edo State, Nigeria	January – March 2020 (Dry season)	1.20 ± 0.23	0.87 – 1.53						0.29 ± 0.06	0.21 – 0.37
				Micrococcus spp.	18/188	9.57	0.12 ± 0.02	0.09 – 0.15		
				Bacillus spp.	124/188	65.96	0.79 ± 0.15	0.57 – 1.01		
				Staphylococcus simulans	4/188	2.13	0.03 ± 0.01	0.02 – 0.04		
				Staphylococcus pseudintermedius	21/188	11.17	0.12 ± 0.03	0.09 – 0.17		
				Staphylococcus aureus	3/188	1.60	0.02 ± 0.00	0.01 – 0.03		
				Staphylococcus epidermidis	18/188	9.57	0.12 ± 0.02	0.09 – 0.15		
	July – September 2020 (Rainy season)	4.00 ± 3.59	2.56 – 5.44						3.09 ± 2.78	1.97 – 4.21
				Micrococcus spp.	38/181	20.99	0.84 ± 0.75	0.54 – 1.15		
				Bacillus spp.	3/181	1.66	0.07 ± 0.06	0.05 – 0.09		
				Staphylococcus aureus	48/181	26.52	1.06 ± 0.95	0.68 – 1.44		
				Staphylococcus epidermidis	7/181	3.87	0.16 ± 0.14	0.10 – 0.22		
				Staphylococcus saprophyticus	1/181	0.55	0.02 ± 0.02	0.01 – 0.03		
				Staphylococcus pseudintermedius	82/181	45.30	1.81 ± 1.63	1.15 – 2.47		
				Staphylococcus simulans	2/181	1.11	0.04 ± 0.04	0.02 – 0.06		
Delta State, Nigeria	January – March 2020 (Dry season)	1.17 ± 0.10	0.88 – 1.46						0.27 ± 0.02	0.22 – 0.34
				Micrococcus spp.	15/190	7.90	0.09 ± 0.01	0.07 – 0.11		
				Bacillus spp.	131/190	68.95	0.81 ± 0.07	0.61 – 1.01		
				Staphylococcus pseudintermedius	20/190	10.53	0.12 ± 0.01	0.09 – 0.15		
				Staphylococcus simulans	5/190	2.63	0.03 ± 0.00	0.02 – 0.04		
				Staphylococcus epidermidis	15/190	7.90	0.09 ± 0.01	0.07 – 0.11		
				Staphylococcus saprophyticus	4/190	2.11	0.03 ± 0.00	0.02 – 0.04		
	July – October 2020 (Rainy season)	4.21 ± 3.68	2.74 – 5.68						2.77 ± 2.43	1.30 – 3.24
				Micrococcus spp.	44/182	24.18	1.02 ± 0.89	0.66 – 1.38		
				Bacillus spp.	18/182	9.89	0.42 ± 0.36	0.27 – 0.57		
				Staphylococcus epidermidis	11/182	6.04	0.25 ± 0.22	0.16 – 0.34		
				Staphylococcus pseudintermedius	72/182	39.56	1.67 ± 1.46	1.09 – 2.25		
				Staphylococcus simulans	4/182	2.20	0.09 ± 0.08	0.06 – 0.12		
				Staphylococcus aureus	33/182	18.13	0.76 ± 0.67	0.49 – 1.03		

Amongst the staphylococci that were found to colonize the companion dogs during the dry season, *S. pseudintermedius* was the most abundant, with a relative occurrence of 11.17% and a mean count of 0.12 ± 0.03 log_{10} CFU cm^{-2} while *S. aureus* was the least abundant, with a relative occurrence of 1.60% and a mean count estimated at 0.02 ± 0.00 log_{10} CFU cm^{-2}. The mean count of presumptive staphylococci and confirmed staphylococci were respectively estimated at 1.20 ± 0.23 log_{10} CFU cm^{-2} and 0.29 log_{10} CFU cm^{-2} during the dry season. The count datasets of presumptive and confirmed staphylococci were non-normally distributed ($p = 0.01$ for both presumptive and confirmed staphylococci) with unequal variances ($p = 0.04$ for presumptive staphylococci and $p = 0.03$ for confirmed staphylococci). Kruskal-Wallis ANOVA tests indicated no significant difference in the median counts of presumptive and confirmed staphylococci ($p = 0.49$ for presumptive staphylococci and $p = 0.42$ for confirmed staphylococci).

Unlike the findings reported on companion dogs that were harboured in homes situated in Edo State, Nigeria during the dry season, the staphylococci were the most abundant bacteria on the companion dogs during the rainy season, with *S. pseudintermedius* accounting for 45.30% of the total viable bacteria on the fur of companion dogs and *S. aureus* accounting for 26.52%. Mean counts of presumptive staphylococci and confirmed staphylococci during the rainy season were estimated at 4.00 ± 3.59 $\log_{10}$ CFU cm^{-2} and 3.09 ± 2.78 $\log_{10}$ CFU cm^{-2}, respectively. The count datasets of presumptive and confirmed staphylococci were non-normally distributed ($p = 0.00$ for both presumptive and confirmed staphylococci) with equal variances ($p = 0.70$ for presumptive staphylococci and $p = 0.43$ for confirmed staphylococci). Kruskal-Wallis ANOVA tests showed that there was no significant difference in the median counts of presumptive and confirmed staphylococci ($p = 0.41$ for presumptive staphylococci and $p = 0.34$ for confirmed staphylococci). The mean count of presumptive staphylococci on companion dogs harboured in homes situated in Delta State, Nigeria during the dry season was estimated at 1.17 ± 0.10 $\log_{10}$ CFU cm^{-2} while the mean count of confirmed staphylococci was estimated at 0.27 ± 0.02 $\log_{10}$ CFU cm^{-2}. The presumptive and confirmed staphylococci count datasets were normally distributed ($p = 0.06$ for presumptive staphylococci and $p = 0.08$ for confirmed staphylococci) with equal variances ($p = 0.52$ for presumptive staphylococci and $p = 0.43$ for confirmed staphylococci). Fisher one-way ANOVA tests indicated that there was no significant difference in the mean counts of presumptive and confirmed staphylococci ($p = 0.85$ for presumptive staphylococci and $p = 0.65$ for confirmed staphylococci). *Bacillus* spp. was also the most abundant bacterium on companion dogs harboured in homes situated in Delta State, Nigeria during the dry season. *S. pseudintermedius*, with a relative occurrence of 10.53% and a mean count of 0.12 ± 0.01 $\log_{10}$ CFU cm^{-2}, was also found to be the most abundant staphylococcum on companion dogs harboured in homes situated in Delta State, Nigeria during the dry season. Unlike companion dogs harboured in homes situated in Edo State, Nigeria, no *S. aureus* was seen in samples obtained from companion dogs harboured in homes situated in Delta State, Nigeria during the dry season.

In Delta State, the staphylococci were also the most abundant bacteria seen on the fur of the companion dogs during the rainy season, with *S. pseudintermedius* accounting for 39.56% of the total viable bacteria on the fur of companion dogs. Mean counts of presumptive and confirmed staphylococci during the rainy season were estimated at 4.21 ± 3.68 $\log_{10}$ CFU cm^{-2} and 2.77 ± 2.43 $\log_{10}$ CFU cm^{-2} respectively. The count datasets of presumptive and confirmed staphylococci were non-normally distributed ($p = 0.00$ for both

presumptive and confirmed staphylococci) with equal variances ($p = 0.30$ for presumptive staphylococci and $p = 0.21$ for confirmed staphylococci). Kruskal-Wallis ANOVA tests showed that there was no significant difference in the median counts of presumptive and confirmed staphylococci ($p = 0.94$ for presumptive staphylococci and $p = 0.71$ for confirmed staphylococci). Student's t-test showed that the count datasets of presumptive and confirmed staphylococci on companion dogs in Edo State during the dry season did not significantly differ ($p = 0.49$ for presumptive staphylococci and $p = 0.63$ for confirmed staphylococci) from those that were obtained from companion dogs in Delta State, Nigeria. No significant difference ($p = 0.16$ for presumptive staphylococci and $p = 0.24$ for confirmed staphylococci) was also observed during the rainy season samplings. However, there was a significant difference in the counts of presumptive and confirmed staphylococci when the count datasets obtained during the dry and rainy seasons were compared ($p = 0.01$ and $p = 0.03$ for counts of presumptive and confirmed staphylococci in Edo State; $p = 0.01$ and $p = 0.00$ for counts of presumptive and confirmed staphylococci in Delta State).

Occurrence and counts of methicillin-resistant staphylococci and their virulent strains in companion dogs

The occurrence and counts of bacteria seen on MSA plates with oxacillin are presented in Tab. 4. Due to the absence of bacterial growth on the Petri plates, no counts of presumptive methicillin-resistant staphylococci were reported on the fur of the companion dogs harboured in homes situated in Edo State, Nigeria during the dry season. However, during the rainy season in which bacteria was seen on the Petri plates, methicillin-resistant *S. pseudintermedius* was the most abundant methicillin-resistant bacterial species, with a relative occurrence of 43.59% and a relative mean count of 1.38 ± 1.31 $\log_{10}$ CFU cm^{-2}. The least frequently occurred methicillin-resistant species was reported as *S. simulans* and *S. epidermidis*, with relative occurrences of 1.28% and relative mean counts of 0.04 ± 0.04 $\log_{10}$ CFU cm^{-2}. Mean counts of presumptive and confirmed methicillin-resistant staphylococci were respectively estimated at 3.16 ± 3.00 $\log_{10}$ CFU cm^{-2} and 2.63 ± 2.50 $\log_{10}$ CFU cm^{-2} during the rainy season. The count datasets of presumptive and confirmed staphylococci were non-normally distributed ($p = 0.00$ for both presumptive and confirmed staphylococci) with unequal variances ($p = 0.00$ for both presumptive and confirmed staphylococci). Kruskal-Wallis ANOVA tests showed that there was no significant difference in the median counts of presumptive and confirmed staphylococci ($p = 0.72$ for presumptive staphylococci and $p = 0.64$ for confirmed staphylococci).

No counts of presumptive methicillin-resistant staphylococci were reported on companion dogs that were sampled in Delta State, Nigeria during the dry season. Methicillin-resistant *S. pseudintermedius* were also the most abundant methicillin-resistant bacterial species seen on the fur of the companion dogs during the rainy season. Mean counts of presumptive methicillin-resistant staphylococci during rainy season was estimated at 3.17 ± 3.01 $\log_{10}$ CFU cm^{-2} while the mean count of confirmed methicillin-resistant staphylococci was estimated at 2.48 ± 2.37 $\log_{10}$ CFU cm^{-2}. The count datasets of presumptive and confirmed staphylococci were non-normally distributed (p = 0.00 for both presumptive and confirmed staphylococci) with equal variances (p = 0.33 for presumptive staphylococci and p = 0.51 for confirmed staphylococci). Kruskal-Wallis ANOVA tests showed that there was no significant difference in the median counts of presumptive staphylococci (p = 0.69) and median counts of confirmed staphylococci (p = 0.48). Student's t-test indicated that the count datasets of presumptive and confirmed staphylococci obtained during rainy season sampling of companion dogs in Edo State did not significantly differ (p = 0.49 for presumptive staphylococci and p = 0.61 for confirmed staphylococci) from those obtained from companion dogs in Delta State.

Table 4. Occurrence and counts of methicillin-resistant staphylococci (MRS) and its virulent strains on MSA plates containing 4 µg per millilitre

Sampling locations	Period of sampling	Counts of presumptive MRS (*CPMS*)		Counts of identified methicillin-resistant bacteria					Counts of confirmed MRS (*CMS*)		PVL-producing MRS			Enterotoxigenic-producing MRS		
		Mean ± SE (Log_{10} CFU cm^{-2}) (N = 35)	95% CI (Log_{10} CFU cm^{-2}) (N = 35)	Identified bacterial colonies	Relative occurrence of bacterial colonies		Relative counts		Mean ± SE (Log_{10} CFU cm^{-2}) (N = 35)	95% CI (Log_{10} CFU cm^{-2}) (N = 35)	(P)	Relative counts		(O)	Relative counts (*CES*)	
					(*H*)	(%)	Mean ± SE (Log_{10} CFU cm^{-2}) (N = 35)	95% CI (Log_{10} CFU cm^{-2}) (N = 35)				Mean ± SE (Log_{10} CFU cm^{-2}) (N = 35)	95% CI (Log_{10} CFU cm^{-2}) (N = 35)		Mean ± SE (Log_{10} CFU cm^{-2}) (N = 35)	95% CI (Log_{10} CFU cm^{-2}) (N = 35)
Edo State, Nigeria	January – March 2020 (Dry season)			None												
	July – September 2020 (Rainy season)	3.16 ± 3.00	1.91 – 4.41						2.63 ± 2.50	1.59 – 3.67						
				Micrococcus spp.	11/78	14.10	0.45 ± 0.42	0.27 – 0.63			NP			NP		
				Bacillus spp.	2/78	2.56	0.08 ± 0.08	0.05 – 0.11			NP			NP		
				Staphylococcus aureus	29/78	37.18	1.17 ± 1.11	0.71 – 1.63			2/78	0.07 ± 0.06	0.04 – 0.10	11/78	0.37 ± 0.35	0.19 – 0.55
				Staphylococcus pseudintermedius	34/78	43.59	1.38 ± 1.31	0.84 – 1.92			29/78	0.98 ± 0.93	0.52 – 1.44	0/78	0.00 ± 0.00	0.00 – 0.00
				Staphylococcus simulans	1/78	1.28	0.04 ± 0.04	0.02 – 0.06			0/78	0.00 ± 0.00	0.00 – 0.00	0/78	0.00 ± 0.00	0.00 – 0.00
				Staphylococcus epidermidis	1/78	1.28	0.04 ± 0.04	0.02 – 0.06			0/78	0.00 ± 0.00	0.00 – 0.00	0/78	0.00 ± 0.00	0.00 – 0.00
Delta State, Nigeria	January – March 2020 (Dry season)			None												
	July – October 2020 (Rainy season)	3.17 ± 3.01	1.92 – 4.42						2.48 ± 2.37	1.50 – 3.46						
				Micrococcus spp.	20/102	19.61	0.62 ± 0.59	0.38 – 0.87			NP			NP		
				Bacillus spp.	2/102	1.96	0.06 ± 0.06	0.04 – 0.08			NP			NP		
				Staphylococcus epidermidis	3/102	2.94	0.09 ± 0.09	0.05 – 0.13			0/102	0.00 ± 0.00	0.00 – 0.00	0/102	0.00 ± 0.00	0.00 – 0.00
				Staphylococcus pseudintermedius	40/102	39.22	1.24 ± 1.18	0.75 – 1.73			37/102	0.90 ± 0.86	0.45 – 1.35	0/102	0.00 ± 0.00	0.00 – 0.00
				Staphylococcus simulans	5/102	4.90	0.16 ± 0.15	0.10 – 0.22			0/102	0.00 ± 0.00	0.00 – 0.00	0/102	0.00 ± 0.00	0.00 – 0.00
				Staphylococcus aureus	32/102	31.37	1.00 ± 0.94	0.61 – 1.38			1/102	0.02 ± 0.02	0.01 – 0.03	9/102	0.22 ± 0.21	0.11 – 0.33

N: total number of dogs examined during the dry or rainy season; MRS: methicillin-resistant staphylococci; SE: standard error of the mean; CI: confidence interval of the mean; NP: PVL or enterotoxin B detection not performed on bacterial isolates.

Amongst the isolated methicillin-resistant staphylococci, methicillin-resistant *S. pseudintermedius* was the most prevalent PVL-producing methicillin-resistant staphylococci. Of the 34 isolated methicillin-resistant *S. pseudintermedius* in Edo State, 29 methicillin-resistant *S. pseudintermedius* was found to produce PVL toxin, thus, constituting 85.29% prevalence. In Delta State, 37 methicillin-resistant *S. pseudintermedius* was found to produce PVL toxin out of the 40 methicillin-resistant *S. pseudintermedius* isolated from the companion dogs, with an estimated prevalence of 92.50%. Only a few methicillin-resistant *S. aureus* were found to produce PVL toxin in both Edo and Delta States samples.

Twenty-nine methicillin-resistant *S. aureus* were reported in companion dogs from Edo State, with only 2 of these isolates producing PVL; corresponding to a 6.90% prevalence. Of the 32 isolated methicillin-resistant *S. aureus* reported in Delta State samples, only 1 methicillin-resistant *S. aureus* produced PVL, with an estimated prevalence of 3.13%. The mean count of PVL-producing methicillin-resistant staphylococci on the fur of the companion dogs harboured in homes situated in Edo State, Nigeria was estimated at 1.05 ± 0.99 $\log_{10}$ CFU cm^{-2}, and in Delta State, an estimate of 0.92 ± 0.88 $\log_{10}$ CFU cm^{-2} was reported. Representative PVL toxins produced by some of these strains and their respective translated gene were deposited in the GenBank under accession numbers QWX21626, QWX21629, QWX21628, QZW25256 for the PVL toxins, and MZ230623, MZ230626, MZ230625, MZ682632 for the translated gene.

Methicillin-resistant *S. aureus* accounted for all of the enterotoxigenic-producing staphylococci on the fur of companion dogs selected from both Edo and Delta States. In Edo State, 11 methicillin-resistant *S. aureus* produced enterotoxin B, out of the 29 methicillin-resistant *S. aureus* isolated from the companion dogs, with an estimated prevalence of 37.93%. In Delta State, 9 methicillin-resistant *S. aureus* produced enterotoxin B, out of the 32 methicillin-resistant *S. aureus* that was isolated from the companion dogs, thus, given prevalence estimates of 28.13%. The mean count of enterotoxin-producing methicillin-resistant staphylococci on the fur of the companion dogs harboured in homes situated in Edo State, Nigeria was estimated at 0.40 ± 0.38 $\log_{10}$ CFU cm^{-2} and in Delta State, it was estimated at 0.22 ± 0.21 $\log_{10}$ CFU cm^{-2}. Representative enterotoxin B produced by some of these strains were also deposited in the GenBank under accession numbers QZW25259, QZW25258 and their respective translated gene deposited under accession numbers MZ682635, MZ682634.

Exposure estimates

The prevalence of companion dogs colonized by *Staphylococcus* species is presented in Tab. 5. During the dry season, humans, especially dog owners, were mostly exposed to *S. pseudintermedius* carried by companion dogs harboured in homes situated in Edo State (17.14%) and Delta State (20.00%), Nigeria.

During the dry season in Edo State, the likelihood of exposure of humans to coagulase-positive *S. aureus* was estimated at 5.71%. In the rainy season, exposure to *S. pseudintermedius* was estimated at 80.00% in Edo State and 71.43% in Delta State; while the likelihood of exposure to *S. aureus* was estimated at 51.43% in Edo State and 34.29% in Delta State.

Unlike the dry season, it was found that humans were likely exposed to methicillin-resistant staphylococci, as well as PVL- and enterotoxigenic-producing staphylococci, carried on companion dogs during the rainy season. In Edo State, exposure to methicillin-resistant *S. pseudintermedius* and methicillin-resistant *S. aureus* was 31.43% and 25.71%, respectively, as well as 37.14 ± 8.29% and 28.57 ± 7.75% in Delta State. Only *S. aureus* was found to produce enterotoxins, and the likelihood of exposure of humans to enterotoxigenic-producing methicillin-resistant *S. aureus* was estimated at 8.57 ± 4.80% in Edo and Delta States, respectively. Exposure to PVL-producing methicillin-resistant *S. aureus* and PVL-producing methicillin-resistant *S. pseudintermedius* was respectively estimated at 5.71% and 25.71% in Edo State, as well as 2.86% and 34.29% in Delta State.

Table 5. Prevalence of companion dogs colonized by *Staphylococcus* species

Period of sampling	Identified *Staphylococcus* species	Companion dogs			
		Prevalence of colonized companion dogs from Edo State N = 35		Prevalence of colonized companion dogs from Delta State N = 35	
		Mean ± SE (%)	95% CI (%)	Mean ± SE (%)	95% CI (%)
January – March 2020 (Dry season)	*Staphylococcus simulans*	5.71 ± 3.98	0.00 – 13.51	5.71 ± 3.98	0.00 – 13.51
	Staphylococcus pseudintermedius	17.14 ± 6.46	4.47 – 29.81	20.00 ± 6.86	6.56 – 33.44
	Staphylococcus aureus	5.71 ± 3.98	0.00 – 13.51		
	Staphylococcus epidermidis	14.29 ± 6.00	2.53 – 26.05	14.29 ± 6.00	2.51 – 26.07
	Staphylococcus saprophyticus			5.71 ± 3.98	0.00 – 13.51
July – October 2020 (Rainy season)	*Staphylococcus aureus*	51.43 ± 8.57	34.63 – 68.23	34.29 ± 8.14	18.34 – 50.25
	Staphylococcus epidermidis	8.57 ± 4.80	0.00 – 17.98	11.43 ± 5.46	0.74 – 22.12
	Staphylococcus saprophyticus	2.86 ± 2.86	0.00 – 8.46		
	Staphylococcus pseudintermedius	80.00 ± 6.86	66.56 – 93.44	71.43 ± 7.75	56.24 – 86.62
	Staphylococcus simulans	2.86 ± 2.86	0.00 – 8.46	5.71 ± 3.98	0.00 – 13.51
	Methicillin-resistant *Staphylococcus aureus*	25.71 ± 7.50	11.02 – 40.40	28.57 ± 7.75	13.38 – 43.76
	Methicillin-resistant *Staphylococcus pseudintermedius*	31.43 ± 7.96	15.83 – 47.03	37.14 ± 8.29	20.90 – 53.38
	Methicillin-resistant *Staphylococcus simulans*	2.86 ± 2.86	0.00 – 8.46	5.71 ± 3.98	0.00 – 13.51
	Methicillin-resistant *Staphylococcus epidermidis*	2.86 ± 2.86	0.00 – 8.46	2.86 ± 2.86	0.00 – 8.46
	PVL-producing methicillin-resistant *Staphylococcus aureus*	5.71 ± 3.98	0.00 – 13.51	2.86 ± 2.86	0.00 – 8.46
	PVL-producing methicillin-resistant *Staphylococcus pseudintermedius*	25.71 ± 7.50	11.02 – 40.40	34.29 ± 8.14	18.34 – 50.25
	Enterotoxigenic-producing methicillin-resistant *Staphylococcus aureus*	8.57 ± 4.80	0.00 – 17.98	8.57 ± 4.80	0.00 – 17.98

N: total number of dogs examined during the dry or rainy season;
SE: standard error of the mean; CI: confidence interval of the mean

Antibiotic resistance profile

Tab. 6 shows the antibiotic resistance profile of methicillin-resistant staphylococcal colonies obtained from the companion dogs. Methicillin-resistant staphylococci that exhibited resistance to at least two other antibiotics from the different antibiotic classes examined were termed multidrug-resistant. Thirty-eight methicillin-resistant staphylococci were found to be multidrug-resistant out of the 141 methicillin-resistant staphylococci that were isolated from the companion dogs selected from Edo and Delta States. Coagulase-positive methicillin-resistant *S. aureus* and *S. pseudintermedius* accounted for all the multidrug-resistant staphylococci. Methicillin-resistant *S. aureus* was mostly resistant to ciprofloxacin, as indicated by percentage resistance of 13.79% and 13.33%, in isolates obtained from Edo and Delta States, respectively. Methicillin-resistant *S. aureus* also had the highest prevalence of resistance to all antibiotics tested, except for erythromycin. The coagulase-negative methicillin-resistant staphylococci were mostly susceptible to all the classes of antibiotics tested.

Table 6. Antibiotic resistance profile of methicillin-resistant staphylococcal colonies obtained from the companion dogs

Sampling locations	Period of sampling	Identified methicillin-resistant staphylococci on the MRSA plates	Relative count of methicillin-resistant staphylococci	Prevalence of antibiotic resistance										Relative count of multidrug-resistant staphylococci
				CIP 5 µg		PF 5 µg		C 30 µg		E 15 µg		GM 10 µg		
				Q	T (%)	Q	T (%)	Q	T (%)	Q	T (%)	Q	T (%)	
Edo State, Nigeria	January – March 2020 (Dry season)	No identified methicillin-resistant staphylococci												
	July – September 2020 (Rainy season)	Coagulase-positive *Staphylococcus aureus*	29	4/29	13.79	4/29	13.79	11/29	37.93	7/29	24.14	13/29	44.83	13
		Coagulase-positive *Staphylococcus pseudintermedius*	34	2/34	5.88	3/34	8.82	11/34	32.35	1/34	2.94	11/34	32.35	6
		Coagulase-negative *Staphylococcus simulans*	1	0/1	0.00	0/1	0.00	0/1	0.00	1/1	100.00	0/1	0.00	0
		Coagulase-negative *Staphylococcus epidermidis*	1	0/1	0.00	0/1	0.00	0/1	0.00	0/1	0.00	0/1	0.00	0
Delta State, Nigeria	January – March 2020 (Dry season)	No identified methicillin-resistant staphylococci												
	July – October 2020 (Rainy season)	Coagulase-positive *Staphylococcus aureus*	30	4/30	13.33	7/30	23.33	14/30	4.67	6/30	20.00	12/30	40.00	10
		Coagulase-positive *Staphylococcus pseudintermedius*	38	1/38	2.63	3/38	7.90	16/38	53.33	5/38	13.16	13/38	34.21	9
		Coagulase-negative *Staphylococcus epidermidis*	3	0/3	0.00	0/3	0.00	0/3	0.00	0/3	0.00	0/3	0.00	0
		Coagulase-negative *Staphylococcus simulans*	5	0/5	0.00	0/5	0.00	0/5	0.00	0/5	0.00	0/5	0.00	0

CIP: Ciprofloxacin; PF: Pefloxacin; C: Cotrimoxazole; E: Erythromycin; GM: Gentamycin; Z: fractional prevalence of resistance; I: percentage prevalence of resistance

Discussion

Bacterial transmissions associated with companion dogs are largely dependent on the cleanliness of the shelter/homes where these pet animals are kept (Song *et al.*, 2013). Upon examination of swabbed fur samples that were inoculated on MSA Petri plates with and without oxacillin, both *Staphylococcus*

and non-*Staphylococcus* species were found to colonize the fur of companion dogs that were harboured in Nigerian homes (Tables 1 and 2). *Bacillus* and *Micrococcus* species were the dominant non-*Staphylococcus* species, while the main *Staphylococcus* species that were carried on the fur of companion dogs included *S. pseudintermedius*, *S. aureus*, *S. epidermidis*, *S. simulans* and *S. saprophyticus*. Amongst the *Staphylococcus* species, *S. pseudintermedius* was the most frequently detected (up to 39.56% in Delta State and 45.30% in Edo State) followed by *S. aureus* (up to 18.13% in Delta State and 26.52% in Edo State). Suepaul *et al*. (2021) also reported that *S. pseudintermedius* was the most frequently detected species on companion dogs, accounting for 87.40% of the isolates that they examined. The findings of the present study were also consistent with the work of Janos *et al.* (2021) that detected *S. pseudintermedius* and *S. intermedius* in 48.83% and 27.90% of all canine isolates, followed by *S. aureus* in 11.62% of all isolates carried by the skin of healthy companion dogs.

Some *Staphylococcus* species that colonized the fur of the healthy companion dogs selected for this study were found to express resistance phenotype to methicillin, as confirmed by *MecA* PCR (Tab. 1 and 2), and were then regarded as methicillin-resistant *Staphylococcus* species. The effects of seasonal variations may have also resulted in the absence of methicillin-resistant staphylococci on the fur of the companion dogs during the dry season (Tab. 4). Amongst the *Staphylococci*, the expression of methicillin resistance was mostly exhibited by *S. pseudintermedius* and *S. aureus* during the rainy season. Interestingly, one of the earliest reports on methicillin resistance in companion dogs was recorded in Nigeria in 1972 (Ojo, 1972). The relative occurrence of methicillin-resistant *S. pseudintermedius* carried by the companion dogs harboured in Nigerian homes (39.22% in Delta State and 43.59% in Edo State) was higher than those reported in Australia [11.80%] (Saputra *et al.*, 2017) and even significantly higher when compared to those reported in Sweden, estimated at 0.4% (SWEDRES/ SVARM, 2015), and Norway, estimated at 0.5% (Simonsen and Urdahl, 2017).

26.95% of all the coagulase-positive methicillin-resistant *S. aureus* and *S. pseudintermedius* were found to account for all the multidrug-resistant *Staphylococcus* species carried by the fur of the companion dogs examined in the present study (Tab. 6). Coagulase-positive *Staphylococcus* species accounted for most of the multidrug-resistant staphylococci (25.40%) in canine samples examined by Suepaul *et al*. (2021), thus agreeing with the values reported in the present study. Unlike the study of Chah *et al*. (2014) that found a high rate of multidrug resistance (81.3%) amongst the coagulase-negative *Staphylococci* carried by clinically healthy dogs in Enugu State, Nigeria, no coagulase-negative *Staphylococci* obtained from the present study were regarded as multidrug-resistant.

In the present study, only the methicillin-resistant *S. aureus* was found to produce enterotoxin (Tab. 4), with an estimated prevalence of 37.93% and 28.13% in Edo and Delta States, respectively. In a study carried out by Abdel-Moein and Samir (2011), enterotoxigenic *S. aureus* was also detected at an estimated prevalence of 10.00% in pet dog samples collected from Egypt. The high prevalence of enterotoxigenic *S. aureus* on the fur of companion dogs in the present study is a pointer to a probable zoonotic transmission to human contacts.

PVL-producing methicillin-resistant *S. aureus* and *S. pseudintermedius* were also carried on the fur of companion dogs sampled in the present study (Tab. 4). This was consistent with the study of Findik *et al*. (2018) that reported the carriage of PVL-producing methicillin-resistant *S. aureus* and *S. pseudintermedius* on healthy dogs in Turkey. Futagawa-Saito *et al*. (2004) also reported the colonization of pet dogs harboured in Japanese homes with PVL-producing methicillin-resistant *S. intermedius*. These PVL-producing strains could pose a probable risk of transmission between humans and dogs that share the same household. The PVL toxins produced by these strains are pore-forming toxins that are capable of necrotizing plasma membranes, thus, resulting in cell lysis (Prévost *et al*., 2001; Reyes-Robles *et al*., 2013; Spaan *et al*., 2013; Maali *et al*., 2018).

The present study revealed that amongst staphylococci species, humans were mostly exposed to *S. pseudintermedius* during dry [17.14%] and rainy [80.00%] seasons (Tab. 5). Exposure of humans to methicillin-resistant *Staphylococci*, as well as PVL- and enterotoxigenic-producing *Staphylococci*, was reported only during the rainy season (Tab. 5). The companion dogs carried methicillin-resistant *S. pseudintermedius* at the rates of 31.43% in Edo State and 37.14% in Delta State, Nigeria. Rates ranging from 1.5% to 2.1%, as it relates to the prevalence of healthy dogs colonized by methicillin-resistant *S. pseudintermedius* and methicillin-resistant *S.aureus*, have been reported by several researchers (Griffeth *et al*., 2008; Hanselman *et al*., 2008; Loeffler *et al*., 2011; Chah *et al*., 2014; Suepaul *et al*., 2021); thus, revealing a significant difference from the rates reported during the rainy season in the present study. In the present study, the colonization of companion dogs by methicillin-resistant staphylococci during the rainy season is worrisome. This is because these staphylococcal strains could potentially transfer the resistance genes between dogs and humans.

The prevalence of companion dogs (8.57%) that carried enterotoxigenic *S. aureus* in the present study was similar to that reported by Abdel-Moein and Samir (2011). In the present study, 25.71% and 34.29% of companion dogs from Edo and Delta States, Nigeria were respectively colonized by PVL-producing

methicillin-resistant *S. pseudintermedius* (Tab. 5). These colonization rates reported in the present study appeared to be lower than that reported by Futagawa-Saito *et al*. (2004) where all 8 healthy dogs (100%) examined were colonized by PVL-producing *S. intermedius*.

Conclusions

This study revealed the high exposure of humans to PVL toxins and enterotoxins on the fur of companion dogs, mainly during the rainy season. The high prevalence of toxigenic-producing isolates seen on the fur of companion dogs, especially during the rainy season, could pose a risk for humans, particularly those that harbour pet dogs at their homes.

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