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Original Article

Phenotypic Resistance of *Staphylococcus aureus* to Antibiotics in Dogs of Tamale Metropolis, Ghana

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ABSTRACT

Introduction: *Staphylococcus aureus* is an important bacterium which induces a wide range of diseases. Its presence in dogs and resistance to antibiotics is a threat to public health due to the close association of humans with dogs. The objective of the present study was to determine the phenotypic resistance of *Staphylococcus aureus* (*S. aureus*) to antibiotics in dogs without any clinical manifestation of diseases in Tamale Metropolis, Ghana. The current study also examined microbial load in these dogs.
Materials and methods: A total of 120 samples from various parts of dogs, including the mouth, nose, anus, inner ear, and outer ear, were examined. Isolation and antibiotic resistance of *S. aureus* were determined using the USA Bacteriological Analytical Manual and the Disc Diffusion method, respectively.
Results: The presence of *S. aureus* in the dogs ranged from 8.3% (anus) to 58.3% (nose), averaging 40%. The microbial load also ranged from 2.9 log cfu/cm^2 (mouth) to 3.4 log cfu/cm^2 (outer ear) with an average of 3.2 log cfu/cm^2. There were significant differences among the examined samples regarding the presence of *S. aureus*, but not the microbial load. The overall resistance, intermediate resistance, and susceptibility of *S. aureus* were 46.2%, 12.9%, and 42.2%, respectively. The *S. aureus* was highly resistant to teicoplanin (88.0%) and susceptible to chloramphenicol (72.0%). The multiple antibiotic indexes ranged from 0 to 0.9, and 89.1% of the isolates exhibited multidrug resistance.
Conclusion: The findings of the current study revealed that healthy dogs in Tamale Metropolis, Ghana, were carriers of *S. aureus* as well as other bacteria, and *S. aureus* exhibited different resistance patterns to antibiotics.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive cocci-shaped bacterium that tends to be arranged in clusters and is a member of the family *Micrococcacae*[1,2]. It is an important bacterium due to its capacity to induce a wide range of diseases and its ability to adapt to different environmental conditions[2]. The emergence of multidrug-resistant strains of *S. aureus* has contributed to the importance of this pathogen. *Staphylococcus aureus* could be acquired from human and animal hosts. Approximately 30% of humans bear *S. aureus* in their nasal cavities, which is the primary reservoir and the primary source of infection[3]. Strains of *S. aureus* have been reported to cause mastitis in cattle, botryomycosis in horses, dermatitis in dogs, septicemia, and arthritis in poultry[4,5,6,7]. Case reports of human infection or colonization from household pets have shown the high likelihood of animals acting as reservoirs for transmission of this pathogen[8,9,10].

Pets can develop a social and emotional relationship with humans and their environment[11]. This has led to a strong attachment between dogs and their owners or caretakers, and therefore, pets are treated as family members[11]. Dogs have been reported to be potential sources of various zoonotic pathogens, such as *S. aureus*, *Escherichia coli, Salmonella enterica, Klebsiella species,* and *Bacillus* species[9,12,13,14] that are resistant to various antibiotics, including ampicillin, cephalosporin, gentamicin,

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enrofloxacin, methicillin, and tetracycline[10,15,16,17].

In Ghana, dogs are kept for different purposes, such as security, hunting, pets, and even food (meat). Although keeping dogs in Ghana is now rampant and currently raised due to the aforementioned reasons, the closeness between dogs and humans makes them potential sources for the transmission of zoonotic pathogens, such as *S. aureus*, to humans. However, information on the occurrence of *S. aureus* in dogs and their antibiotic resistance patterns in Ghana is limited. As these animals are in close contact with humans, the presence of antibiotic-resistant *S. aureus* can pose a health threat to humans. Therefore, the present study was conducted to determine the presence and antibiotic resistance of *S. aureus* in apparently healthy dogs in the Tamale Metropolis, Ghana. Furthermore, the microbial load of the various parts of the dogs was determined. To the best of the researchers' knowledge, the current study is one of the first reports on the prevalence of antibiotic resistance *S. aureus* in dogs of Ghana.

2. Materials and Methods

2.1. Ethical approval

The present study did not cause any harm to humans or animals and was approved by Project Supervisors of the Department of Veterinary Science, University for Development Studies, Ghana.

2.2. Study area

The present study was carried out at the Tamale Metropolis of Ghana. Geographically, the Tamale Metropolis lies between latitudes 9°16 and 9°34 North and longitudes 0°36 and 0°57 South[18]. The Metropolis has an estimated total land area of 646.90180 sq km, and a population of 223252[18].

2.3. Sample collection

A total of 120 samples were randomly collected from dogs without any clinical manifestation of diseases in the Tamale Metropolis, confirmed by a veterinarian. Houses that owned dogs were numbered, and the researchers randomly picked the numbers from a box. Houses picked were visited, and with the help of dog owners, such as the restrainers and a veterinary officer, swabs were taken from the various parts of the dogs. The dogs were randomly selected without emphasis on selecting a particular breed. Sterile cotton swabs were used to swab the anus, (n=24), mouth (n=24), nose (n=24), inner ear, (n=24), and outer ear (n=24) of the dogs at their homes with the help of dog owners and a veterinary officer. The swabbed samples were placed in an ice chest box containing ice blocks and transported to the Spanish Laboratory of the University for Development Studies, Nyankpala Campus, Ghana, where they were analyzed for *S. aureus*. Sampling was carried out from December 2019 to March 2020.

2.4. Enumeration of aerobic plate count

Enumeration of aerobic plate count was performed using a slightly modified procedure as previously done[19,20]. Swabs were soaked in 9 ml of 1% buffered peptone water (BPW), and serial dilutions from 10^{-1} to 10^{-5} were made using one ml of 1% BPW. It was then spread plated (0.1 ml) unto plate count agar and incubated at 37°C for 24 hours. Afterwards, the colonies were counted and expressed in colony-forming units. All media used were purchased from Oxoid, Basingstoke, the UK.

2.5. Isolation and identification of Staphylococcus aureus

Isolation and identification of *S. aureus* were performed using a slightly modified procedure[21,22]. Briefly, swabbed samples from healthy dogs were pre-enriched in BPW and incubated at 37°C for 24 hours. They were then streaked with mannitol salt agar (MSA) and incubated at 37°C for 24 hours. Presumptive *S. aureus* colonies formed yellow colonies on MSA surrounded by a yellow area. Two or three colonies were picked and purified on Trypticase soy agar and incubated for 24 hours at 37°C. Gram staining and Staphylase test were used to confirm the purified *S. aureus* colonies. All media and reagents used were purchased from Oxoid, Basingstoke, the UK.

2.6. Antibiotics susceptibility of Staphylococcus aureus

The disk diffusion method was used to determine the antimicrobial resistance of *S. aureus* against some antibiotics, including Amoxycillin/clavulanic acid (Amc, 30 µg), chloramphenicol (C, 30 µg), gentamicin (Cn, 10 µg), ceftriaxone (Cro, 30 µg), ciprofloxacin (Cip, 5 µg), azithromycin (Azm, 15 µg), sulphamethoxazole/ trimethoprim (Sxt, 22 µg), tetracycline (Te, 30 µg), and teicoplanin (Tec, 30 µg)[23]. Pure cultures of *S. aureus* were grown overnight in tryptic soy broth at 37°C, and the concentration was adjusted to 0.5 McFarland turbidity. About 0.5 ml of the culture was spread plated on Mueller Hinton agar. Four and five antimicrobial disks were placed on the surface of the agar plate at a distance to avoid overlapping inhibition zones. The plates were incubated at 37°C for 24 hours, and the results were interpreted according to a previous study[24]. All media and disks used were purchased from Oxoid, Basingstoke, the UK. The multiple antibiotic resistance (MAR) index was calculated and interpreted to use the formula a/b, where a represents the number of antibiotics to which a particular isolate was resistant, and b denotes the total number of antibiotics tested[25].

2.7. Data analysis

The data obtained from microbial load was analyzed using ANOVA of GenStat Software 12.1 Edition. Data on the presence of *S. aureus* were analyzed using binary logistic of IBM Statistical Package for the Social Sciences (SPSS) Software Version 17. The statistical difference test was

done using the Wald test in Chi-square. All differences were determined at a 5% significance level.

3. Results and Discussion

3.1. Microbial loads of healthy dogs in the Tamale Metropolis, Ghana

Table 1 shows the microbial load of the anus, mouth, nose, inner ear, and outer ear of apparently healthy dogs in the Tamale Metropolis. The microbial load was 3.2 log cfu/cm^2 (anus), 2.9 log cfu/cm^2 (mouth), 3.2 log cfu/cm^2 (nose), 3.1 log cfu/cm^2 (inner ear), and 3.4 log cfu/cm^2 (outer ear). There were no significant differences in microbial load among the anus, mouth, nose, inner ear, and outer ear ($p > 0.05$).

In Ghana, the increase in the number of dog owners has caused close contact between humans and dogs. Such close contact enhances the transmission of pathogens. The current study showed that bacteria were present in the outer ear, anus, nose, inner ear, and mouth of the dogs studied. These bacteria could be naturally present in the parts of the dogs examined, or the dogs might pick them from their food, environment, and humans. In support of this, various types of bacteria in dog feces collected from urban streets, could be considered a risk factor for the transmission of microorganisms among humans, the environment, and dogs[26]. In another study, diverse bacterial species in the mouth of dogs were observed[27]. Dog foods have also been reported as potential sources of bacteria that can cross-contaminate the mouth and other parts of the body[28].

Table 1. Total aerobic plate count of apparently healthy dogs in the Tamale Metropolis, Ghana

Organ	Bacteria load (log cfu/cm^2)
Anus	3.2
Mouth	2.9
Nose	3.2
Inner ear	3.1
Outer ear	3.4
Sed	0.497
P-value	0.898

3.2. The presence of Staphylococcus aureus in healthy dogs of the Tamale Metropolis, Ghana

The presence of *S. aureus* in the apparently healthy dogs in the Tamale Metropolis is presented in Table 2. The overall presence of *S. aureus* in the dogs was 40% (48/120). *Staphylococcus aureus* was most common in the nose (58.3%), followed by the mouth (54.2%), outer ear (50.0%), inner ear, (29.2%), and anus (8.3%). Significant differences were observed in the presence of *S. aureus* in the dog samples ($p < 0.05$). Mouth, nose, and outer ear did not differ significantly ($p > 0.05$) from each other but were significantly higher ($p < 0.05$) than the anus. The inner ear did not differ significantly ($p > 0.05$) from the anus, mouth, and outer ear except the nose.

Table 2. The presence of *Staphylococcus aureus* in apparently healthy dogs in the Tamale Metropolis, Ghana

Organ	Number of samples tested	Positive samples	Percentage
Anus	24	2	8.3
Mouth	24	13	54.2
Nose	24	14	58.3
Inner ear	24	7	29.2
Outer ear	24	12	50.0
Overall	120	48	40.0

Similar to the microbial load, *S. aureus* was also present in all the various parts of the healthy dogs examined. The contamination of *S. aureus* was generally higher in the dogs' mouth, nose, and outer ear. The sources of *S. aureus* include the environment (air, soil, water) as well as skin and nose[1,9]. This pathogen can be transferred from these sources by direct or indirect means. For instance, it is common to find dogs licking themselves or their owners, potentially transferring S. aureus to other body parts. The licking of dog owners also serves as a potential means of transmitting *S. aureus* between humans and dogs and vice versa. Another study supports this by indicating few dog-to-dog and dog-to-human transmissions of *S. aureus*[17]. Other authors reported the presence of S. aureus in dogs in different countries. In Nigeria, 14% of domestic dog stools were contaminated with *S. aureus*[8]. Furthermore, 16% of dogs in Bangladesh[16], 15.4% in Portugal[17], 7.9% in Columbia[12], 6.6% in Lithuania[29], and 4.5% of dogs in Trinidad[10] were contaminated with *S. aureus*. In Australia, 67.3% of dogs were infected with *Staphylococcus* spp.[15] Differences in the location of examined organs, and handling methods of dogs accounted for the differences in the prevalence rates.

3.3. Antibiotic susceptibility of Staphylococcus aureus obtained from healthy dogs in the Tamale Metropolis, Ghana

The antibiotic resistance of *S. aureus* in healthy dogs is shown in Table 3. The overall resistance, intermediate resistance, and susceptibility were 46.2%, 12.9%, and 42.2%, respectively. The highest resistance occurred for teicoplanin (88.0%), followed by ceftriaxone (68%), azithromycin (52%), and tetracycline (52%). The isolates showed susceptibility to chloramphenicol (72%), ciprofloxacin (64%), sulphamethoxazole/trimethoprim (64%), gentamicin (60%), and amoxicillin/clavulanic acid (52%). Relatively higher intermediate resistances were observed for azithromycin (20.0%), gentamicin (28.0%), and tetracycline (32.0%). Intermediate resistances are those isolates that are not entirely resistant or susceptible[30,31], and such isolates can alter treatment patterns when they are involved in infections[32].

Dogs can put humans at health risk because they can be carriers of zoonotic antibiotic-resistant bacteria [33]. In the current study, *S. aureus* was resistant to teicoplanin, ceftriaxone, azithromycin, and tetracycline (> 50%). They were susceptible to chloramphenicol, sulphamethoxazole/trimethoprim, ciprofloxacin, and gentamicin (≥ 60%). This suggests that teicoplanin and tetracycline will not

Table 3. Antibiotic resistance of *Staphylococcus aureus* isolated from healthy dogs in the Tamale Metropolis, Ghana

Antimicrobials	Resistant (%)	Intermediate resistant (%)	Susceptibility (%)
Amoxocillin/clavunic acid	48.0	0.0	52.0
Azithromycin	52.0	20.0	28.0
Ceftriaxone	68.0	16.0	16.0
Chloramphenicol	24.0	4.0	72.0
Ciprofloxacin	24.0	12.0	64.0
Gentamicin	12.0	28.0	60.0
Teicoplanin	88.0	4.0	8.0
Tetracycline	52.0	32.0	16.0
Sulphamethoxazole/trimethoprim	36.0	0.0	64.0
Overall	46.2	12.9	42.2

Amoxycillin/clavulanic acid (30 µg), Chloramphenicol (30 µg), Gentamicin 10 µg, Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Azithromycin (15 µg), Suphamethoxazole/trimethoprim (22 µg), Tetracycline (30 µg) and Teicoplainin (30 µg)

be the antibiotic of choice for treating *S. aureus* infections associated with dogs in the Tamale Metropolis. However, chloramphenicol could be used to manage infections in dogs caused by *S. aureus*. It was observed that *S. aureus* obtained from dogs was resistant to gentamicin (40.2%), tetracycline (75%), and ciprofloxacin (7.7%)[12]. The present study found lower resistances to gentamicin and tetracycline except for ciprofloxacin. Moreover, coagulase-positive *Staphylococci* from 112 dogs sampled exhibited 23.2%, 21.4%, 9.8%, and 2.7% resistances to tetracycline, trimethoprim/sulfamethoxazole, ciprofloxacin, and chloramphenicol, respectively, which were higher in the current study[10]. High resistance to tetracycline (87.5%) and chloramphenicol (75.0%) for *S. aureus* isolated from dogs were recorded in another study, compared to the current study[34]. Differences in the extent to which antibiotics were used and handled in the study areas contributed to differences in the results obtained.

3.4. Antibiotic resistance profile and multiples antibiotic resistance index of individual Staphylococcus aureus recovered from healthy dogs in the Tamale Metropolis, Ghana

The antibiotic resistance profile and MAR index of *S. aureus* from the healthy dogs are shown in Table 4. Two, four, and three *S. aureus* isolates were resistant to eight, seven, and five different antibiotics, respectively. Resistance to three or more different antibiotics (multidrug resistance) was recorded for 90 isolates (89.1%). Multidrug resistance *S. aureus* isolates have also been reported in dogs[5,12]. Multiple antibiotic resistance ranged from 0 (resistant to 0 antibiotics) to 0.9 (resistant to 8 antibiotics). *Staphylococcus aureus* with a MAR index of greater than 0.2 originates from sources where antibiotics are frequently used, while those with MAR less than 0.2 originates from sources where antibiotics use is uncommon[35]. Based on this, 72% of the isolates originated

Table 4. Antibiotic resistance profile and multiple antibiotic resistance index of individual *Staphylococcus aureus* isolated from healthy dogs in the Tamale Metropolis, Ghana

Code	Sources	No. of antibiotics	Antibiotics resistance profile	MAR index
8OE	Outer ear	8	Cip-Azm-Tec-Cn-Te-C-Cro-Sxt	0.9
15OE	Outer ear	8	Cip-Amc-Azm-Tec-Te-C-Cro-Sxt	0.9
2N	Nose	7	Cip-Azm-Tec-Cn-Te-Cro-Sxt	0.8
3OE	Outer ear	7	Cip-Amc-Tec-Te-C-Cro-Sxt	0.8
18OE	Outer ear	7	Cip-Amc-Tec-Cn-Te-C-Sxt	0.8
17M	Mouth	7	Amc-Azm-Tec-Te-C-Cro-Sxt	0.8
15M	Mouth	5	Amc-Azm-Tec-Cro-Sxt	0.6
24M	Mouth	5	Amc-Azm-Tec-Cro-Sxt	0.6
2OE	Outer ear	5	Tec-Te-C-Cro-Sxt	0.6
8A	Anus	4	Amc-Azm-Tec-Te	0.4
9M	Mouth	4	Amc-Azm-Tec-Te	0.4
11N	Nose	4	Amc-Tec-Te-Cro	0.4
22IE	Inner ear	4	Azm-Tec-Te-Cro	0.4
24N	Nose	3	Amc-Tec-Cro	0.3
2M	Mouth	3	Azm-Tec-Te	0.3
11M	Mouth	3	Azm-Tec-Cro	0.3
23M	Mouth	3	Amc-Tec-Cro	0.3
10N	Nose	3	Cip-Amc-Tec	0.3
6N	Nose	2	Tec-Cro	0.2
24OE	Outer ear	2	Azm-Cro	0.2
1IE	Inner ear	2	Tec-Cro	0.2
16IE	Inner ear	2	Azm-Te	0.2
24IE	Inner ear	2	Tec-Cro	0.2
21N	Nose	1	Tec	0.1
20OE	Outer ear	0	All susceptible	0

No: Number, MAR: Multiple antibiotic resistance, Amc: Amoxycillin/clavulanic acid (30 µg), C: Chloramphenicol (30 µg), Cn: Gentamicin (10 µg), Cro: Ceftriaxone (30 µg), Cip: Ciprofloxacin (5 µg), Azm: Azithromycin (15 µg), Sxt: Suphamethoxazole/trimethoprim (22 µg), Te: Tetracycline (30 µg) and Te: Teicoplainin (30 µg)

from sources where they were frequently exposed to antibiotics. The *S. aureus* isolates exhibited 19 different phenotypic antibiotic-resistant profiles. The resistant profile Tec-Cro was the most common and was exhibited by three different *S. aureus* isolates. *Staphylococcus aureus* of the anus (8A) and month (9M) shared the same phenotypic resistance pattern (Amc-Azm-Tec-Te). Similarly, *S. aureus* with codes 24N and 23M shared the same phenotypic resistance pattern (Amc-Tec-Cro). These suggest possible cross-contamination; nonetheless, molecular characterization is required to authenticate this observation.

4. Conclusion

Bacteria were present in healthy dogs' anus, mouth, nose, inner ear, and outer ear. Furthermore, *S. aureus* was recovered from these parts. The *S. aureus* exhibited some resistance and intermediate resistance to some antibiotics. Therefore, healthy dogs in the Tamale Metropolis, Ghana, are potential sources for the transmission of *S. aureus* resistant to some antibiotics.

Declarations

Competing interests

The authors declared that they have no conflict of interest.

Authors' contribution

Frederick Adzitey conceived the idea, supported it with funds, was involved in data analysis, and wrote the first draft. Nicholas Prah and David Yidana were involved in data collection, and analysis of raw data and reviewed/edited the study.

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