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ANTIMICROBIAL RESISTANCE AND PHENOTYPIC CHARACTERIZATION OF STAPHYLOCOCCI ISOLATED FROM SICK AND APPARENTLY HEALTHY GOATS IN NSUKKA ZONE, NIGERIA

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ABSTRACT

Staphylococci are known to cause numerous diseases of animals and have acquired resistance to different antibacterial agents making treatment extremely difficult. This study was carried out to determine the phenotypic virulence and antimicrobial resistance attributes of staphylococci from upper respiratory tracts of sick and apparently healthy goats in Nsukka zone. Three hundred nasal swab samples from 100 sick and 200 apparently healthy goats from 3 local government areas were collected equally from both sexes. Samples were cultured on mannitol salt agar after enrichment on nutrient broth supplemented with 6.5% Nacl. Isolates were confirmed through microscopic morphology and biochemical tests. They were screened for coagulase and haemolysin production. Antimicrobial susceptibility test was carried out using disc diffusion method against a panel of 15 antimicrobials. Data were subjected to statistical analysis. Ninety one (30%) goats haboured staphylococci out of which 63(31%) and 28(28%) of the apparently healthy and sick goats respectively and 62(41%) and 29(19%) of the males and females respectively were positive for staphylococci. Four percent and 12% of the isolates were coagulase and haemolysin producers respectively. The isolates

were most resistant to streptomycin, tetracycline and ceftazidine and most sensitive to enrofloxacin and gentamycin. Goats in Nsukka zone were found to be highly colonized by multi-drug resistant coagulase positive and coagulase negative staphylococci which could constitute serious health hazard to animals and man.

Keywords: Bacteria, Goat, Nsukka, Resistance, *Staphylococci*

INTRODUCTION

Staphylococcus species are facultative anaerobic, gram positive cocci and are catalase positive with some strains haemolytic **[1]**. They are pathogens of great concern because of their virulence **[2]**, with ability to cause a diverse array of life threatening infections and ability to adapt to different environmental conditions **[3]**. Staphylococci have been incriminated to be part of the normal *flora* of the upper respiratory tract of small ruminants **[4]**, and was reported to cause both nosocomial and community-acquired infections **[5]**. *S. aureus* has been found to be the most frequently isolated pathogen causing bloodstream infections, skin and soft tissue infections, pneumonia, mastitis and metritis **[6-8]**.

Sheep and goats form very significant aspect of the livestock economy in developing countries in the humid tropics **[9]**. More than 80 % of rural families especially women and children keep these ruminants **[10, 11]**. In Nigeria, 37.4% and 8.3% of households keep goats and sheep respectively with an average number per owner being 6.5 (sheep) and 5.2 (goats) **[12]**. Over the past 15 years however, there has been a steady growth in goat production. Goats provide 30-36% of the total meat consumption of the Nigerian populace annually **[13]**. Nigeria has a small ruminant population of about 34.5 million goats and 22.1 million sheep and of these, 25% are the West African Dwarf breed that is resident in the humid zone of the country **[14]**. Due to poor management, inbreeding and inadequate nutrition, these goats are usually predisposed to a range of health problems **[15]**.

Antibiotics are used to treat diseases of cattle, sheep, goat, water buffalo and other animals and as well as used as preservatives for milk **[16]**. The indiscriminate use of antibiotics could lead to the development of multiple antibiotic resistances thereby rendering the antibiotic treatment ineffective **[17]**. Resistant bacteria occur in soil, water, plants and animals **[18]**; and are a major public health concern in many countries due to the persistence and circulation of these resistant strains of bacteria in the environment and the possible contamination of food and water **[19]**.

In Nsukka, goats are reared both intensively and extensively. The intensively managed goats are kept in close contact with humans and infections can be transmitted from animal to man by contact or inhalation. The nasal carriage has been implicated as an important factor in introducing *Staphylococcus* species onto the skin or other sites of humans and other animals **[20, 21]**. The objectives of the study was to determine the prevalence and antibiotic resistance profile of staphylococci isolated from sick and apparently healthy goats in Nsukka zone.

MATERIALS AND METHODS

Study Area and sample collection

This study was carried out at Nsukka Zone in Enugu State, Nigeria. Nsukka is in the savanna region of South East Nigeria. Samples were collected from three local government areas (LGA) of the zone, namely Udenu, Igbo-Eze South and Nsukka LGAs. A total of 300 samples were collected from sick and apparently healthy goats comprising of 150 males and 150 female goats. One sterile swab stick was moistened with sterile normal saline and used to sample both nostrils of each randomly selected goat gently. The samples were transferred to the Microbiology laboratory of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, within 2 hours of collection for analysis. The media used were Nutrient broth [Oxoid®], Mannitol salt agar[Oxoid®], Blood agar base[Oxoid®] and Iso-sensitest agar [Oxoid®] and were prepared according to the manufacturers' recommended procedures.

Culture and Isolation

The nasal swabs were inoculated in nutrient broth and incubated at 37^{o}C overnight. After overnight incubation, the samples were streaked onto Mannitol salt agar plates and incubated at 37^{o}C for 24 hours. Growth was observed and colonial morphologies were noted.

Characterization of the Isolates

The isolate were Gram stained and observed under simple light microscope. All the Gram positive cocci in bunches were tested for catalase production and the catalase positive samples were stocked for further analysis. Isolates were evaluated for haemolytic activity on blood agar following the procedure described by Boerlin **[22]** and for coagulase production on rabbit plasma using tube coagulase test as described by Singleton **[23]**.

Antibiogram of the isolates

Antimicrobial susceptibility test was conducted using disc diffusion method as described by Bauer **[24]** and in accordance with procedures of Clinical Laboratory Standards Institute **[25]**, against a panel of fifteen (15) antibiotics. These include:

chloramphenicol (30µg), ciprofloxacin (10 µg), amikacin (30 µg), streptomycin (10 µg), gentamicin (10 µg), tetracycline (30 µg), cefotaxime (30 µg), ceftazidine (30 µg), cefuroxine (30 µg), ceftriaxone (30 µg), levofloxacin (5 µg), norfloxacin (10 µg), enrofloxacin (5 µg), cefpodoxime (10 µg), and ofloxacin (5 µg). The inhibition zone diameters of the isolates were measured and recorded. Each test isolate was classified as resistant or sensitive to the test antibiotics in accordance with the guidelines given by **[25]**.

Data Presentation and Statistical Analysis

Data generated were presented in form of tables and percentages

RESULTS

A total of 300 goats were sampled comprising of 200 and 100 nasal swabs from the upper respiratory tract of sick and apparently healthy goats respectively of equal number of males and females. The result of the isolation rate from sick and apparently healthy goats from each LGA are shown in **Table 1**. The results of the distribution of staphylococcal isolates from male and female goats in Nsukka zone are shown in **Table 2** while the results of coagulase and haemolysin production among staphylococci from sick and healthy goats in Nsukka zone is shown in **Table 3**.

Out of the total sampled, 91 (30%) comprising of 63 (31%) from apparently healthy and 28 (28%) from sick goats were positive for staphylococci. Hence the prevalence of nasal colonization of *Staphylococcus spp* among goats in Nsukka was 30.0%. There was no significant association between the different LGAs sampled in isolation of staphylococci among the apparently healthy and sick goats.

The antibacterial resistance profile of the 63 strains from apparently healthy goats is shown in **Table 4** while the resistance profile of the 28 strains from sick goats is shown in **Table 5**. The result showed that the isolates from the sick goats exhibited more resistance to the antibacterials tested than the isolates from apparently healthy goats. Highest resistance was shown to streptomycin, tetracycline and ceftazidine while lowest resistance was shown to enrofloxacin and gentamycin.

The antibacterial resistance patterns of the isolates from sick and apparently healthy goats are shown in **Table 6**. There were 35 resistance patterns observed among the isolates. The most frequent patterns among the isolates were TE (5), TE-C-S (4) and CAZ (4). Two isolates exhibited resistance to 11 antibacterial agents while 4 isolates showed resistance to different set of 6 antibacterial agents.

Table 1: Rate of colonization of the upper respiratory tract of sick and apparently healthy goats with staphylococci in Nsukka zone

Status of the goats sampled	Number of goats sampled	No (%) positive for *staphylococci*
Healthy	200	63 (31%)
Sick	100	28 (28%)
Total	300	91 (30.3%)

Table 2: Rate of staphylococcal colonization of anterior nares of apparently healthy and sick goats according to LGAs in Nsukka zone

LGA	No of sick goats sampled	No (%) of sick goats positive for staphylococci	No of apparently healthy goats sampled	No (%) of apparently healthy goats positive for staphylococci
Nsukka	50	15(30)	80	29(36)
Udenu	30	8(27)	65	19(29)
Igbo-Eze South	20	5(25)	55	15(27)
Total	100	28 (28)	200	63(31)

Table 3: Distribution of Staphylococci isolated from sick and apparently healthy goats according to sex in Nsukka zone

Sex	Status	No of goats sampled	No (%) of sample positive for staphylococci
Male	Healthy	100	46 (46)
	Sick	50	16 (32)
	Total	150	62 (41.3)
Female	Healthy	100	17 (17)
	Sick	50	12 (12)
	Total	150	29 (19.3)
	Grand total	300	91 (30.3)

Table 4: Prevalence of coagulase and haemolysin production among staphylococci isolated form goats in Nsukka zone

	No of staphylococci	No of strains positive (%)
Coagulase	91	4 (4.4)
Haemolysin	91	11 (12.1)

Table 5: Antibiogram of staphylococci isolated from apparently healthy goats in Nsukka zone

Antibacterial agent	No of sensitive isolates (%)	No (%) of intermediate sensitive isolates	No of resistant isolates (%)
Tetracycline	15 (24.2)	10 (16.1)	36 (58.1)
Amikacin	58 (93.5)	1 (1.6)	3 (4.8)
Gentamicin	58 (93.5)	1 (1.6)	3 (4.8)
Streptomycin	31 ((50)	10 (16.1)	22 (35.5)
Chloramphenicol	26 (41.9)	13 (21)	23 (37.1)
Ciprofloxacin	54 (87.1)	4 (6.5)	4 (6.5)
Ofloxacin	55 (88.7)	1 (1.6)	6 (9.7)
Norfloxacin	55 (88.7)	4 (6.5)	3 (4.8)
Levofloxacin	58 (93.5)	0 (0)	4 (6.5)
Enrofloxacin	54 (87.1)	5 (8.1)	3 (4.8)
Cefuroxime	44 (71)	6 (9.7)	12 (19.4)
Cefpodoxime	28 (45.2)	23 (37.1)	11 (17.7)
Ceftazidine	25 (40.3)	8 (12.9)	29 (46.8)
Ceftriaxone	33 (53.2)	27 (43.5)	2 (3.2)
Cefotaxime	34 (54.8)	22 (35.5)	6 (9.7)

Table 6: Antibiogram of staphylococci isolated from sick goats in Nsukka zone n=28

Antibacterial agent	No of sensitive isolates (%)	No (%) of intermediate sensitive isolates	No of resistant isolates (%)
Tetracycline	7(25)	3(11	18(64)
Amikacin	22(79)	0(0)	6(21)
Gentamicin	18(64)	6(21)	4(14)
Streptomycin	2(7)	1(4	25(89
Chloramphenicol	23(82)	2(7	3(11)
Ciprofloxacin	13(46)	3(11)	12(43)
Ofloxacin	18(64)	1(4)	9(32)
Norfloxacin	20(71)	2(7)	6(21)
Levofloxacin	21(75)	3(11)	4(14)
Enrofloxacin	19(68)	8(29	1(4)

Cefuroxime	**9(32)**	**15(54)**	**4(14)**
Cefpodoxime	**19(68)**	**0**	**9(32)**
Ceftazidine	**21(75)**	**0**	**7(25)**
Ceftriaxone	**13(46)**	**10(36)**	**5(18)**
Cefotaxime	**12(43)**	**12(43)**	**4(14)**

Table 7: Antibiotics resistance patterns of staphylococci isolates from goats in Nsukka zone

S/No	Resistant Pattern	No of Isolates
1.	TE	5
2.	TE-C	2
3	TE-C-S	4
4	TE-C-CPO	1
5	TE-C-CAZ	2
6	TE-C-S-OFX	1
7	TE-C-S-CAZ	2
8	TE-C-CN-LEV	1
9	TE-C-AK-CAZ	1
10	TE-CXM-CPO	1
11	TE-C-CN-CAZ-CXM	1
12	TE-C-CAZ-CTX-CPO-ENR	1
13	TE-C-S-CAZ-CPO-NOR	1
14	TE-C-S-ENR-OFX-LEV	1
15	TE-CN-CAZ-CXM-CPO	1
16	TE-C-S-AK-CAZ-CRO-CTX-CXM-CPO-OFX-LEV	1
17	TE-S	1
18	TE-S-AK	1
19	TE-S-CXM-CIP	1
20	TE-S-CAZ-CTX-CXM-CPO	1
21	TE-S-CAZ-CXM-CPO	1
22	TE-CAZ	3
23	TE-CAZ-CPO	1
24	TE-CAZ-CXM-CIP	1
25	CAZ	4
26	CAZ-CPO	3
27	CAZ-CPO-LEV	1
28	CXM	1
29	CPO	1
30	C-CAZ-CPO	1
31	C-CAZ-CPO-CIP	1
32	C-S-CAZ-CTX-CXM-CPO-CIP-NOR-ENR-OFX-LEV	1
33	S	1
34	S-CAZ	1
35	S-CAZ-CTX-CXM-CPO	1

Tetracyclin (TE), Chloramphenicol (C), Streptomycin (S), Amikacin (AK), ceftazidine (CAZ), ceftriaxone (CRO), cefotaxime (CTX), cefuroxime (CXM), cefpodoxime (CPO), ofloxacin (OFX), norfloxacin (NOR), enrofloxacin (ENR), levofloxacin (LEV), ciprofloxacin (CIP), gentamycin (CN)

DISCUSSION

The prevalence of staphylococci among goats in Nsukka was 30%. This is similar to the report of Aher **[26]** who reported a prevalence of 30% among goats in India and also in agreement with the report of Adamu **[27]** who recorded 30% prevalence of staphylococci among goats in Maiduguri, Nigeria. However it is lower than 73.2% reported by Obi **[28]**. Apparently healthy goats recorded a slightly higher (31.5%) prevalence than the sick goats (28%) in this study. This could be attributed to the competition in the nasal cavity between staphylococci and other more established nasal organisms in goat which includes streptococci, Pastuerella and some other bacteria. It could also be attributed to the fact that the sick goats may have received

antibacterial treatment which will eliminate the bacteria in some of the goats.

The rate of isolation of staphylococci from male goats (62%) is significantly ($P<0.05$) higher than isolation from female goats (19.3%). This shows that sex is a factor in the staphylococcal colonization of goats in Nsukka. To our knowledge there have not been much work comparing isolation rate of staphylococci from male and female goats. The high rate of colonization in the males could probably be due to the higher activity of the male goats and their association with different female goats from different places. In their sexual drive, the nostrils are usually employed which could make them vulnerable to higher colonization.

From the study, it has been shown that *Staphylococcus species* from goats in Nsukka, Enugu state, Nigeria habours various virulence characteristics which includes haemolysin and coagulase production. Out of the 91 isolates from this study, 12.1% were coagulase and haemolysin producers respectively. This shows that the isolated organisms may be pathogenic. Ability to lyse red blood cells is a common attribute of staphylococci especially *S. aureus* which has been recognized as highly virulent human pathogen. Production of different types of haemolysis (α, β, δ or ɣ) has been attributed to species of staphylococci which contributes significantly to the pathogenicity of the organism.

From the study, 4.4% of the isolates were coagulase positive staphylococci (CPS) which are known to be highly pathogenic while the rest of goats harbour coagulase negative staphylococci (CoNS) which are mainly opportunistic pathogens and less dangerous. The known CPS includes *S aureus*, *S. hycus* and *S. intermedius* **[1]**; *S. aureus anaerobius*, *S. delphini* **[29, 30]**.

From the study, it was recorded that most of the isolates were resistant to at least one antibacterial agent. Seventy-seven percent of the isolates were resistant to more than 3 antibacterials thereby making them multi-drug resistant. Isolates from the apparently healthy goats exhibited highest resistance to tetracycline (58.1%), ceftazidine (46.8%), chloramphenicol (37.1%) and streptomycin (35.5%) while the isolates from sick goats showed highest resistance to streptomycin (89%), tetracycline (64%), ciprofloxacin (43%) and cefpodoxime (32%). The high resistance to tetracycline and streptomycin is understood, being likely due to widespread use of the antibacterial agents in animal treatment in the area. This is in agreement with the report of Ibezim **[31]** who attributed the development of resistance to indiscriminate use of antibiotics. The resistance to tetracycline is in line with the report of Han **[32]**, who

recorded a resistance of 14-72% among staphylococci. The high resistance to chloramphenicol, ceftazidine, cefpodoxime and ciprofloxacin is not attributable to any known factor since these antibacterial agents are mostly used for human treatments in the study area. It suggests that antibacterial agents produced for human therapy are widely used for animal treatment. Also, resistant organisms could be transferred from humans to these animals through faulty management practices. This is very possible as the animals are usually housed in close proximity to humans in households. There is also the possibility of acquisition of resistance attributes through genetic transfer especially in a multi-culture environment. It is also worthy to note that the high resistance to some of the beta-lactam antibacterial agents tested may possibly be due to the likelihood of the organisms being methicillin-resistant. This requires further investigation as this group of staphylococci has been reported worldwide among different animal species including goats. It is also implicated in numerous diseases of both humans and animals and has been adjudged the greatest potential pathogen **[33]**. The least resistance among the apparently healthy goats was shown to ceftriazone (3.2%), enrofloxacin, norfloxacin, gentamycin and amikacin (4.8%) while those for the sick goats was shown to enrofloxacin (4%), chloramphenicol (11%), cefotaxime, cefuroxime, levofloxacin and gentamycin (14%). The sensitivity to fluoroquiolones recorded in this study is in total contrast to the 38% and 85% fluoroquinolone resistance recorded by MacDougall **[34]** and Udo **[35]** in US and Kuwaiti hospitals among staphylococci. The staphylococcal strains were sensitive to aminoglycosides group made up of gentamycin (93.5%), amikacin (90.3%), streptomycin (60%). This is in line with the finding of Dewaele **[36]** who reported high aminoglycoside sensitivity in one of the farms groups they tested. The sensitivity of the isolates to some aminoglycoside may be related to unavailability of these drugs for animal treatment in the study area and genetically by the absence of the transposon carrying the aac(6')-aph(2") genes responsible for the resistance to aminoglycosides.

The level of resistance recorded could be attributed to the indiscriminate use of antibiotics for therapeutic use mainly. And also the genetic causes due to gene transfer, example, in an environment like the nasal cavity, various organisms grow together and lying side by side, genetic elements conferring resistance like plasmids, transposon (jumping gene) could be transferred to otherwise sensitive strain.

There was no distinct resistance pattern exhibited among the isolates which suggests that the organisms were faced with numerous challenges in the environment. The factors that led to development of resistance were multifaceted. Bearing in mind that most of the isolates were coagulase negative staphylococci, it is in agreement with the report of Chambers **[36]** who observed that CoNS presents hetero-resistance pattern.

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