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Prevalence and antibiogramofcoagulase positive Staphylococciisolated from farm animals in Michael OkparaUniversity of Agriculture, Umudike, South-Eastern Nigeria

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Abstract: The prevalence and antibiogram of coagulase positive Staphylococci isolated from farm animals in Michael Okpara University of Agriculture, Umudikewas investigated. A total of 200 samples from five farm animals; Goat (56), Sheep (16), Rabbit (42), Pig (58) and Cattle (50) were analyzed.108 (54%) coagulase positive Staphylococci and 43 (21.5%) coagulase negative Staphylococci were isolated, while 49 (24.5%) of the samples showed no growth. Of the 108 coagulase positive Staphylococci isolates, 40% were from goats, 26.9% from cattle, 21.3% from pigs, 12% from rabbits and 9.3% from sheep. All the 108 Staphylococcus aureus isolates were subjected to in vitro antibiotic susceptibility test using the modified Kirby- Bauer susceptibility testing technique. Sensitivity discs containing Ceftazidime (30µg), Cefuroxime, (30µg) Gentamicin (10µg), Ceftriazone (30µg), Erythromycin (5µg), Cloxacillin (5µg), Ofloxacin (5µg) and Augmentin (30µg) were used. Ofloxacin was found to be the most effective chemotherapeutic agent with 60(55.6%) sensitivity, while the highest resistance was recorded against Cloxacillin90 (83.3%). This study therefore identifies the presence of multiple drug resistant Staphylococcus aureus in farm animals which is of a major public health concern because the farm animals can serve as a reservoir for the dissemination of multiple drug resistant Staphylococcus aureus.

Keywords –Antibiogram, coagulase positive, farm animals, Staphylococci, Nigeria

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I. INTRODUCTION

Staphylococcus aureus belongs to the family Micrococcaceae and is part of the genus Staphylococcus which contains more than thirty (30) species such as Staphylococcus epidermidis, Staphylococcus saprophyticusand Staphylococcus haemolyticus. AmongtheStaphylococcus species, Staphylococcus aureus is by far the most virulent and pathogenic for human and animals. Staphylococcus aureus is a Gram-positive bacterium which appears in pairs or in grape-like clusters. It is characterized as being coagulase and catalase positive, non-motile, non-spore forming and as a facultative anaerobe. It grows in yellow colonies on nutrient rich media and is referred to as the "yellow Staphylococci"[1].

Staphylococcus aureus is a widely distributed bacterium found in the air, soil, water and dust because Staphylococcus aureus has the ability to adapt to different environments, and it may colonize the human skin, nails, nares and mucus membrane and thereby disseminate among recipient host population through physical contact and aerosols [2]. Colonization with Staphylococcusaureus is an important factor for subsequent Staphylococcus aureus infection [3-4]. Numerous investigators have reported nasal carriers in normal adults varying from 30% to 80% [5].

Farm animals such as sheep, cattle, goats, pig and rabbits and even man are known to harbor potentially pathogenic Staphylococcus in their body without any apparent clinical signs of illness [6], but diseases results only when the defensive mechanism of the host animal is broken. As a pathogen of domestic animals, Staphylococcus aureus has been found associated with cases of abortion in cattle as well as

documented cases of clinical and subclinical mastitis in dairy farms worldwide [7] and pneumonic lesions in goat [8].

Staphylococcus aureus causes a wide range of infections in humans, from a variety of skin, wound and deep tissue infection to more life threatening conditions such as pneumonia, endocarditis, septic arthritis and septicemia. The bacterium is also one of the most common species in nosocomial infections, however little is known about the virulence factor behind all these conditions. In addition, Staphylococcus aureus may also cause food poisoning, scalded skin syndrome and toxic shock syndrome through production of different toxins [1]. Widespread interchange of Staphylococci between animals and human has been observed possibly due to closeness of animals to human population in their environment [9].

Antibiotics are used to control bacterial infection in farm animals. Staphylococcusaureus in general are sensitive to many antibiotics but strains from different patients and carriers differs in the pattern and degree of the sensitivity to different drugs and many strains are now resistant to some of the drugs [10-11]. This has been attributed to the multiple uses of antibiotics in agriculture as prophylactic agents and as growth promoter has led to the emergence of resistant bacteria in the environment. As is apparent, the use or misuse of antimicrobial agents to control animal infection in farm animals has resulted in development of resistance among microorganisms [1]. Moreover, there remains the possibility that resistance may be transmitted from antibiotic resistance bacteria to the susceptible ones [12]. In line with the above, Staphylococcus aureus is one of the bacteria that has remained resistant to antibiotics.

At first, Penicillin was used to treat Staphylococcus aureus infections.Soon afterwards, resistance emerged when strains acquired a genetic element coding for beta-lactamase production and today over 80% of all Staphylococcus aureus strains are resistant to penicillin. The next drug to be introduced for treating infections with Staphylococcus aureus was the semi synthetic, penicillinase resistant penicillin named Oxacillin or Methicillin, but shortly after its introduction, the first isolate with resistance was detected [1].With the emergence of resistance to Penicillinase-resistant Penicillin, the glycopeptide agent Vancomycin became the treatment of choice for infection with Methicillin resistant Staphylococcus aureus (MRSA) and in the year 1996, the first isolate with intermediate Vancomycin resistance was detected [13]. Resistance to Methicillin is considered the most important for Staphylococcus aureus. Methicillin resistant Staphylococcus aureus has become endemic in hospitals as well as in health care settings globally [14].This study is therefore aimed at establishing the occurrence of Staphylococcus aureus in the ear and nostrils of farm animals in the University farm and to determine the antibiogram of the Staphylococcus aureus isolates, to assess the prevalence of Methicillin resistant Staphylococcus aureus (MRSA) from among the S .aureus isolates and to provide information on the best choice of antibiotics to be used for empirical treatment of Staphylococcus aureus infections.

II. MATERIALS AND METHODS

2.1 Study area

Michael Okpara university of Agriculture, Umudike is located in the well-known Agricultural training and research community of Umudike in Ikwuano L.G.A. about 10 kilometers East of Umuahia town, the Abia state capital, along Umuahia-IkotEkpeneroad, Umudike; and is 140km North of Port-Harcourt International airport, 135km South of Enugu airport and 80km East of Owerri airport with a latitude of 05°, 29'N and Longitude of 07°, 3'E with rainforest type of vegetation.

2.2 Sample collection

A total of 200 samples were collected randomly from the ear and nostrils of 100 farm animals; Goat (56), Sheep (16), Rabbit (42), Pig (58) and Cattle (50) in Michael Okpara University of Agriculture, Umudike, using sterile swab sticks soaked in sterile normal saline.All theswab specimens were returned to the laboratory in their respective labeled swab sleeves to avoid contamination and were cultured for bacteria isolation within 1-2 hours of collection.

2.2 Bacterial isolation and enumeration

Nutrient agar (Titan Biotech Ltd. Rajasthan, India), Mannitol salt agar (Titan Biotech Ltd. Rajasthan, India), Mueller Hinton agar (Titan Biotech Ltd. Rajasthan, India) and Peptone water (Titan Biotech Ltd. Rajasthan, India) were used. The media were prepared according to the manufacturers' instructions and were brought to boiling before sterilization at 121°C for 15 minutes at 15psi.The media (apart from Peptone water) were then allowed to cool to 45-46°C before being aseptically dispensed into the sterile Petri dishes [15]. After media solidification, the swab samples were then aseptically inoculated by streaking the swab samples on the solidified media and were incubated aerobically for 24 hours at 37°C.Pure cultures of the isolates were obtained by sub-culturing in fresh medium using the streak plate method. The final cultures containing discrete colonies

were transferred onto McCartney bottles containing agar slants and were stored in the refrigerator at 4°C for further studies

2.3 Identification of the isolates.

The bacterial isolates were identified based on standard microbiological cultural, morphological and biochemical characteristics as described by [16] and [17].

2.4 Antimicrobial susceptibility testing

The bacterial isolates were subjected to in vitro antibiotic susceptibility test using the modified Kirby-Bauer susceptibility testing technique as described by [16]. The antibiotics and their different concentrations in the sensitivity discs (Abtek Biological limited, Liverpool, United Kingdom) used in the sensitivity testing include; Ceftazidime (30µg), Cefuroxime, (30µg) Gentamicin (10µg), Ceftriazone (30µg), Erythromycin (5µg), Cloxacillin (5µg), Ofloxacin (5µg) and Augmentin (30µg).

2.5 Statistical analysis

T-test was used to compare the mean incidence values of the isolates obtained from the ear and nostrils of the farm animals at both 95% and 99% probability levels respectively as described by [18].

Table 1: Percentage occurrence of coagulase positive and coagulase negative Staphylococciin the animal samples

S/N	Types of animal	No. of samples collected	Coagulase positive Staphylococci	Coagulase negative Staphylococci
A)	Goat			
i)	West Africa dwarf	30(100%)	24 (80%)	6 (20%)
ii)	Sokoto red	26(100%)	17(65.4%)	4 (15.3%)
B)	Sheep			
i)	Yankasa	10(100%)	7 (70%)	3 (30%)
ii)	Sothern sheep	6(100%)	3(50%)	1 (16.7%)
C)	Rabbit			
i)	Dutch Breed	20(100%)	6(30%)	3 (15%)
ii)	New Zealand Breed	22(100%)	7(31.8%)	2 (9.1%)
D)	Pig			
i)	Durock	6(100%)	3(50%)	1(16.7%)
ii)	Hampshire	26(100%)	10 (38.5%)	1(3.8%)
iii)	Large white	26(100%)	10 (38.5%)	5 (19.2%)
E)	Cattle			
i)	Cross white N'dama	15(100%)	10(66.7%)	6(40%)
ii)	breed	11(100%)	8(72.7%)	4 (36.3%)
iii)	N'dama breed	24(100%)	11(45.8%)	7(29.2%)
	White Fulani breed			
	Total	200(100%)	108 (54%)	43 (21.5%)

T**able 2**: Percentage occurrence of Staphylococcus aureus isolated from the ears and nostrils of the farm animals

S/N	Types of samples	No. of samples tested	Ear (%)	Nostril (%)	Total (%)	Percentage (%) distribution of S. aureus
A)	Goat					
i)	West Africa dwarf	30	8(26.6)	16(53.3)	24(80)	22.2
ii)	Sokoto red	26	10(38.5)	7(26.9)	17(65)	15.7
	Total					37.9
B)	Sheep					
i)	Yankasa	10	4(40)	3(30)	7(70)	6.5
ii)	Sothern sheep	6	1(16.6)	2(33.3)	3(50)	2.8
	Total					9.3

C)	Rabbit					
i)	Dutch Breed	20	2(10)	4(20)	6(30)	5.6
ii)	New Zealand Breed	22	2(9.0)	5(22.7)	7(31.8)	6.5
	Total					12.1
D)	Pig					
i)	Durock	6	1(16)	2(33.3)	3(50)	2.8
ii)	Hampshire	4	1(25)	1(25)	2(50)	1.9
iii)	Large white	26	4(15.3)	6(23.0)	10(34)	9.3
	Total					14.0
E)	Cattle					
i)	Cross white N'dama breed	15	6(40.0)	4(26.6)	10(66.6)	9.3
ii)	N'dama breed	11	3(27.2)	5(45.4)	8(72.7)	7.4
iii)	White Fulani breed	24	6(25.0)	5(25.0)	11(45.8)	10.2
	Total					17.6
	Total	200	48(24)	60(30)	108(54)	100
	Mean ± SD		4 ± 2.95	6 ± 4.03		

Table 3: Antibiogram of the Staphylococcus aureus isolates

		Number of S. aureus strains		
S/N	Antimicrobial drug tested	Sensitive	Intermediate	Resistant
1	Ceftazidime	8(7.4%)	20 (18.5%)	80(74.1%)
2	Cefuroxime	13(12.0%)	25 (23.1%)	70(64.8%)
3	Gentamicin	55(50.9%)	20(18.5)	33(30.6%)
4	Ceftriaxone	25(23.1%)	39(36.1%)	44(40.7%)
5	Erythromycin	20(18.5%)	23(21.3%)	65(60.2%)
6	Cloxacillin	6(5.6%)	12 (11.1%)	90(83.3%)
7	Ofloxacin	60(55.6%)	17(15.7%)	31(28.7%)
8	Augmentin	40(37.0%)	36(33.3%)	32(29.6%)

Table 4**: Occurrence of Multiple drug resistance Staphylococcus aureus isolated from animal samples (%)**

Animal sample	Number of isolates	S. aureus resistant to more than two antibiotics
Goat	41	35 (85.4%)
Sheep	10	9(90%)
Rabbit	13	9 (69.2%)
Pig	15	12(80%)
Cattle	29	20(69%)
Total	108	85(78.7%)

Table 5: Prevalence of Cloxacillin (Methicillin) Resistant Staphylococcus aureus isolated from animal samples

S/N	Type of farm animals	Number of samples tested	Number of samples positive for S. aureus	Number Resistant to cloxacillin (%)
1	Goat	56	41	39 (95.1)
2	Sheep	16	10	8(80.0)
3	Rabbit	42	13	7(53.8)
4	Pig	36	15	11(73.3)
5	Cattle	50	29	25(86.2)
	Total	200	108	90(83.3)

III. DISCUSSION

Staphylococcus aureus, the principal etiological agent of Staphylococcosis, occurs as normal flora of the skin, nose, throat and mucous membrane of man and a wide variety of animals [19]. The disease

Staphylococcosisis a highly infectious bacterial zoonosis of global significance which causes considerable morbidity and mortality among the susceptible subjects [19].

The results of the study shows that out of 200 samples analyzed, 108 (54%) were positive for Staphylococcus aureus. This result shows high prevalence of Staphylococcus aureus in the farm animals investigated. The high incidence of Staphylococcus aureusmight be due to the ubiquitous nature of Staphylococciwhichoccurs as a commensal on the body of animals and man [20].The high frequency of distribution of Staphylococcus aureus is of clinical significance with respect to the fact that the organism is the leading cause of a variety of infections in animals such as Mastitis, pneumonic lesions and others. The result of this study is in agreement with those of [21] and [22] who isolated S. aureus from clinical and subclinical infections.

The occurrence of Staphylococcus aureus was highest (53.3%) in the nostrils of West African Dwarf goat,followed bythe nostrils of N'dama cattle (45.4%). This result is similar to those of [23] and [24] who isolated this organism from farm animals. The high occurrence of Staphylococcus aureus in the nostrils (30%) than ear (24%) can be attributed to the fact that the anterior nares are in continuous contact with the environment and moreover, the nose is an air passage and this organism is usually trapped in the mucous [25]. Studies, so far have shown that eradicating Staphylococcus aureus from the nose can eradicate the load of Staphylococcus aureus from other sites[26].

Antimicrobial susceptibility testing revealed that one hundred and eight (108) isolates of Staphylococcus aureus showed one or more resistance to the antimicrobial drugs [Cloxacillin (83.3%), Ceftazidime (74.1%),Cefuroxime (64.8%) and Erythromycin (60.2%)] used. A major reason for this development of multiple drug resistance (MDR) is because of indiscriminate use of antibiotics in feeds of animals for prophylaxis and as growth promoters [27-28]. Alternatively, resistance also emerges as a result of degrading enzymes and also as a result of acquisition of plasmid which carry the resistance gene.The Multiple Drug Resistance (MDR) pattern observed in this study is in agreement with previous studies carried by [29], who reported the emergency of multiple drug resistant Staphylococcus aureus strain to Cloxacillin and other antibiotics. The organism was sensitive to Ofloxacin (55.6%) and Gentamicin (50.9%) respectively. The high rate of sensitivity toOfloxacin may be due to the fact that the drug is not frequently used as a growth promoter and in prophylaxis for farm animals which may not be unrelated due to its high cost. This result is in agreement with the work of [30] who recorded high level of sensitivity with Gentamicin and Ofloxacin with percentage values of 92.4% and 76.6% respectively on Staphylococcus aureus isolated from clinical samples, but in contrast with [24], who recorded high level of sensitivity (54.29%) to Tetracycline on Staphylococcus aureus isolated from animals. The highest intermediate results were recorded against ceftriazone 39(36.1%) and augmentin 36(33.3%); this may be as a result of the organisms being in the intermediate phase of acquiring resistance.

The zoonotical importance of Staphylococcusaureus cannot be overemphasized as it has the capacity to colonize a wide range of host [31] hence animals may serve as a reservoir and transmit the infection to other animals and human handlers [32-35]. According to [36] and [37], healthy animals carry resistant Staphylococcus aureus without exhibiting symptoms and thus pose a risk to handlers. Research shows that humans who interact frequently with certain animals are at a high risk of contracting resistant Staphylococcus aureus as compared to those who do not encounter animals on a routine basis [38-40]

Finally, with regard to the statistical analysis, the test values (mean ± standard deviation) of Staphylococcus aureus isolated from the nostrils was higher (5±15.09) than that isolated from the ears (4±8.73) of the farm animals. Although there was no significant difference, between the Staphylococcus aureus isolates from the nostrils and ear of various animals screened at both 95% and 99% probability levels($P>0.05$; $P>0.01$).

This study has therefore provided important information on antimicrobial drugs suitable for use in the empirical treatment of infection in animals caused by Staphylococcus aureus.Ofloxacin is the drug of choice followed by Gentamicin and Augmentin on the medical and veterinary management of Staphylococcosis

IV. CONCLUSION

In conclusion, the findings of this study has therefore established the fact that the ears and nostril of farm animals are the true reservoirs of Staphylococcus aureus and thus, suggests that Staphylococcus aureus plays an important role in the pathogenesis of mastitis and other infections associated with farm animals. From the antimicrobial susceptibility pattern of Staphylococcus aureusisolates, the study has therefore revealed that most of the Staphylococcus aureus isolates showed multiple drug resistance (MDR) to the antimicrobial drugs tested. The study therefore suggests that Ofloxacin is the drug of choice in the treatment and veterinary management of Staphylococcosis.It is therefore recommended that proper management practices and adequate personal hygiene should be observed in the course of breeding of farm animals to avoid the prevalence of Staphylococcus aureus; and the use of indiscriminate antibiotics in animal feed as growth and prophylaxis

should be discouraged to help reduce the development of multiple drug Resistant (MDR) organisms especially Staphylococcus aureus.

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