Research Article ISSN 2639-9458

Microbiology & Infectious Diseases

Prevalence and Antibiogram of *Staphylococcus Aureus* Isolated from Herd of Goat, Cow and Ram at Obinze, Imo State, Nigeria

Chijioke A. Nsofor* and Ozurumba O. Patience

Department of Biotechnology, Federal University of Technology Owerri, Nigeria.

***Correspondence:**
Dr. Chijioke A. Nsofor, Department of Biotechnology, School of Biological Sciences, Federal University of Technology Owerri, Nigeria.

Received: 11 April 2019; **Accepted:** 05 May 2019

Citation: Chijioke A. Nsofor, Ozurumba O Patience. Prevalence and Antibiogram of *Staphylococcus Aureus* Isolated from Herd of Goat, Cow and Ram at Obinze, Imo State, Nigeria. Microbiol Infect Dis. 2019; 3(2): 1-5.

ABSTRACT

This study focused on the prevalence and antibiotic susceptibility pattern of Staphylococcus aureus isolated from livestock in Obinze, Owerri Imo State Nigeria. A total of 100 nasal swabs sample were collected, 86 samples were from goat, 9 samples were from cow and five samples were from ram. The samples were inoculated onto mannitol salt agar and the culture plates were incubated at 37 °C for 24 hours. The colonies showing yellow colour on mannitol salt agar were presumably identified as Staphylococcus spp; S. aureus was identified by biochemical tests. Antibiotics susceptibility pattern was determined by the disc diffusion method. The result of overall prevalence of the Staphylococcus aureus was 64%, with 62.79% isolates from goat sample, 66.67% isolates from cow sample and 80% isolates from ram samples. The frequency of resistance to antibiotics as observed in the animals were Sulphamethoxazole Trimethoprim 6 (12.77%), Nalidixic acid 32 (68.09%), Erythromycin 9 (19.15%), Amoxicillin/Clavulanic acid 16 (34.04%), Amoxicillin 17 (36.17%), Oxacillin 26 (55.32%), Gentamycin 9 (19.15%), Ciprofloxacin 6 (12.77%), Norfloxacin 20 (42.55%), Ampicilin19 (40.43%), Streptomycin 17 (36.17%). Sulphamethoxazole-Trimethoprim and Ciprofloxacin were the most sensitive antibiotics. The antimicrobial resistant pattern of Staphylococcus aureus isolate from the animals showed that 7 (14.89%) of the isolate were resistant to only one antibiotic and 2 (4.26%) isolates were resistant to 2 antibiotics and multidrug resistance was observed in 24 (51.07%) isolate of from goat, cow and ram giving a total of 33 (70.22%) resistant to antimicrobial agent respectively. The high prevalence of resistant strains of Staphylococcus aureus observed in this study may play a potential role of disseminating antibiotics resistant traits between animals and humans in the study area. Therefore, continued surveillance of this resistant strain of Staphylococcus aureus is very important for early prevention and control of community acquired infection.

Keyword

Staphylococcus aureus, Antibiotic resistance, Nigeria.

Introduction

Domestic livestock serve as a main source of food for man, as so many of their products such as milk and meat are essential raw materials in food production industries and in the preparation of many other consumables. The ability of *Staphylococcus aureus* to grow and produce staphylococcal enterotoxins (SEs) under a wide range of conditions is evident from the variety of foods implicated in the staphylococcal food poisoning (SFP) [1]. Similarly, there have been some reports indicating that domestic animals are the source of human infections, particularly humans in close contact with the animals either through the nature of their occupation or keeping the animals as pet. In this regard, the bacterial pathogen *Staphylococcus aureus* is one of the most important bacteria; particularly its methicillin resistant strain [2]. There is also evidence of the role of food animals in human methicillin resistant *Staphylococcus aureus* (MRSA) in some countries and of pets as a possible source of human infection. Some group of individuals who work closely with animals, such as veterinarians have high MRSA colonization rates [3,4].

The use of antibiotics growth medication has expanded as the meat and livestock industry moved to more mass production. The drugs are added to animal feed to help them grow larger for slaughter, lower fat in the livestock and boost protein content. They are also meant to prevent bacteria including *Escherichia coli, salmonella*

enterococci from infecting the animals, but the controversial practice promotes antibiotic resistant bacteria strain to form in the animals internal systems [5,6]. The widespread reliance on these antimicrobials in food animals has resulted in a considerable rise of antimicrobial-resistant strains of bacteria, complicating the treatment of infectious diseases in livestock, companion animals, and humans. This has led to important changes in the perception and priorities of regulatory agencies with regards to antimicrobial usage particularly the use of antimicrobials as growth promoters and prophylactic agents. The selective pressure from the use of antimicrobial agents at sub therapeutic levels in dairy cattle could result in the selection of those strains that contains genes for antimicrobial resistance [7-9].

Antimicrobial resistance is a major public health concern in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water and food [10]. In developed countries, the extensive use of antibiotics in agriculture, especially for prophylactic and growth promoting purposes, has generated much debate as to whether this practice contributes significantly to increase frequencies and dissemination of resistance gene into the ecosystem. According to [11] and [12], in developing countries such as Nigeria, antibiotics are used only when the animals fall sick, and only the sick ones are treated in such cases. However, even in the absence of heavy use of antibiotics, it is important to identify and monitor susceptibility profiles of bacterial isolates, particularly pathogenic organisms and this will provide information on resistance trends including emerging antibiotic resistance which are essential for clinical practice.

Staphylococcus aureus is present in a wide range of animal species including dogs, cats, rabbits, horses, cattle, pigs, poultry and exotic species both as a cause of infection and in healthy carriers. *Staphylococcus aureus* in cattle and its impact on human health are still only superficially understood, but it is clear that *Staphylococcus aureus* is a potentially important veterinary and public health concern that needs a great deal of more study to enhance its understanding and effective response. It is also a critically important human pathogen that is also an emerging concern in veterinary medicine [2]. This study, therefore, was carried out to evaluate the prevalence and antibiotics susceptibility pattern of *Staphylococcus aureus* isolated from apparently healthy Cattle, Goats and Ram in Obinze, Owerri, Imo State; where there is absence of extensive use of antibiotics for prophylaxis and growth promotion.

Materials and Method

Study area

This study was carried out in Imo state which is located in the South Eastern part of Nigeria. Imo state is bordered by Abia state on the East, River Niger and Delta state in the West, Anambra state on the North and Rivers state in the south. The sampling site is located at Obinze, Owerri in Imo state, where animals are sold and reared. The specimens were obtained from herds of goats, cow and ram. All the animals included in this study were (at the time of this specimen collection) not showing any sign of ill-health and there was no documented evidence of antibiotics use in the area from which the specimen were collected.

Sample collection, cultivation and identification of Staphylococcus aureus

A total of 100 samples (86 goat's samples, 9 cow's samples and 5 ram's samples) were randomly collected from various spots within the area (market) and all the samples were collected from Obinze, Owerri, in Imo state, Nigeria. They were collected by inserting sterile swab sticks into the anterior nares (nostrils) of the animal and then rotating the swab stick against the nasal mucosa [13,14]. The properly packed samples were transported to the laboratory immediately after collection where the microbial examination was performed within one hour of collection. All sampling procedure was in accordance with guidelines of the National Health Research Ethics Committee of Nigeria (www.nhrec.net), and the Ethics Committee of Federal University of Technology Owerri, Nigeria, approved this study.

The samples were processed upon arrival using aseptic measures and techniques. The swab samples collected were cultured on Mannitol salt agar (selective media for *Staphylococcus aureus*) according to the conventional technique. The culture plates were incubated in an inverted position at 37°C for 24 hours. Golden yellow colonies suspected to be *Staphylococcus aureus* showing mannitol fermentation were further confirmed by conventional microbiological techniques (biochemical tests: catalase and coagulase tests, Gram staining) which were carried out as described by [13].

Antimicrobial susceptibility testing

One isolate from each of *Staphylococcus aureus* positive sample was selected for susceptibility tests. The antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) according to the Clinical Laboratory Standards institutes, [15]. The following antibiotics impregnated discs were used: Gentamycin (30 µg), Nalidixic acid (30 µg), Erythromycin (15 µg), Oxacillin (1 µg), Ampicillin (10 µg), Streptomycin (10 µg), Norfloxacin (10 µg), Ciprofloxacin (5 µg), Sulphamethoxazole-Trimethoprim (25 µg), Amoxicillin/Clavulanic acid (30 µg) and Amoxicillin (10 µg) (Oxoid UK). After 16 hours incubation at 37°C, the diameter of inhibition zones was measured and interpreted using the European Committee on Antimicrobial Susceptibility Testing break point [16].

Results and Discussion

Sample sources	Total number of samples	Total number of positive samples	% of positive samples
Goat nasal swab	86	54	62.79
Cow nasal swab	9	6	66.67
Ram nasal swab	5	4	80
Total	100	64	64

Table 1: Total distribution of *Staphylococcus aureus* in the animals.
*Total number of assayed samples (n) = 47.

This table shows that a total of 100 samples were analyzed which comprises of 86 samples from goat, 9 samples form cow and 5 samples from ram nasal swabs. Among them, 54 (62.79%) goats, 6 (66.67%) cows and 4 (80%) rams were positive for *Staphylococcus aureus*, respectively. The overall prevalence was 64%.

Table 2 show the frequency of resistance and sensitivity to antibiotics by the *Staphylococcus aureus* isolates. The isolates were subjected to eleven antimicrobial agents from different antibiotics used in this study. 6 (12.77%) isolates were resistant to Streptomycin; Nalidixic acid had the highest frequency of resistance with a total of 32 (68.09%) isolates, followed by Oxacillin with a total of 26 (42.55%) isolates, Norfloxacin with a total of 20 (42.55%) isolates, Ampicillin 19 (40.43%) isolates, Streptomycin and Amoxicillin have equal resistance of 17 (36.17%) each and Amoxicillin/Clavulanic acid 16 (34.04%) isolates.

Antibiotics	SXT	NA	E	AMC	AML	OX	CN	CIP	NOR	AMP	S
Total no of resistance	6	32	9	16	17	26	9	6	20	19	17
Total % of resistance	12.77	68.09	19.15	34.04	36.17	55.32	19.15	12.77	42.55	40.43	36.17
Total no of sensitive	41	15	38	31	30	21	38	41	27	28	30
Total % of sensitive	87.23	31.91	80.85	65.96	63.83	44.68	80.85	87.23	57.45	59.57	63.83
Total %	100	100	100	100	100	100	100	100	100	100	100

Table 2: Frequency of resistance to antibiotics in the animal samples.

Number of antibiotics	Antimicrobial agents	Number of isolates	Percentage (%)
No resistance		14	29.78
1	Na	7	14.89
2	NaOx	2	4.26
3	NaOxAmp	1	2.13
4	NaAmpOxS NaOxCnNor EOxAmpS AmcAmlOxAmp	1 1 1 1	8.51
5	NaAmcAmlCipNor SxtNaOxCipNor NaOxCnNorS	1 1 1	6.38
6	NaAmcAmlOxNorAmp	2	4.26
7	NaAmcAmlOxNorAmpS NaAmcAmlCipNorAmpS NaOxCnCipNorAmpS NaEAmcAmlOxAmpS	2 1 1 1	10.64
8	NaEAmcAmlOxNorAmpS SxtNaAmcOxCnCipNorS NaEAmlOxCnNorAmpS NaEAmcAmlOxCipNorAmp SxtNaAmcAmlOxNorAmpS	1 1 1 1 1	10.64
9	NaEAmcAmlOxCnNorAmpS SxtNaAmcAmlOxNorAmpS	2 1	6.38
10	SxtNaAmcAmlOxCnNorAmpS	1	2.13
		47	100

Table 3: Antibiotics resistant pattern of *Staphylococcus aureus* isolated from herds of goat cow and ram in Nigeria.

In this table, the antibiotics resistance pattern of *Staphylococcus aureus* isolates from goats and rams revealed that most of the isolates, 33 (70.22%) were resistant to one or more antibiotics agent. Seven (7) isolates (14.89%) were resistant to a single antibiotic and 2 isolates (4.26%) showed resistance to two antimicrobial agents. Multi-resistance was observed in 24 (51.07%) of *Staphylococcus aureus* isolates.

Result of antimicrobial susceptibility is shown in Figure 1. In total *Staphylococcus aureus* originating from goat and ram nasal swabs were more resistant than those of cow origin.

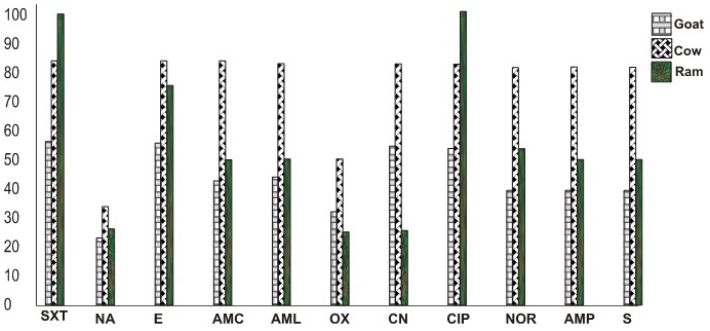

Figure 1: Comparison of sensitivity to antibiotics in *Staphylococcus aureus* from goats, cow and ram nasal swabs.

In this study, the overall prevalence rate of *Staphylococcus aureus* isolated from goat, cow, and ram nasal swabs obtained at Obinze was 64%. Nalidixic acid had the highest frequency of resistance with a total of 32 (68.09%) samples, followed by Oxacillin with a total of 26 (42.55%) samples, Streptomycin and Amoxicillin had equal resistance of 17 (36.17%) each. Inversely several isolates were found susceptible to Ciprofloxacin, Sulphamethoxazole-Trimethoprim, Gentamycin and Erythromycin. In contrast to the data generated from this study, lower prevalence rates of *Staphylococcus aureus* has been observed in other studies, though on milk samples as observed in the work of [17], whose study shows 18.80% prevalence, [18] with a report of 6.6% prevalence.

The antimicrobial susceptibility showed highest sensitivity to Ciprofloxacin and Sulphamethoxazole-Trimethoprim in almost all the animals samples, this result is closely related to those obtained in the works of [1]; this implies that any of the two drugs or their combinations could be used in treatment of animals infected with *Staphylococcus aureus* as it inhibits the growth and survival of the organism. On the other hand, Nalidixic acid which showed the highest resistivity among the eleven antibiotics used in this study therefore cannot be used in the treatment of animals infected with *Staphylococcus aureus* as its effects is being inhibited by the organism thereby encouraging the bacteria to survive and even multiply in the host animal.

In the results of the antimicrobial susceptibility pattern as shown in this study, *Staphylococcus aureus* originating from goat nasal swab were more resistant compared to those of cow and ram origin. The antimicrobial resistance pattern of *Staphylococcus aureus* isolates originated from nasal swabs of goat and ram of this study also revealed that most of the isolates that is 33 (70.22%) were resistant to one or more antimicrobial agent. Seven (7) isolates (14.89%) were resistant to a single antibiotic and 2 isolates (4.26%) showed resistance to two antimicrobial agents. Multi-drug resistance was observed in 24 (51.07%) of *Staphylococcus aureus* isolates.

Although there are few studies on the nasal carriage rate of *Staphylococcus aureus* in these animals with most studies carrying reports on *Staphylococcus aureus* isolates from milk and skin of these animals but some of their results can be compared to those obtained from nasal swabs. Gulani et al., [14], showed that samples that were positive to *Staphylococcus aureus* and MRSA were mostly originated from nasal and skin sources and distribution of MRSA and *Staphylococcus aureus* were higher in the nasal samples as compared to those from skin. This is due to the fact that *Staphylococcus aureus* is ubiquitous and can be transmitted which makes the nose and skin the primary invaded sites of the organism in most instances and this finding was in agreement with the report of [19]. The results from this study can also be compared to that of [20] in Saudi Arabia, with prevalence rates of 20%, 15.5% from goat and cow respectively and also the results from [14], with prevalence rates of 12.3%, 13.5% from cow and goats samples respectively in Maiduguri, Nigeria.

In this study, the highest incidence of *Staphylococcus aureus* was confirmed repeatedly in goats and this is similar to the reports of [21]. While the results of [20] carried out in Saudi Arabia reported that camels showed high unexpected rate of MRSA colonization, however, sheep, goats and cows had low colonization rates. According to [2], MRSA infection fluctuates in animals depending on ecological locations. Also, the disparity of the results may be as a result of the differences in the design of study, study environment, sample size, type of sample collected, and method of *Staphylococcus aureus* isolation and of antibiotics.

In general observation, the large percentage of Oxacillin, Ampicillin, and Amoxicillin/Clavulanic acid resistant *Staphylococcus aureus* were isolated from the study area. These were also resistant to several other antibiotics. Therefore it can be said that these are methicillin resistant *Staphylococcus aureus* (MRSA) [22]. The presence of MRSA in animals and humans is of public health concern where the multidrug resistance MRSA strains disseminate from animals to humans and vice versa in a community [23,24]. Screening the prevalence of MRSA will be of much use in early prevention and control of community acquired infections.

Since *Staphylococcus aureus* is transmitted from animals to humans and vice versa [25], and skin to skin contact is probably the main route of transmission between humans, humans to animal, and between animals, however contaminated materials, surfaces, food or dust can play a role in transmitting the agent [26,27]. Therefore, simple hygiene should be duly observed by animal handlers to also reduce the risk of infection of *Staphylococcus aureus*.

Conclusion

The resistant *Staphylococcus aureus* strain found in this study is of great public health concern, because there is risk of transmission and dissemination of this resistant strains to between the animals and humans which may successively spread and can be passed to the food chain. The high prevalence of resistant strains of *Staphylococcus aureus* observed in this study suggests the need for improved education and communication on the issue of antibiotic use in humans and veterinary medicine.

Since *Staphylococcus aureus* has become a major cause of disease and death, it is important to educate the public on the risks of infection and ways of combating its spread. Since drug abuse is a leading cause of drug resistance, it should be discouraged among animal handlers as this will play a vital role in mitigating the increase in the prevalence rate of the drug resistant infections. Therefore, livestock handlers should be advised on appropriate antibiotic use, that is to say that only sick animals should be should be given the appropriate antibiotics and only when necessary.

Acknowledgment

We wish to thank the farm owners for giving us permission to collect sample from their animals.

References

1. Fagerlund A, Granum PE, Håvarstein LS. *Staphylococcus aureus* competence genes: mapping of the SigH, ComK1 and ComK2 regulons by transcriptome sequencing. Molecular Microbiology. 2014; 94: 557-579.
2. Alzohairy M. Incidence of Macrolides-Lincosamide-streptograminb resistant *Staphylococcus aureus* (MRSA) among farm animals in Saudi Arabia. Research Journal of Microbiology. 2012; 7: 256-262.
3. Weese JS. Methicilin Resistant *Staphylococcus Aureus* in Animals. ILAR Journal. 2010; 51: 233-244.
4. Nsofor CA, Odom CM. Distribution and Antibiotics Susceptibility Pattern of *Staphylococcus aureus* Isolated from Pigs in Ahiara. Imo State Nigeria. MOJ Cell Sci Rep. 2018; 5: 78-81.
5. Moodley A, Nightingale EC, Stegger M, et al. High Risk for nasal carriage of Methicilin Resistant *Staphylococcus aureus* among Danish Vertinary Practitioners: Scand J Work Environ Health. 34: 151-157.
6. Nsofor CA, Umeorah UE. Antibiotics susceptibility pattern of Staphylococci isolated from poultry and poultry environment in Owerri, Nigeria. Asian Journal of Research in Pharmaceutical Sciences and Biotechnology. 2018; 6: 87-94.
7. Salmon SA, Watts JL, Case CA, et al. Comparison of MICs of ceftiofur and other antimicrobial agents against bacterial pathogens of swine from the United States, Canada, and Denmark. J Clin Microbiol. 1995; 33: 2435-2444.
8. Angulo FJ, Nargund VN,Chiller TC. Evidence of an association between use of anti-microbial agents in food

animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. J Vet Med. 2004; 51: 374-379.
9. Molbak K. Spread of resistant bacteria and resistance genes from animals to humans-the public health consequences. J Vet Med. 2004; 51: 364-369.
10. Normanno TG, La Salandra G, Dambrosio A, et al. Occurrence, Characterization and Antimicrobial Resistant of Enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. International J Food microbiology. 2007; 115: 290-296.
11. John J, Fishman N. Programmatic role of the infectious diseases physician in controlling antimicrobial costs in the hospital. Clin Infect Dis. 1997; 24: 471-485.
12. Chikwendu CI, Nwabueze RN, Anyanwu BN. Antibiotic resistance profile of *Escherichia coli* from clinically healthy pigs and their commercial farm environments. African Journal of Microbiology Research. 2008; 2: 12-17.
13. Cheesbrough M. District Laboratory Practice in Tropical Countries, Microbiology 2nd Edition Cambridge University Press. 2006; 582195.
14. Gulani IA, Geidam YA, Adamu L, et al. Prevalence and Phenotypic Detection of Methicilin Resistance *Staphylococcus Aureus* between Ruminants Butchered for Humanoid Intake and Animal Handlers in Maiduguri, Nigeria. Journal of Advanced Veterinary and Animal Research. 2016; 3: 152-159.
15. https://clsi.org/media/2663/m100ed29_sample.pdf
16. http://www.eucast.org
17. Santana EHW, Cunha MLRS Oliviera Moraes LB, Alegro LCA. Assessment Risk of Raw milk consumption related to staphylococcal food poisoning. Ciencia Animal Brasileira. 2010; 11: 643-652.
18. Kumar R, Prasad A. Detection of E coli and Staphylococcus in milk and milk products in and around pantnagar. VetWord. 2010; 3: 495-496.
19. Mai-Siyama IB, Okonko, Adamu NB, et al. Colonizations among Animant. Methicilin (Oxacilin) -Resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. App Environ Microbiology. 2014; 69: 6489-6494.
20. Alzohairy M. Colonization and Antibiotic Susceptibility Pattern of Methicilin *Staphylococcus Aureus* (MRSA) among farm animals in Saudi Arabia. 2011.
21. Masalha M, Borovok I, Schreiber R, et al. Analysis of transcription of the *Staphylococcus aureus* aerobic class Ib and anaerobic class III ribonucleotide reductase genes in response to oxygen. Journal of Bacteriology. 2001; 183: 7260-7272.
22. Batabyal B, Kundu GKR, Biswas S. Methicilin-resistant Staphylococcus sure us: A brief review. Int ResJ Biol Sci. 2012; 1: 65-71.
23. De Neeling A, Van Den Broek M, Spalburg E, et al. High Prevalence of methicillin resistant *Staphylococcus aureus* in pigs. Vet Microbiology. 2007; 122: 366-372.
24. Persoons D, Van Hoorebeke S, Hermans K Butaye P, et al. Methicilin Resistant *Staphylococcus aureus* in poultry. Emerg infect Dis. 2009; 15: 452-453.
25. Roberson JR. The Epidemiology of *Staphylococcus aureus* on dairy farms. National Mastitis Council 38th Annual Meeting. USA. 1999.
26. Asoh N, Masaki H, Watanabe K, et al. Molecular characterization of the transmission between the colonization of methicilin-resistant *Staphylococcus aureus* to human and environmental contamination in geriatric long-term care wards. Inten Med. 2005; 44: 41-45.
27. Angaw M. A Review on Assessment of Public Health Hazards of Methicillin Resistance *Staphylococcus Aureus* (MRSA) in Animals and Foods. Journal of Natural Sciences Research. 2015; 5: 240-245.