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Distribution and antibiotics susceptibility pattern of *staphylococcus aureus* isolated from pigs in Ahiara, Imo state Nigeria

Abstract

One hundred (100) nasal swab samples were collected from one hundred pigs in four farms in Ahiara Nigeria. The samples were subjected to standard microbiological and biochemical test (catalase, coagulase, gram staining) to identify *Staphylococcus aureus*. The isolates were tested for susceptibility against 11 antibiotics using the disk diffusion method. Out of the hundred pigs sampled, a total of 99 isolates of Staphylococcus spp. were recovered representing 99% of total samples. In all, 59.6% (59/99) were identified to be *S.aureus*. The antibiotic susceptibility profile of the isolates to the commonly used drugs shows high resistance to Nalidixic acid (59%), Streptomycin (55%) and Oxacillin (39%). Nineteen (19) isolates were susceptible to all the antibiotics, representing 32.3% (19/59). The prevalence of antibiotics resistant *S.aureus* stands at 67.7% among pigs in this region. This call for urgent intervention because pigs can serve reservoir through which this multi-drug resistance organisms can spread to other animals and humans at large. Therefore, proper hygiene practices, control of indiscriminate use of antibiotics is recommended bother for prevention and control of *S. aureus* infection.

Keywords: *Staphylococcus aureus*, antibiotic resistance, pig

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Introduction

Staphylococcus aureus is an important pathogen and is regarded as one of the most versatile and devastating zoonotic pathogens responsible for causing widespread outbreaks of serious infections, food poisoning, colonization, animal mastitis and pneumonia affecting animals.[1,2] *S. aureus* can also cause a number of infections in ruminant animals such as abortion.[3] In the various farms where such infections have been diagnosed, cumbersome preventive and control measures is undertaken on *S. aureus,* but despite all these, new infections continue to occur, treatment is often associated with poor success. One of the reasons for these failures is that infections originate from both exogenous and endogenous sources to the host; more importantly, it is due to the ability of *S. aureus* pathogen to rapidly develop resistance to antimicrobial agents commonly employed for intervention by farmers.[4–6] Due to the high rate of antibiotic resistant *S. aureus* encountered in pig farms, there is the need to continue investigate and check the underlining cause of this resistance and also compare the antibiotics in use to check for those still effective in tackling *S. aureus* infections. This will help reduce economic loses for farmers who lose money and herd of swine's on yearly bases to *S. aureus* related infections due to ignorance or antibiotic abuse. It will also help the farmers to curb and ameliorate the rising cases of zoonotic diseases and appropriate antibiotics will be administered in the right dosage to reduce to a minimum the microbial load of *S. aureus* in pigs. This study was conducted to determine the prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from pigs in Ahiara, Ahiazu Mbaise Imo State, Nigeria. The findings from this study will help farmers in the study area and the general population to checkmate the rising menace of multi drug resistance often encountered as a result of consistent or indiscriminate use of antibiotics.

Materials and method

Sample collection, bacterial cultivation and identification

One hundred (100) nasal swab samples were collected from pigs from four (4) different farms by swabbing both anterior nares with sterile swab sticks. The farms housed between 40 and 90 pigs of different groups (suckling, piglets, weaning pigs, grower-finisher pigs, and sows). 20 samples were collected from farm A, 30 samples from farm B, 30 from farm Cand 20 from farm D. The swab specimens were cultured onto mannitol salt agar and incubated at 37°C for 24hours. Identification of *Staphylococcus aureus* was based on colony morphology, Gram staining and appropriate biochemical tests. *Staphylococcus aureus* is Gram positive, beta hemolytic, catalase and coagulase positive and mannitol fermenter.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of isolates was performed using disk diffusion method on Mueller-Hinton agar plates. Sterile wire loop was used to touch 3 to 5 well isolated colonies and emulsified in 3ml sterile normal saline solution in a sterile test tube. The turbidity of the suspension was then adjusted to 0.5 McFarl and standard in order to standardize the size of inoculums. A sterile cotton swab was dipped into the standardized suspension of the bacterial culture, squeezed against the sides of the test tube to remove the exceeded fluid and inoculated onto Mueller-Hinton agar. Thereafter, antimicrobial discs were placed on the agar with forceps and gently pressed down to ensure contact. The plates were then allowed to stand for 30 minutes for diffusion of active substance of the agents. Plates were inverted and incubated at 37°C for 18hours. After the incubation, the diameter of inhibition zones was measured and interpreted using the European Committee on Antimicrobial Susceptibility Testing

break point.[7] The isolates were tested against 11 antibiotics, namely; Gentamycin (30µg), Nalidixic acid (30µg), Erythromycin (15µg), Oxacillin (1µg), Ampicillin (10µg), Streptomycin (10µg), Norfloxacin (10µg), Ciprofloxaxin (5µg), Sulphamethoxazole-Trimetroprim (25µg), Amoxicillin/Clavulanic acid (30µg) and Amoxicillin (10µg) (Oxoid UK).

Results

Out of the one hundred Nasal swab samples collected from one hundred pigs in four forms in Ahiara, a total of 99 isolates of Staphylococcus spp. were recovered representing 99% (99/100). A total of 59.6% (59/100) were identified to be *Staphylococcus aureus* (Table 1); 67.7% (40/59) of isolated *S. aureus* were antibiotic resistant and 2.2% (19/59) were antibiotic sensitive. The antibiotic susceptibility profiles of the isolates to the commonly used antibiotics showed high resistance to Nalidixic acid (59%), Streptomycin (55%), Oxacillin (39%); while the isolates were susceptibleto ciprofloxacin (96%), Norfloxacin, Gentamycin and Erythromycin having (94%) respectively, Trimethoprim sulfamethoxazole (90%) (Table 2). The prevalence of antibiotic resistant *S.aureus* isolates were highest in farm B and C. Among the 100 samples taken from the nostrils of 100 pigs, only one did not grow *Staphylococcus spp* (Table 3).

Table 1 Number of Pigs sampled and the percentage of *Staphylococcus aureus* isolation

	Total no of samples	Total no positive	Percentage (%)
FARM A	20	14	70
FARM B	30	20	66
FARM C	30	15	50
FARM D	20	10	50
TOTAL	100	59	59

Table 2 Antibiotics Susceptibility Patterns of *Staphylococcus aureus* isolates against the 11 antibiotics tested
NB: Antibiotic Assay was carried out on the 59 isolates

	SXT		N/A		E		AMC		AML		OX		GEN		CIP		NOR		AMP		S	
	S*	%	S*	%	S*	%	S*	%	S*	%	S*	%	S*	%	S*	%	S*	%	S*	%	S*	%
FARM1	14	100	12	86	14	100	14	100	14	100	14	100	13	93	14	100	13	93	14	100	13	93
FARM2	18	90	11	55	18	90	20	100	14	70	14	70	18	90	17	85	18	90	16	80	11	55
FARM3	10	71	3	21	12	86	14	100	14	100	10	71	13	93	14	100	13	93	14	100	3	21
FARM4	10	100	NIL	0	10	100	NIL	0	2	20	NIL	0	10	100	10	100	10	100	2	20	1	10
TOTAL	52	90	26	40	54	94	48	75	44	73	38	60	54	94	55	96	54	94	46	75	28	45

NB: S* Denotes sensitive

Table 3 Antibiotics Susceptibility Patterns of *Staphylococcus aureus* isolates against the 11 antibiotics tested

	SXT		N/A		E		AMC		AML		OX		GEN		CIP		NOR		AMP		S	
	R	%	R	%	R	%	R	%	R	%	R	%	R	%	R	%	R	%	R	%	R	%
FARM1	Nil	0	2	14	Nil	0	Nil	0	Nil	0	Nil	0	1	7	Nil	0	1	7	Nil	0	1	7
FARM2	2	10	9	45	2	10	Nil	0	6	30	6	30	2	10	3	15	2	10	4	20	9	45
FARM3	4	28	11	78	2	14	Nil	0	Nil	0	4	28	1	7	Nil	0	1	7	Nil	0	11	78
FARM4	Nil	0	10	100	Nil	0	10	100	8	80	10	100	Nil	0	Nil	0	Nil	0	8	80	9	90
TOTAL	6	9.5	32	59	4	6	10	25	14	27	20	39	4	6	3	3.7	4	6	12	25	30	55

N: B - R Denotes resistant

Key: SXT = Sulphamethoxazole-Trimethoprim, NA = Nalidixic acid, E = Erythromycin, AMC = Amoxicillin/Clavulanic acid AML =Amoxicillin, OX = Oxacillin, GEN = Gentamycin, CIP = Ciprofloxacin, NOR =Norfloxacin, AMP = Ampicillin, S= Streptomycin

Multi drug resistance S. aureus isolates

Multi-drug resistance (resistance to ≥ 2 or more antibiotics) was recorded in 35 (87.5) of the *S. aureus* isolates. About 9 (22.5%) were demonstrated to be resistant to two antibiotics, 3 (7.5%) resistant to 3 antibiotics, 8 (20%) resistant to 4, 5 (12.5%) resistant to 5, 9 (22.5%) resistant to 6 and 5 (12.5%) resistant to 1 antibiotic respectively. None of the *Staphylococcus aureus* isolates were sensitive to all the11 tested antibiotics (Table 4)

Discussion

S. aureus is an important opportunistic pathogen colonizing humans and animals. Antibiotic resistant *S. aureus* has been reported in various animals and livestock farms.[8–11] The prevalence of antibiotic resistance among pigs in this study was 67.7% which was lower than results obtained in Netherland (80%) in 2005, Spain (83%) in 2008 and 85.7% in 2010 in Spain but slightly higher than the report of Lewis et al.[12] Vanderhaeghen et al.[13] Vanderhaeghen, et al.[14] who reported resistant rate of 46%, 40%, and 43.9% respectively. The difference in the lower results could be as a result of antibiotics being used, poor hygiene and exposure of the pigs to the reservoirs. The source of acquisition of antibiotic resistant in this study was not known but might be due to indiscriminate use of antibiotics.

Since molecular analysis of the antibiotic resistant isolates in the study was not carried out, it is difficult to establish the origin of the colonizing type which would have given insight to the likely route of transmission.

Herd size could also be associated with antibiotic resistance prevalence. The results obtained in this study showed varying

prevalence among the pigs farms used in this study. Farm A with 14 *S. aureus* isolates gave the lowest antibiotic resistance prevalence result with only 2 isolates showing resistance (14.2%). The resistance prevalence increased in farm B having 20 *S. aureus* isolates of which 13 were resistant giving rise to a65% prevalence. Farm C had 15 *S. aureus* isolates all of which were resistant 100% while farm D had 10 *S. aureus* isolates all of which were resistant to at least two antibacterial (100%). The reason for the increase in antibiotic resistant *S. aureus* as we go down the farm is unknown.

Table 4 resistance pattern of *staphylococcus aureus* isolates on each antibiotics

No. of antibiotics	Antibiotics	No of isolates	Percentage (%)
	S	1	
1	N/A	3	12.5
	OX	1	
	N/AS	8	
2	N/A AML	1	22.5
	SXT GEN S	1	
3	N/A CIP NOR	1	7.5
	N/A E S	1	
	N/A GEN NOR S	1	
	N/A AML AMP S	1	
4	AML OX NOR S	1	
	AML OX CIP S	1	20
	AML OX AMP S		
	SXT N/A OX S	1	
	SXT N/A E S		
	N/A AMC OX S	1	
	SXT N/A OX CIP S	1	
	N/A E AML OX AMP	1	
5	N/A AMC AML OX S	1	12.5
	N/A AMC AML OX AMP	1	
	N/A AMC OX AMP S	1	
	N/A E OX GEN AMP S	1	
	SXT N/A E OX GEN S	1	
	SXT AML OX CIP NOR S	1	22.5
6	N/A AMC AML OX AMP S	6	
8	SXT N/A AML OX GEN NOR AMP S	1	2.5
TOTAL		40	100

Conclusion

The data cumulated from this study unraveled that prevalence of antibiotic susceptible *S. aureus* in pigs in Ahiara is high. Of the 40 antibiotic resistant *S. aureus* isolates, 87.5% (35/40) were multi drug resistant. Every antibiotic resistant *S.auras* isolate was resistant to at least one or all of either Nalidixic acid, Oxacillin and Streptomycin, showing that these antibiotics are increasingly becoming ineffective in the treatment or suppression of *S.aureus* infections in pigs. Norfloxacin, Ciprofloxacin, Gentamycin and Erythromycin remain effective but there is the worry that the continuous use or abuse of these antibiotics could render the bacterial resistant to them in the near future. The rate of spread of this pathogen and its unique ability to acquire and transfer antibiotic resistance calls for attention and a coordinated surveillance programme to combat this pandemic outbreak.

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None.

Conflict of interest

The authors declare that there is no conflict of interest.

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