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Original Research

Distribution and antibiogram of Staphylococci from pigs and contact humans in Abia State

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ABSTRACT

Staphylococcus species are among the dominant groups of saprophytic bacteria; however, lately, they are globally known for being opportunistic pathogens and prime cause of community-associated and hospital-acquired infections in humans and animals. This study was conducted to determine the phenotypic characteristics of *Staphylococcus* isolated from pigs and in-contact humans in Abia Central Senatorial Zone, Nigeria. Using standard microbiological methods 1084 nasal swab samples were collected from 968 pigs and 116 in-contact humans. Kirby-Bauer's disc diffusion method was used to determine the antibiogram of the isolates, and the findings were interpreted using CLSI interpretive values. *Staphylococcus* species were recovered from 38 (3.9%) of the 968 pigs screened and 2 (1.7%) of the 116 humans. The recovered species distributions were *S. aureus* (1), *S. intermedius* (10), *S. hyicus* (21), *S. chromogens* (1), *S. haemolyticus* (5), *S. warneri* (1) and *S. xylosus* (1). Most of the isolates were resistant to at least one antimicrobial agent. Resistance to ampicillin in 33(86.8%) of the isolates while 31(81.6%), 30(78.9%), 24(63.2%) and 23(60.5%) were resistant to vancomycin, oxacillin, penicillin and tetracycline, respectively. Thirty one unique resistance patterns were identified among the staphylococci isolates. The multiple antibiotics resistance index (MARI) for all the isolates was greater than 0.2 indicating that the isolates may have originated from an environment where antibiotics were frequently used. All the staphylococcal isolates that were resistant to vancomycin by disc

Keywords: Opportunistic pathogen, phenotypic characteristics, resistant patterns, saprophytic bacteria, staphylococcus

INTRODUCTION

Staphylococci include coagulase-positive staphylococci (CoPS), almost exclusively represented by *Staphylococcus aureus*, and coagulase-negative staphylococci (CoNS). The *Staphylococcus* genus comprises 49 species and 26 subspecies (Han *et al*., 2015). Most Studies on staphylococcal pathogenicity have focused on *Staphylococcus aureus* (*S. aureus*), and little attention has been paid to CoNS (Chajecka- Wierzchowska *et al*., 2015).

Staphylococcus aureus is an important pathogen that causes serious and potentially fatal infections in both humans and animals (Stewart-Johnson *et al*., 2019). *Staphylococcus aureus* is among the important pathogens contributing to foodborne illnesses. It easily cross contaminates foods from hands that come in contact with the nostrils and mouth. It causes endocarditis, impetigo, cellulitis and scalded skin (Adzitey *et al*., 2019). The anterior nares has remained the major site of staphylococcal colonization in humans, they are the primary reservoir for replication and spread to other body sites (Nsofor *et al*., 2015, Abdulazi & Olayinka, 2016). Nasal carriage of staphylococcus species has been reported in Nigeria (Abdulaziz & Olayinka, 2016), so also were infections associated with staphylococcus species have also been reported from various parts of the country (Momoh *et al*., 2016; Ugwu *et al*., 2015a; Ugwu *et al*., 2015b).

Reports of methicillin resistant strains among coagulase negative staphylococcus (CoNS) are challenging due to the large proportion of methicillin-resistant strains and increasing numbers of isolates reinforcing the need to revise their importance to food safety (Bhargava & Zhang, 2014; Osman *et al*., 2016a,b). Coagulase negative staphylococcus of both animal and human origins are believed to serve as important reservoirs of antimicrobial resistance genes (Becker *et al*., 2014) which can transfer and integrate into the *S. aureus* genome leading to the emergence of new, potentially more resistant strains thus enhancing its potential to resist antimicrobial treatment (Rossi *et al*., 2016). Recently, reports on multi-resistant CoNS are increasing,

with the indiscriminate use of antibiotic in children (Pourakbari *et al*., 2018). The increasing antibiotic resistance of CoNS also limits the drug choices for treatment of CoNS infections (Gkentzi *et al*., 2016). CoNS exist in places where antibiotics are widely used, such as hospitals and animal farms (Zlatian *et al*., 2018), which accelerate the spread of resistant genes.

MATERIALS AND METHODS

STUDY AREA

This study was carried out in Abia State. Abia State is one of the States in South-East Nigeria. It is made up of 17 Local Government Areas (LGAs) and is divided into three Senatorial zones namely; Abia Central, Abia South and Abia North. Sampling for this study covered six Local Government Areas (Isiala Ngwa North, Isiala Ngwa South, Umuahia North, Umuahia South, Osisioma and Ikwuano) representing Abia Central Senatorial Zone. This zone was chosen due to prominence of pig farms in these areas. Abia state occupies about 6320 square kilometer. It is bounded on the North and North East by Anambra, Enugu and Ebonyi; Imo State to the West; Cross- River and Akwa-Ibom to the East and South East. Rivers state also borders Abia to the South. The Southern part of the state lies within the riverine part of Nigeria. It is a low-lying tropical rain forest with some oil palm brush. The Southern portion gets heavy rainfall of about 2400 millimetres per year and is especially intense within the months of April and October. The rest of the state is moderately high plain and woody savanna (Hoiberg, 2010). The study and protocol was approved by the Health Research Ethics Committee of Ministry of Health, Abia State.

STUDY DESIGN AND SAMPLE COLLECTION

A cross-sectional study targeting pigs in commercial farms, pigs at slaughter and the in-contact humans was conducted. Sample size was calculated (as described by Thrusfield, 2005) on the basis of the following criteria:

Prevalence: 50%, Desired confidence level: 95%
Allowable error (e): 5%,
N = sample size, Z = 1.96 (constant)
Using the formula:

$$N = \frac{Z^2P(1-P)}{e^2}$$

$$N = \frac{1.96^2 \times 0.50 \times 0.50}{0.05^2} = 384$$

However, 968 samples were collected from pigs in this study to increase precision. A purposive selection of Abia Central Senatorial Zone was based on high production, sale and consumption of pork by the residents. A random selection of 30 piggeries in the six Local Government Areas was employed in no definite order because there was no sampling frame and from the Umuahia pig slaughter slab. The study was conducted between March 2017 and September 2018. The farm attendants restrained each animal samples were to be collected from. Sterile cotton swabs were used to collect nasal swab samples from the anterior nares of pigs. Nasal swab Samples were collected by inserting a sterile swab stick in both anterior nares of each pig gently rubbing the swab against the mucosa surface for approximately 5-10 seconds. These were placed in 5ml Trypticase Soy broth (Oxoid, UK) supplemented with 6.5% sodium chloride. The same protocol was followed in humans. One hundred and sixteen nasal swab samples from humans were collected based on consent and availability from each of the sampled locations. For each animal and human sampled, the following information were recorded; date, age and sex. All samples obtained were properly labeled, stored on ice pack and transported immediately to the Department of Veterinary Microbiology Laboratory, Michael Okpara University of Agriculture Umudike for processing.

SAMPLE COLLECTION

Nasal swab Samples were collected by inserting a sterile swab stick in both anterior nares of each pig and human and gently rubbing the swab against the mucosa surface for approximately 5-10 seconds. These were placed in 5ml Trypticase soy broth (Oxoid, UK) supplemented with 6.5% sodium chloride. All the samples were transported immediately to the Department of Veterinary Microbiology Laboratory, Michael Okpara University of Agriculture, Umudike for processing.

IDENTIFICATION AND CONFIRMATION OF STAPHYLOCOCCUS

A loopful of the inoculum from the enrichment broth above was streaked on mannitol salt agar and incubated at 37 °C for 24 h.

MICROGEN IDSTAPH® (MICROGEN BIOPRODUCTS, U.K) CONFIRMATION

The test was carried out and interpreted based on the instructions of the manufacturer. The Microgen Staph_ID system consists of a single microwell test strip with 12 standardized biochemical substrates that were chosen after extensive computer analysis. These substrates are found in each well: nitrate, sucrose, tetrahalose, mannitol, N-acetyl glucosamine, mannose, turanose, β-glucosidase, β-glucoronidase urease, arginine, and 1-pyrolidonyl-α-naphthylamide. By combining 2-5 isolated colonies with the 3ml staphylococci suspending medium included in the kit a homogeneous suspension was made (Microgen Bioproducts, U.K), then four drops of the bacterial suspension were added to each well with a Pasteur pipette; well no 7 indicated by a black circle on the test strip coated with arginine was overlaid with two drops of mineral oil. The inoculated test strips were incubated at 35 °C ± 2°C for 24 h. After incubation, two drops of fast blue reagent was added to well

no 12 indicated by a green circle on the test strip (Beta galactosidase). A colour change within 5-10seconds to plum purple was observed for positive test. All results were recorded on the organism ID report forms provided in the kit and interpreted using the microgen identification package.

ANTIBIOTIC SUSCEPTIBILITY TEST

The Kirby-Bauer method was used for the antimicrobial susceptibility testing on Mueller Hinton agar (Bauer *et al*., 1996) using 12 antimicrobial agents and interpreted according to the recommendations of Clinical and Laboratory Standards Institute (CLSI, 2020). The *Staphylococcus* isolates were tested using a panel of twelve antimicrobials namely, ampicillin (10µg), cefoxitin (30µg), ceftriaxole (30µg), erythromycin (15µg), gentamicin (10µg), oxacillin (1µg), penicillin (10units), sulphamethoxazole/trimethoprim (25µg), tetracycline (30µg), chloramphenicol (30µg), ciprofloxacin (5µg) and vancomycin (30µg). Each *Staphylococcus* isolate was inoculated into trypticase soy broth (Oxoid Ltd, Basingstoke, UK) and incubated at 37^0C for 24h before testing. The turbidity of the actively growing culture was adjusted to correspond with that of a barium sulphate (0.5 MacFarland) standard. Subsequently 0.1ml of the suspended culture was inoculated onto Mueller Hinton agar plates (90mm diameter disposable Petri dishes) and spread over the surface with sterile cotton swabs. Six antimicrobial disks were then placed on the surface of each plate by means of antibiotic disk dispenser and incubated at 37^0C for 18h. Inhibition zone diameters were measured using a transparent ruler, and the interpretative breakpoints for resistance determined by comparing zone diameters according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines.

MINIMUM INHIBITORY CONCENTRATION (M.I.C) DETERMINATION

Minimum inhibitory concentration of Vancomycin was determined using the Episolmeter strip (E-test strip) (Biomerieux, France). This was carried out as described by the manufacturer and interpreted using the CLSI guidelines (CLSI, 2020). From an overnight culture, 0.5 McFarland standard bacterial suspension was made, plated on Mueller Hinton agar using a bend glass rod on which the E-test strips were placed with a forcep one on each plate. The plates were incubated for 24 h at 37ºC. After the period of incubation, an eclipse corresponds to the antimicrobial concentration no longer inhibitory to the growth of the organism. The corresponding concentration of antibiotic at the point of intersection on the strip was read as the MIC in µg/ml. If the growth intersects on the line between two sections, the MIC was read as the value in the lower section. If there was growth along the entire length of the strip, the MIC was read as greater than the highest value on the M.I.C.E scale. MIC values were interpreted as susceptible (S), intermediate (I) and resistant (R) (CLSI, 2020).

RESULTS

Out of the 968 samples collected from pigs, 38 (3.9%) *Staphylococcus* isolates were recovered. The proportion of the different *Staphylococcus* species were *S. hyicus* (55.3%), *S. intermedius* (23.7%), *S. haemolyticus* (13.16%), *S. chromogenes*, *S. warneri and S. xylosus* (2.6%) respectively (Table I). Of the 38 isolates from pigs 32 (84.2%) were from farms while 6 (15.8%) were from an abattoir. The distribution were *S. hyicus* 19 (90.5%) and 2 (9.5%), *S intermedius* 7(77.8%) and 2 (22.2%), *S. haemolyticus* 4 (80%) and 1 (20%) from farms and abattoir respectively (Table II). Out of the 116 samples collected from humans only 2 (1.7%) *Staphylococcus* isolates were recovered. The species identified were *S. aureus* and *S. intermedius*. Both isolates from humans were from farm attendants and none from abattoir workers.

The result of antibiotic susceptibility showed that most of the isolates were resistant to at least one or more of the antibiotics tested (Table III). Multiple drug resistance is antimicrobial resistance shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories. Majority of the isolates were resistant to ampicillin 33 (86.8%), cefoxitin and vancomycin 31 (81.6%), oxacillin 30 (78.9%), penicillin 24 (63.2%) and tetracycline 23 (60.5%) (Table IV). Thirty-one unique susceptibility patterns were identified among the

Table 1: Isolation of *Staphylococcus* species from pigs in Abia Central Senatorial Zone

***Staphylococcus* species**	**Number**	**Percentage**
S. intermedius	9	23.68
S. hyicus	21	55.26
S. chromogenes	1	2.63
S. haemolyticus	5	13.16
S. warneri	1	2.63
S. xylosus	1	2.63
Total	38	100

Table II: Distribution of different *Staphylococcus* species in Pigs from farms and slaughter

Staphylococcus Species	**Farm (%)**	**Slaughter (%)**	**Total**
S. hyicus	19 (90.5)	2 (9.5)	21
S intermedius	7 (77.8)	2 (22.2)	9
S. haemolyticus	4 (80.0)	1 (20.0)	5
S. chromogenes	0 (0)	1 (100)	1
S. warneri	1 (100)	0 (0)	1
S. xylosus	1 (100)	0 (0)	1
Total	32 (84. 2%)	6 (15.8%)	38

staphylococci isolates with twenty-four being resistant to multiple clinically important antimicrobial classes (Table V). All the staphylococci isolates had MAR index values ≥ 0.2 (Table VI). Minimum Inhibitory Concentration values for vancomycin were within the range of 1-2ug/ml.

Table III: Susceptibility of staphylococci species isolates from pigs and humans in Abia Central Senatorial District to 12 antimicrobial agents

Antibiotics	Pigs (n=38)		Humans (n=2)	
	R (%)	S (%)	R (%)	S (%)
Oxacillin	30 (78.9)	8 (21.1)	2 (100)	0 (0)
Tetracycline	23 (60.5)	15 (39.5)	2 (100)	0 (0)
Ceftriaxone	11 (28.9)	27 (71.1)	1 (50)	1 (50)
Ampicillin	33 (86.8)	5 (13.2)	2 (100)	0 (0)
Penicillin	24 (63.2)	14 (36.8)	2 (100)	0 (0)
Sulphamethoxazole/trimethoprim	3 (7.9)	35 (92.1)	1 (50)	1 (50)
Cefoxitin	31 (81.6)	7 (18.4)	1 (50)	1 (50)
Vancomycin	31 (81.6)	7 (18.4)	2 (100)	0 (0)
Erythromycin	6 (15.8)	32 (84.2)	1 (50)	1 (50)
Gentamicin	6 (15.8)	32 (84.2)	1 (50)	1 (50)
Chloramphenicol	4 (10.5)	34 (89.5)	2 (100)	0 (0)
Ciprofloxacin	2 (5.3)	36 (94.7)	1 (50)	1 (0)

Table V: Multiple antibiotics resistance (MAR) index of staphylococci species

MAR Index	No. of isolate s	% of isolates
0.3	6	15
0.4	9	22.5
0.5	12	30
0.6	8	20
0.7	4	1.6
0.8	1	0.4
Total	40	100

DISCUSSION

This study presented an analysis of 38 isolates of *Staphylococcus* species recovered from nasal swab samples from 968 pigs. The recovery rate of 3.9% (38/968) for the staphylococci from pigs in this study is lower than the 11.1% (47/425) reported by Otalu *et al*. (2020) in Kogi-State, Nigeria. Also, the recovery rate of 1.7% (2/116) for humans

Table IV: Antibiotic resistance patterns of *Staphylococcus* species from pigs and humans in Abia Central Senatorial Zone.

Resistance Phenotypes	Number of Isolates	
	Human	Pigs
OX		1
OX, P		1
AMP, VA		1
OX, FOX, VA		1
OX, TE, AMP		1
OX, AMP, P, VA		1
TE, AMP, P, FOX		1
TE, AMP, FOX, VA		1
OX, C, FOX, VA		1
OX, AMP, P, FOX, VA		3
TE, AMP, P, FOX, CIP		1
TE, AMP, P, FOX, VA		2
TE, AMP, FOX, VA, C		1
OX, TE, CRO, AMP, P		1
TE, AMP, P, FOX, VA, CN		1
OX, TE, AMP, P, FOX, VA		3
OX, CRO, AMP, P, FOX, VA		3
OX, TE, CRO, AMP, P, FOX		1
OX, TE, AMP, P, VA, CN		1
OX, AMP, P, SXT, C, VA		1
OX, TE, CRO, AMP, P, FOX, VA		3
OX, TE, AMP, P, FOX, VA, E		1
OX, CRO, AMP, P, FOX, VA, C		1
OX, TE, CRO, AMP, FOX, VA, E		1
OX, TE, AMP, P, FOX, E, CN		1
OX, TE, AMP, FOX, VA, C, CIP		1
OX, TE, AMP, P, FOX, VA, E, CN		1
OX, TE, AMP, SXT, C, VA, E, CN		1
OX, TE, AMP, SXT, VA, E, CN		1
OX, TE, CRO, AMP, P, SXT, VA, C	1	
C, OX, TE, AMP, P, FOX, VA, E, CN,	1	
TOTAL	2	38

Table VI: Values of Vancomycin Minimum Inhibitory Concentration (MIC) for *Staphylococcus* from Humans and Pigs using Etest strip. S; sensitive, S.: staphylococcus

Staphylococcus species	Number of Isolates	MIC (µg/ml)	Interpretation
S. hyicus	19	1	S
		1	S
		2	S
		2	S
		2	S
		2	S
		2	S
		2	S
		2	S
		1	S
		1	S
		1	S
		2	S
		1	S
		1	S
		2	S
		2	S
		1	S
		1	S
S. intermedius	10	2	S
		2	S
		2	S
		2	S
		2	S
		2	S
		2	S
		2	S
		1	S
		1	S
		1	S
S. aureus	1	2	S
S xylosus	1	1	S
S. haemolyticus	2	1	S
		1	S

in this study is lower than the 10% (34/255) reported by Otalu *et al*. (2020) in Kogi-State, Nigeria. The recovered species were *S. aureus* (1), *S. intermedius* (10), *S. hyicus* (21), *S. chromogens* (1), *S. haemolyticus* (5), *S. warneri* (1) and *S. xylosus* (1). Most of the staphylococci species isolated in this study have previously been reported in Nigeria (Ugwu *et al*., 2015a; Momoh *et al*., 2016; Otalu *et al*., 2020). Most of the staphylococci species from pigs in this study were from farms 32(84.2%) while 6 (15.8%) were from an abattoir. Abunna *et al*. (2016) reported 53.2% isolation rate from pigs at slaughter and 44.8% from pig farms in Ethiopia. The difference in the recovery rate of staphylococci amongst the farm and abattoir could be attributed to the contamination of the pigs, hygiene level practiced on the farms and the personal hygiene of in-contact humans. *S. hyicus* was the predominant species found among the pig *isolates* in this study followed by *S. intermedius*. This finding differs from those of Momoh *et al*. (2016) who reported *S. xylosus* to be the predominant species in pigs in Jos; Ugwu *et al*. (2015a) who reported *S. scuiri* to be the predominant species in their study in Nsukka and Tulinski *et al*., (2011) who reported *S. cohnii* to be the most predominant in Netherlands. *Staphylococcus aureus* and *S. intermedius* recovered from humans in this study were from farm attendants.

Staphylococcus species identified in this study showed multidrug resistance to the 12 antimicrobial agents tested. Most of the *Staphylococcus* isolates in this study were resistant to beta lactams 33 (86.8%), ampicillin 31(81.6%) to cefoxitin, 30 cefoxitin, 30 (78.9%) to oxacillin, 24 (63.2%) to penicillin and is in agreement with the report of Momoh *et al*. (2016) and Detweiller *et al*. (2013) in Michigan who reported most *Staphylococcus* species to be resistant to beta-lactams. Besides the beta-lactams, 37 of the 40 isolates were in addition resistant to 3 or more classes of antimicrobial agents and were termed multi-drug resistant. Multi-drug resistance of isolates as observed in this study is in agreement with the findings of Huber *et al*. (2011) who reported that *Staphylococcus* species that were methicillin resistant exhibited co-resistance to other classes of antibiotics. That 33 (86.8%) of the *Staphylococcus* species in this study were resistant to ampicillin is slightly lower than the findings of Effah *et al*. (2018) who reported 100 %, resistance of isolates to ampicillin. This observed difference may be attributed to the fact that resistance mechanisms for this class of antibiotics are similar and usually carried with the genetic elements responsible for beta-lactam resistance on the same plasmids (Karam *et al*., 2016). The level of resistance to antimicrobial agents recorded in this study may be attributed to the fact that the isolates may have originated from animals that have been pre-exposed to different antibiotics (Christopher *et al*., 2013). Also, the use of antibiotics as growth promoters, prophylaxis and at inaccurate dosages administered to animals by farm attendants may result in plasma concentrations that are inconsistent with the desired objectives which can influence resistance (Suleiman *et al*., 2013).

CONCLUSION

There is low occurrence of 3.9% (38/968) of staphylococci species among pigs and 1.7% (2/116) in humans in the study area. Only one S. *aureus* isolate was recovered in the study area from a human sample. However, other staphylococci species comprising *S. intermedius*, *S. hyicus*, *S. chromogens*, *S. haemolyticus*, *S. warneri* and *S. xylosus* were recovered

from both humans and pigs. Thirty-one unique susceptibility patterns were identified among the staphylococci isolates with twenty-four resistant to multiple clinically important antimicrobial classes. All the staphylococci isolates had MAR index values ≥ 0.2. Individuals that frequently come in contact with animals should be educated on the possibility of the transmission of resistant genes from pathogens infecting animals to humans and vice versa. This is because the transmission of resistant genes is dynamic and involves both humans and animals as well as their surroundings.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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