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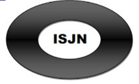

Research Paper

Morphological and Biochemical Characterization of Staphylococci Isolated from Food-Producing Animals in Northern Nigeria

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Staphylococcus aureus (*S. aureus*) is an important pathogen of clinical significance, gram positive coccus, with colonies measuring 1mm in diameter. Some strains, especially the oxacillin-resistant strains pose a global threat to both humans and animals. Food-animals could be a vehicle for the transmission and spread of the bacteria in humans. In Nigeria, routine enumeration of staphylococci in animals by veterinary microbiological laboratories has less been reported. This study was therefore a bid to observe the morphological, cultural and biochemical characteristics, as well as, oxacillin-resistance ability of staphylococci isolated from food-producing animals including cattle, sheep, goats and chickens, in north-eastern Nigeria, sub-Saharan Africa. A total of 1080 specimens collected from these animals were analyzed for the presence of staphylococci, by culture and biochemical tests. Isolates which formed yellow colonies on mannitol salt agar plates and coagulase-positive, were presumptively suspected to be *S. aureus* and were screened for oxacillin resistance on oxacillin-resistance screening agar (ORSA). Results revealed that isolates formed round, white-gray or yellow colonies on Columbia blood agar plates (Figure 1, plate A) and 84% of the isolates produced smooth, raised, glistening, yellow or golden yellow colonies on mannitol salt agar plates (Figure 1, plate B). On conventional biochemical tests, 100% were catalase positive, 28.7% were found coagulase positive, 89.5% produced acetoin and 91% possessed carbohydrates hydrolyzing activity. Prevalence amongst the species indicated cattle (90.5%), sheep (77.1%), goats (89.2%) and chickens (80%). Some isolates (10.9%) formed colonies with intense blue colouration on ORSA medium (Figure 1, plate C), indicative of oxacillin (meticillin) resistance; prevalence being high in cattle (15.8%), sheep (11%) and chickens (10%) than in goats (6%). The presence of oxacillin-resistant staphylococci at this percentage level in food-animals may portend danger to humans, and calls for caution in the livestock industry. The need for regular surveillance and screening for oxacillin-resistant staphylococci in animals is imperative, with a view to recommend appropriate therapy.

Key words: Staphylococcus, morphology, biochemical, characteristics, oxacillin-resistance, food-animals, Nigeria.

INTRODUCTION

Staphylococci are Gram-positive cocci, usually commensal organisms that are found occurring on the skin and mucosa of humans and animals. *Staphylococcus aureus* (*S. aureus*) is an important

pathogen of clinical significance, causing variety of illnesses in both humans and animals worldwide (Chakraborty *et al*., 2011; Böhme *et al*., 2012; Petinaki and Spiliopoulou, 2012; Markey *et al*., 2013). It causes superficial skin infections and life-threatening diseases including endocarditis, sepsis and soft tissues, urinary tract, respiratory, intestinal tract, and bloodstream infections (Rallapalli *et al*., 2008). Close association has been observed to enhance spread of staphylococcal strains among livestock and veterinary care-givers and animal handlers through contact or aerosol (Ajuwape and Aregbesola, 2001). *S. aureus* is a major food-borne pathogen due to its production of eneterotoxins that cause serious intoxications (Wu *et al*., 2011; Liu *et al*., 2014). Fast identification of *S. aureus* and its toxins in food is crucial to determine microbial risk and assure food quality.

Oxacillin-resistant *S. aureus* (ORSA) is mediated by the presence of PBP2a, which is expressed by an exogenous gene, called *mec*A (Hiramatsu, 20*01)*. Oxacillin resistance in *S. aureus* may be attributed by the following: (i) inactivation of antibiotic as a result of structural modification by enzymatic action, (ii) prevention of access to target site by altering the outer membrane permeability, (iii) alteration of the antibiotic target site, (iv) efflux pump which pumps out the antibiotic and (v) bypass or overproduction of target enzyme (Chakraborty *et al*., 2011). Animals can act as a reservoir and source for the emergence of ORSA clones in humans (Gercia-Alvarez *et al*., 2011) due to increased animal population and antibiotics usage (Petinaki and Spiliopoulkou, 2012). Until recently, reports of ORSA in food-producing animals were mainly limited to occasional detections in dairy cattle mastitis. Many aspects of its prevalence in animals like pigs has long been unclearly elucidated, and in other livestock including cattle, sheep and goats, colonizing capacity and reservoir status are still requiring elucidation (vanderhaeghen *et al*., 2010), as the increasing prevalence of ORSA in animals represents a significant health implications (Maddox *et al*., 2011).

According to Bautista-Trujillo *et al* (2013) rapid isolation and identification of the *S. aureus* pathogen is a major goal of diagnostic microbiology. A variety of selective and /or differential culture media have been used to isolate and identify the organism (Thakar *et al*., 2013). The use of culture media for *S. aureus* isolation in combination with coagulase activity and haemolysis determination as secondary tests have improved the accuracy of identification, and was in consonance with rrs gene sequence analysis compared with the use of the culture media alone (bautista-Trujillo *et al*., 2013).

Information on characterization of animal strains of staphylococci in Nigeria is very scanty. Earlier researches have however, centred on humans, and some animals including cattle and goats (Adegoke, 1981, 1985, 1987; Adegoke and Ojo, 1982; Adegoke *et al*., 1985; Ajuwape and Akinyede, 2000; Oyenkunle and Adetosoye, 1988), mainly from the western angle of Nigeria. It has been reported that characteristics of staphylococci isolates from certain hosts vary between geographical regions and strains derived from different animal species also vary from one animal to another (Devriese and Oeding, 1976).

This paper was an attempt to study morphological and biochemical characteristics of staphylococci isolated from cattle, sheep, goats and chickens in north-eastern Nigeria, with the aim of determining prevalence amongst such animal species.

MATERIALS AND METHODS

Sample processing

Samples were collected using sterile swabs, and all samples were conveyed to the laboratory on ice in a sterile container and kept at 4°c until processed. Each swab was homogenized in 250 µl of sterile normal saline or physiological buffered saline (PBS) and tissues (trachea and lungs) samples, where collected without swabbing were separately homogenized in a sterile mortar in 250 µl sterile PBS according to Collee and Mar (1996).

All homogenized samples were transferred into 5 ml nutrient agar broth No 2 (Oxoid, Basingstoke, UK), and left to stand for 48 h to enrich the growth, before inoculating onto agar media. Nutrient agar is a basic medium which supplies essential nutrients for the growth of non-fastidious bacteria (Quinn et al., 2011). All samples were processed within 36 h on receipt.

Inoculation of samples

The enriched broth was vortexed and then streaked onto Blood Agar (BA) or Columbia blood agar (CAB; Oxoid, Basingstoke, UK) supplemented with 5% sheep blood (Kolar *et al*., 2010; Quinn *et al*., 2011) for primary isolation of the organisms, as it supports the growth of most pathogens (Quinn *et al*., 2011).

One growth or colony from each plate was further sub-cultured onto Mannitol salt agar (MSA; Oxoid, Basingstoke, UK) and incubated overnight at 37°c (Wedley *et al*., 2014).

All streaking were done using 5 µl sterile disposable loops. Where present, colonies morphologically resembling staphylococci and yielding small to medium, pink or yellow or golden yellow colonies were presumptively selected as *S. aureus* (Bannerman, 2003) from all plates using 5µl sterile disposable loops, and these were sub-cultured onto tryptic soy agar (TSA)(Oxoid, Basingstoke, UK) plates by aerobic overnight incubation at 37°c to obtain a pure culture of the organism before subjecting to Gram staining.

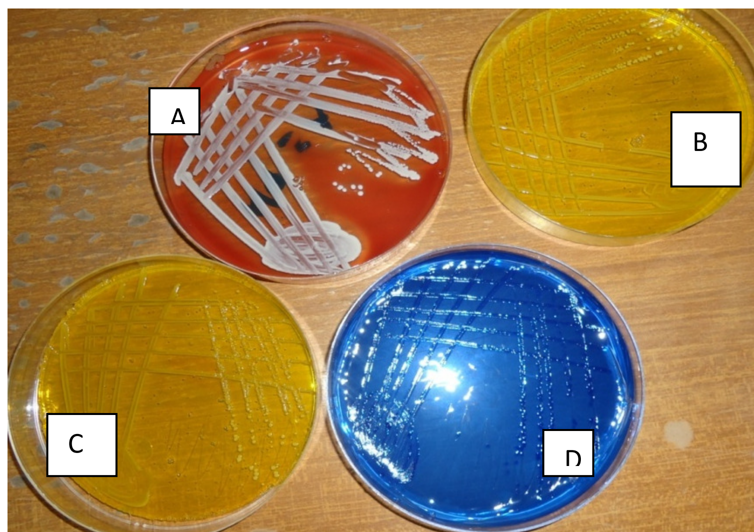

Figure 1. Presumptive *Staphylococcus aureus* (plates A, B and C) and meticillin-resistant *Staphylococcus aureus*, MRSA (plate D) isolated from food-animals in northeast Nigeria.

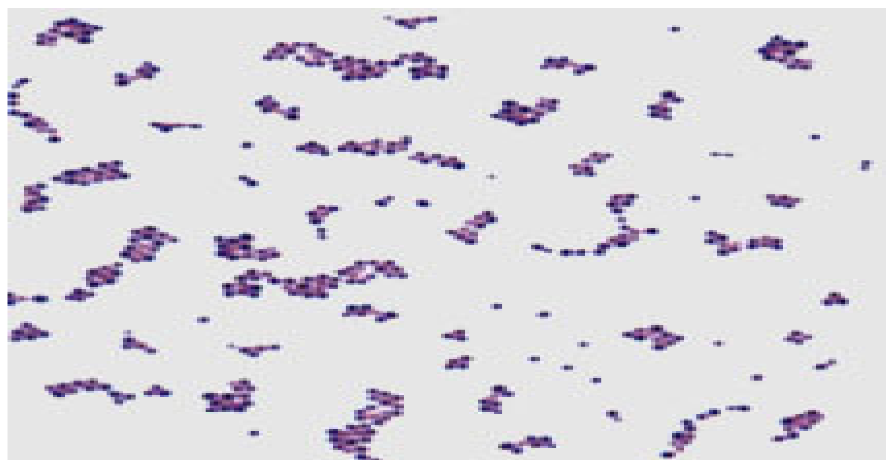

Figure 2. Staphylococci in cluster, chains, and singles arrangements isolated from sheep nasal swab.

Biochemical characterization

Fresh cultures of the presumptive colonies from TSA plates were Gram-stained (Chandra and Mani, 2011; Quinn *et al*., 2011; Ochei and Kolhatkar, 2008) to select for Gram-positive cocci. Gram-positive organisms stained purple or blue (Figure 2). All Gram-positive isolates were sub-cultured onto MSA (Oxoid, Basingstoke, UK) and incubated aerobically overnight at 37°c (Zunita *et al*., 2008), and then subjected to conventional biochemical tests including catalase, coagulase, acetoin production, methyl red, indole production, urease production, oxidase and sugar fermentation as described previously (Ochei and Kolhatkar, 2008; Quinn *et al.,* 2011; Chakraborty *et al*., 2011; Thakar *et al*., 2013) to differentiate *S. aureus* from streptococci and coagulase-negative staphylococci (CoNS). Oxacillin-resistance screening agar base (ORSAB) test Where present isolates presumptively typical of *S. aureus* were selected and rubbed onto an Oxacillin-Resistance Screening Agar (ORSA, Oxoid, Basingstoke, UK) plates in one set of streaks near the plate perimeter. The sample material was then streaked across the plate using the diminishing sweep technique. Plates were incubated at 37°c for 24 h and examined after 24 h for blue colonies (Zunita *et al*., 2008; Schmidt *et al*., 2014). Colonies that appeared blue or deep blue colour (Figure 1, plate C) were considered Oxacillin (meticillin)-Resistant *S. aureus*. Negative plates were re-incubated for further 24 h when necessary. *S aureus* ATCC 29213 (oxacillin-susceptible) was used as negative

Table 1. Percentage of samples that yielded growth of organisms.

Source of sample	Number tested	Number (%) growth
Cattle	270	48 (17.8)
Sheep	270	41 (15.2)
Goats	270	43 (15.9)
Chickens	270	58 (21.5)
Total	1080	190 (17.6)

Table 2. Morphology and Cultural Characteristics of presumptive Staphylococci isolated from food-producing animals in Northeast Nigeria.

Characteristics observed	Number (%) positive isolates				
	Cattle (n = 48)	Sheep (n = 41)	Goats (n = 43)	Chickens (n = 58)	Total (n = 190)
Round, white – gray, or yellow colonies, on BA/CAB plates	48 (100)	41 (100)	43 (100)	58 (100)	58 (100)
Gram-positive cocci in clusters pairs, tetrads or chains on Gram-staining	42 (87.5)	35 (85.4)	37 (86.0)	50 (86.2)	164 (86.3)
Smooth, raised glistening, gray, pink, pale yellow or deep yellow colonies on MSA plates	38 (90.5)	27 (77.1)	33 (89.2)	40 (80.0)	138 (84.1)[a]
Typical colonies with intense blue colour on ORSAB plates	6 (15.8)	3 (11.1)	2 (6.1)	4 (10.0)	15 (10.9)[b]

[a] N=164 (cattle, n 42; sheep, n 35; goats n 37; chickens n 50) [b] N =138 (cattle n 38; sheep n 27; goats n 33; chickens n 40)

control.

Statistical analysis

Descriptive statistics (percentage proportions) was used to describe the prevalence of *S. aureus* and X^2 was used to determine statistical difference in the prevalence of oxacillin-resistant *S. aureus* amongst food-animals, where possible, using SPSS version 16 software package (www.spss.com, 2007). A P value ($P < 0.05$) was considered statistically significant.

RESULTS

A total of 1,080 samples were collected from different animals including cattle, sheep, goats and chickens out of which only 190 (17.6%) yielded growth of organisms (Table 1). The results showed significant variation in the isolation of organisms amongst the animal species, with samples from cattle 48 (17.8%), sheep 41(15.2%), goats 43(15.9%) and from chickens 58 (21.5%) were positive for growth of organisms. These isolates were morphologically and biochemically characterized by standard methods for the isolation and identification of *S. aureus*.

Table 2 shows the morphological and culture characteristics of the isolates. The results showed that all isolates 190 (100%) produced round, white–gray colonies, some with haemolysis, on Blood Agar/or Columbia Blood Agar plates (Figure 1 plate A), distributed amongst the species of animals as 48/48 (100%), 41/41 (100%), 43/43 (100%) and 58/58 (100%) isolates from cattle, sheep, goats and chickens respectively. When Gram-stained, 164 (86.3%) of the isolates were Gram-positive cocci either in clusters, pairs, single, tetrads or chains (Figure 2). The percentage distribution among the animal species indicates that 87.5% from cattle, 85.4% from sheep, 86.0% from goats and 86.2% from chickens were gram positive cocci. The gram positive isolates were inoculated onto MSA and incubated overnight aerobically at 37°c, and the results showed that a total of 138 (84.1%) isolates produced smooth, raised, glistening colonies with characteristic gray, pink, pale yellow or deep golden yellow pigments on Mannitol Salt Agar plates (Figure 1, plate B). These isolates were suspected to be Staphylococci, and the prevalence amongst the animal species was cattle (90.5%), sheep (77.1%), goats (89.2%) and chickens (80.0%). To select for oxacillin resistance, the 138 suspected staphylococci isolates were streaked onto ORSAB agar plates, and we obtained 10.9% of the isolates formed typical colonies with intense blue colouration on a colourless ORSAB agar plates (Figure 1C). Prevalence being 15.8% in cattle, 11.1% in sheep, 6.1% in goats and 10.0% in chickens respectively formed deep blue colouration with the ORSAB media (Table 2).

Table 3 shows the biochemical characteristics of the suspected staphylococci isolates obtained from food-animals. Result shows that all the 138 (100%) isolates were catalase – positive and 49 (35.5%) were positive for coagulase test (Figure 3). Some isolates 45 (32.6%) were negative for indole production test, while 111 (80.4%) isolates were positive for methyl red, acetoin production (Voges-Proskuaer), urease production and citrate utilization, and were oxidase–negative, whereas, all 138

Table 3. Biochemical Characterization of suspected Staphylococci isolated from food Animals.

Biochemical test	Isolate that showed characteristic reaction of *S. aureus*				
	Cattle (n = 38)	Sheep (n = 27)	Goats (n = 33)	Chickens (n = 40)	Total (n = 138)
Catalase (+ve)	38 (100)	27 (100)	33 (100)	40 (100)	138 (100)
Coagulase (+ve)	12 (27.9)	17 (45.9)	7 (17.9)	13 (25.0)	49 (35.5)
Indole production (-ve)	11 (28.9)	14 (51.9)	7 (21.1)	13 (32.5)	45(32.6)
Methyl Red (+ve)	30 (78.9)	22 (81.5)	26(78.8)	33 (82.5)	111 (80.4)
Voges-Proskauer (+ve)	30 (78.9)	22 (81.5)	26(78.8)	33 (82.5)	111 (80.4)
Urease production (+ve)	30 (78.9)	22 (81.5)	26(78.8)	33 (82.5)	111 (80.4)
Citrate utilization (+ve)	30 (78.9)	22 (81.5)	26(78.8)	33 (82.5)	111 (80.4)
Oxidase (-ve)	30 (78.9)	22 (81.5)	26(78.8)	33 (82.5)	111 (80.4)
Glucose (+ve)	38 (100)	27 (100)	33 (100)	40 (100)	138 (100)
Lactose (+ve)	38 (100)	27 (100)	33 (100)	40 (100)	138 (100)
Sucrose (+ve)	38 (100)	27 (100)	33 (100)	40 (100)	138 (100)

Figure 3. Coagualase-positive isolates (top 3 tubes); Coagulase-negative isolate (bottom tube).

(100%) isolates fermented glucose, lactose and sucrose. Based on species distribution, all 38 (100%) isolates from cattle, 27 (100%) isolates from sheep, 33 (100%) isolates from goats and 40 (100%) from chickens were positive to catalase and carbohydrate (glucose, lactose and sucrose) fermentation tests, whereas, 12 (31.6%) of the isolates from cattle, 17 (63.0%) from sheep, 7 (21.2%) from goats and 13 (32.5%) from chickens were positive for coagulase tests (Figure 3), these were presumptively considered *S. aureus*. Further results indicate that 11 (28.9%) isolates from cattle, 14 (51.9%) from sheep, 7 (21.2%) from goats and 13 (32.5%) from chickens were negative for indole production test. However, 30 (78.9%) isolates from cattle, 22 (81.5%) from sheep, 26 (78.8%) from goats and 33 (82.5%) from chickens showed positive reactions with methyl red, Voges-Proskauer, urease production and citrate utilization tests, and were negative for oxidase test, respectively.

DISCUSSION

The present paper studied the morphological and biochemical characteristics of *Staphylococcus aureus* isolated from food–producing animals (cattle, sheep, goats and chickens) in north-eastern Nigeria. Staphylococci are spherical cells about 1µm in diameter arranged in irregular clusters. Staphylococci grow readily on many types of media and active metabolically, fermenting carbohydrates and producing pigments that vary from white to yellow; colonies on solid media are round, smooth, raised and glistening (Brooks *et al*., 2007). Mannitol salt agar is a medium for the isolation of staphylococci, and about 84% of the isolates in this study when grown on it, produced colonies which were smooth, raised, and glistening, with characteristic gray, pink, and pale or deep yellow colouration or pigments. On blood agar, they produced round, white-gray, or golden yellow colonies. Staphylococci colonies on most media are round, smooth, raised and measuring 1 – 2mm in diameter (Ochei and Kolhatkar, 2008). These were the characteristics of staphylococcal isolates in this study, and were presumptively suspected of being *S. aureus* (Bannerman, 2003). *S. aureus* but not other staphylococci ferment mannitol, and usually form gray to deep golden yellow colonies on mannitol salt agar (Brooks *et al*., 2007). The isolates which demonstrated characteristic appearance of *S. aureus* in this study were

found to be Gram – positive, appearing in clusters, singles, pairs, tetrads or chains. Young cocci stain strongly Gram – positive, and on aging, many cells become Gram – negative; typical staphylococci appear as Gram positive cocci in clusters in Gram stain smears (Brooks *et al*., 2007). However, according to Ochei and Kolhatkar, the morphology of the stained bacteria, in many cases, is not definitive (Ochei and Kolhatkar, 2008).

Staphylococci produce catalase enzyme which converts hydrogen to water and oxygen. All the isolates presumptively suspected of being *S. aureus* (138; 100%) in this study were found to be positive for catalase test. Catalase test is used to detect the presence of cytochrome oxidase enzymes which is absent in streptococci and this differentiates them from streptococci (Brooks *et al*., 2007). El-Hadadey and EL-Nour (2011) reported 85% of *S. aureus* isolates that were positive for catalase test compared to our result. However, differentiation of staphylococci into *S. aureus* usually is dependent on the ability of bacteria to produce coagulase, whether free or bound coagulase, as well as clumping factor. *S. aureus* produces coagulase, an enzyme-like protein that clots oxalated or citrated plasma (Lowy, 1998; Walvogel, 2006). Previously, a proof of free plasma coagulase in the tube coagulase test was the gold standard of *S. aureus* identification (Bannerman, 2003). The presence of coagulase – positive *S. aureus* in food animals is of grave public health concern, and is viewed as health hazard; for coagulase production is considered synonymous with invasive pathogenic potential (El-Hadadey and EL-Nour, 2011). Pathogenic staphylococci often haemolyse blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins, and produce a yellow pigment (Brooks *et al*., 2007). The result of this study showed that of 138 presumptive staphylococci, about 36% were found positive for coagulase. This percentage could likely be of potential threat to humans associated with these animals in the study area, e.g. butchers, livestock farmers and traders. Higher percentages than that reported here were previously reported in rabbit isolates in western Nigeria (Ajuwape and Aregbesola, 2001), and in human isolates elsewhere (El-Hadadey and EL-Nour, 2011) respectively. In the present paper, about 80% of the presumptive staphylococci isolates were found positive for methyl red, urease production, citrate utilization and acetoin production tests, and negative for oxidase tests, whereas, 32.5% were found negative for indole-production. Carbohydrate fermentation was observed by 100% of the isolates, as glucose, lactose and sucrose were completely hydrolyzed. These characteristics have previously been used to confirm *S. aureus* (Holt *et al*., 1993; Bannerman, 2003). El-Hadadey and EL-Nour (2011) in their study report 85% of isolates found positive for methyl red, Voges-Proskauer and negative for oxidase and indole tests, and in addition, had glucose, lactose and sucrose fermentation activities, and were approved to be *S. aureus*. These studies are in agreement with our findings. Previous studies in food-producing animals, Ajuwape and Aregbesola (2001), Adegoke and Ojo (1982) and Oyekunle and Adetosoye (1988) have reported complete fermentation of sugar including sucrose by 100% of staphylococci isolated from the various animals studied. These findings are in concordance with our findings in which we report 100% isolates exhibited characteristic sugar fermentation. In a standard biochemical tests carried out on human clinical isolates of *S. aureus*, Chakraborty *et al* (2011) reported that none of the isolates had sucrose fermentation activity, perhaps implying possible variant strains from animal isolates or inherent sugar hydrolyzing ability.

Our finding also reveals that *S. aureus* is more prevalent in sheep (63%) than in chickens (32.5%), cattle (31.6%) and goats (21.2%). However, the frequency of isolation of oxacillin-resistant *S. aureus* appeared to be more in cattle (16%) than in sheep (11%), chickens (10%) and goats (6%) in this order, as determined using ORSAB agar. ORSAB agar is a special media containing aniline blue dye. It can detect mannitol fermentation in staphylococci. Its formulation includes a dual antibiotic supplement (1mg oxacillin and 25,000IU polymixin B) and 5.5% concentration of sodium chloride salt incorporated into 500ml of the agar solution, these combine to reduce the growth of non-staphylococcal organisms and select for MRSA (Manufacturer: Oxoid, Basingtone, UK). About 11% of the staphylococcal isolates in our study exhibited the characteristic appearance of intense blue colouration on the ORSAB agar plates, indicating that they are oxacillin–resistant. The presence of oxacillin–resistant *S. aureus* to this percentage level in animals might portend danger to humans in closed association with these animals, and calls for caution in the livestock production industry. Chakraborty *et al* (2011) reported about 47% *S. aureus* isolated from human clinical infections that exhibited the characteristic intense blue colour on oxacillin-screening agar. Thaker *et al* (2013) reported *S. aureus* isolates from cow milk that exhibited resistance against oxacillin, in addition to ampicillin and tetracycline. The development and spread of bacterial strains that are oxacillin – resistant is a growing global health problem, as MRSA infections are a global health issue due to the severity of the illnesses they may cause (Byströn *et al*., 2009), and effective treatment and ability to control infectious diseases in both human and animals may be jeopardized.

Conclusion

In conclusion, this paper described the morphological and biochemical characteristics, as well as, oxacillin-resistant ability of staphylococci isolated in specimens collected from food-animals in north-eastern Nigeria. The presence

of coagulase positive and oxacillin-resistant staphylococci at the level observed in this study in food-animals may portend danger to humans, and calls for caution in the livestock industry. The need for regular surveillance and screening for oxacillin-resistant staphylococci in animals is imperative with a view to recommend appropriate therapy.

AUTHORS` DECLARATION

We declare that this study is an original research by our research team and we agree to publish it in the journal.

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