

Research Article

Prevalence and Antimicrobial Susceptibility Patterns of Bovine and Ovine *Staphylococcus aureus* Isolates in Maiduguri, Nigeria

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ABSTRACT

A study was conducted to determine the prevalence and antibiotic susceptibility patterns of *Staphylococcus aureus* from Bovine and Ovine species in Maiduguri metropolis. Two hundred and eighty one ($n=281$) clinical and non-clinical swab samples from Bovine ($n=133$) and Ovine ($n=148$) species were collected; the samples were screened for incidence of *Staphylococcus aureus* and susceptibility of the isolates to various antimicrobial agents using standard procedures. Eighty two (61.7%) of the Bovine and 91 (61.5%) of the Ovine samples were *S. aureus* positive. The isolates were further tested for haemolysin production and subjected to antibiotic sensitivity tests against 16 antibiotics representing various classes of antimicrobial agents, 10 antibiograms (Groups A – K) were obtained and the antimicrobial resistance index (MARI) showed that as high as 86.1% (149/173) of the screened isolated pathogens had MARI above 0.2 (> 0.2). The haemolysin tests showed 34.1% of the isolates to be alpha-haemolytic. The MARI value is an indication of misuse or abuse and the haemolysin test results suggests a possibility of cross-species infections or acquisition of mobile genetic elements from other sources by the pathogens. There is widespread misuse of antimicrobials and signs of resistance development by the pathogen in Maiduguri, Nigeria. The use of antibiotics should be regulated to arrest this development and such regulation should be enforced by appropriate authority.

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is an important pathogen of domestic ruminants and man (Bukharie et al., 2001; Grundmann et al., 2006; Fitzgerald, 2012), and is regarded as one of the most versatile and devastating zoonotic pathogen responsible for causing widespread outbreaks of serious infection, food poisoning, colonization, animal mastitis and pneumonia affecting man and animals (Fthenakis, 1998; Simoons-Smit et al., 2000; Kesah et al., 2003; Pengov and Ceru, 2003; Eftekhari and Dadaei, 2011; Ateba Ngoa et al., 2012; Dhama et al., 2013). *S. aureus* can also cause a number of infections in animals, such as abortions in Ewes (Edwards et al., 2008), staphylococcosis in rabbits (Hermans et al., 1999, 2003), pneumonia and osteomyelitis complex in poultry (Akbar and Anal, 2013; Monecke et al., 2013). In the various farms where such infections have been diagnosed, cumbersome preventive and control measures had to be undertaken on *S. aureus*, and despite all these measures, new infections continued to occur, treatment is often associated with poor success (Zecconi and Scali, 2013a) and attempts at producing a viable vaccine have failed and more research is needed in this aspect (Proctor, 2012; Sadeghi, 2012). One of the reasons for these failures are not only because infections originate from both exogenous and endogenous sources to the host, but more importantly due to the ability of the pathogen to rapidly develop resistance to antimicrobial agents commonly employed for intervention by

the clinicians (Grundmann et al., 2006; Hasman et al., 2010; Gharsa et al., 2012; Porrero et al., 2012). Besides their role as commensals on the skin and mucosal surfaces, their occurrence on the respiratory and gastrointestinal tracts of worm blooded animals; they have also been involved in a wide variety of animal diseases (Werckenthin et al., 2001). These pathogens are responsible for disease when the host defense or immunity is compromised or lacking. Several manifestations of disease such as pyemic abscesses, suppurative arthritis and urinary tract infections in domestic and wild animals could be attributed to numerous virulent factors, such as the production of toxins and enzymes (Pengov and Ceru, 2003; Gharsa et al., 2012). *S. aureus* has several factors that contribute to the pathogenesis of infection (Gharsa et al., 2012). Several reports of drug resistant *S. aureus* isolated from animals have been documented; few reports of *S. aureus* species are from horses (Werckenthin et al., 2001; Alves et al., 2009; van Duijkeren et al., 2010), dairy herds (Fthenakis, 1998; Normanno et al., 2007; Alves et al., 2009), pet dog (Fitzgerald, 2012; Yamamoto et al., 2013), avian species, cats, rabbits, pigs, goats and sheep (Van Duijkeren et al., 2010; de la Fuente et al., 2011; Zecconi and Scali, 2013b). However, some studies (Seguin et al., 1999; Zecconi and Scali, 2013b) emphasized the possibility of exchange of the organism between species of animals.

Previous workers have reported studies on *S. aureus* in different parts of Nigeria in both human and animal subjects

(Chigbu and Ezeronye, 2004; Isara et al., 2010; Olajubu et al., 2012). Maiduguri, the capital of Borno State, Nigeria is located in the extreme north of the Sahel region and accounts for about one quarter of the total ruminant population in Nigeria (Majiyagbe and Lamorde, 1997). Despite the large population of these animals in the region, the prevalence and the antibiotic susceptibility pattern of *S. aureus* among Bovine and Ovine species in Maiduguri metropolis have not been adequately studied and documented. The ability to isolate *S. aureus* and the determination of the antibiograms is very crucial for clinicians to select empiric antimicrobial therapy, rational formulation of public health policies and providing useful information on the global surveillance of this pathogen (Shittu et al., 2006).

Present study was designed to comparatively evaluate trends in colonization or infections and determined the extent of *S. aureus* prevalence as a widespread problem among Bovine and Ovine species in Maiduguri. The study also accessed the antibiograms of the isolates to determine their sensitivity to commonly used antibiotics.

MATERIALS AND METHODS

Sample collection

The Bovine and Ovine clinical and non-clinical samples for analysis in this study were collected from septic wounds, abscess, nasal and skin swabs from animals in Maiduguri. The study lasted between February 2006 to November, 2007. Two hundred and eighty one (281) specimens; Bovine (n =133) and Ovine (n = 148) clinical and non-clinical samples were collected from different animal species using sterile swab sticks (Evepon sterile, Anambra State, Nigeria). University of Maiduguri Veterinary Teaching Hospital (UMVTH) and Modu Sheriff (State) Veterinary Clinic both located in Maiduguri, Northeastern Nigeria served as the sources of clinical specimen while various commercial farms and house hold farm animals served as non-clinical subjects. Each specimen was accompanied by details of the species from which the samples was collected as described by Cook and Pezzlo (Cook and Pezzlo, 1992). Verbal consent was sort prior to sample collection and farm/herds owner's permission or consents were obtained before sampling of non-clinical subjects. The labelled samples were transported to the laboratory as soon as possible for processing or stored at refrigeration temperature (4°C) where processing was not possible.

Sample analysis

Collected swabs samples were streaked over blood and nutrient agar plates (Oxoid) and were incubated at 37°C for 24-48 hours. After incubation, colony types were visualized for characteristic morphology of *S. aureus* subcultured on mannitol salt agar (LAB

M, Landashire, UK) and identified phenotypically by conventional methods as Gram's staining, catalase test, coagulase test as described by Cowen and Steel (Cowen and Steel, 1993) and Bello (Bello, 2002).

Antibiotic susceptibility test

A light suspension for each of the isolates was made in peptone water and was incubated, the inoculum was standardized to appropriate density of recommended 0.5 McFarland, which is equivalent to the turbidity of a Barium chloride standard (Brown et al., 2005; CLSI, 2008). The suspension was poured over 4mm thick Mueller-Hinton agar plates. The agar surface was allowed for few minutes and antibiotic impregnated discs (Mast Diagnostics, UK) containing: Ofloxacin (5µg), Erythromycin (15µg), Gentamycin (10µg), Ciprofloxacin (5µg), Clindamycin (2µg), Penicillin (10Units), Fusidic acid (10µg), Chloramphenicol (30µg), Nalidixic acid (30µg), Streptomycin (30µg), Tetracycline (30µg), Rifampicin (5µg), Mupirocin (5µg), Oxacillin disc (1µg) and Neomycin (30µg) were placed on the agar (about 10mm apart), over-turned and incubated at 37°C for 24 hours, then the zones of inhibition pattern were read following the CLSI guidelines (CLSI, 2008). Interpretative zone diameters or susceptibility breakpoints for resistance to Fusidic acid, Neomycin and Streptomycin which were not stated in the CLSI guidelines were considered as follows; ≤ 14 mm- Fusidic acid (Daxboeck et al., 2004), ≤ 16 mm- Neomycin and ≤ 14 mm- Streptomycin (Kim et al., 2004). Growth within a 14mm zone of inhibition with the 5µg Mupirocin disc indicated low-level resistance (Udo et al., 1999).

Multiple Antibiotic Resistance Index (MARI)

Determination of multiple antibiotic resistance index (MARI) was determined for each isolate by dividing the number of antibiotic to which the organism was resistant by the total number of antibiotics tested (Krumperman, 1983; Paul et al., 1997; Olayinka et al., 2006).

RESULTS

A total of two hundred and eighty one (281) specimens; Bovine (n =133) and Ovine (n = 148) clinical and non-clinical samples were collected from the animal species, Table 1 shows the details of prevalence of *S. aureus* according to different sources. Bovine clinical samples accounted for 30/38 (78.9%) isolates while 52/95 (57.7%) *S. aureus* isolates were obtained from the non-clinical samples. Ovine clinical samples showed 50/75 (66.7%) positive and 41/73 (56.2%) were positive from non-clinical samples. Hence, of the total 281 samples examined for *S. aureus*, 173 (61.6%) were positive.

Sample source	SAMPLES EXAMINED			POSITIVE SAMPLES (%)		
	Clinical	Non clinical	Total Samples Examined	Clinical	Non clinical	Total Samples Positive
Bovine	38	95	133	30 (78.9)	52 (57.7)	82 (61.7)
Ovine	75	73	148	50 (66.7)	41 (56.2)	91 (61.5)
Total	113	168	281	80 (70.8)	93(55.4)	173 (61.6)

Table 1: Incidence of *S. aureus* in Bovine and Ovine samples collected in Maiduguri

Antimicrobial susceptibility testing (AST)

Antimicrobial Susceptibility Testing (AST) on the 173 isolates was done by disc diffusion method only, using various concentrations of antimicrobial agents representing various antimicrobial classes. AST results detailed in Table 2, showed that the highest level of resistance (100%) by all the isolates were found against Nalidixic acid. Test against Penicillin

showed that 67/82 (81.7%) of Bovine isolates were resistant and 88/91 (96.7%) of Ovine isolates were resistant.

Tetracycline tests against the isolates indicated very high resistance among Ovine 63 (69.3%) isolates, while Bovine isolates showed remarkably higher susceptible population of 66 (80.5%). All the isolates were 100% susceptible to Fusidic acid, Neomycin, Oxacillin, Rifampicin and Vancomycin.

Comparatively, Ovine isolates had lower 47 (51.7%) Ciprofloxacin resistant population than Bovine isolates which showed 62 (75.6%) Higher sensitivity to Clindamycin was observed among Bovine isolates as they showed sensitivity in above 50% of the population while Ovine isolates test showed that <50% were sensitive to the drug. 72.5% isolates from Ovine showed high resistance to Erythromycin, but Bovine isolates had lower resistant population of 28.1%. Less than 50% of the Bovine, and Ovine isolates were resistant to Gentamicin. Only 7 (8.5%) of Bovine isolates showed resistance to Chloramphenicol, although Ovine isolates showed 29.9%. All the isolates showed high sensitivity to Streptomycin. Generally, *S. aureus* isolates from Ovine samples indicated higher antimicrobial resistance than the Bovine isolates.

Multi antimicrobial resistance index (MARI)

The multi antimicrobial resistance index (MARI) gives a direct suggestion of the probable sources of an microorganism. According to previous workers, MARI greater than 0.2 indicates that an organism must have originated from an environment where antibiotics are often used or abused (Krumperman, 1983; Paul *et al.* 1997; Olayinka, *et al.* 2006). A careful look at the results (Table 3) shows that all of the isolates in group A, were resistant to only three of the sixteen antimicrobial agents tested. The calculation (not shown) proved that all the isolates in the group had MARI equal to 0.2. Hence, only 5.2% (9/173) fall within this group. 86.1% (149/173) of the isolates are within Group B – J, and all had MARI above 0.2 (MARI > 0.2), while 8.7% (15/173) of the isolates (Group K) were susceptible to all the antibiotics tested, hence their MARI = 0.

Table 2: Antibiotic susceptibility of *S. aureus* isolated from Bovine and Ovine in Maiduguri metropolis

Antimicrobial agent	BOVINE n = 82			OVINE n = 91			TOTAL n = 173
	Number of isolates that were:			Number of isolates that were:			Number of isolates that were:
	S	R	R/rate	S	R	R/rate	R/rate (%)
Penicillin	15	67	81.7	3	88	96.7	155 (89.6)
Ciprofloxacin	20	62	75.6	44	47	51.7	109 (63.0)
Clindamycin	48	34	41.5	38	53	58.2	87 (50.3)
Erythromycin	59	23	28.1	25	66	72.5	89 (51.4)
Fusidic acid*	82	0	0	91	0	0	0
Gentamicin	52	30	36.6	60	31	34.1	61 (35.3)
Mupirocin*	82	0	0	91	0	0	0
Chloramphenicol	75	7	8.5	72	19	20.9	26 (15.0)
Nalidixic acid	0	82	100	0	91	100	173 (100)
Neomycin*	82	0	0	91	0	0	0
Ofloxacin	41	41	50	53	38	41.8	79 (45.7)
Oxacillin	82	0	0	91	0	0	0
Rifampicin	82	0	0	91	0	0	0
Streptomycin *	79	3	3.7	83	8	8.8	11 (6.4)
Tetracycline	66	16	19.5	28	63	69.2	79 (45.7)
Vancomycin	82	0	0	91	0	0	0

S = sensitive; R = resistant; R/rate = resistance rate; * = Interpretative zone diameters or susceptibility breakpoints were not stated in the CLSI guidelines, but were considered as follows; ≤ 14 mm- Fusidic acid (Daxboeck *et al.*, 2004), ≤ 16 mm- Neomycin and ≤ 14 mm- Streptomycin (Kim *et al.*, 2004). Growth within a 14mm zone of inhibition with the 5µg Mupirocin disc indicated low-level resistance (Udo *et al.*, 1999).

ANTIBIOGROUP	ANTIMICROBIAL AGENTS	SOURCE OF ISOLATE		
		BOVINE n = 82 (%)	OVINE n = 91 (%)	TOTAL n = 173 (%)
Group A	PEN, ERY, NA.	2 (2.4)	7 (7.7)	9 (5.2)
Group B	ERY, TET, CIP, NA.	7 (8.5)	4 (4.4)	11 (6.4)
Group C	PEN, ERY, TET, NA.	15 (18.3)	8 (8.8)	23 (13.3)
Group D	PEN, ERY, TET, CIP, NA.	7 (8.5)	12 (13.2)	19 (11.0)
Group E	PEN, ERY, TET, CIP, CLN, NA.	9 (11.0)	22 (24.2)	31 (17.9)
Group F	PEN, ERY, TET, CIP, GEN, NA.	8 (9.8)	10 (11.0)	18 (10.4)
Group G	PEN, ERY, TET, CIP, GEN, CLN, NA.	6 (7.3)	5 (5.5)	11 (6.4)
Group H	PEN, ERY, TET, CIP, GEN, CLN, CHL, NA.	3 (3.7)	4 (4.4)	7 (4.0)
Group I	PEN, ERY, TET, CIP, CLN, CHL, NA.	7 (8.5)	6 (6.6)	15 (8.7)
Group J	PEN, CIP, TET, CLN, NA.	7 (8.5)	8 (8.8)	15 (8.7)
Group K	Susceptible to All Antimicrobial the Agents	10 (12.2)	5 (5.5)	15 (8.7)

Table 3: Antibio groups and resistance patterns of *S. aureus* isolates from human and animals in Maiduguri metropolis

Ofloxacin (OFX) 5µg, Erythromycin (ERY) 10µg, Gentamicin (GEN) 10µg, Ciprofloxacin (CIP) 5µg, Clindamycin (CLI) 2µg, Streptomycin (STR) 10µg, Penicillin (PEN) and Tetracycline (TET) 30µm, Nalidixic acid (NA) 30µg, Chloramphenicol (CHL).

Table 4: Analysis of haemolysin production test associated with *S. aureus* strain from human and animals in Maiduguri

SPECIES (Number of isolates)	HAEMOLYSIN PRODUCTION TEST									
	Number Positive for α		Number Positive for β		Number Positive for γ		Number negative for haemolysin production		TOTAL (n = 173)	
	No.	%	No.	%	No.	%	No.	%	No.	%
BOVINE (n=82)	29	35.4	32	39.0	9	11.0	12	14.6	82	47.4
OVINE (n= 91)	30	33.0	41	45.1	13	14.3	7	7.7	91	52.6
TOTAL (n=173)	59	34.1	73	42.2	22	12.7	19	11.0	173	100

Antibiograms

Ten antibiograms (Groups A – K) were detected in this study based on the resistance pattern of isolates. Bovine and Ovine isolates showed their highest level of resistance in Groups C-18.3%, and E-24.2%. Lowest resistance population in bovine isolate was 2.4% (Group A), while Ovine had a population of 4.4% in both Group B and H as the lowest resistant population. In Group L, 15 (8.7%) of the total isolates were susceptible to all the eight classes of antimicrobial agents tested. Comparatively, Bovine isolates presented more isolates 10 (12.2%) that were susceptible to all the antimicrobial agents tested, Ovine isolates were 5 (5.5%) (Table 3).

Hemolysin production test

Twenty nine (35.4%) of the Bovine isolates were α -hemolytic, 32/82 (39.0%) were β -hemolytic 9/82 (11.0%) produced γ -hemolysins and only twelve (14.6%) were non-hemolytic. Among the Ovine isolates, the α -hemolytic strains were 30/91 (33.0%), β -hemolysin positive isolates were 41/91 (45.1%) and 13/91 (14.3%) of γ -hemolytic strains were found among ovine isolates, 7/91 (7.7%) were negative for haemolysin production. (Table 4). Overall haemolysin production test result showed that Ovine isolates had the higher prevalence rate of 92.3% (84/91) of haemolytic strains compared to Bovine isolates with 85.4% (70/82) prevalence rate.

The β -hemolytic strains were 42.2% (73/173) which were the most prevalent among all the isolates tested, followed by α -hemolytic strains that accounted for 34.1% (59/173). The γ -hemolytic strains were 12.7% (22/173) while the non-hemolytic group gave 11.0% (19/173). Of the 173 isolates from Bovine and Ovine tested, 89.0% (154/173) were either α , β or γ -hemolytic.

DISCUSSION

The results of this study demonstrated a high prevalence of *S. aureus* among the animals investigated, the clinical and non-clinical samples of both animals revealed >50% positive for *S. aureus*. These results are similar to those reported from Umudike, Nigeria (Achi and Ugbo, 2006), where *S. aureus* incidence among sheep, goat, cattle, pigs and rabbits were reported to be high in healthy animals. Thus both clinically healthy and unhealthy animals can carry *S. aureus* with zoonotic and pathogenic potentials. The result is also an indication that *S. aureus* is one of the most common pathogen in most infections which is in agreement with the report of earlier workers (Werckenthin et al., 2001; Oranusi et al., 2003; Olaoye and Onilude, 2009; de la Fuente et al., 2011) were they explained that the pathogen begins with colonization of the host, which usually culminate into clinical conditions in the animals and is suspected to be the source of most resistant strains infecting both man and animals (Seguin et al., 1999; Simoons-Smit et al., 2000; Lee, 2003). AST of the isolates tested against different

classes of antibiotics showed that great populations of resistant strains were found among both animal species. All of the isolates were resistant to Nalidixic acid, this could be due to the fact that Nalidixic acid is an antibiotic used against gram negative bacteria which explains why there seems to be high level of resistance on the isolates.

This study indicated that a greater percentage (86.1%) of the isolates had MARI above 0.2, this underscores the need for effective antibiotic policy in both veterinary and human consumption in Maiduguri. Susceptibility testing serves as a guide to the physician in selecting appropriate drug to which a particular microorganism is susceptible, thereby producing effective therapy. The observation of high population of resistant strains among these animals poses a great public concern for humans and a risk to other animal population in and around Maiduguri owing to the fact that people rely on the animals for food, labour, transport and have constant and prolonged contact with the animals in the area of study.

Many of the Bovine isolates (35.4%) were α -hemolytic, this also applies to the Ovine isolates where 33.0% (30/91) were recorded as α -hemolysin positive. Alpha-hemolytic (α -hemolysin positive) strains of *S. aureus* are known to be more of human biotypes (Dinges et al., 2000), the isolation of these strains from animals in such a high number is an indication that a cross species infection or contamination had occurred between human and animals. Equally, as many as 39.0% and 45.2% Bovine and Ovine isolates were discovered to be β -hemolytic, these β -hemolytic biotypes are known to predominantly be of animal origins (Dinges et al., 2000; Achi and Ugbo, 2006; Hasman et al., 2010; de la Fuente et al., 2011; Espinosa-Gongora et al., 2012). These results suggest that the close association of human and animals in Maiduguri may have led to the transfer or exchange of different specie specific biotypes. This is in agreement with previous reports that constant contact with animals could result into exchange or just jumping of these pathogens (Umoh et al., 1991; Oranusi et al., 2006).

Ten resistance patterns (antibiograms) were found (table 3). The predominant pattern was in group E where resistance to six antibiotics (penicillin, erythromycin, tetracycline, ciprofloxacin, Clindamycin and Nalidixic acid) was observed. This is evidence that *S. aureus* isolates of animal origin are relatively resistant to many commonly used antibiotics which is similar but slightly higher than results reported from Umudike, Nigeria (Achi and Ugbo, 2006). This high resistance could be attributed to extensive use of antibiotics in Veterinary establishments to combat infections or in subtherapeutic doses in feeds. Most of the antibiotics tested for are commonly used in both human and veterinary interventions in clinical cases or as prophylaxis, the high resistance patterns are also not entirely strange since there is the possibility of resistant strains originating from other sources and spread to the animals

through close contact. The presence of multidrug-resistant isolates in these animals constitutes a reservoir of antibiotic resistant genes, which can spread to other pathogenic species. These strains might have acquired the genes from other species living in the surrounding environment through conjugational genetic transfer (Forbes and Schaberg, 1983) and through mutation of an already existing bacterial gene or horizontal transfer of a resistance gene from another bacterium (Grundmann et al., 2006).

It has been significantly proven that the use of antimicrobial agents for enhancing growth, chemoprophylaxis and treatment of animals meant for food increases the possibility of resistance in human pathogens (Singer et al., 2003; Ternak, 2005; Martinez, 2009). Therefore, the reduction or strict regulation and judicious use of antibiotics use in veterinary medicine is important to minimize the emergence of resistant strains. In this study, we recommend that the choice of antibiotics used for intervention by clinicians (both human and veterinary) and feed formulators must take into account the emergence of drug resistant strains. Constant evaluation of the antibiotic susceptibility patterns for commonly used antimicrobial agents in the study environment is carried out to be informed and take rational decisions on the most efficacious agents to employ during infections.

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