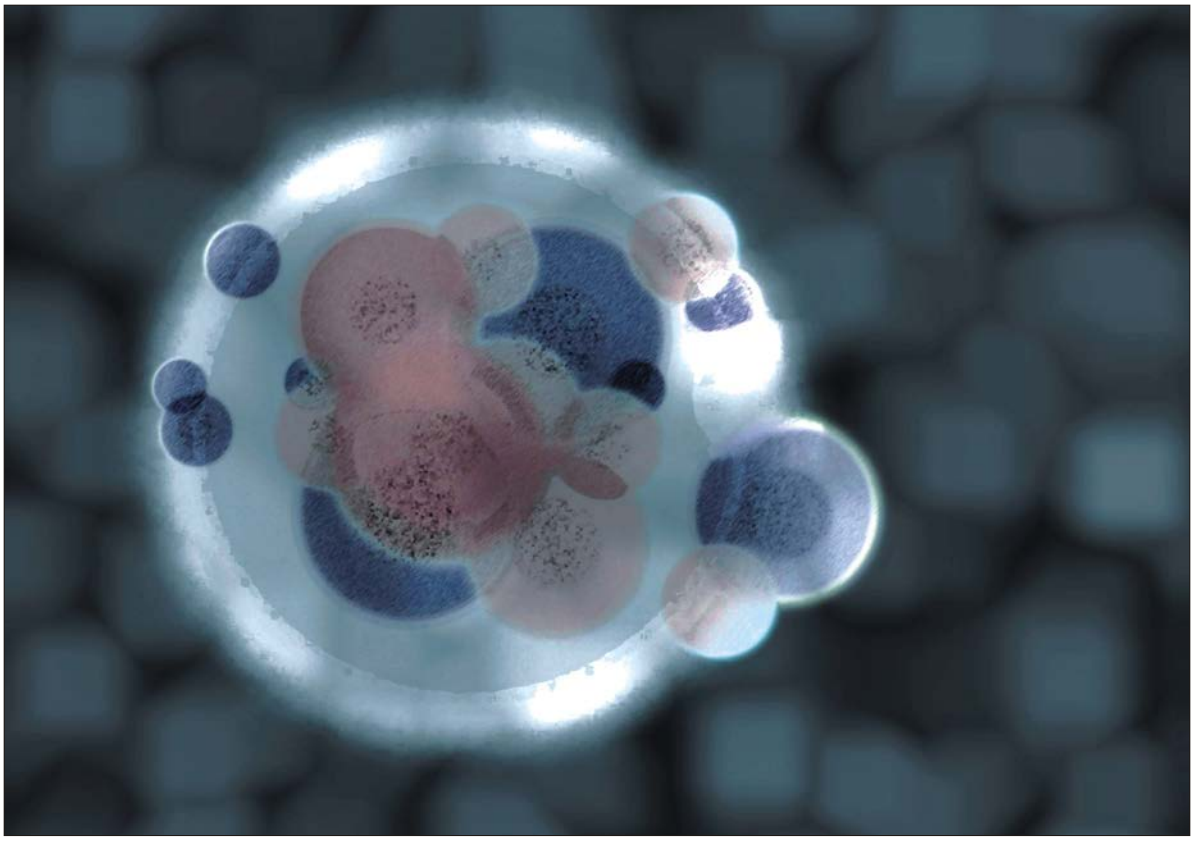

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Characterization of Drug Resistant *Staphylococcus aureus* of Animal Origin

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Abstract: One hundred and eighty samples for culture of ear and nose swabs collected from livestock at a local animal farm at Umudike were examined to determine the incidence of *Staphylococcus aureus*. The susceptibility of the isolates to 15 antimicrobial agents allowed for human and animal therapy were also evaluated. The results show that these animals frequently harbour *S. aureus* strains. In addition, a high number (79%) of the isolates were resistant to one or more antimicrobial agents and 10 different resistance profiles were recorded. Resistance to amoxycillin (71%) and Cotrimoxazole (62.2%) was demonstrated for the isolates. The result indicate that the occurrence of multi-resistant *S. aureus* strains in farm animals may constitute a reservoir for disseminating antibiotic-resistance in the community and the need for the prudent drugs use to diminish the development and spread of antimicrobial resistance.

Key words: *Staphyloccus aureus,* antibiotic resistance, farm animals, incidence

INTRODUCTION

Increased antibiotic resistance in human bacterial pathogens continues to be a major pubic health concern in all countries of the world (Levy, 1984). With some organisms, *in vitro* susceptibility testing has shown complete or varying degrees of resistance to all classes of currently used antibiotic. The reasons for such increased resistance appear to hinge largely on inappropriate, indiscriminate and incomplete courses of antibiotic therapy use in the treatment of human diseases, as well as the used of these agents in veterinary medicine, animal husbandry, agriculture and aquaculture (Tenover and Hughes, 1996). Transfer of antibiotic resistance genes by conjugation among bacteria in the environment must also be regarded as significant factor (Kessie *et al*., 1998; Shoemaker *et al*., 2001). This has led to an intensification of discussion about the prudent use of anti-microbial agents, especially in veterinary medicine, nutrition and agriculture (Caprioli *et al*., 2000).

The use of antimicrobial drugs as regular supplements for prophylaxis, growth promotions in the feed of animal herds and poultry flocks results in the exposure of a large number of animals, irrespective of their health, to frequently subtherapeutic concentrations of antimicrobials (Du Pont and Steels, 1987; Franco *et al*., 1990). In addition, antibiotics to animals and closely related compounds used in human therapy exert selective pressure on their target organisms and can generate a reservoir of antimicrobial resistant bacteria (Endtz *et al*., 1991; Smith *et al*., 2002). Although the problem appears to be related mainly to subtherapeutic applications of antimicrobial drugs in feed of food animals and poultry, all drug use poses a possible threat (Collins-Thompson *et al*., 1988).

Staphylococcus sp. is important microorganisms in meat products. Animal farmers are all aware of the existence of clinical mastitis caused by *Staphylococcus aureus*. The microorganism is usually present on the skin of 40% of all humans and almost 100% of all animals (Synder and Poland, 1991). It produces a heat-resistant enterotoxin that is not inactivated by boiling for 25 min or longer (Bergdoll, 1989). Ingestion of foods containing this toxin causes illness.

Staphylococcus aureus continues to be a major cause of community-acquired and health-care related infections around the world (Lowy, 1998). The emergence of high levels of penicillin resistance followed by development and spread of strains resistant to the macrolides, tetracyclines and aminoglycosides has made the therapy of staphylococcal disease a global challenge (Lowy, 1998; Maranan *et al*., 1997).

Surveillance of antimicrobial resistance therefore is essential for providing information to doctors and national governments relative to restricting antibiotic usage to those cases where human health is threatened by virulent pathogens (WHO, 2001; Cole *et al*., 2003).

Few studies have been reported about antimicrobial resistance in *Staphylococcus* isolates from Nigeria. The purpose of this study was to determine and to compare

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antimicrobial-resistant *S. aueus* of animal origin and to characterize the isolates by antimicrobial susceptibility. The study was carried out at the animal farm of the Michael Okpara University of Agriculture, Umudike, Abia State.

MATERIALS AND METHODS

Collection of samples: The study was carried out using 180 samples collected from the ear and nasal swabs of 90 randomly selected animals at the Michael Okpara University of Agriculture Umudike farm between April to August 2003. This comprise of 44 goats, 38 sheep, 22 cattle, 28 pigs and 48 rabbits. Samples were obtained by swabbing the ear and nostrils of the animals using the method of Clark (1965) with modifications. Each site was swabbed twice. The swabs from each site were transferred in 100 mL of sterile 0.1% alkaline peptone water.

Microbiological analysis: All the samples were analysed within one hour of collection or refrigerated at 5°C before being analysed, but never longer than 2 days. Serial dilutions of all the samples were made with phosphate buffered saline. The dilutions (0.I mL) were surface plated on Mannitol salt Agar (Oxoid Ltd, Bassingstoke, UK) and incubated for 24 to 48 h at 37°C.

Identification of isolates: Two to four representative colonies were subcultured from each positive sample plate and were purified on nutrient agar slants incubated at 37°C. Only coagulase positive strains (Collins *et al*., 1990) were tested further in accordance with standard procedures (Speck, 1984). Cultures considered *Staphylococcus aureus* were subjected to the following tests; catalase activity (Harrigan and McCance, 1976) growth in nutrient agar supplemented with 7.5% (w/w) and 10% (w/w) NaCl, nitrate reduction in nitrate broth and coagulase activity by the tube assay (Baird-Parker, 1979). Further characterization included production of acid from glucose, arabinose, mannitol, mannose, lactose, sucrose and xylose (Devrises *et al*., 1985).

Antimicrobial susceptibility testing: Antimicrobial susceptibility was tested according to the guideline of the National Committee for clinical laboratory standards (NCCLS, 2001) for the diffusion technique.

The antibiotics and their concentrations in micrograms tested were ampicillin 20 (Amp), cloxacillin 10 (Clo), erythromycin 25 (Ery), peflacine 10 (Pef), chloramphenicol 25 (Chl), lincomycin 30 (Lin), Rifampin 10 (Rif) ciprofloxacin 10 (Cpx), streptomycin 30 (Stp), gentamicin 10 (Gen), Penicillin 30 (Pen), Amoxycillin 30 (Amx), Cotrimoxazole 25 (Cot) and Ampiclox 30 (Amc).

The actual test procedure employed the direct colony suspension method. One colony of each isolate was subcultured on Trypticase Soy Agar (TSA) with 5% sheep blood and incubated at 37°C for 24 h. Isolated colonies were then suspended in Trypticase Soy Broth (TSB) and the suspension adjusted to a 0.5 McFarland turbidity standard, resulting in a suspension containing 10^6 cfu mL^{-1}. Within 15 min after adjusting the turbidity, a sterile swab was dipped into the adjusted suspension for inoculation and then uniformly streaked over the entire surface of a Mueller-Hinton Agar (MHA) plate. The drug-impregnated discs were placed on the surface of the MHA plates and incubated at 37°C for 24 h.

The zone of inhibition around each of the discs was measured to the nearest millimeter using sliding calipers. The zone margin was defined as the area showing no obvious visible growth detected by the unaided eye (Cole *et al*., 2003). The inhibition zones were scored as sensitive, intermediate susceptibility and resistant according to NCCLS recommendations. *S. aureus* ATCC 12600 was used as reference strain for antibiotic disc control.

Statistical analysis: All the results were expressed as Means±Standard Deviation (SD) of three parallel measurements and the statistical significance was assessed by student's t-test. The confidence limit were added at ($p<0.05$).

RESULTS AND DISCUSSION

One hundred and eight different samples were investigated for the presence of *S. aureus* strains by direct plating methods. These samples included 44 goats, 38 sheep 22 cattle, 28 pigs and 48 rabbits sample. Table 1 shows the frequency of isolation of the *S. aureus* strains according to the anatomical sites. Thirteen isolates were from goat, 31 from sheep, 19 from cattle, 21 from pigs and 33 from rabbits. With the direct plating method, 12 (54.5%) and 19(50%) of nasal samples from cattle and sheep, respectively examined were found to contain *S. aureus* strains (Table 1).

Table 1: Incidence of *S. aureus* in animal samples

Type of samples	No. of samples examined	Positive samples after isolation	
		Ear	Nostril
Sheep	38	12 (31.8)	19 (50.0)
Goat	44	14 (31.6)	17 (38.6)
Cattle	22	7 (31.8)	12 (54.5)
Pig	28	9 (32.1)	12 (42.9)
Rabbit	48	14 (29.2)	19 (39.6)
Total	180		

*: Figures in parenthesis represent percentage of positive samples

Table 2: Antimicrobial susceriptibility resistance in *S. aureus* strains isolated from different animal sources

Samples	No. of strains	% Susceptibility resistance to antimicrobial agent tested														
		Cpx	Pen	Clx	Gen	Tet	Amp	Stp	Amx	Amc	Rfp	Peel	Ery	Chl	Cot	Lin
Goat	31	100	61.3	100	83.9	67.7	38.7	77.4	22.6	61.3	93.5	93.5	45.8	38.7	45.2	83.9
Sheep	31	83.9	67.7	83.9	67.7	45.2	45.8	83.9	22.6	36.8	11.1	70.4	83.9	35.7	45.2	83.9
Cattle	19	73.7	100	89.5	10.6	56.6	73.7	100	36.8	63.2	100	100	63.2	63.2	26.3	73.7
Pig	21	90.5	90.5	100	90.5	90.5	90.5	90.5	57.1	57.1	100	96.2	90.5	66.7	76.2	90.5
Rabbit	33	51.5	78.8	57.6	36.4	57.5	36.4	63.6	15.2	42.4	84.4	78.8	42.4	21.2	6.7	42.4
Total1	35	79.3	77.0	70.4	71.9	60.0	54.8	80.7	281	563	94.8	79.3	65.2	42.2	378	73.3

Table 3: Resistance of *S. aureus* strains of different origins to a variety of antimicrobials[a]

Animal samples	No. of isolates	No. resistant to (>2) antibiotics	(%)
Goat	31	24	77.4
Sheep	31	28	90.3
Cattle	19	14	73.7
Pig	21	11	52.4
Rabbit	33	30	90.9
Total	135	107	79.3

[a]Isolates from different animal sources were grouped according to the number that were resistant to more than two antimicrobial agents

Table 4: Antimicrobial resistance patterns in *S. aureus* isolated from different animal sources

Resistant pattern	Goat (%) n = 31	Sheep (%) n = 31	Cattle (%) n = 19	Pig (%) n = 21	Rabbit (%) n = 33	Total 135
Amx	2(20)	2(16.7)	1(12.5)	0	3(15)	
Cot	1(10)	1(8.3)	2(25)	0	4(20)	
Amc, Cot	2(20)	1(8.3)	1(12.5)	0	2(10)	
Amc, Amx	1(10)	2(16.7)	2(25)	3(27.2)	1(5)	
Amx Gen Cot	0	1(8.3)	1(12.5)	1(9.1)	0	
Amc, Cot Chl	1(10)	2(16.7)	0	4(36.4)	3(15)	
Amc Ery Chl Cot	1(10)	0	0	0	315	
Amp Amx Chl Cot	1(10)	1(8.3)	0	1(9.1)	1(5)	
Amp Tet Amc Rfp	0	1(8.3)	0	0	1(5)	
Amx, Amc Efo Chl Cot	1(10)	1(8.3)	1(12.5)	2(18.2)	2(10)	
Total strains/samples group	10	12	8	11	20	61

Results of this study demonstrated a high incidence of *S. aureus* strains in the farm animals investigated. These results are similar to those of Oyekunle and Adetosoye (1998) who isolated these organisms from among N'Dama cattle. Thus clinically healthy animals can carry potentially pathogenic staphylococci in their ears and nostrils.

In the present study, *S. aureus* strains was isolated from all the animals examined. However there was higher incidence in the free-range animals such as cattle and sheep than from goats and rabbit, which are somehow confined in the farmhouses. The prevalence of *S. aureus* in the samples is consistent with their ubiquity in nature, occurring as commensals on the body of animals and man (Synder and Poland, 1991; Devrises *et al*., 1985). Animal and inanimate environment have been suspected as sources for some resistant clinical isolates (Bates *et al*., 1994; Holmerg *et al*., 1984).

Antimicrobial susceptibility of *S. aureus* strains (n = 135) isolated from different animals sources was determined. A great proportion of susceptible strains were found, 94.8% showing susceptibility to at least one antimicrobial drug. The highest levels of susceptibility were found for rifampicin, 100% each of sheep, cattle and pigs. High susceptibility was also found to streptomycin (80.7%). High levels of resistance were found for cotrimoxazole (62.25) and amoxycillin (71.9%)(Table 2).

A total of 80.7% of *S. aureus* strains were multiresistant (resistance to two or more antimicrobial agents) (Table 3). Among these, 90.9% of strains isolated from rabbits were fully susceptible or showed resistance to one antimicrobial agent. Lower resistance was observed among the strains isolated from pigs, 52.4% showing resistance to two or more antimicrobial agents (Table 3).

Ten resistance patterns were found (Table 4). The predominant pattern was resistance to ampiclox and. cotrimoxazole. The patterns varied from 9 in rabbit to 4 in pigs. From the above data, it can generally be concluded that *S. aureus* isolates are relatively resistant to some commonly used antibiotics. This is mainly because of the extensive application of antibiotics in farm animals either as therapeutic agents to combat bacterial infections or in subtherapeutic doses in feeds.

The present results of resistance to penicillins, ampiclox and ampicillin for the isolates were as high as that found by Shakibaie *et al.* (1999) in Iran for clinical isolates of *S. aureus*. These results are expected, since penicillins is broadly used in veterinary medicine. The main determinants perhaps, appear to be the prevalence of resistant genes and the extent of antibiotic use.This indicates that the resistance pattern may have originated from one source (organisms) and spread to other animals through close contact.

In the present study the resistance to tetracycline was low (40%) although it is one of the most used antibiotics therapeutically for production animals. Similarly 42.2% of the isolates were susceptible to chloramphenicol. It has been shown that the strains of *S. aureus* isolated in the present study are capable of colonizing and infecting humans. The occurrence of multi-resistant *S. aureus* in farm animals constitutes a reservoir of antibiotic resistant genes, which may spread to other pathogenic species. These strains might have received the genes from other species living in the surrounding environment through conjugational genetic transfer (Forbes and Schaberg, 1983).

There is therefore a need to find strategies to minimize the risk of spreading antimicrobial resistance between animal and human populations. There is considerable evidence that the use of antimicrobials for growth promotion, prophylaxis and treatment of food animals increases the prevalence of resistance in human pathogens (Singer *et al*., 2003). Therefore the reduction of antimicrobial use in agriculture may be important to minimize this problem. This does not obviate the primary means of preventing the transmission of antimicrobial resistance through the food chain, which is through proper food handling and food preparation practices.

The choice of appropriate antimicrobial agents for suspected *S. aureus* infections in animal populations must now take into account the emergence of antimicrobial resistant strains. Health care providers in animal husbandry should be aware that several available antimicrobial agents should be effective in treating these infections. This finding provides a rational basis for the targeted use of efficacious antimicrobial agents to combat a spectrum of emerging and often antibiotic resistant bacterial pathogens that can present significant healh risks in the human environment.

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