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Anti-Microbial Susceptibility Profile of *Staphylococcus aureus* Isolates from Ear and Nostrils of Farm Animals at Michael Okpara University of Agriculture, Umudike Abia-State

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Abstract: A total of 180 ear and nasal swabs were collected from clinically healthy goats, sheep, cattle, pigs and rabbits at the University Farm and were examined for *staph. aureus* using mannitol salt agar. The antibiotic susceptibility profiles of the Isolates were determined using ciprofloxacin, penicillin, cloxacillin, ampicillin, chloramphenicol, Lincomycin, Septrin, tetracycline, gentamycin, rifampicin and erythromycin. Rifampicin 94.3% and streptomycin 80.7% were the most sensitive of all the fifteen antibiotics used. Amoxycilin 72.9% and septrin 62.2% were the most resistance drugs. Out of the 135 Isolates, 107 79.3% showed multiple resistance ranging from two to thirteen drugs. The increasing antibiotic resistance of *Staph. aureus* isolates of livestocks in the University Farm should be a serious cause for concern to clinicians to devise ways of reducing the prevalence of this bacterium in farm animals. This will inadvertently prevent epidermic of life threatening infection among the animals in the Farm.

Key words: Anti-microbial susceptibility, *Staphylococcus aureus*, farm animal

INTRODUCTION

Antibiotics are used to control bacterial infections of Farm Animals. *Staphylococcus aureus* in general are sensitive to many antibiotics but strains from different patients and carriers differ in the pattern and degree of the sensitivity to different drugs and many strains are now resistant to some of these antibiotics[1].

As is apparent, the use or misuse of antimicrobial agents to control animal infection in dairy farms has resulted in development of resistance among micro-organisms. Similarly, multiple use of antibiotics in agriculture as prophylactic agents and as growth promoters has led to the emergence of resistance bacteria in the environment. Moreover, there remain the possibility that resistance may be transmitted from antibiotic resistance bacteria to the susceptible ones[2]. In line with the above, *Staph. aureus* is one of the bacteria that has remain resistance to antibiotics.

Since many bacterial isolates show resistance to antmicrobial agents, Isolation of an infectious agents from animal disease is often not sufficient for determining proper treatment[3]. Also since susceptibility patterns of bacteria are constantly changing, it is essential for microbiologist to know the antibiogram of selected bacterial isolate before the initiation of treatment by a veterinarian[4,5].

However, not much work have been done on the antibiotic resistance patterns of *Staph. aureus* isolates from livestock at Michael Okpara University of Agriculture, Umudike. Such data will be necessary in order to develop rational strategies for therapy of *Staph. aureus* infections. Hence the present study was undertaken to determine antibiotic resistance and sensitivity patterns and provide information on the best choice of antibiotics to be used for empirical treatment of *Staph. aureus* infections in farm animals.

MATERIALS AND MATHODS

Collection of samples: Samples were collected from ears and nostrils of some farm animals using sterile swab sticks (Evepon Industries Limited, Anambra State, Nigeria) at Michael Okpara University of Agriculture, Umudike Farm. The swab samples were inoculated and strealed on Mannitol Salt Agar (DIFCO) containing 7.5% of sodium chloride for isolation of discrete colonies. The plates were incubated aerobically for 24 h at 37°C.

Preparation of antibiotic discs: The antibiotics used in this study were fifteen which include Ciprofloxacin (CFN); cloxacillin (Clox), Chloramphenicol (CHL),Gentamycin (GN); Erythromycin (ERY), Penicillin (PN), Streptomycin

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Table 1: Comparative antibiotic susceptibility patterns of 135 staphylococcus aureus isolates of ear and nostrils from animal sp. in Umudike

	Number sensitive (%)						
Antimicrobial agent	Goat n = 31	Sheep n = 31	Cattle n = 19	Pig n = 21	Rabbit n = 33	All sources % n = 135	No (%) resistant isolates
Ciprofloxacin	31 (100)	26 (83.9)	14 (73.7)	19(90.5)	17 (51.5)	107(79.3)	28(20.7)
Penicillin	19(61.3)	21(67.7)	19(100.0)	19(90.5)	26(78.8)	104(77.0)	31(23.0)
Cloxacillin	31(100)	26(83.9)	17(89.5)	21(100)	19(57.6)	95(70.4)	40(29.6)
Tetracycline	21(67.7)	14(45.2)	10(52.6)	19(90.5)	17(51.5)	81(60)	54(40)
Gentamycin	26 (83.9)	21(67.77	19(100)	19(90.5)	12.36.4)	97(71.9)	38(28.1)
Ampicillin	12(38.7)	17(54.8)	14(73.7)	19(90.5)	12(36.4)	74(54.8)	61(45.2)
Streptomycin	24(77.4)	26(83.9)	19(100.0)	19(19.5)	21(63.6)	109(80.7)	26(19.3)
Amoxycillin	7(22.6)	7(22.6)	7(36.8)	12(57.1)	5(15.2)	38(28.1)	97(71.9)
Ampiclox	19(61.3)	19(61.3)	12(63.2)	12(57.1)	14(42.4)	76(56.3)	59(43.7)
Rifampicin	29(93.5)	31(100.0)	19(100.0)	21(100.0)	28(84.8)	128(94.8)	7.5.2)
Peflacine	29(93.5)	24(77.4)	19(100.0)	16(76.2)	19(78.8)	107(79.3)	28(20.7)
Erythromycin	17(54.8)	26(83.9)	12(63.2)	19(90.5)	14(42.4)	88(65.2)	47(34.8)
Chloramphenicol	12(38.7)	12(38.7)	12(63.2)	14(66.7)	7(21.2)	57(42.2)	78(57.8)
Septrin	14(45.2)	14(45.2)	5(26.3)	16(76.2)	2(6.1)	51(37.8)	84(62.2)
Lincomycin	26(83.9)	26(83.9)	14(73.7)	19(90.5)	14(42.4)	99(73.3)	36(26.7)
% mean no of antibiotic active against strains	68.2±23.8	68.7±20.9	74.4±23.2	83.8±13.5	47.3±22.4		

Table 2: Percentage of isolates that are resistance to 2 or more antibiotics

		Multiple resistance		
	Category	No. of isolates	Number resistant to (>2) Antibiotics	(%)
1	Goat	31	24	77.4
2	Sheep	31	28	90.3
3	Cattle	19	14	73.7
4	Pig	21	11	52.4
5	Rabbit	33	30	90.9
	Total	135	107	79.3%

($p<0.05$)

(STR); Tetracycline (TET); Peflacin (PEF), Lincomycin (LN). Septrin (SXT), Ampiclox (APX). Rifampicin (RIF); Amoxycillin (AXN and Ampicillin (AMP).

Disc measuring 6mm in diameter was used. The discs were labeled with a dark pencil according to the code of the antibiotic. Intended to be impregnated. The discs were sterilized by autoclaving in different bottles at 15 lbs pressure for 15 minutes. The dilution of antibiotics were done to get the required concentration and it was incorporated into the discs being punched. The antibiotic discs were left to dry for 24 h in the incubator and then pack in sterile injection bottles and were stored in the refrigerator at 4°C. All the materials used in the procedure were sterile.

Antibiotic sensitivity test: The disc diffusion method of Bauer *et al.*,[6] was adopted using Nutrient Agar (0xoid) and single-disc, containing CFN (10 μg), STR (30 μg), GEN (10 μg), PN (30 μg), AXN (10 μg), SXT (25 μg), APX (30 μg), ERY (25 Mg), PEF (10 μg), CHL (25 μg), LN (30 Mg), RIF (10 μg), TET (30 μg), AMP (AMP(25 μg) and CLOX (10 μg). A 0.2 Ml Volume of Standardized culture of *Staph. aureus* grown in peptone water (LAB M) at 37°C for 12 h was used to flood the surfaces of a dry sterile Nutrient agar. The plates were rocked such that the standardized culture covered the entire surface. Excess was drained off, plates were allowed to evaporate dry for 15 min and antibiotic disc paper was placed aseptically on the plate using sterile forcep. Each disc was placed far from each other such that their zone of inhibition may not coalesce in order to obtain acceptable Inhibition Zone Diameter (I Z D) measurement. All plates were incubated at 37°C for 18 h after which they were examined for zones of bacterial inhibition by each antibiotics. Inhibition Zone Diameter (I Z D) of each drug that showed activity was measured and recorded.

RESULTS

Table 1 shows the sensitivity patterns of the various bacterial isolates. The percentage susceptibility of the isolates per antibiotics varied from 38 28.1% for Amoxycillin to 128 94.8%. For Rifampicin regardless of the source of isolation. While the mean number (%) of drugs active against strains ranged from (47.3±13.5) against isolates from rabbits to (83.8±13.5) against isolates from pigs Table 1. All the *Staph. aureus* show significant resistance to Amoxycillin 71.9%, septrin 62.2% and chloramphenicol 57.8% but generally sensitive to Rifampicin, cloxacillin, penicillin, Peflacine, Lincomycin, ciprofloxacin, gentamycin, streptomycin etc.

One hundred and seven 79.3% of the 135 coagulase positive tested were resistant to two or more of the 15 antimicrobial agent used Table 2. The Multiple Drug Resistance (MDR) shown by the staphylococcal isolates ranged from 52.4% in pigs to 90.0% in rabbits Table 2. There was no significant difference in MDR. of isolates from the various animal species ($p<0.05$). No isolate was resistant to all 15 anti microbial agent.

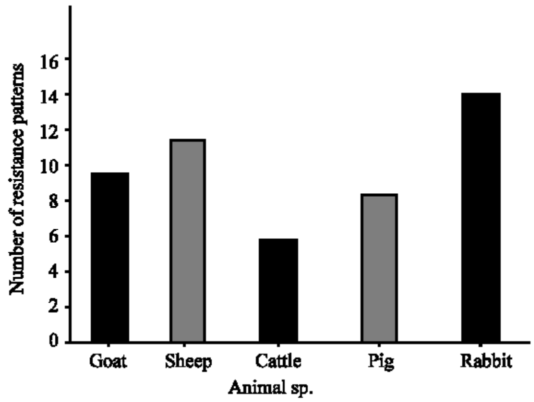

Fig. 1: Number of *Staph. aureus* resistant in different animal pattern

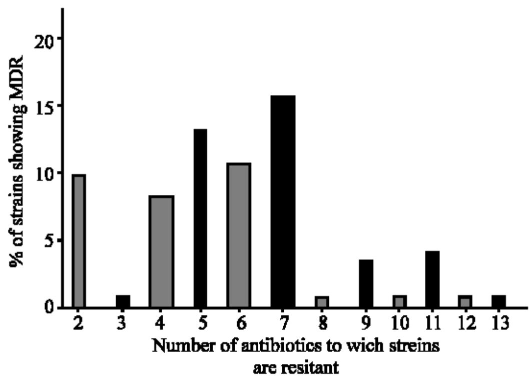

Fig. 2: Percentage of strains with MDR Regardless of the source

A number of anti-microbial resistance patterns were observed. The pattern varied from seven in cattle to fourteen in rabbits Fig. 1. Ampiclox resistance was most common among the various patterns observed.

Figure 2 indicates the multiplicity rate of drug resistance which ranged from 2 drugs by 10.4% strains regardless of the source of isolation. The highest proportion of strains showing multiplicity of MDR was 15.6% to 7 drugs.

DISCUSSION

The 135 isolates of *S. aureus* had 97 71.9% resistance to Amoxycilin. Resistance to penicillin 23.0% and other related antibiotics, cloxacillin 29.6%; ampicilin 45.2% and ampiclox 43.7%, respectively, may have resulted in part due to indiscriminate use of these drugs as the majority of *S. aureus* isolates may be beta-lactamase producers. Alternatively, transmissible resistance *Staph. aureus* may have entered the bacterial population of most of the animals studied. The 23.8% resistance penicillin observed in this study agrees with 25.0% reported by Adekeye[7] on *S. aureus* isolated from human and animals but is in contrast to the 97.8% resistance to penicillin and ampicillin reported by Paul *et al.*, (1982) in a study in Ile-Ife. Resistance was also more frequently amongst other most commonly used antibiotics for example, tetracycline 40.0%, chloramphenicol 57.8%. Septrin 62.2%, erythromycin 34.8% as reported by chah and Nwaeze[8].

The high rate of sensistivity recorded for ciprofloxacin and peflacine, 79.3% each with concentration of 10 μg each as compared to cloxacillin, gentamycin, lincomycin and penicillin with concentration of 10 μg - 30 μg which were equally sensitive may be due to their expensive nature and relative newness in the system compared to others. Rifampicin is not in common use and this may explain why it showed greatest susceptibility with 94.8%, followed by streptomycin 80.7%. The survey showed that none of the Isolates was susceptible to all the antibiotics used. Thus, this account for the multiple resistance to majority of the antibiotics observed. Generally, the antimicrobials to which high rates of resistance were demonstrated like Amoxycilin, septrin and chloramphenicol are the ones that are most commonly requested, inappropriately prescribed and administered in veterinary and human medical practice[8]. Similarly, the low cost and availability of these agents as well as poor compliance with treatment and the use of low quality (sometimes counterfeit drugs[9] and the use of drugs as feed addictives, all contribute to the selective pressure in our environment.

CONCLUSION

In conclusion, this research work has shown that there are rather high prevalence of multi-drug resistant *Staph. aureus* among the farm animals and this should be of concern. More so, the study have provided important information on antimicrobial drugs suitable for use in the empirical treatment of infection caused by *Staph. aureus* in the study area. Finally, containment strategy should be aimed at minimizing any unnecessary, inappropriate or irrational use of anti-microbial drugs. Until these are adopted the prevalence of multiple drug resistance of *Staph. aureus* will be on the increase in the study area.

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