
**ANTIMICROBIAL SUSCEPTIBILITY OF STAPHYLOCOCCI
ISOLATED FROM URETHRA OF MALE FARM ANIMALS REARED
AT THE MICHAEL OKPARA UNIVERSITY OF AGRICULTURE,
UMUDIKE, NIGERIA**

Ndubueze Amaechi, Ikechi G. Erumaka and Magdalene O. Okoronkwo

Department of Veterinary Microbiology, College of Veterinary Medicine, Michael Okpara
University of Agriculture, Umudike. Abia State, Nigeria.

ABSTRACT

This study was undertaken to ascertain the presence of Staphylococcus species in the urethra of bucks, rams and boar and to determine their in vitro susceptibility to antibiotics. We hypothesized that Staphylococcus aureus is a urinary tract pathogen and that colonized urinary tract could be a source of opportunistic staphylococcal infections. Sterile cotton swabs were used to collect samples from the urethra and were inoculated into blood agar and nutrient agar and sub-cultured into Mannitol Salt Agar for the isolation of Staphylococcal species. Identification was based on colonial morphology, Gram staining reaction, haemolysis on blood agar and biochemical tests. The results showed that coagulase-positive Staphylococcus species were the most common group. In the ram, 75% of all the nine isolates were Staphylococcus aureus. Coagulase negative Staphylococcus species were the most common group isolated in bucks (64.7%) and boars (54.6%). Susceptibility studies revealed that all isolates from rams and bucks were highly susceptible to rifampicin (100%), streptomycin (100%), ciprofloxacin (100% in sheep only) and levofloxacin (100%) while isolates from the boar had 90.9% susceptibility to erythromycin. A high resistance to norfloxacin (100%) were recorded in isolates from rams, bucks and boars. This study confirmed the presence of Staphylococcus in the urethra of clinically healthy bucks, rams and boars; with some isolates being resistant to some antibiotics. This study contributes to a better knowledge of the possible role of Staphylococcus species in the urethra of farm animals and their antimicrobial susceptibility, collaborating for a better treatment of infection caused by these bacteria.

Keywords: Antimicrobial susceptibility, *Staphylococcus* isolates, urethra, farm animals, Nigeria.

INTRODUCTION

There are few studies that have been performed to characterize the normal microbial flora of the urethra of adult male farm animals. Many of these studies have relied on urine culture and the most commonly recognized species thought to inhabit the urethra include *Staphylococcus epidermis*, *Corynebacterium* spp., alpha haemolytic *Streptococcus*, *Lactobacilli* *Enterococcus* spp, etc, [1].

Spaine *et al.* [2] stated that coagulase negative *Staphylococcus species*, group viridans alpha haemolytic streptococci, *Corynebacterium species* and *Enterococcus species* were the bacteria more frequently isolated from the external urethral orifice, navicular fossa and penile urethra. The bacteria affect humans, companion and food animals causing both community acquired and nosocomial infections.

The knowledge of the normal bacterial flora of the urethra is of great importance for the proper diagnosis and treatment of pathogens and abnormalities of the lower urinary tract (urethra) in various species. Majority of cases of *S. aureus* bacteriuria are not usually associated with symptoms of urinary tract infection [3] because bacteriuria almost universally occurs concomitantly with long-term urinary catheterization [4]. Consequently, the clinical significance of isolation of *S. aureus* from the urine is undefined in such patients.

Staphylococci are frequently implicated in opportunistic infections [5]. *Staphylococcus aureus* affects humans, companion and food animals causing both community acquired and nosocomial infections. *Staphylococcus aureus* are sensitive to many antibiotics, but strains from different patients and carriers differ in the pattern and degree of sensitivity to different drugs and many strains are now resistant to some of these antibiotics [6]. The emergence of livestock associated methicillin resistant *Staphylococcus aureus* (MRSA) strain has raised the profile of *S. aureus* in pigs; a wide variety of non specific presentations including septicaemia, abscesses and osteomyelitis are associated with the organism [7,8].

Antimicrobial susceptibility tests are used to determine which specific antibiotic a particular bacterium is sensitive to [9] and can guide the physician in drug choice and dosage for difficult-to-treat infections [10]. Since susceptibility patterns of bacteria are constantly changing, it is, thus, essential to determine the antibiogram of bacteria isolates before initiation of antibacterial treatment of bacterial infection [11].

MATERIALS AND METHODS

Study Area

The study was carried out in Umudike, a community in Abia State, Nigeria about 10 kilometres Southeast of Umuahia, the capital of the State. It is home to Michael Okpara University of Agriculture (MOUAA) and National Root Crops Research Institute. The coordinates for Umudike are: 5°28' and 33° north, 7°32' and 56° East/5.47583°, 7.548889°.

Animal and Management System

Animals sampled were in the College of Animal Science and Animal Production Teaching and Research Farm, MOUAA and included bucks, rams and boars. The total number of animal in the farm were goats 120 (28 males, 42 females and 50 kids), sheep 90 (18 males, 30 females and 42 lambs) and pigs 125 (25 males, 45 females and 55 piglets). They were maintained under intensive system of management with food and water provided *ad libitum*.

Sample Collection

Samples were collected from the urethra of the animals. A total of 40 samples were collected made up of 10 boars, 12 rams and 18 bucks. Animals for sampling were selected based on the ones with suspended and pendulous scrotum in a random sampling. Each animal was restrained on lateral recumbence and the prepuce pulled backwards to expose the penile urethra. A sterile swab was used to collect sample from the penile urethra by gently inserting it into the urethra and rotating it a bit to pick the sample. Samples collected were immediately transported in an ice pack to the Department of Veterinary Microbiology Laboratory for bacterial culturing.

Bacterial Isolation

All the media used were prepared in the laboratory according to the manufacturer's direction. Each sample was cultured once. Samples were inoculated on both Blood agar and Nutrient agar and incubated at 37°C aerobically for 24 hours. Growth that occurred after 24 hours of incubation were

Gram stained and examined microscopically. Colonies with morphology consistent with the genus *Staphylococcus* species (ie appearing white, opaque and up to 4 mm in diameter) were sub cultured into Mannitol Salt Agar (MSA), a selective medium for staphylococcal organism containing 7.5% NaCL and incubated for another 24 hours.

Bacterial Identification

Colonies on selective culture media (MSA) were identified based on colonial morphology, Gram staining reaction and haemolysis on blood agar. Biochemical identification included catalase test and coagulase test. They were classified as Gram positive when they retained the blue stain and as Staphylococci when they appeared in clusters and resembled bunches of grapes. *Staphylococcus aureus* are non-motile, catalase positive, coagulase positive and haemolytic on sheep blood agar. Catalase activity was done by collecting discrete colony with a sterile wire loop. This was added unto a slide containing 3% hydrogen peroxide, immediate gas bubbling was observed which indicate positive catalase test. Coagulase test (slide coagulation test) was done by collecting discrete colony which was emulsified on a clean glass slide. A drop of human plasma was added and rocked gently. A positive reaction was indicated by the clumping of the bacteria within 1 – 2 minutes.

Antibiotics Sensitivity Test

Two types of antibiotic disks were used: a foreign single cellular disk (Oxoid) which comes as a single disk and a local disk (Optu disc®) which is usually joined but separated as branched. The foreign disk used comprised of five antibiotics: Penicillin (P), Gentamicin (CN), Nalidixic acid (NA), Streptomycin (S), and Cephalothin (CRO).

The local disk consist of 10 antibiotics namely; Gentamicin(CN), Ciprofloxacin (CPX), Norfloxacin (NB), Amoxil (AML), Streptomycin (S), Rifampicin (RD). Erythromycin (E), Chloramphenicol (CH), Ampiclox (APX) and Levofloxacin (LEV). These discs were procured to be used in testing selected isolates that will show coagulase positive reaction and coagulase negative reaction.

The disk diffusion method of Bauer *et al.* [12] was adopted using Nutrient Agar (Oxoid) and antibiotic disk containing the above mentioned antibiotics.

The test organism was collected from MSA using a sterile wire loop and streaked on the culture plate in a back and forth motion very close together across and down the plate. The plate was rotated about 60° and the streaking action was repeated until the whole surface was streaked. This was done to ensure even distribution of the inoculums that will result in a confluent lawn of growth.

The disks were aseptically placed on the inoculated media and the plates incubated at 37°C for 18 hours after which they were examined for zones of inhibition by each antibiotic. The isolates were categorized according to the standards as being susceptible, intermediate or resistant to the drugs [13]. All the isolates were tested for antibiotic sensitivity using the local disk while 5 isolates already classified as coagulase positive (2 isolates) or coagulase negative (3 isolates) were randomly selected from each animal species and tested for susceptibility using the foreign disk.

RESULTS

Coagulase Reaction

Out of the grand total of 40 samples collected from the farm animals, 38 (95.0%) isolates made up of 17 from bucks, 12 from rams and 9 from boars were obtained that had the typical characteristics of the genus *Staphylococcus* (Table 1). Out of the 38 isolates, 17 (44.8%) were coagulase positive while 28 (52.6%) were coagulase negative. One sample (2.6%) was non specific. Out of the 17 isolates from bucks, 5 (29.4%) were coagulase positive while 11 (64.7%) were coagulase negative. One sample (5.9%) was non specific. In rams, there were 9 (75%) coagulase positive and 3 (25%) coagulase negative *Staphylococcus* isolates. Among the boars, 3 (33.3%) and 6 (66.7%) isolates were coagulase positive *Staphylococcus* and coagulase negative *Staphylococcus* respectively.

Table 1. Coagulase reaction of *Staphylococcus* species isolated from farm animals reared at the Michael Okpara University of Agriculture, Umudike, Nigeria.

Coagulase Reaction	Bucks	Rams	Boars	All animals
Coagulase positive	5 (29.4%)	9 (75%)	3 (33.3%)	17 (44.8%)
Coagulase negative	11 (64.7%)	3 (25%)	6 (66.7%)	20 (52.6%)
Undefined reaction	1 (5.9%)	0	0	1 (2.6%)
Total	17	12	9	38

Antimicrobial Susceptibility

The results of antimicrobial susceptibility test are presented in Table 2. The results from bucks showed that the most effective antimicrobial agents were Gentamicin, Rifampicin, Streptomycin and Levofloxacin to which all isolates were 100% susceptible (Table 2). On the other hand, the isolates were resistant to Ampiclox (11.8%), Amoxil (47.1%), Norfloxacin (100%) and Chloramphenicol (5.8%). Variable susceptibility was seen with the other antimicrobial agents used.

Among the rams, the most effective antibiotics to which all isolates were 100% susceptible included Gentamicin, Rifampicin, Amoxil, Streptomycin, Ciprofloxacin, Erythromycin and Levofloxacin (Table 2). Variable susceptibility was seen with other antimicrobial agents used while varying levels of resistance was observed with Norfloxacin, Ampiclox and Chloramphenicol.

The most effective (100% sensitive) antibiotics in boars were Ampiclox, Rifampicin and Streptomycin while Norfloxacin (100%), Amoxil (81.8%), Chloramphenicol (9.1%) and Ciprofloxacin (9.1%) showed varying levels of resistance.

In general, Rifampicin and Streptomycin were the only antibiotics that were very effective with 100% susceptibility in all species while Norfloxacin is the only antibiotic that showed 100% resistance in all animal species.

The common antibiotics between the local and foreign disks were Streptomycin and Gentamicin to which all isolates were susceptible when tested using either the local or foreign disk. On the foreign disk, coagulase positive isolates were all susceptible to penicillin while all coagulase negative isolates were resistant to penicillin (Table 3). All the isolates were resistant to Nalidixic acid (Table 3).

DISCUSSION

Occurrence of members of the genus *Staphylococcus* in the animals used in this study was an expected finding, since Gram Positive bacteria are common residents of the external genitalia [14,15]. Coagulase-Positive *Staphylococcus* species were the most common group in ram, representing 75% of the isolates (nine strains); all of them classified as *Staphylococcus aureus*. This is in line with a similar report in which pathogenic bacteria were present in association with vaginitis in ewes [16]. Coagulase negative *Staphylococcus* species were also the most common group isolated from the other species (bucks and boars) during this study (64.7% and 54.6% respectively in bucks and boars).

With regard to the antimicrobial susceptibility of the isolates obtained in this study, some unexpected and alarming results were observed. Since only apparently healthy animals with no history of recent antimicrobial treatment were included, it was expected that the members of the normal urethral flora would be highly susceptible to the antibiotics used in the test. Nevertheless, 100% of all the isolates showed resistance to a particular antibiotic (Norfloxacin) while 47.1%, 25%, 9.1% showed resistance to at least one drug in bucks, rams and boars respectively.

Table 2. Antibiotic susceptibility of *Staphylococcus* species isolated from bucks, rams and boars reared at the Michael Okpara University of Agriculture, Umudike, Nigeria.

Drugs	No. (%) susceptibility of bucks			No. (%) susceptibility of rams			No. (%) susceptibility of boars		
	Sensitive	Intermedi- ate	Non- sensitive	Sensitive	Intermedi- ate	Non- sensitive	Sensitive	Intermedi- ate	Non- sensitive
Gentamicin	17 (100%)	-	-	12(100%)	-	-	6(54.6%)	5(45.5%)	-
Ampiclox®	10 (58.8%)	5 (29.4)	2 (11.8%)	11(91.7%)	-	1(8.3%)	11(100%)	-	-
Rifampicin	17 (100%)	-	-	12(100%)	-	-	11(100%)	-	-
Amoxil®	9 (52.9%)	-	8 (47.1%)	12(100%)	-	-	2(18.2%)	-	9(81.8%)
Streptomycin	17 (100%)	-	-	12(100%)	-	-	11(100%)	-	-
Norfloxacin	-	-	17 (100%)	-	-	12(100%)	-	-	11(100%)
Chloramphenicol	16 (94.1%)	-	1 (5.8%)	9(75%)	-	3(25%)	10(90.9%)	-	1(9.1%)
Ciprofloxacin	8 (47.1%)	9 (52.9%)	-	12(100%)	-	-	9(81.8%)	1(9.1%)	1(9.1%)
Erythromycin	9 (52.9%)	8 (47.1%)	-	12(100%)	-	-	10(90.9%)	1(9.1%)	-
Levofloxacin	17 (100%)	-	-	12(100%)	-	-	10(90.9%)	1(9.1%)	-

Table 3. Antibiotic susceptibility of *Staphylococcus* species isolates using the foreign disks.

Drug	Coagulase Sensitive	Positive (n=2) Resistant	Coagulase Sensitive	negative (n=3) Resistant
Penicillin	2 (100%)			3 (100%)
Cephalothin	2 (100%)		3 (100%)	
Streptomycin	2 (100%)		3 (100%)	
Gentamicin	2 (100%)		3 (100%)	
Nalidixic acid		2 (100%)		3 (100%)

The drugs that were effective against all isolates were rifampicin, streptomycin and ciprofloxacin (in rams). This was in contrast to the findings of Astal [17] who observed an increased ciprofloxacin resistance among prevalent urinary tract bacteria isolates. There was also high susceptibility to levofloxacin and erythromycin by isolates from the ram.

Earlier studies in the sheep [16] showed a high susceptibility of these bacteria to Cephalosporin and this agrees with the present findings with *Staphylococci* originating or isolated from rams. The susceptibility of *Staphylococcus* species of bovine origin to antimicrobials was studied by Nunes *et al.* [18], and they reported high resistance to penicillin and low resistance to gentamicin. This was similar to the results of this study where all the isolates from all animal species were also susceptible to gentamicin.

It is important to note that *Staphylococci* isolated from different species and in different countries may present a very similar resistance pattern to antimicrobials, which suggests that this resistance may be inherent to the urethral staphylococcal population of ruminants and porcine. It is necessary that Veterinary practitioners bear in mind the occurrence of these similarities and possible variations when dealing with infectious diseases of the urinary tract, particularly urethritis, in bucks, rams and boars.

CONCLUSION

In conclusion therefore, the findings of this study suggest that *Staphylococcus* isolates were recovered from the urethra of bucks, rams and boars and that resistance to antimicrobials was an infrequent finding on such isolates. The results further suggest that rifampicin and streptomycin may be the most effective drugs against infections due to these isolates.

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