

Isolation and Antibiogram of Aerobic Nasal Bacterial Flora of Apparently Healthy West African Dwarf Goats

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Keywords

West African Dwarf goat – Microbial flora – Antibiotic – Drug resistance – Disease control.

Summary

Goats are important in the livestock economy by their adaptability to adverse environmental conditions as they are good sources of protein and income for the rural poor. Studies conducted on the bacterial flora of the respiratory tract in goats focused on the pneumonic lungs, with fewer studies on the apparently normal nasal passage and antibiogram of isolated organisms. This study was carried out on 60 apparently healthy West African Dwarf goats. The nasal swab from each goat was analyzed using standard methods. The disc diffusion technique was used for the antibiotic sensitivity test. Three hundred and twenty-eight isolates were obtained. The most frequently isolated species was *Streptococcus* spp., while *Escherichia coli* and *Staphylococcus aureus* were the second dominant bacteria. Other species were isolated at relatively lower rates. The isolation of *Mannheimia haemolytica* and *Pasteurella multocida* from the nasal cavity of apparently healthy goats in this study reflects their possible role in most common respiratory diseases encountered in small ruminants. Most of the bacteria were found to be susceptible to streptomycin, quinolones (perfloracin, ciprofloracin and oflaxacin) and gentamicin, while they were resistant to tetracycline, augmentin and erythromycin. This study shows the relationship between misuse or unrestricted use of antibiotics and drug resistance. Therefore, there is a need for practitioners and researchers to be informed of the appropriate antibiotics to be used in respiratory infections and during control programs.

INTRODUCTION

Goats have gained importance in the Nigerian livestock economy as a result of their remarkable adaptability to adverse environmental conditions (19). They are good sources of protein and income for the rural poor, especially women and children (14), and a good source of foreign exchange earnings. Hence, increase in goat production is needed to maintain food security and increase earnings (10).

In spite of the fact that every household keeps goats, their production is not well developed because of factors such as inadequate nutrition, poor management and prevailing diseases. Of all the diseases of goats, those affecting the respiratory tract [*peste des petits*

ruminants (PPR), contagious caprine pleuro-pneumonia (CCPP) and pasteurellosis] cause substantial loss through high morbidity and mortality. Bacterial pneumopathies are commonly attributed to *Mannheimia haemolytica* which causes severe damage to the lung. In addition, bacterial agents such as *Actinomyces pyogenes* also inflict damage on pulmonary tissues in goats (4).

It is increasingly difficult to make an etiological diagnosis in most infectious pneumopathies as a viral agent may be a primary invader, and, when the local resistance of respiratory mucosae is lowered, bacterial agents growing in the nose and throat develop downward producing multiple bacterial infections. Most of the infectious agents that cause respiratory diseases are usually normal inhabitants of the system.

Studies conducted on the bacterial flora of the respiratory tract of domestic animals in various parts of the world mainly focus on the pneumonic lungs of sheep and goats (1, 13) with fewer studies on the microbial flora of the apparently normal nasal passage (17) and

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on the antibiogram of isolated organisms (2, 11). This study aimed at isolating and characterizing bacteria from the nasal passageways of apparently healthy West African Dwarf goats. The results of the antibiotic sensitivity of the isolates are also presented.

■ MATERIALS AND METHODS

Animals

The study was carried out from November 2005 through March 2007 on 60 apparently healthy goats purposely selected for experiments. These animals were bought in a batch of 20 goats from individuals and households in and around Ibadan. They were kept in the small ruminant pens of the Veterinary Pathology Department, in the experimental animal unit of the Faculty of Veterinary Medicine at the University of Ibadan. They were not allowed to graze. They were fed with concentrates and water was provided *ad libitum*. The animals were less than one year old and were apparently healthy at the time of purchase.

Sample collection

The nasal samples were collected a day after arrival by inserting sterile cotton-tipped applicator sticks or swab into the nasal passage after proper cleaning and disinfection of the external nares. Each nasal swab was carefully cut and put into a labeled bottle containing 2 mL brain heart infusion broth. The swabs were transported in a cool box to the laboratory for bacterial culture.

Bacteriological examination

Each nasal swab was removed from the bottle and streaked over the plates containing blood agar base supplemented with 7% sheep blood and McConkey agar. The streaking was further spread with inoculating loop to aid colony isolation. The plates were labeled and incubated aerobically at 37°C for 24-48 h (9). After taking note of cultural growth characteristics, positive cultures were subjected to Gram's staining properties and cellular morphology observed with a

light microscope (x100). Mixed colonies and Gram negative bacteria were subcultured on both blood and McConkey agars and further incubated aerobically for 24 h. Pure culture of single colony type from both blood and McConkey agars were transferred onto nutrient agar slants for a series of biochemical tests including catalase, oxidase and fermentative/oxidative tests for final identification following standard procedures (21). For the antibiotic sensitivity test, the disc diffusion technique was used and inhibition observed as clear zones around the antibiotics. Inhibition zones were measured and measurements greater than 0.5 cm were regarded as susceptible.

Data analysis

Descriptive statistics were used to summarize the data generated by the study. The relative abundance of each species and genus were expressed as a percentage of the total number of isolates.

■ RESULTS

Three hundred and twenty-eight bacterial isolates were obtained. Table I shows the bacteria isolated from the nasal passages of goats and Table II shows the results of the antibiotic sensitivity tests. The most frequently isolated species was *Streptococcus pyogenes*, while *Escherichia coli* and *Staphylococcus aureus* were the second dominant bacteria isolated. *Mannheimia haemolytica* and *Pasteurella multocida* were the common important respiratory pathogens of ruminants obtained from the samples. Other species isolated were at relatively lower rates.

■ DISCUSSION

This study showed that a variety of bacterial flora inhabited and colonized the nasal passageways of apparently healthy West African Dwarf goats. Several authors reported similar bacteria from pneumonic lungs in goats and sheep (1, 13) with fewer reports in apparently healthy goats (17). The isolation of *S. aureus* from

Table I
Nasal bacteria isolates from apparently healthy West African Dwarf goats

Bacterial organism	20 goats	20 goats	20 goats	Total 60 goats	Bacterial organisms/ total isolates (%)
<i>Streptococcus</i> spp.	17	21	12	50	15.2
<i>Escherichia coli</i>	6	21	16	43	13.1
<i>Streptococcus pyogenes</i>	17	21	1	39	11.9
<i>Staphylococcus aureus</i>	15	21	–	36	11.0
<i>Klebsiella</i> spp.	4	12	15	31	9.5
<i>Staphylococcus</i> spp.	4	12	10	26	7.9
<i>Mannheimia haemolytica</i>	5	21	–	26	7.9
<i>Enterococcus</i> spp.	4	5	12	21	6.4
<i>Pasteurella multocida</i>	3	18	–	21	6.4
<i>Pseudomonas</i> spp.	3	1	14	18	5.5
<i>Proteus</i> spp.	2	4	4	10	3.1
<i>Bacillus</i> spp.	–	5	–	5	1.5
<i>Micrococcus</i> spp.	–	–	1	1	0.3
<i>Streptococcus viridians</i> spp.	–	–	1	1	0.3
Total	80	162	86	328	100

Table II
Antibiotic sensitivity of nasal bacteria isolate from apparently healthy West African Dwarf goats

Organism	Antibiotic tested													Total sensitivity
	Amoxicillin (25 µg)	Ofloxacin (5 µg)	Streptomycin (10 µg)	Chloramphenicol (30 µg)	Ceftriaxone (30 µg)	Gentamicin (10 µg)	Cotrimoxazole (25 µg)	Ciprofloxacin (10 µg)	Nitrofurantoin	Tetracycline	Augmentin	Erythromycin (5 µg)	Perifloxacin (5 µg)	
<i>Escherichia coli</i>	+	+	+	+	+	+	+	+	R	R	R	+	+	10
<i>Klebsiella</i> spp.	+	+	+	+	+	+	+	+	R	R	R	+	+	10
<i>Pseudomonas</i> spp.	+	+	+	R	R	+	+	+	+	+	R	+	+	10
<i>Staphylococcus aureus</i>	R	+	+	R	+	+	+	+	+	+	R	+	+	9
<i>Streptococcus</i> spp.	+	+	+	R	R	+	+	+	+	+	R	+	+	9
<i>Streptococcus pyogenes</i>	+	R	+	R	R	+	+	+	+	+	R	+	+	8
<i>Streptococcus viridans</i>	+	+	R	+	+	+	+	+	R	R	R	+	+	9
<i>Mannheimia haemolytica</i>	R	+	+	+	+	+	+	+	R	R	R	+	+	8
<i>Proteus</i> spp.	R	+	+	+	+	+	R	+	R	R	R	+	+	7
<i>Micrococcus</i> spp.	R	+	+	R	+	+	+	+	R	R	R	+	+	7
<i>Pasteurella multocida</i>	R	+	R	R	R	+	R	+	+	+	R	+	+	6
<i>Enterococcus</i> spp.	R	+	R	R	+	+	+	+	R	R	R	+	+	6
<i>Bacillus</i> spp.	R	+	R	+	R	+	+	+	R	+	R	+	+	6

R = resistance; + Susceptible/sensitive

the nasal passage is consistent with other findings obtained from caprine (25) and ovine (7) lungs. Robbins et al. (23) report that *S. aureus* resides in the upper respiratory tract and is involved in disease processes only when stress conditions prevail. The detection of *E. coli* in nasal samples of goats is also consistent with findings by other authors (20). *E. coli*, which is known to be usually harmless in its normal habitat, can cause pulmonary and urogenital tract infection (20). This may also be associated with possible fecal contamination due to the sniffing nature of goats, especially those on heat and during courting before mating. Although *Micrococcus* spp. isolated from this study was considered to be non pathogenic (9), its ubiquity may be primarily due to skin contamination. The isolation of *Bacillus* spp. in an apparently normal animal was at variance with other authors' finding who observed the bacterium in goats' tracheas and lungs in a disease condition (22), whereas it is supposed to be absent in a healthy animal. The constant isolation of *M. haemolytica* from the lungs of various animal species either healthy or having different respiratory syndromes may indicate their possible role in infectious pneumopathies (16). Hence the isolation of *M. haemolytica* and *P. multocida* in higher proportions (7.9% and 6.4%, respectively) from the nasal cavity of apparently healthy West African Dwarf goats in this study reflects their possible role in most common respiratory diseases in small ruminants (8).

M. haemolytica was isolated in the nasopharynx and tonsils of apparently healthy animals, where, interestingly, serotype A2 is most commonly isolated from both sheep and cattle (24). The organism can be isolated from lambs soon after birth (5) and this carriage has been shown by Pass and Thompson (18) to fluctuate over time. The presence of the organism in the nasopharynx of sheep has been shown to coincide with the occurrence of infections. In the nasal passages of calves the bacterial flora has been shown to fluctuate in both species and numbers and, although *M. haemolytica* can dominate the flora, it can also be absent for weeks at a time (15). There has also been failure to culture consistently *M. haemolytica* in swabs taken daily from known colonized animals (18). The mechanisms that *M. haemolytica* possesses to survive in the upper respiratory tract are unknown (24).

Stress factors with or without viral infection have been reported to suppress the mucociliary clearance mechanism which allows the proliferation of bacterial commensals in the respiratory tract (8). They also cause an abrupt shift from commensal to pathogen especially in *M. haemolytica* where serotype 2 shifts to serotype 1, which is known to be pathogenic to animals (12). This shift has made *M. haemolytica* to assume greater prominence in caprine pneumonia (16).

Considering the stress of weather, disease and poor management conditions to which the animals are constantly subjected, the pathogenic role of several bacterial species, and especially *M. haemolytica*, that inhabit the upper respiratory tract of apparently normal West African Dwarf goats could be enormous.

Apart from the possible pathogenic role of the normal nasal bacterial flora, drug resistance of some the pathogenic bacteria has become a rampant, proven, serious problem to both animal and human health care providers. Aghomo and Ojo (2) reported a high level of resistance of *M. haemolytica* to streptomycin while the organism was found to be susceptible to ampicillin, oxytetracycline, and chloramphenicol. In this study, however, *M. haemolytica* was found to be susceptible to streptomycin, quinolones (perifloxacin, ciprofloxacin and ofloxacin) and gentamicin, while it was resistant to tetracycline, augmentin and erythromycin. This represents a shift of resistance from streptomycin to tetracycline. This may be associated with the present lesser usage or misuse of streptomycin for animal diseases unlike tetracycline which is

commonly used for most animal diseases today. This study shows the relationship between misuse or unrestricted use of an antibiotic and drug resistance (11).

The antibiotic sensitivity test on the normal nasal flora of goats also revealed resistance to cheap and easily accessible antibiotics (tetracycline, amoxicillin) which are easily bought off the shelf without appropriate prescription from practicing veterinarians. Apart from ease of accessibility, these drugs have been found to be adulterated and therefore used at a very low dosage. The drugs to which *M. haemolytica* was found to be sensitive are expensive and may not be used frequently. They are also said to be less prone to adulteration.

Since most bacteria complicating PPR in goats are normal commensals of the nasal passages, the results of this study may further explain the relationship between the antibiotics employed and the outcome of treatment management in the reported cases of PPR virus infection (2, 25). In the studies where penicillin, streptomycin and chloramphenicol were used in the treatment of bacterial complications in PPR, less than a 46% recovery rate was recorded (26), while with oxytetracycline and chloramphenicol, a

14% recovery was obtained (6). However, Ajala et al. (3) obtained a 80% recovery rate using Advocin, a newer antibiotic, and a quinolone. Although the survivability of animals affected by the PPR virus depends on the timing of the commencement of treatment, fluid replacement therapy, and curtailing of secondary bacterial complications, the use of quinolones, to which most normal nasal bacteria in this study were susceptible, may be very effective in reducing losses during outbreaks as a result of bacterial complications in PPR.

Therefore, there is a need for practitioners and researchers to be aware of the aerobic nasal bacterial flora of the West African Dwarf goats and of their antibiotic sensitivities so as to be informed of the appropriate antibiotics to be used in the course of respiratory infections and control programs.

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Résumé

Emikpe B.O., Oyero O.G., Akpavie S.O. Isolation et antibiogramme de la flore bactérienne aérobie des cavités nasales de chèvres Naines d'Afrique de l'Ouest apparemment saines

Les chèvres jouent un rôle important dans l'économie de l'élevage parce qu'elles sont une source de protéines et de revenus essentiels pour les pauvres paysans, et parce qu'elles s'adaptent à des conditions environnementales difficiles. Les études sur la flore bactérienne de l'appareil respiratoire des chèvres ont principalement porté sur les lésions pneumotiques ; peu d'études ont été réalisées sur les cavités nasales apparemment normales et peu d'antibiogrammes des organismes isolés ont été effectués. La présente étude a été menée sur 60 chèvres Naines d'Afrique de l'Ouest apparemment saines. Un écouvillon nasal a été effectué sur chaque chèvre et analysé avec les méthodes standard. La méthode de diffusion en gélose a été utilisée pour le test de sensibilité des antibiotiques. Trois cent vingt-huit isolats ont été obtenus. *Streptococcus* spp. a été la bactérie rencontrée le plus fréquemment, suivie d'*Escherichia coli* et de *Staphylococcus aureus*. D'autres espèces ont été isolées à des taux relativement plus faibles. La présence de *Mannheimia haemolytica* et de *Pasteurella multocida* dans les cavités nasales de chèvres apparemment saines indiquerait leur rôle potentiel dans la plupart des pathologies respiratoires courantes des petits ruminants. La majorité des bactéries ont été sensibles à la streptomycine, aux quinolones (péfloxacin, ciprofloxacine et ofloxacine) et à la gentamicine, alors qu'elles ont été résistantes à la tétracycline, à l'augmentin et à l'érythromycine. Cette étude a montré la relation entre le mauvais usage ou l'abus d'antibiotiques, et la résistance aux médicaments. Il est ainsi essentiel d'informer les professionnels de santé et les chercheurs sur les antibiotiques adaptés à utiliser lors d'infections respiratoires et au cours de programmes de lutte.

Mots-clés : Caprin – Chèvre Naine d'Afrique de l'Ouest – Flore microbienne – Antibiotique – Résistance aux médicaments – Contrôle des maladies.

Resumen

Emikpe B.O., Oyero O.G., Akpavie S.O. Aislamiento y antibiograma de la flora bacteriana aeróbica nasal en cabras Enanas Oeste Africanas aparentemente sanas

Las cabras son importantes en la economía pecuaria porque son una buena fuente de proteína y de ingresos para los menesterosos rurales y porque se adaptan bien a las condiciones adversas del medio ambiente. Los estudios conducidos sobre la flora bacteriana del tracto respiratorio de cabras se han enfocado en los pulmones con neumonía con menos estudios en vías nasales aparentemente normales y antibiogramas de organismos aislados. El presente estudio se llevó a cabo en 60 cabras Enanas Oeste Africanas aparentemente sanas. El frotis nasal de cada cabra se analizó usando métodos estándar. La técnica de difusión en disco se empleó para el test de sensibilidad de antibióticos. Se obtuvieron 328 aislamientos. La bacteria más frecuentemente aislada fue *Streptococcus* spp. mientras que *Escherichia coli* y *Staphylococcus aureus* fueron las segundas especies dominantes. Otras especies fueron también aisladas en cantidades relativamente menores. El aislamiento de *Mannheimia haemolytica* y *Pasteurella multocida* en la cavidad nasal de cabras aparentemente sanas en este estudio refleja su posible papel en enfermedades respiratorias comunes encontradas en pequeños ruminantes. La mayoría de las bacterias fueron susceptibles a estreptomycina, quinolonas (péfloxacin, ciprofloxacina y eritromicina) y gentamicina, mientras que fueron resistentes a tetraciclina, augmentin y eritromicina. El presente estudio muestra la relación entre el mal uso o uso no restringido de antibióticos y la resistencia a drogas. En consecuencia, hay una necesidad de informar al personal de salud e investigadores sobre los antibióticos apropiados, a usar durante el curso de infecciones respiratorias y durante programas de control.

Palabras clave: Caprino – Cabra Enana Africa occidental – Flora microbiana – Antibiótico – Resistencia a medicamentos – Control de enfermedad.