Isolation and antibiogram of aerobic nasal bacterial flora of apparently healthy West African dwarf goats and sheep in Abeokuta Area, Ogun State
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Studies conducted on the bacterial flora of the respiratory tract in goats or sheep focused on the clinically ill, with fewer studies on the apparently healthy goats and sheep and the antibiogram of isolated organisms. This study was carried out on apparently healthy 54 goats and 43 sheep. A total of seven different bacterial species were isolated from the upper respiratory tracts of the apparently healthy small ruminants using colonial morphology, gram staining and biochemical characterization. Antibiotic sensitivity of the bacterial isolates was performed against 10 commonly prescribed antimicrobial agents and minimum inhibitory concentration (MIC) of the antibiotics was conducted. The overall occurrence rate of bacteria isolated are Pseudomonas spp (42.0% in caprine and 27.3% in ovine); Bacillus spp (36.9%, caprine; 40%, ovine); Mannheimia spp, (9.2%, caprine; 23.6%, ovine); Escherichia coli (7.6%, caprine; 9.1%, ovine); Staphylococcus spp (2.5%, caprine); Pasteurella spp (0.8%, caprine) and Streptococcus spp (0.8%, caprine). The isolation of Pasteurella multocida and Mannheimia haemolytica from the nasal cavity of apparently healthy goats and sheep in this study reflects their possible role in most common respiratory diseases encountered in these small ruminants. All the 174 (100%) isolates were resistant to Amoxicillin and 161 (92.5%) were resistant to Ceftriaxone. One hundred and sixty-eight (96.6%) isolates were sensitive to Ofloxacin and 140 (80.5%) were sensitive to both Gentamycin and Ciprofloxacin and 135 (77.6%) were sensitive to Perfloxin. Staph. aureus was resistant to all the antibiotics used except Amoxicillin hence only Amoxicillin can be used for its treatment, while most isolates were susceptible to the antimicrobials tested, as demonstrated by higher MIC value. The emergence of antibiotic resistance to these pathogens may increase infectious disease burdens and make therapeutic treatment more expensive.

Keywords; Antibiogram, Nasal bacterial Flora, apparently healthy Goats and Sheep, Minimum Inhibitory Concentration

Introduction
The population of sheep in Nigeria is currently estimated at 33.9 million making up 3.1% of the world's total (FAOSTAT, 2011) and an estimated 72.5 million goats. These small ruminants provide the easiest and most readily accessible source of credit available to meet immediate social and financial obligations mostly for rural women who are usually involved in raising or rearing them around homes. The rural women feed them with kitchen wastes and most times leave them to graze on surrounding herbs and shrubs. Sheep and goats have shorter reproductive cycle and they recover from rapid resumption of breeding following harsh environmental factors and devastating disease infestation. In spite of their relatively high population, sheep and goats production is not well developed because of factors such as inadequate nutrition, poor management and prevailing diseases. Of all the diseases of these small ruminants, those affecting the respiratory tract (peste des petits ruminants (PPR), contagious caprinepleuropneu-
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 (Ikede, 1977). In addition, bacterial agents such as *Actinomyces pyogenes* also inflict damage on pulmonary tissues in goats (Akpavie and Emikpe, 2000). There are different studies conducted on the pathogens of small ruminants with pneumonia in different parts of the country (Ikede, 1977; Adekeye, 1984; Ugochukwu, 1985). Most of the infectious agents that cause respiratory disease are usually common inhabitants of the respiratory system (Emikpe et al., 2009). When the immune resistance of the small ruminants is reduced most commonly due to stress of transportation or poor weather and managerial conditions, the non-pathogenic flora usually becomes pathogenic. The unrestricted use of antibiotics as feed additives among farmers also has adverse effects on the common microflora populations of the nasal passages of these small ruminants. There is a dearth of information available on the population and antibiogram of the nasal microflora of apparently healthy small ruminants in Ogun State. This study is on the isolation, characterization, antibiogram of normal nasal microflora of the West African dwarf goats and sheep in Abeokuta, Ogun State.

**Materials and methods**

**Study areas**

Abeokuta is the largest city and capital of Ogun State in Southwest Nigeria situated at the east bank of Ogun State River, with the coordinate: 7089"N, 3020'54"E and elevation of 66m (217ft). Three Local Government Areas (LGAs) were selected in Abeokuta for this study. The locations in the LGAs include Directorate of University Farms, Federal University of Agriculture ('DUFARMS'), Olugbo, Eweje and Odeda from Odeda LGA; Kemta from Abeokuta South Local Government Area and Elega from Abeokuta North Local Government Area. These locations are between 2.0 and 50.0 kilometers from each other.

**Sampling design**

Cross sectional study design method was employed, ensuring that samples were taken from apparently healthy animals.

**Study animals**

The study animals were 97 (both males and females) comprising 54 goats and 43 sheep. The animals were raised in traditional management practices. Records of the exact age of the animals were not available; however, the small ruminants were clinically healthy upon examination.

**Sample collection**

Nasal swabs were collected in sterile test tubes after cleaning and disinfecting the external part of the nose using 70% alcohol. A total of 97 swab samples from the upper respiratory tract of the nasal passages of apparently healthy goat and sheep were collected as follows: twenty-eight samples were collected from the Directorate of University Farms (Dufarms), 15 from Eweje, 14 from Olugbo, 12 from Odeda. Thirteen (13) were from Elega in Abeokuta North LGA while 15 from Kemta, Abeokuta South LGA (Table 1). The swab samples were collected and transported in Icebox to the Veterinary Microbiology Laboratory of the Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta for analysis.
Table 1: Distribution of animals sampled per locations

| Location | No of Animals Sampled | Sex | Age |
|----------|------------------------|-----|-----|
|          | Caprine | Ovine | Male | Female | Kids | Adults |
| DUFARMS  | 14 | 14 | 12 | 16 | 10 | 18 |
| Eweje    | 11 | 4 | 5 | 10 | 0 | 15 |
| Olugbo   | 1 | 13 | 7 | 7 | 1 | 11 |
| Odeda    | 5 | 7 | 7 | 5 | 1 | 11 |
| Elega    | 13 | 1 | 5 | 9 | 7 | 7 |
| Kemta    | 10 | 4 | 5 | 9 | 2 | 12 |

Isolation of bacteria

In the laboratory, the samples were immediately transferred into buffered peptone water for the growth of the organisms that may be present and incubated at 37°C for 20 hours (Quinn et al., 2002). A loopful from the overnight buffered peptone culture was streaked directly on 5% sheep blood agar and MacConkey agar respectively and incubated under aerobic condition for 24 hrs at 37°C as described by (Quinn et al., 2002).

Identification of isolates

Identification was based on standard protocols (Cater, 1984). After taking note of cultural growth characteristics, positive cultures were subjected to Gram's staining to study staining properties and cellular morphology under 100x objective of light microscope. Mixed colonies and Gram negative bacteria were sub-cultured 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, heamolysis on blood agar and Gram staining technique (Quinn et al., 2002).

Antibiotics sensitivity test

A previously described Kirby-Bauer disc diffusion method (Masud et al., 2012) was used to determine the susceptibility of the bacterial isolates against antibiotic agents. The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2009). Nine antimicrobial discs of antibiotics routinely used on the field, engaged in this antibiotic sensitivity test study include Cotrimoxazole, Gentamycin, Pefloxacin, Chloramphenicol, Streptomycin, Amoxicillin, Ciprofloxacin, Ofloxacin and Erythromycin. Fresh nutrient agar plate was prepared which was then inoculated from the old culture plate after which the paper disc containing specific concentration of antibiotics was placed and incubated at 37°C for 24 hrs. The diameter of inhibition zone surrounding each antibiotic disc was also measured and subsequently matched with the standard inhibition zone diameters of respective antibiotic disc. On the basis of size of inhibition zone of various antibiotics, the isolates were classified as sensitive, intermediately sensitive or resistant (Quinn et al., 2004)

Minimum inhibition concentration

Minimum inhibitory concentration (MIC) testing using micro broth dilution was performed on all the 174 bacteria isolates.

Statistical analysis

Programs Excel version 2003 (Microsoft * Office Excel 2003) was used for management and analysis of data. Descriptive statistics were used to describe the prevalence analyses and antibiogram was presented in percentages.

Results

The distribution of animals sampled per
Aerobic nasal bacterial flora of apparently healthy West African dwarf goats and sheep

location is as shown in Table 1. Seven different bacterial species that were isolated from the upper respiratory tracts of apparently healthy goats and sheep are shown in Table 2 while the biochemical reaction of each bacterial species isolated is shown in Table 3. The Antibiotic susceptibility pattern of the bacteria isolated was studied and the results are illustrated in percentages in Table 4.

Table 2: Overall occurrence rate of bacteria isolated

| Bacteria Isolated | Caprine N (%) | Ovine n(%) |
|-------------------|---------------|-----------|
| Pseudomonas spp   | 50 (42.02)    | 15 (27.3) |
| Bacillus spp      | 44 (36.97)    | 22 (40)   |
| Mannheimiasspp    | 11 (9.24)     | 13 (23.6) |
| Escherichia coli  | 9 (7.56)      | 5 (9.1)   |
| Staphylococcus spp| 3 (2.52)      | 0(0.0)    |
| Pasteurellaspp    | 1 (0.84)      | 0(0.0)    |
| Streptococcus spp | 1 (0.84)      | 0(0.0)    |

Table 3: Characterisation pattern of the biotyped isolated Organism

| Bacteria | Catalas e | Oxidase | Coagulase | TSI Slant/Butt | TSI H2S | TSI Gas Production |
|----------|-----------|---------|-----------|----------------|--------|--------------------|
| Pseudomonas spp | +ve       | +ve     | -ve       | A/K            | -ve    | -ve                |
| Bacillus spp    | +ve       | -ve     | -ve       | A/K            | -ve    | -ve                |
| Staphylococcus spp | +ve | -ve | -ve | A/K | -ve | +ve |
| Mannheimiasspp | +ve       | -ve     | -ve       | A/K            | -ve    | +ve                |
| Escherichia coli | +ve       | -ve     | -ve       | A/A            | -ve    | +ve                |
| Pasteurellaspp  | +ve       | +ve     | -ve       | A/K            | +ve    | -ve                |
| Streptococcus spp | +ve | -ve | -ve | A/A | -ve | +ve |

Key:
A - Acidic       K - Alkaline       TSI - Triple sugar iron agar
+ve - Positive   -ve - Negative

Table 4: Antibiogram of isolated nasal bacteria agents

| Antibiotics | Sensitive | Intermediate | Resistant |
|-------------|-----------|--------------|-----------|
| Amoxicillin | 0(0.0)    | 0(0.0)       | 174 (100%) |
| Ofloxacin   | 168 (96.6%) | 0(0.0)     | 6 (3.5%)  |
| Streptomycin | 40 (22.9%) | 74 (42.5%)  | 60 (34.5%) |
| Chloramphenicol | 88 (50.6%) | 46 (26.4%)  | 40 (22.9%) |
| Ceftriaxone | 0(0.0)    | 13 (7.5%)    | 161 (92.5%) |
| Gentamycin  | 140 (80.5%) | 4 (2.3%)    | 30 (17.2%) |
| Pefloxacin  | 135 (77.6%) | 25 (14.4%)  | 14 (8.1%)  |
| Cotrimoxazole| 84 (48.3%) | 33 (18.9%)  | 57 (32.8%) |
| Ciprofloxacin | 140 (80.5%) | 28 (16.1%)  | 6 (3.5%)   |
| Erythromycin| 8 (4.6%)  | 103 (59.2%)  | 63 (36.2%) |

*:- The interpretation of antibiotic susceptibility was done according to Clinical and Laboratory Standard Institute (CLSI) 2009

The antimicrobial susceptibility of isolates per bacterial species from the nasal samples of the ruminants was studied and results are presented in Table 5 and the Minimum Inhibitory Concentration (MIC) at breakpoint mic≥8 ug/ml is shown in Table 6.
| **Organisms** | AMX | OFX | STR | CHL | CFZ | GN | CPX | ERY | PEF | COT |
|---------------|-----|-----|-----|-----|-----|----|-----|-----|-----|-----|
| Pseudo***(27) | 4   | 3   | 20  | 3   | 3   | 5   | 1   | 6   | 7   | 15  |
| Micro(10)     | 2   | 4   | 4   | 7   | 2   | 1   | 5   | 2   | 3   | 3   |
| Stapha (2)    | 0   | 0   | 2   | 0   | 2   | 0   | 0   | 1   | 1   | 0   |
| Staphpp (2)   | 1   | 0   | 1   | 2   | 0   | 1   | 1   | 0   | 1   | 0   |
| Bac (31)      | 9   | 5   | 17  | 27  | 1   | 3   | 1   | 6   | 1   | 4   |
| Past (2)      | 0   | 1   | 1   | 1   | 0   | 1   | 1   | 0   | 2   | 0   |
| EC (8)        | 1   | 3   | 4   | 4   | 3   | 1   | 2   | 0   | 6   | 2   |
| Strp (1)      | 0   | 0   | 1   | 1   | 0   | 0   | 1   | 0   | 0   | 0   |
| Mahem (8)     | 3   | 1   | 4   | 4   | 1   | 3   | 2   | 2   | 4   | 1   |

**Key:**
- *Pseudo*: Pseudomonas Spp
- *Micro*: Micrococcus Spp
- *Stapha*: Staphylococcus aureus
- *Staphpp*: Staphylococcus pyogenes
- *Bac*: Bacillus Spp
- *Past*: Pasteurella Spp
- *EC*: Escherichia coli
- *Strp*: Streptococcus Spp
- *Mahem*: Mannheimia spp
- **Amx**: Amoxicillin
- **Ofx**: Ofloxacin
- **Cpx**: Ciprofloxacin
- **Cot**: Cotrimoxazole

**Number of bacterial isolates per species in brackets. S = Sensitive. I = Intermediate. R = Resistant**
Aerobic nasal bacterial flora of apparently healthy West African dwarf goats and sheep

Table 6: Minimum inhibitory concentration of isolates from nasal swab sample showing mic8 ug/ml

| Organisms   | AMX | OFX | STR | CHL | CTZ | GN | CPX | ERY | PEF | COT |
|-------------|-----|-----|-----|-----|-----|----|-----|-----|-----|-----|
| Pseudo (27) | 1   | 16  | 7   | 11  | 4   | 8  | 12  | 3   | 2   | 2   |
| Micro (10)  | 1   | 4   | 6   | 3   | 7   | 4  | 2   | 3   | 1   | 1   |
| Staph (2)   | 0   | 0   | 0   | 2   | 0   | 2  | 0   | 2   | 1   | 1   |
| Bac (2)     | 1   | 10  | 16  | 15  | 21  | 10 | 17  | 16  | 8   | 23  |
| Paste (2)   | 0   | 0   | 0   | 0   | 0   | 0  | 0   | 0   | 1   | 12  |
| Ec (8)      | 0   | 8   | 4   | 3   | 5   | 4  | 2   | 6   | 7   | 4   |
| Strp (1)    | 0   | 1   | 1   | 0   | 0   | 0  | 0   | 1   | 0   | 1   |
| Mann (8)    | 3   | 3   | 5   | 5   | 1   | 7  | 4   | 4   | 3   | 5   |

NOTE: MIC <8u g/ml are susceptible strains and >8 ug/ml are resistant strains

Key: Pseudo- Pseudomonas spp; Micro- Micrococcus spp; Stapha- Staphylococcus aureus; Staphpp- Staphylococcus pyogenes; Bac- Bacillus spp; Past- Pasteurella spp; Echo- Escherichia coli; Strp- Streptococcus spp; Mann- Mannheimia spp

Discussion

This present study shows the variety of bacterial flora that colonise the nasal passages of apparently healthy Goats and Sheep. The work of Emikpe et al. (2009) were on nasal bacterial flora of apparently healthy goats in Ibadan (Oyo State of Nigeria), to the best of our knowledge, this is the first study on nasal bacterial flora of apparently healthy Goats and Sheep in Abeokuta Area (Ogun State, Nigeria). This study indicated that several bacterial species inhabit the respiratory passageways of apparently normal goats. Considering the extremes of weather and other poor managerial conditions, which subject the animals to a considerable stress under this environment, the pathogenic role of these apparently commensals and otherwise organisms could be enormous.

The more pathogenic Mannheimia spp was isolated in higher proportion than Pasteurella spp in this study and is consistent with the reports of several authors (Megra et al., 2006; Seker et al., 2009; Emikpe et al., 2009). The isolation of this organism from nasal cavity at a higher rate compared with Pasteurella spp might indicate that the organism lives there as opportunistic pathogens capable of causing infections if they pass through normal defences of the host (Omer et al., 2002). The mechanisms that M. haemolytica possesses to survive in the upper respiratory tract are unknown (Rowe et al., 2001). Pseudomonas species was isolated in higher proportion in this study. This finding is consistent with the work of Ajuwape and Aregbesola (2002) who recorded a high rate of Pseudomonas species from the nasal cavity of normal rabbits. However Yimer and Asseged (2007) reported that P. aeruginosa is more prevalent in the trachea, while Quinn et al. (1994) reported it is more prevalent on the skin, mucous membranes and feaces of Sheep and Goats. Omer et al. (2012) recognized that most of the nasal microflora isolated from apparently healthy animals can act as primary pathogens. In this study, Bacillus species isolated was rather high viz: 36.97% goats and 40% sheep. This is at variance with previous works that isolated Bacillus species only from camel's lung (Shemsidin, 2002) and goat's trachea and lung (Mohammed, 1999) under disease conditions. On the other hand, Ajuwape and Aregbesola (2002) could not isolate the agent from the nasal swab of normal rabbits implying that the organism might preferentially colonize diseased organs. Although E. coli is considered to be transient in the respiratory tract when inhaled with dust particles and do not play a pathogenic role (Collier 1964). Its isolation from clinically healthy goats (7.6%) and Sheep (9.1%) in this study in the absence of clinical
enteritis is noteworthy. Megra et al. (2006) recorded 22% in healthy goats while Ajuwape et al (2002) could not isolate E. coli in rabbits, suggesting that E. coli, which is usually harmless in their normal habitat, could cause pulmonary and urogenital tract infections. Pelczar et al. (1986) associated these systemic infections with possible fecal contamination due to the sniffing nature of these small ruminants especially those on heat and during courting before mating. The overall occurrence rate of Staphylococcus species in this study is low - Goats (2.5%) and Sheep (0%). This result is consistent with the work of Mork et al. (2012). However the nasal carriage of Staphylococcus species in apparently healthy cattle recorded by Rahimiet al. (2015) is higher (5.6%). This variation could partly be due to differences in nasal physiology (Rahimi et al., 2015). Robbins et al. (1981) reported that S. aureus resides in the upper respiratory tract and is involved in disease processes only when stress conditions prevail. In addition, this study attempted to demonstrate the pattern of resistance or susceptibility to commonly used antibiotics in Abeokuta, Ogun State. Overall, the 174 bacterial isolates show 100% resistance to Amoxillin and a very high resistance, 92.5% to Ceftriaxime especially among Pseudomonas, Bacillus, E. coli and Mannheim species. Also, 59.2% and 42.5% of the isolates show intermediate resistance to erythromycin and streptomycin respectively among Pseudomonas, Micrococcus, Staph. pyogens, Mannheim, and Bacillus species. These observations of high rates of bacterial resistance to antimicrobials are supported by the previous studies of Goosens (2000) in Belgium; El-Astal (2004) in Palestine; Okeke et al. (2005) and Okesola and Oni (2009) in Nigeria, among human populations; and Emikpe et al. (2009) and Akpavie et al. (2000) in Nigeria among animal populations. The study is also in line with the result of analysis of antimicrobial drug resistance among human and animal populations in Africa by Kimang’a (2012). The emergence of antimicrobial resistance is primarily due to the excessive and often unnecessary use of antibiotics in humans and animals and this has made infectious disease burden to be very high. Cost constraints to prevent the widespread application of newer, more expensive drugs and the management of all these conditions has been critically compromised by the appearance and rapid spread of resistance (Okeke et al., 2005; Eggleston et al., 2010). In addition, the general availability of these routinely used drugs as an over-the-counter medication (Okesola et al., 2009) has also led to the development of serious problems of resistance to older and less expensive antimicrobial agents like penicillin, ampicillin, tetracyclines and chloramphenicol (Eke and Rotimi 1987). The use of MIC in this study also determines precisely the concentration of the antibiotic required to inhibit growth of each isolate. Even though surveillance of resistance in many developing countries is suboptimal, the general picture is one of accelerating rates of resistance spurred by antimicrobial misuse and shortfalls in infection control. Antimicrobial increased resistance has been reported by several authors (Morrissey et al., 2013; Kimang’a, 2012). Recommendations: It is important to recognize that nasal microbes in apparently healthy small ruminants are potential primary and secondary commensals that can predispose small ruminants to infection. Further work is needed to clarify the role of environmental factors, agent, and host determinants to respiratory diseases. It is also considered necessary to standardize
sampling and laboratory procedures. Considerable economic, management and health burdens could decrease the ruminants' bacterial resistance. Research is needed to accurately quantify the problems that could precipitate infections; propose and evaluate practicable solutions.

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Aerobic nasal bacterial flora of apparently healthy West African dwarf goats and sheep

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