ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATED FROM SMALLHOLDER RED SOKOTO AND WEST AFRICAN DWARF BREEDS OF GOATS FAECES IN NASARAWA STATE, NIGERIA

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ABSTRACT
There is a growing risk of antibiotic resistance (AR) in goats farming in North central Nigeria. A total of 56 fresh goats' faecal samples were aseptically collected, randomly, from two breeds of goats (Red Sokoto and West African Dwarf goats). The goats were raised either using ethnoveterinary medicines (n =28) or antibiotics (n =28). Bacterial isolates were characterized and analyzed using standard protocols, and appropriate statistical tools. The Salmonella spp (n= 33, 91.7 %), Pseudomonas spp. (n= 3, 100 %), and Klebsiella spp. (n= 13, 92.9 %) showed the level of susceptibility. In particular, Pseudomonas spp. and Klebsiella spp had higher percentage susceptibility (100%). The goat's susceptibility varied across all the antibiotics usage. All four species were multi-drug resistant and the isolates revealed inter-species dependence with possibility for inter-species gene transfer. These findings provide a background to investigate the metagenomics of Red Sokoto and WAD goats for antibiotics resistance.
Keywords: Red Sokoto, West African Dwarf goat, *Salmonella spp.*, Antibiotics

INTRODUCTION

Antibiotic resistance in bacterial pathogens isolated from livestock is a growing public health concern, particularly in smallholder farming systems where antimicrobial misuse is prevalent. Antibiotic resistance is a significant public health challenge worldwide, that poses a risk to public health and animal welfare (Manyi-Loh *et al*., 2018; Salam *et al*., 2023). The overuse and misuse of antibiotics in both human and veterinary medicine have led to the emergence and spread of antibiotic-resistant bacteria (Caneschi *et al*., 2023). Bacteria present in livestock excreta, can act as a reservoir and a potential source of transmission for antibiotic-resistant bacteria to humans and the environment. Farm management and usage of antibiotics in livestock are known risk factors associated with the increase in global levels of antibiotic resistance (Herawati *et al*., 2023). Smallholder goat farming is a vital component of agriculture in Nigeria, particularly in Nasarawa State, Nigeria, with a significant goat population, including the Red Sokoto and West African Dwarf breeds (Yakubu and Achapu, 2016). However, there is limited information regarding the prevalence and characterization of antibiotic resistance among bacteria isolated from these goat breeds' excreta. Understanding the antibiotic resistance of bacteria in smallholder goat farming systems is crucial for mitigating the potential risks associated with antibiotic resistance transmission.

The emergence and spread of antibiotic resistance can significantly impact the productivity and profitability of smallholder goat farming systems. Antibiotic resistance levels in goat excreta can help identify potential management strategies to preserve the efficacy of antibiotics while ensuring sustainable farming practices. The broad objective of this study was to assess the antibiotic resistance patterns and prevalence of bacteria isolated from smallholder goats' excreta in Nasarawa State.

MATERIALS AND METHODS

Ethics statement

This study was conducted with strict adherence to the ethical guidelines of the Global code of conduct for research in resource poor settings (TRUST, 2018) following the Convention on Biological Diversity and Declaration of Helsinki. Ethics approval was obtained from the Institutional Review Committee of the CGIAR COVID-19 HubNigeria (ILRI Component). Consent was sought from all the participating farmers.

Sampling location and farmers' selection

Faecal samples were collected from smallholder goat house hold farmers located in Villages within Lafia Local Government Area, Nasarawa State, Nigeria. The villages were selected based on the practice of ethnoveterinary medicine and administration of antibiotic to the herd by the farmers. A total of 56 farmers (14 per village) were randomly selected from a pool of 56 farmers (28 per Village) of Red Sokoto and West African Dwarf.

Collection of samples

Excreta were collected from the goats. A total of fifty-six (56) fresh excreta were aseptically (free of contamination) collected using sterile spatulas from randomly selected apparently healthy goats. The samples, which were placed into sterile universal sampling bottles, were kept in a mobile box containing ice packs and immediately transported to the laboratory for microbiological analyses.

Isolation and identification of bacterial isolates

Bacteriological examinations were carried out in the laboratory using standard procedures for aerobic bacteria. For the detection of *Salmonella* spp a representative portion of the excreta was inoculated into Selenite F Broth to prevent the growth of other bacterial species apart from *Salmonella and Shigella*. Then, a loopful of enriched sample was streaked on *Salmonella-Shigella* (SS) agar and incubated at optimum temperature of 36–37°C for 24 h. The presumptive identification of the particular monotype (*Salmonella* spp.) was subjected to morphological and biochemical characteristics such as shape, size, surface texture, edge, elevation and color, motility, Gram staining, and biochemicals (indole production, urease, oxidase, catalase, lactase, and citrate) (Cheesbrough, 2006; Ochei and Kolhatkar, 2008). In order to identify *E. coli*, *Pseudomonas* spp., and *Klebsiella* spp., the samples were grown on MacConkey agar (for mixed growth) and incubated at 36–37°C for 24 hrs. Then, each single colony was sub-cultured to obtain a pure culture. The plates were then examined for growth. Morphological and biochemical characteristics of the bacteria colony were used for confirmation. Microbial counts of *Salmonella* spp., *E. coli*, *Pseudomonas* spp., and *Klebsiella* spp. were conducted using the pour plate technique. In total, 0.1 mL of serially diluted suspensions (10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8}) was mixed in cooled molten agar medium and poured into a petri dish. The plate was then rotated for proper mixing. It was allowed to set and then incubated at 37°C for 2 days. Colonies that appeared throughout the medium were counted and multiplied by the dilution factor to obtain the number of bacteria in the original suspension as follows (Ochei and Kolhatkar, 2008):

$$Colony\ forming\ units\ per\ ml\ (cfu/ml)\ =\ Colonies\ (average)\ \times \frac{Dilution\ factor}{Volume\ plated}$$

Selenite F Broth and SS agar were produced by TM Media (Titan Biotech. Ltd., Rajasthan, India) while MacConkey agar was produced by HIMEDIA (HiMedia Laboratories Pvt. Ltd., Mumbai, India). The biochemicals were purchased from L:S-BIOTECH (LIFESAVE BIOTECH, San Diego, CA, USA). The agar media and biochemicals were prepared in the laboratory according to the manufacturers' instructions.

Antimicrobial Susceptibility Testing

The antibiotic susceptibility test of bacterial pathogen isolates was determined by the disk diffusion method and interpreted as described by Cheesbrough (2006) and Ochei and Kolhatkar (2008). The plates were incubated aerobically at an optimum temperature of 36–37°C for 24 h. The bacterial species were tested against 10 antibiotics (with their respective concentrations) belonging to six different classes, namely, Quinolones (Ciprofloxacin (10 μg), Ofloxacin (10 μg), Nalidixic acid (30 μg), Perfloxacin (10 μg)), β-lactams (Augmentin: amoxicillin and clavulanic (30 μg)), Aminoglycosides (Gentamicin (10 μg), and Streptomycin (30 μg)), Penicillins (Penicillin (10 μg)), Sulfonamides (Co-trimoxazole: sulfamethoxazole-trimethoprim (10 μg)), and Cephalosporins (Ceporex: Cephalexin (30 μg)) as described by WHO. The antibiotics were manufactured by TM Media (Titan Biotech Ltd., Delhi, India). The multiple antibiotic resistance (MAR) index for each isolate was manually calculated as described by Krumperman (1983).

Statistical analysis

The non-parametric Kruskal–Wallis H-test was used to compare the antibiotic resistance rates of the different bacterial species. Significant differences were declared at $\alpha = 0.05$. Prior to analysis, Levene's test of homogeneity of variance and Shapiro–Wilk test of normality were conducted on the data to validate the parameter assumptions. The descriptive and inferential statistical analyses were conducted using IBM-SPSS (2020).

RESULTS AND DISCUSSION

Table 1 represents the antibiotic susceptibility pattern of bacteria. The *Salmonella* spp. (n= 33, 91.7 %), *Pseudomonas* spp. (n= 3, 100 %), and *Klebsiella* spp**.** (n= 13, 92.9 %) showed the level of susceptibility. In particular, *Pseudomonas* spp. and *Klebsiella* spp**.** had higher percentage susceptibility (100%). The goat's susceptibility varied across all the antibiotics usage as seen in Table 1.

The study found that most *Salmonella* isolates (91.7%) were susceptible to the tested antibiotics, indicating effective control over Salmonella infections in goats, though evolving resistance is a concern. Pseudomonas species showed a 100% susceptibility rate, suggesting that antibiotics are currently effective against these infections in goats, which is promising for treating immune-compromised animals. *Klebsiella* species also

exhibited a high susceptibility rate (92.9%), though some strain variability exists. The varying susceptibility across antibiotics indicates the need for targeted treatments based on susceptibility testing. These findings highlight the importance of continuous monitoring and antibiotic stewardship to prevent resistance development, especially in agricultural settings where misuse or overuse of antibiotics can lead to resistance. The present study is similar to that of Herawati *et al.* (2023) who reported that bacteria from goats demonstrated resistance to critical antibiotics such as penicillin, ampicillin, and ciprofloxacin, with resistance levels ranging from 0.4% to 100% for various species.

Table 1: Antibiotic susceptibility pattern of bacteria

	Bacteria species			
Antibiotics	***Salmonella* spp.**	***Pseudomonas* spp**	***Klebsiella* spp.**	***Escherichia coli***
Ciprofloxacin				
Susceptibility	33 (91.7)	2 (100.0)	4 (100.0)	13 (92.9)
Resistance	3 (8.3)	0 (0.0)	0 (0.0)	1 (7.1)
Ofloxacin				
Susceptibility	34 (94.4)	2 (100.0)	3 (75.0)	12 (85.7)
Resistance	2 (5.6)	0 (0.0)	1 (25.0)	2 (14.3)
Nalidixic acid				
Susceptibility	29 (80.6)	2 (100.0)	4 (100.0)	12 (85.7)
Resistance	7 (19.4)	0 (0.0)	0 (0.0)	2 (14.3)
Perfloxacin				
Susceptibility	32 (88.9)	2 (100.0)	4 (100.0)	9 (64.3)
Resistance	4 (11.1)	0 (0.0)	0 (0.0)	5 (35.7)
Gentamicin				
Susceptibility	32 (88.9)	2 (100.0)	4 (100.0)	14 (100.0)
Resistance	4 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
Augmentin				
Susceptibility	32 (88.9)	2 (100.0)	3 (75.0)	12 (85.7)
Resistance	4 (11.1)	0 (0.0)	1 (25.0)	2 (14.3)
Cotrimoxazole				
Susceptibility	31 (86.1)	1 (50.0)	4 (100.0)	11 (78.6)
Resistance	5 (13.9)	1 (50.0)	0 (0.0)	3 (21.4)
Streptomycin				
Susceptibility	31 (86.1)	2 (100.0)	4 (100.0)	14 (100.0)
Resistance	5 (13.9)	0 (0.0)	0 (0.0)	0 (0.0)
Penicillin				
Susceptibility	29 (80.6)	2 (100.0)	2 (50.0)	14 (100.0)
Resistance	7 (19.4)	0 (0.0)	2 (50.0)	0 (0.0)
Ceporex				
Susceptibility	31 (86.1)	2 (100.0)	3 (75.0)	12 (85.7)
Resistance	5 (13.9)	0 (0.0)	1 (25.0)	2 (14.3)

CONCLUSION

This study provided insights into the possible co-existence of *Salmonella* spp., *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp. within the areas studied which could potentially influence horizontal gene transfer between the species, thereby increasing the threat of antibiotic resistance to animals, humans, and the environment. Reduce the use of antibiotics in goat farming, particularly those that have shown high resistance rates. Implementing a more judicious use of antibiotics can help prevent the development of resistant strains. Explore alternative treatments such as probiotics or herbal remedies that can reduce reliance on antibiotics for managing infections.

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