

Antimicrobial resistance surveillance in smallholder mixed livestock farming systems using a Metagenomic approach

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BACKGROUND

Recently, antimicrobial resistance (AMR) associated infections have been perceived as one of the major causes of mortality in Europe (Aguilar *et al.*, 2023). Livestock production systems are a contributor to the emergence and dissemination of AMR. Livestock-associated AMR pathogens such as *Escherichia coli* and *Staphylococcus aureus* were some of the deadliest pathogens reported by Aguilar *et al.* (2023). Advancements in research have seen taxonomics, metagenomics, and transcriptomics being employed to explore the intestinal flora and monitor AMR trends (Wang *et al.*, 2020). A relationship between the AMR isolates from food animals, humans, and the environment has been reported (de Carvalho *et al.*, 2022; He *et al.*, 2019). A study by Sebastian *et al.* (2021) found a significant difference in AMR *E. coli* isolates from areas near the farms compared to areas away from farms, suggesting that farms are the sources of AMR ecological pollution. Hindrance into mitigating AMR in developing countries is impelled by poor documentation of antimicrobial use and surveillance systems (Kimera *et al.*, 2020). Mixed livestock farming systems are used by numerous smallholder farmers to optimize the production chain. This entails that the livestock consume the same feed and are administered the same drugs all within the same environment. Curated knowledge about the presence and prevalence of AMR in smallholder mixed livestock farming systems is crucial in food production and safety.

OBJECTIVE

Use metagenomic analysis to investigate the prevalence and incidence of AMR in smallholder mixed livestock farming systems.

METHOD

Sample collection and DNA extraction

This study was undertaken in the Gauteng province of South Africa. Smallholder farms with mixed livestock were identified in the City of Johannesburg Metropolitan Municipality, Mogale City, and City of Tshwane Metropolitan Municipality districts. Twenty-one fresh fecal samples from cattle, chickens, sheep, pigs, and goats were collected from the ground into 50 mL sterile collection vials. The samples were kept on ice and transported to the Agricultural Research Council Biotechnology platform laboratory for processing. DNA was extracted from 200 mg of fecal samples using the DNA QIAamp Stool Mini Kit (Qiagen, Hilden, Germany). The DNA was stored at -20 °C until further processing.

Library preparation and sequencing
Illumina HiSeq platform (Illumina, San Diego, USA)

Data analysis (16S rRNA)
DADA2

Data analysis (Shotgun)

Quality control – Trimmomatic

Assembly - SPAdes

AMR prediction – CARD and AMR finder

RESULTS

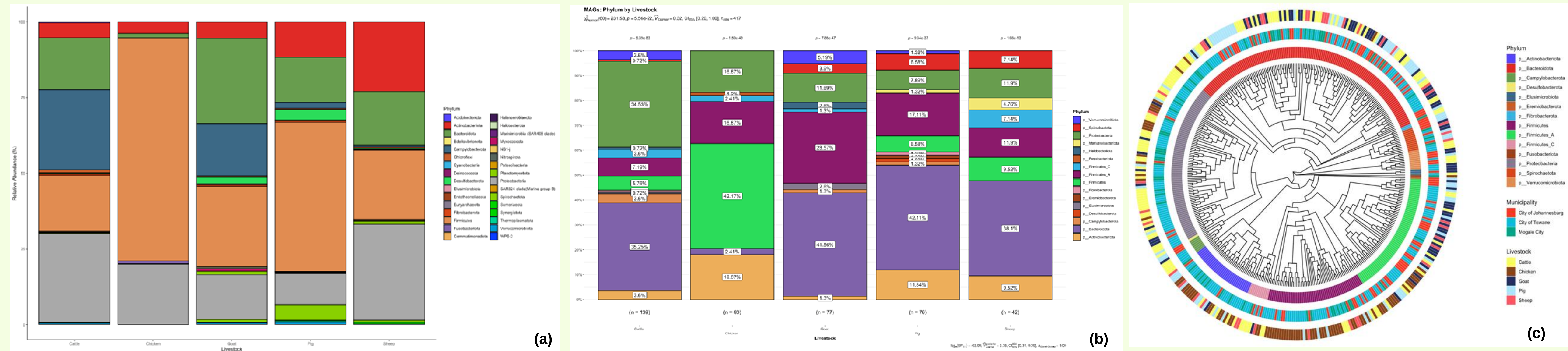


Figure 1: The gut microbiome relative abundances at the phylum level as determined by (a) amplicon sequencing and (b) Metagenome Assembled Genomes after shotgun sequencing. The MAGs also showed a relationship between the phyla identified in each livestock across each location (c). The microbial phyla were distributed in all Municipalities and livestock.

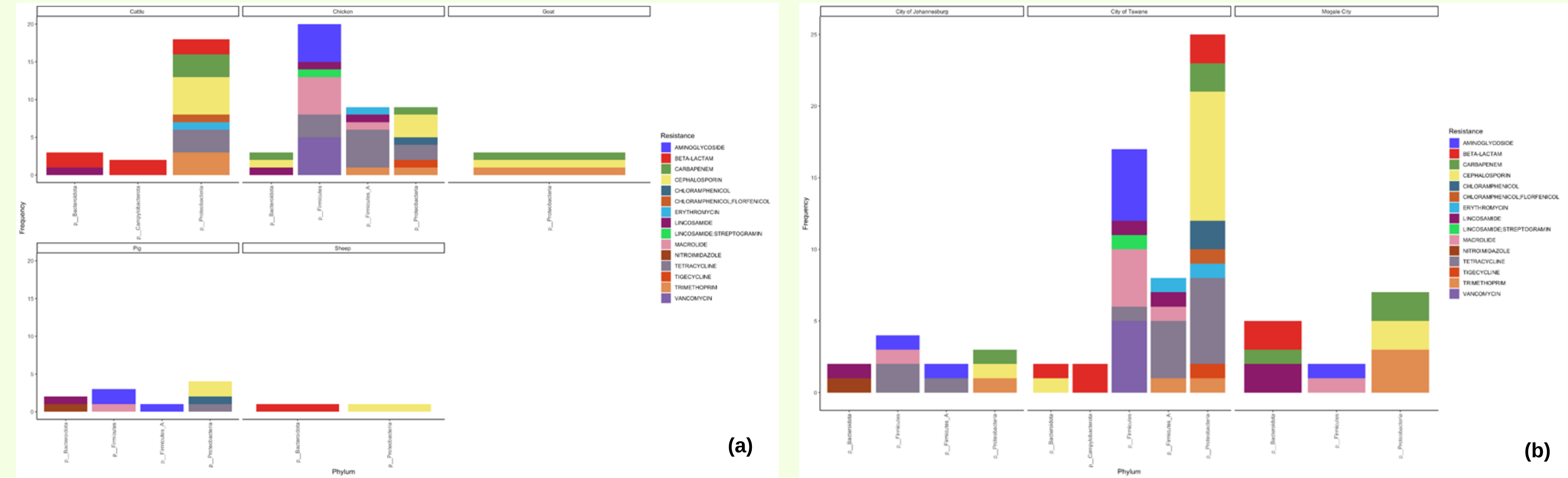


Figure 2: Prevalence of AMR in livestock (a) and different locations (b).

Microbial diversity was the highest in goats and lowest in chickens (fig. 1a) while MAGs showed that the diversity was the lowest in chickens and highest in cattle (fig. 1b). High resistance frequencies were observed in aminoglycoside, tetracycline, macrolide, and vancomycin classes of antibiotics in chicken. AMR was the highest in the City of Tshwane followed by the City of Johannesburg and Mogale City.

DISCUSSION

Analysis of the microbiomes in this study revealed that the gut microbial diversity was the lowest in chickens and highest in cattle and goats (fig. 1). Similar to other previous studies, high abundances were observed in Firmicutes, Proteobacteria, Bacteroides, and Acinetobacteria in this study (de Carvalho *et al.*, 2022; Sabino *et al.*, 2019; Torres Manno *et al.*, 2023). Of concern, is the occurrence of AMR determinants for antibiotics that are of critical and high importance according to the World Health Organization (World Health Organization, 2019). Resistance to vancomycin, macrolide, tigecycline, carbapenem, and beta-lactam by the livestock-associated microbial families in this study calls for urgent attention regarding raising awareness on antimicrobial use in livestock production. Kimera *et al.* (2020) reported high percentages of farms that use antibiotics in African countries, poor antimicrobial use documentation, and AMR surveillance systems, including South Africa.

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