ANALYSIS OF THE GUT MICROBIOME SHEDS INSIGHTS INTO BREED RESILIENCE TO CHALLENGES OF ANTIMICROBIAL RESISTANCE IN DOHNE MERINO SHEEP

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Introduction

Dohne Merino is one of South Africa's leading sheep breeds which is also reared in Australia, New Zealand, and other European countries. In South Africa, sheep and other livestock populations are exposed to multiple diseases and parasites. The efforts to manage diseases and infections while maintaining high productivity has led to a high usage of antimicrobials in sheep production. This has resulted in a high prevalence of antimicrobial resistance (AMR), which is a major global concern that demands surveillance and action. The gut microbiome is of importance to the well-being of ruminant livestock by contributing to nutrition and health of the animals.

Objective

The goal of this study was to investigate the gut microbial environment of South African Dohne Merino sheep by metagenomic sequencing of the rumen, reticulum, omasum, and abomasum of ewes (n=6)

Methods

Animals and sampling: Samples collected from the Wauldby farm in the Eastern Cape province. 6 ewes were selected and slaughtered, and the gastrointestinal tract was extracted. The four stomach compartments of interest were identified, and contents collected from each.

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DNA extraction and Sequencing: DNA was extracted from the samples (200mg each). DNA was amplified and uniquely indexed utilising Illumina® TruSeq® Nano DNA Library Prep kit. Next Generation(NGS) was performed using the HiSeq sequencer.

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Bioinformatics and Visualisation:

A) Trimmomatic : Quality control of the raw sequenced data, by removing host and poor- quality DNA.

B)Spades : Assembly of DNA to contigs (which are longer DNA strands and are better to work with).

C) KRAKEN2 AND GTDB-Tk : Taxonomic classification of the contigs.

D) Finally, the assembled DNA contigs were then ran through ABRicate for AMR screening using NCBI and CARD for references. Data was visualised with R-studio and Excel

Results

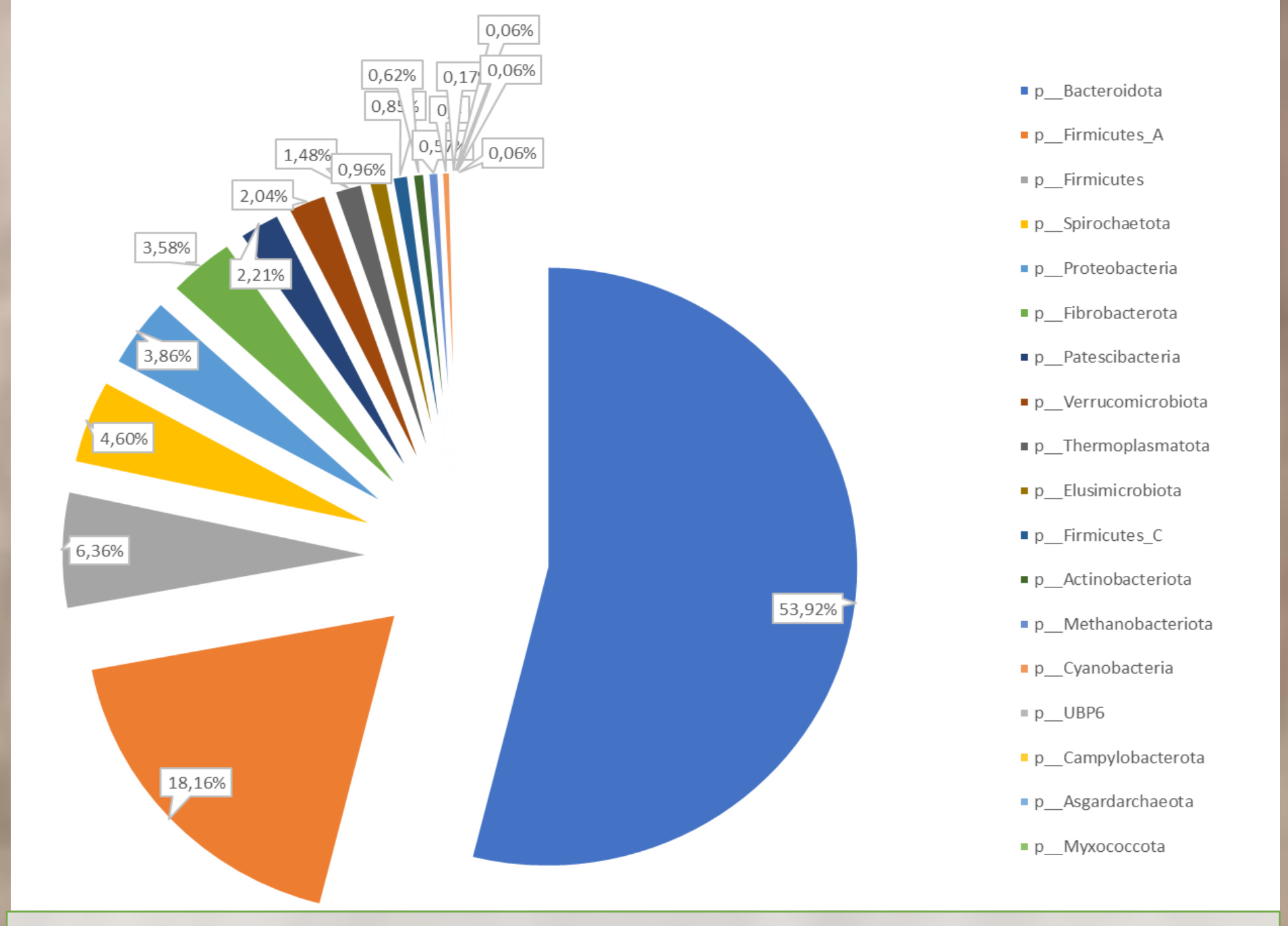

Figure 1: Overall composition of the gut microbial population. A total of 18 phyla were identified . The population is composed of mainly members form the bacterial domain with Bacteroidota and Firmicutes. Contained a small percentage of the archaea.

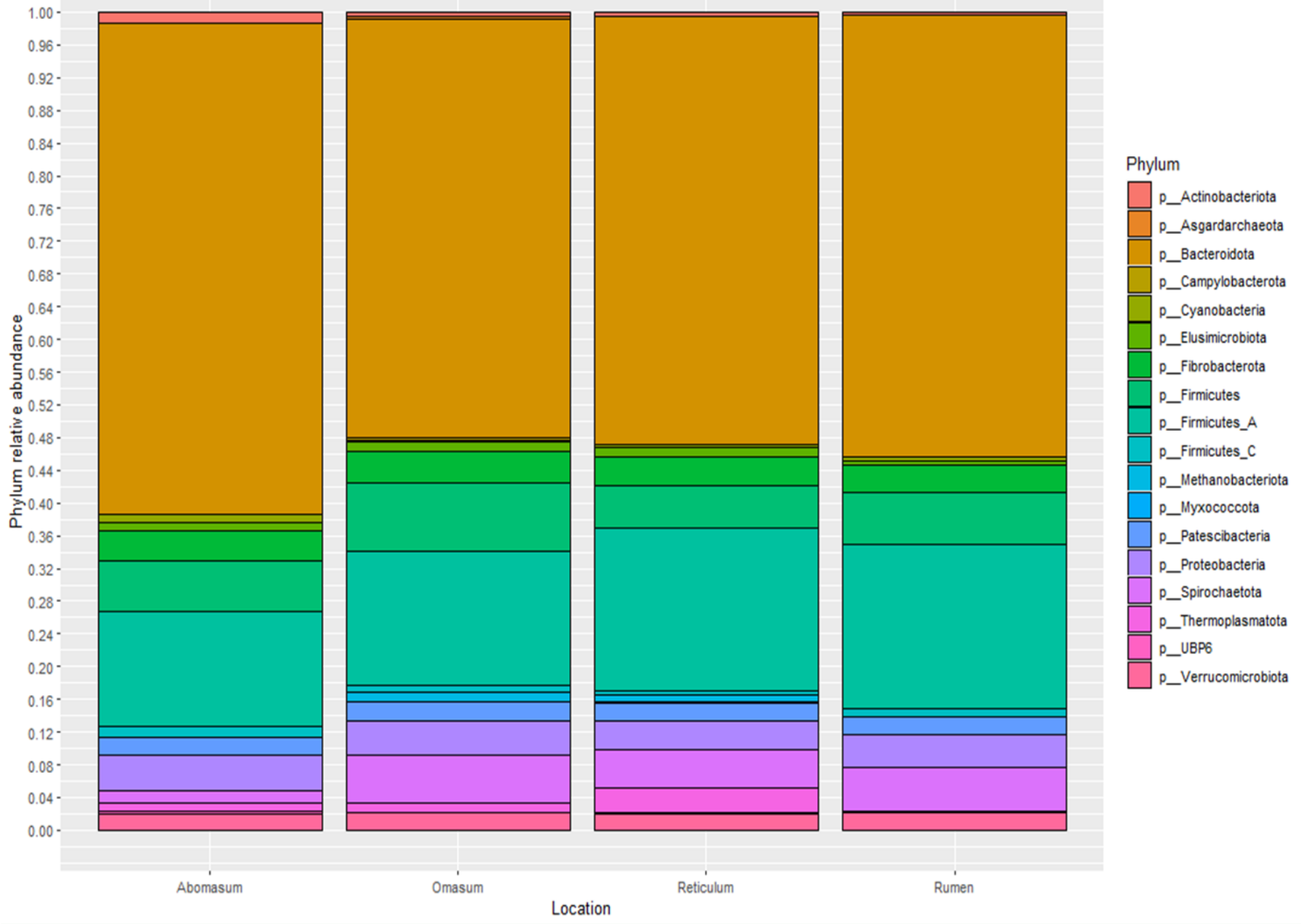

Figure 2: Relative abundance of bacteria and archaea in the four gut compartments at a Phylum level. The composition of the omasum, reticulum and rumen were very similar. Bacteroidota and Firmicutes were found in high abundance across all four chambers.

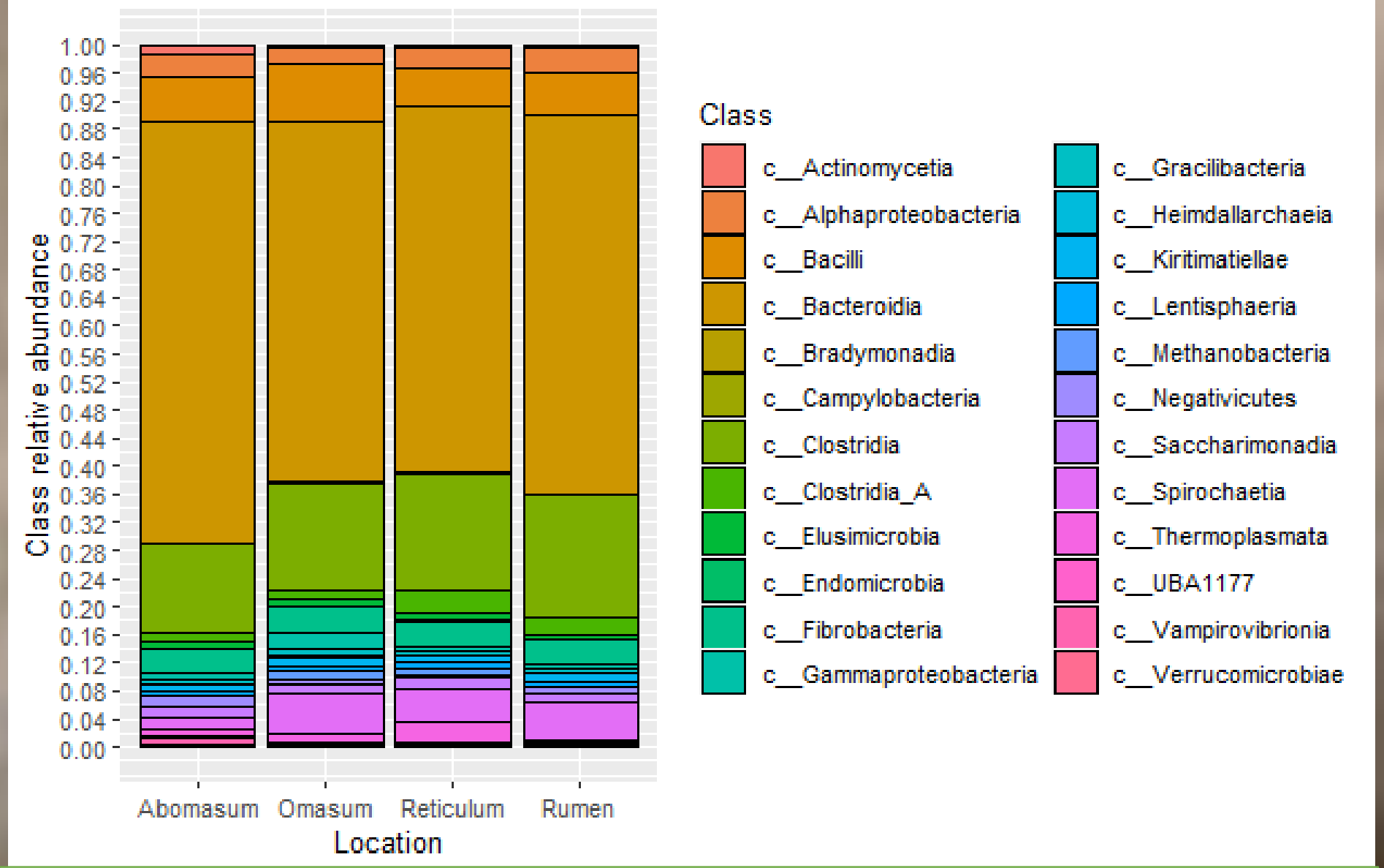

Figure 3: Relative abundance of microbial classes in gut sheep at a class level of classification. The total number of classes was 24 across the entire gut. The classes with the highest abundances included Bacteroida,Clostridia and Bacilli. Which all function to help host digest complex material

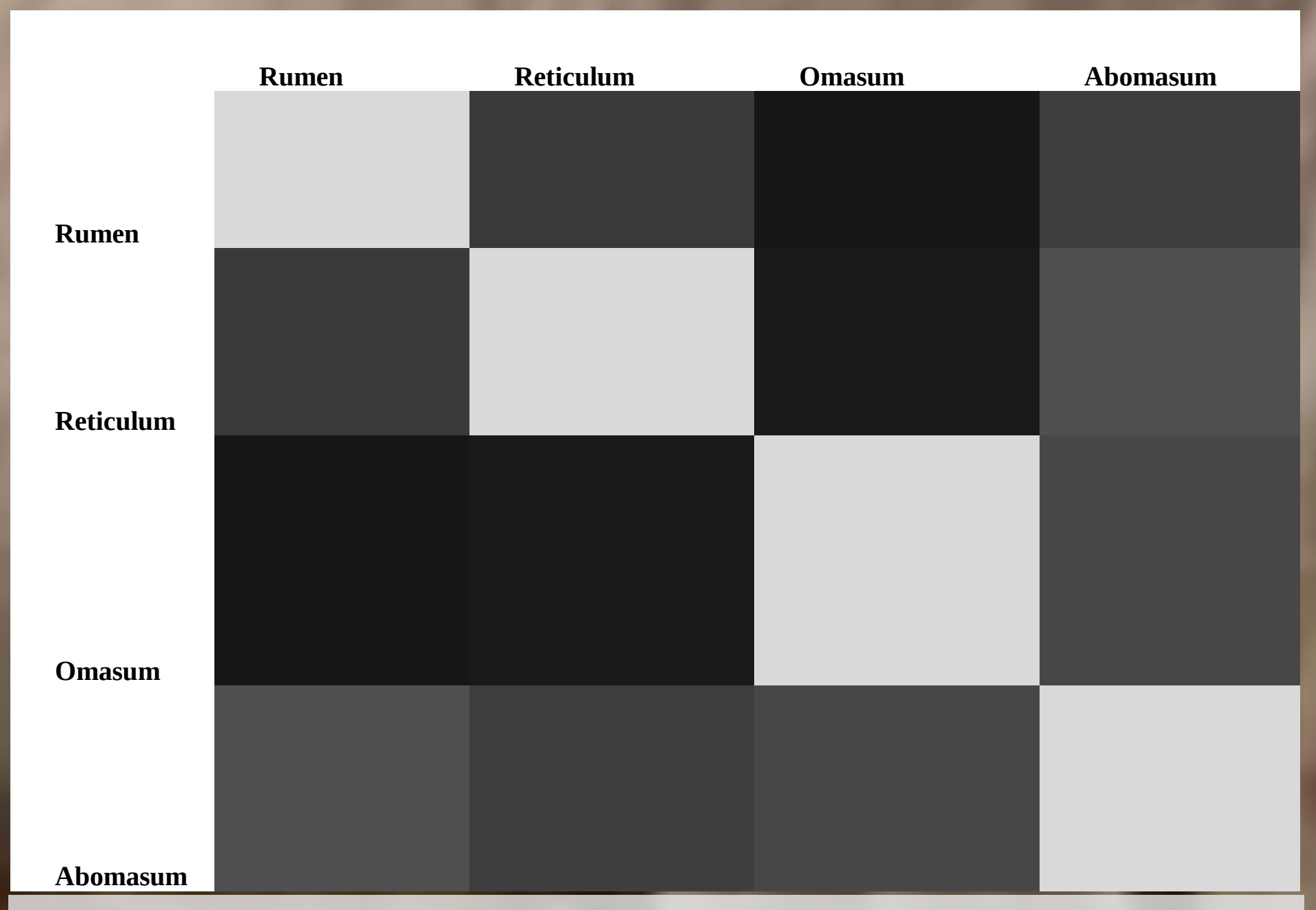

Figure 4: Measure of Beta(β) diversity using the Jaccard distance method. The pair with the highest diversity is the reticulum and omasum and the pair with the lowest measure of diversity is the reticulum and abomasum.

Table 1 Archaeal species

Species	Percentage
Candidatus methanomethylophilus alvus	0,4%
Thermoplasmatales archaeon BRNA1	0,9%
Methanobrevibacter millerae	1,5%
Methanobrevibacter ruminantium	1,3%
Methanobrevibacter sp. YE315	0,1%
Methanobrevibacter alleyae	0,04%
Methanosphaere sp. BMS	0,5%
Methanogenic archaeon ISO4-H5	96,5%

Table 2 Bacterial Genus and Species

Genus	Species
Prevotella	Prevotella ruminicola Prevotella intermedia Prevotella melaninogenica Prevotella dentalis Prevotella fusca Prevotella sp. oral taxon 299 Alloprevotella sp. E39
Bacteroides	Bacteroides coprosuis Bacteroides caecimuris Bacteroides uniformis Bacteroides fragilis Bacteroides heparinolyticus Bacteroides thetaiotaomicron
Fibrobacter	Fibrobacter succinogenes
Ruminococcus	Ruminococcus sp. JE7A12 Ruminococcus albus Ruminococcus bicirculans Ruminococcus champanellensis
Butyrivibrio	Butyrivibrio fibrisolvens Butyrivibrio hungatei Butyrivibrio proteoclasticus

Only 7 known archeal species were identified, most(96,5%) archaea were unknown and classified simply as methanogenic archaea. A total of 99 known bacterial species were found, table 2 only lists those that are known to belong to biologically important genera. With the most abundant genus being Prevotella, followed by Bacteroides, Ruminococcus, Butyrivibrio and lasty Fibrobacter with only one species.

Results continued

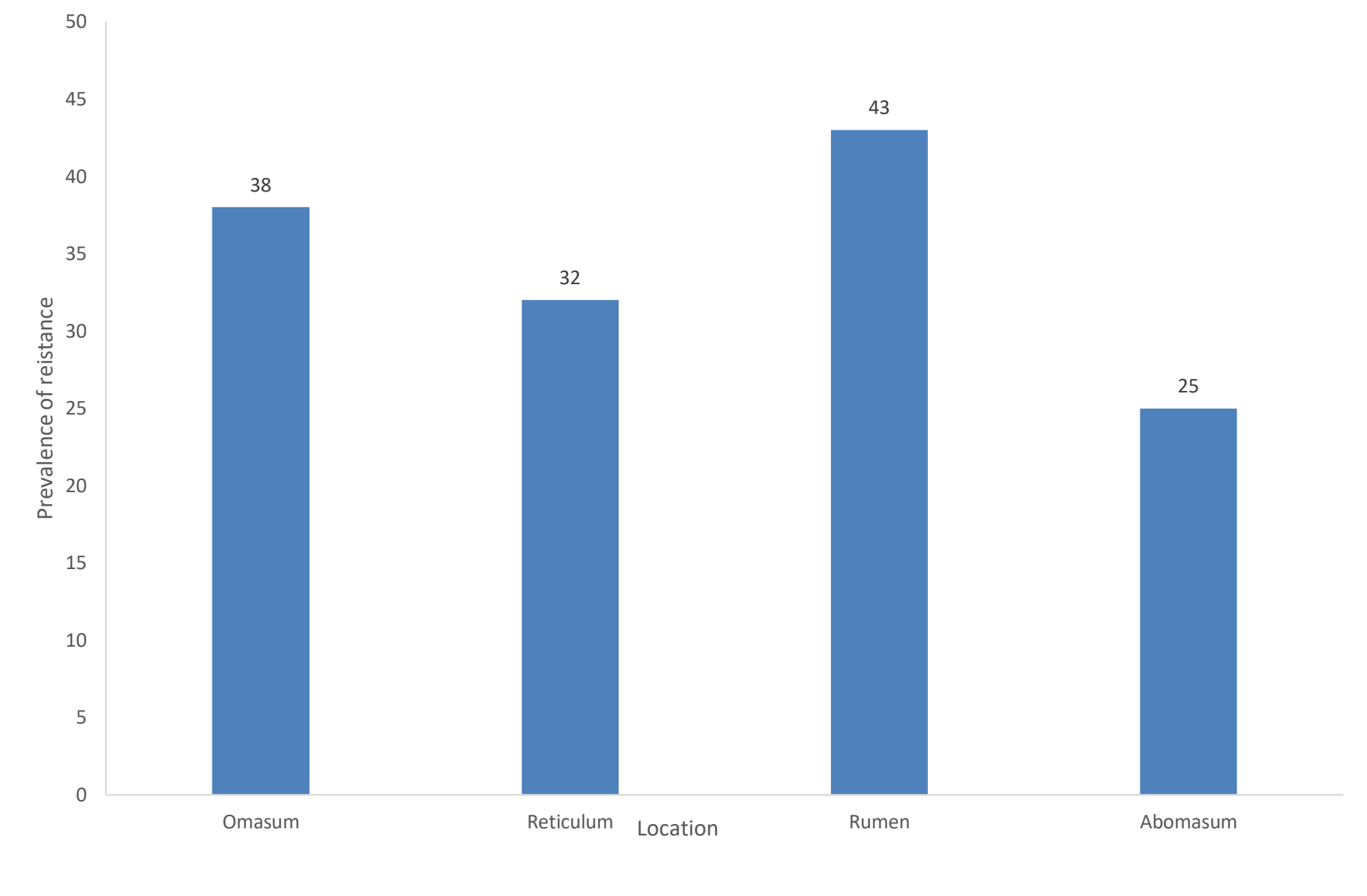

Figure 4 Prevalence of antimicrobial resistance in each of the four stomach compartments of sheep. Number of DNA contigs carrying antimicrobial resistance genes amongst the sampled animals stratified by stomach compartment.

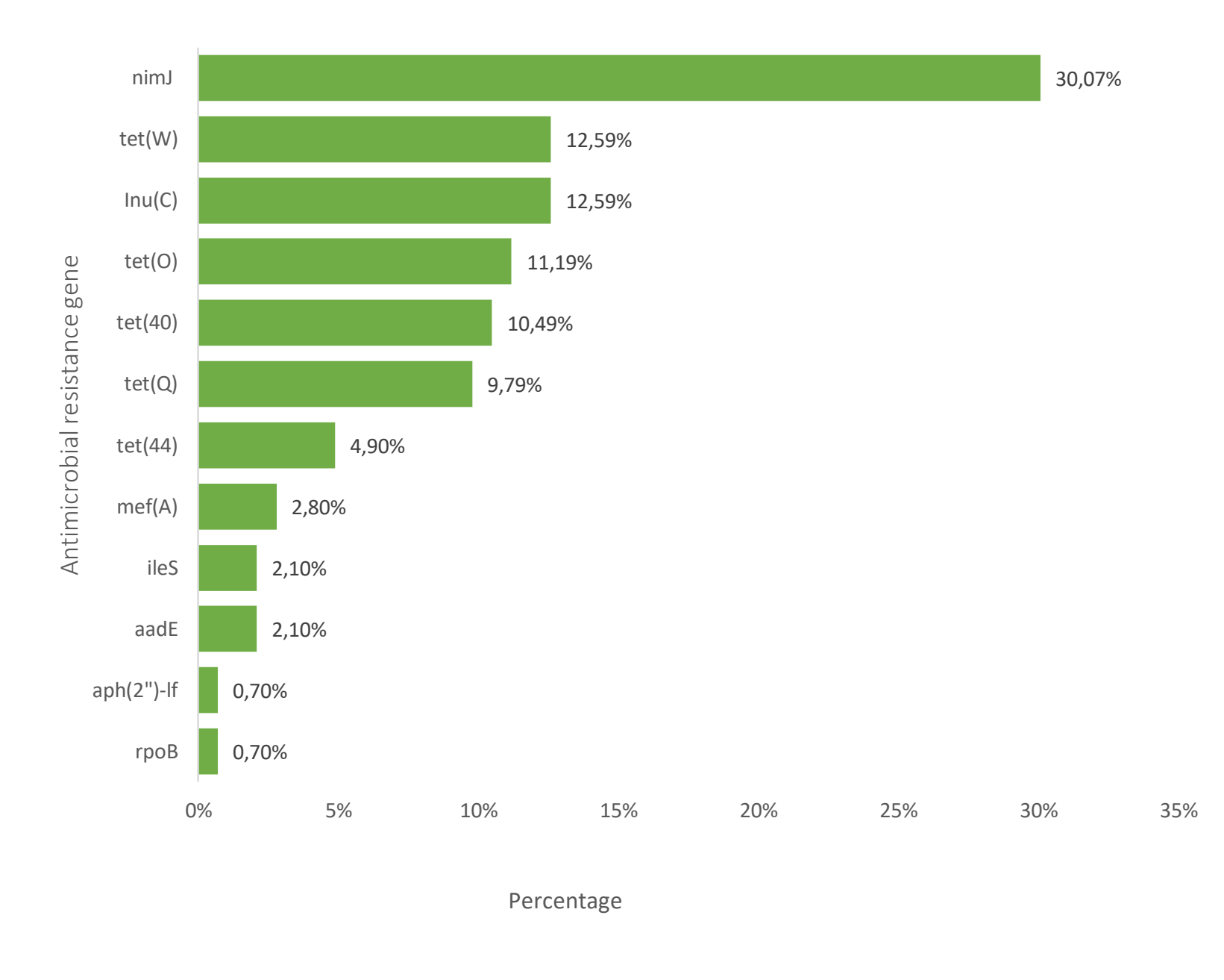

Figure 5 Abundance of antimicrobial genes detected in the gut of sheep. Identity and percentage prevalence of antimicrobial resistance genes present in gut of sheep

Table 3 Conferred resistance and mechanism of resistance for identified genes

GENE	RESISTANCE PHENOTYPES	MECHANISM OF RESISTANCE
NimJ	Nitroimidazole	Impaired drug activation, high expression level of multidrug efflux pumps or DNA repair systems
Tet (O,Q, W,40 and 44)	Tetracycline	Ribosomal protection proteins, Efflux pumps, enzyme inactivation
lnu (C)	Lincosamide	Target-site modification, efflux of the antibiotic, drug inactivation.
aph (2'')-lf	Amikacin; Gentamicin; Kanamycin; Tobramycin	Reduced uptake or decreased cell permeability, alterations at the ribosomal binding sites, target site modification.
ileS	Mupirocin	Target site modification
aadE	Streptomycin	Antibiotic inactivation
Mef(A)	Lincosamide;Macrolide;Oxazolidinone;Phenicol;Pleuromutilin;Streptogramin;Tetracycline	Efflux pumps, Ribosomal methylation.
rpoB	Rifamycin	Antibiotic target alteration, Antibiotic target replacement

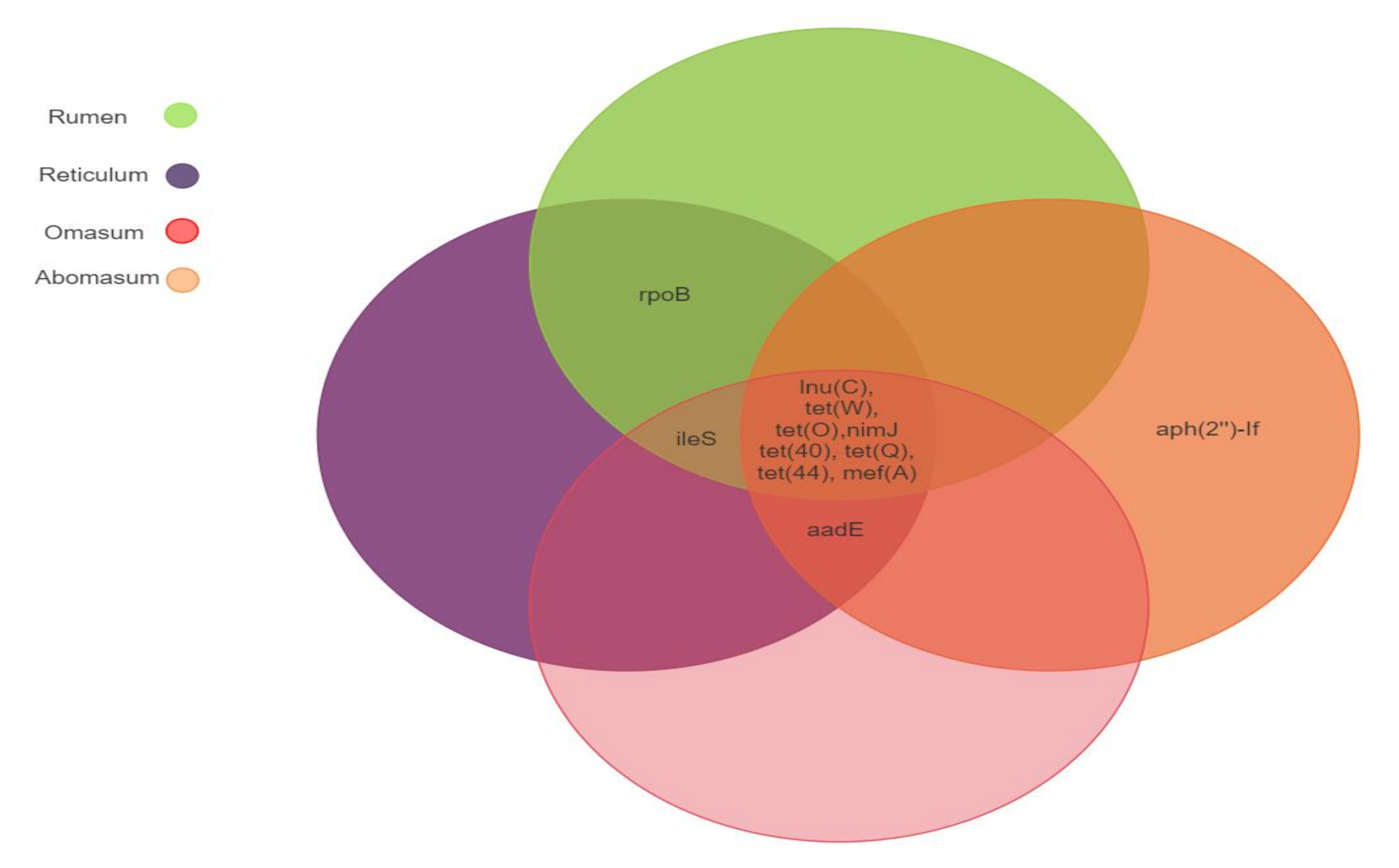

Figure 6 Core resistance genes present in the sheep gut. Eight gene were identified as core resistance gene in this study. These confer resistance to tetracycline, nitroimidazole, lincosamide and macrolides. It is these resistance phenotypes that were found to have high prevalence across all the four compartments with tetracycline resistance having the highest prevalence.

Conclusions

- ❖ The gut has a diverse population of microbes but many of the microbes have overlapping functions
- ❖ Bacteria and Firmicutes are the dominating kingdom in the population
- ❖ The reticulum is the most diverse chamber
- ❖ The gut microbiome does indeed contribute to well-being of the host but not all microbes present in the gut are good microbes
- ❖ The antimicrobial resistance prevalence is moderate
- ❖ Usage of antimicrobials in feed additives increases prevalence of AMR, whilst the gut microbial population influences the observed resistance

References

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