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Isolation and identification of coliform from the Rumen of cows slaughtered at Afor-Oba abattoirs in Anambra

Abstract

This research was conducted to ascertain the coliform resident in the rumen of slaughtered cow. Two samples were aseptically collected and processed. Ten-fold serial dilution was done and 102, 104 and 106 diluents were spread plated on Maconkey and Eosin methylene blue agar. After 24hrs of incubation at 37 0C, (4.0×10^4 , 3.0×10^6, 3.0×10^8;) (6.0×10^4 ; 9.0×10^6, 9.0×10^8)cfu/ml were recorded for Maconkey for sample 1 &2 respectively. Also (2.0×10^4 8.0×10^6 3.0×10^8;),(3.0 ×107, 9.0×10^7, 9.0×10^7) cfu/ml was counted for Eosin methylene blue for samples 1 & 2 respectively. After isolation and identification, *Escherichia coli*, *Klebsiella pneumonia, Enterobacter* spp and *Citrobacter sp were identified* as possible organisms. Of the possible organisms, *Escherichia coli* and *Citrobacter* sp were predominant. Further visit to the ranch revealed that several negative factors were responsible for the presence of the pathogenic organisms. These factors are to be corrected and consumers are advised to cook the meat very well before eating.

Keywords: Abatoir, coliform, colony, incubation, rumen. ruminant

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Introduction

Coliform bacteria are a diverse group of Gram-negative, non-spore-forming rod-shaped bacteria that are predominantly found in the intestines of warm-blooded animals, including ruminants such as cows. They are classified into three primary groups: total coliforms, faecal coliforms, and *Escherichia coli*. The presence of coliforms in the environment is often used as an indicator of faecal contamination, making them significant in food safety and public health contexts.[1] Understanding the ecology of coliforms in the rumen of cows is essential for assessing not only animal health and productivity but also the implications for food safety related to human consumption of animal products.

The rumen is a specialized compartment of the digestive system in ruminants, playing a crucial role in the fermentation of fibrous plant materials. The microbial population in the rumen consists of various microorganisms, including bacteria, archaea, fungi, and protozoa, all of which contribute to the breakdown of complex carbohydrates and proteins.[2] Coliforms, although often considered minor components of the rumen microbiota, play vital roles in this complex ecosystem. They are involved in the fermentation process, facilitating the breakdown of cellulose and other polysaccharides into simpler compounds, including volatile fatty acids (VFAs), which serve as primary energy sources for ruminants.

Furthermore, coliforms can participate in nitrogen metabolism within the rumen, influencing protein synthesis and amino acid availability for the host.[3] Their presence can help maintain a balanced microbial community, promoting optimal digestion and nutrient absorption, which are critical for the overall health and productivity of ruminants. Coliforms are primarily categorized into three groups: total coliforms, faecal coliforms, and *Escherichia coli*. Total coliforms include a broad spectrum of bacteria that may originate from both faecal and non-faecal sources, whereas faecal coliforms are specifically derived from the intestines of warm-blooded animals, indicating faecal contamination.[1] Among these, *E. coli* includes both pathogenic and non-pathogenic strains, making it a significant focus for food safety research. The presence of coliforms in the rumen is not only indicative of faecal contamination but also reflects the overall health of the ruminant and the microbial community's balance. Coliforms contribute to the fermentation process within the rumen, where they assist in breaking down fibrous plant material and converting it into simpler sugars, fatty acids, and gases.[1] These fermentation products are essential for the energy metabolism of ruminants.

Materials and methods

Sample Collection and Processing

Two samples (Rumen) from two slaughtered cows from the Afor-Obah market was collected using two sterilized plastic buckets. The buckets were sterilized by first washing them with detergent, air-dry and rinsed with 95% ethanol. The samples were transported to the laboratory within 2 hrs of collection. The liquid content of rumen was aseptically squeezed into an already sterilized conical flask. Thereafter, 10 ml of each was mixed with 100 ml of sterilized distilled water. This forms a stock solution for serial dilution. Ten-fold serial dilution was done by initially using 1 m of the stock solution. Finally, 10^2, 10^4 and 10^6 diluents were spread plated out on Maconkey and Eosin Methylene blue agar. These were done in duplicates and incubated for 24hrs at 37° C.

Results

After the incubation period of 24hrs, the following results were obtained and biochemical identification analysis conducted on the isolates (Figures 1&2) (Tables 1&2).

Table 1 colony count for the two media and samples

Samples	Total Colonies Count On Macconkey (Cfu/ml)			Total Colonies Count on EMB (Cfu/ml)		
	10^2	10^4	10^6	10^2	10^4	10^6
1	4.0×10^4	3.0×10^6	3.0×10^8	2.0×10^4	8.0×10^6	3.0×10^8
2	6.0×10^4	9.0×10^6	9.0×10^8	3.0×10^7	9.0×10^7	5.0×10^8

Table 2 biochemical identification and possible organism

Isolate	Catalase test	Methyl red test	Oxidase test	Gram-staining test	Morphology	Possible organism
1	+ve	+ve	-ve	-ve	Single rods or short chains	*Escherichia coli*
2	+ve	-ve	-ve	-ve	Large, mucoid rods in pairs	*Klebsiella pneumoniae*
3	+ve	-ve	-ve	-ve	Single rods (occational chains)	Enterobacter spp
4	+ve	+ve	-ve	-ve	Single rods (sometimes pairs)	Citrobacter spp

Figure 1 Agar plates before incubation.

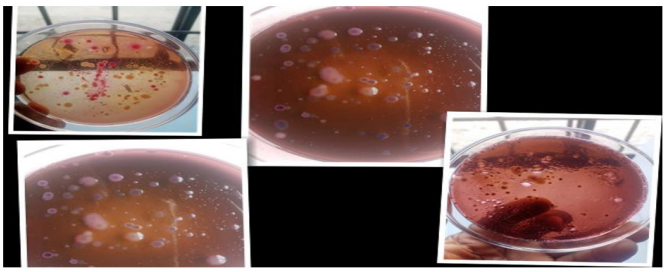

Figure 2 Growth on the plates after incubation.

Discussion

The results show the prevalence of *Escherichia coli* and *Citrobacter* spp. As the dominant coliforms. Colony morphology and biochemical tests confirm the coliform nature of each organism. The Catalase Test confirms that all isolates are catalase-positive. The Methyl Red Test highlights strong acid production in *E. coli* and *Citrobacter spp*. The Oxidase Test results were negative across isolates, as expected for coliforms. Gram Staining identified all isolates as Gram-negative rods, typical of coliform morphology.

Colony counting revealed that *Escherichia coli* and *Citrobacter spp*. were the most prevalent organisms among the coliforms isolated. The use of MacConkey and EMB agars facilitated selective growth and identification based on lactose fermentation and colony morphology, which confirmed coliform presence through distinct colony characteristics on each medium. High counts of *E. coli* and *Citrobacter* spp. suggest their adaptability and possible dominance within the rumen environment, reflecting findings in similar studies that highlight *E. coli* as a resilient member of coliforms in animal digestive systems.[4] All isolates demonstrated catalase positivity and Gram-negative rod morphology, consistent with coliform traits. However, the Methyl Red test showed variability among isolates: *E. coli* and *Citrobacter* spp. produced strong acid reactions, while *Klebsiella pneumoniae* and *Enterobacter aerogenes* exhibited weaker acid production. These results indicate metabolic diversity, particularly in glucose fermentation pathways, which could reflect distinct ecological niches or roles within the rumen microbiome. Such metabolic diversity within coliforms may support the varied nutrient breakdown functions essential for rumen efficiency, especially in fibre and carbohydrate digestion.[5]

Coliform bacteria, though often associated with opportunistic infections, also contribute to the microbiome's balance and digestive processes in the rumen. The predominance of *E. coli* and *Citrobacter* spp., along with the metabolic characteristics observed, may suggest that these coliforms play complementary roles in nutrient cycling, particularly in breaking down simple carbohydrates. This is important for rumen health, as these organisms could facilitate the degradation of substrates that support the broader microbial community. However, high coliform counts also raise concerns about the risk of pathogen transmission or metabolic imbalances that may lead to digestive disturbances.[6]

As potential facilitators of digestion but also as possible sources of pathogenicity. This duality warrants further examination, particularly regarding the role of environmental factors and diet in influencing coliform populations in the rumen.[7]

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Escherichia coli and *Citrobacter* spp. were consistently present in higher colony counts, indicating their dominant presence among rumen coliforms. This suggests they may play essential roles in rumen ecology. The positive results for the Catalase Test and Gram-negative morphology are consistent with typical coliform identification. The variability in Methyl Red results underscores metabolic diversity, with *E. coli* and *Citrobacter* spp. potentially contributing more actively to glucose fermentation.[8]

The presence of these coliforms in moderate-to-high counts could contribute to both the digestive functions and potential pathogenic

risks within the rumen environment. As part of a balanced microbial community, coliforms may support nutrient breakdown but could also act as indicators of microbial shifts or imbalances.[8]

Further findings

The research team further visited the ranch at which the cows are been fed and taken care of. The following were discovered.

1. The environmental conditions of the ranch is not encouraging, as there are enough evidence that everything is highly polluted.
2. The stream from which they get water to feed the cows cut across several markets and these markets use the stream as their final destination of their waste that are generated.
3. The handlers of this ranch do not have good and modern toilet system. They still make use of the near-by bush. This suggest there are high possibility of fecal contamination of solid food which the cow may feed on.
4. There is no proper drainage system around the area to channel waste water away.
5. There is high infestation of house flies. This will surely lead to contamination of every food on which the cows feed on.

Recommendations

The research team recommends as follows

1. While *Citrobacter* spp. was identified as a prevalent coliform in this study, further molecular techniques, such as 16S rRNA sequencing, are recommended to identify specific species. This would clarify the particular roles of *Citrobacter* spp in rumen ecology and determine if any strains possess unique metabolic traits that could be beneficial or detrimental to rumen function.
2. Further biochemical testing should explore other metabolic pathways involved in carbohydrate and nitrogen breakdown to provide a more comprehensive understanding of each organism's contributions to rumen processes. Enzyme assays or metabolomic analyses could reveal additional insights into the ecological functions of these coliforms.
3. Proper toilet system show be constructed within the ranch,
4. Waste disposal system should be provided for the markets to avoid using the river as their dumping site which will pollute the water used by both human and cow.
5. There should be serious environmental clean-up around the ranch to make habitable.

Acknowledgement

None.

Conflict of interests

Authors declare no conflict of interest.

References

1. Bhan GT, Cran TT, Cabral JPS. Water Microbiology: Bacterial Pathogens and Water Quality. Assessment. *Journal of Environmental Health*. 2020;16(2):117–122.
2. Jami CC, Fratamico PM, Bhunia AK, et al. Foodborne Pathogens: Microbiology and Molecular Biology. *Caister Academic Press.* 2023.
3. Hofmann WQ, Leclerc H, Mossel DA, et al. Advances in the Bacteriology of the Coliform Group: Their Suitability as Markers of Microbial Water Safety. *Annual Review of Microbiology*. 2024;55(1):201–234.
4. Zhang X, McDaniel AD, Wolf LE, et al. *Escherichia coli* Strains That Cause Hemorrhagic Colitis and Hemolytic Uremic. Syndrome. *The New England Journal of Medicine*. 2024;342(10):763–769.
5. Sperber WH, Doyle MP. Compendium of the Microbiological Spoilage of Foods and Beverages. *Springer*. 2022.
6. Quinn PJ, Markey BK, Leonard FC, et al. *Veterinary Microbiology and Microbial Disease (2nd ed.).* Wiley–Blackwell. 2022.
7. Paton JC, Paton AW. Pathogenesis and Diagnosis of Shiga Toxin–Producing *Escherichia coli* Infections. *Clinical Microbiology Reviews*. 2022;11(3):450–479.
8. Nataro JP, Kaper JB, Robins–Browne R. Coliforms in Clinical and Environmental Microbiology. *In Bacterial Pathogenesis and Epidemiology. Springer*. 2023;256–278.