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Assessment of the Role of Mutation in Genetic Variations and Speciation among the Strains of *Klebsiella pneumoniae* and *Escherichia coli* Associated with Subclinical Mastitis from the Pastoral Herds in Nigeria

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This study was conducted to evaluate the evolutionary relationship and clonal relatedness of *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from subclinical mastitis cases in pastoral herds in Nigeria. Phylogenetic analysis of gene sequences, performed using Bioedit and FigTree software, was employed to determine the evolutionary relationships among the strains. The analysis revealed that all strains of *K. pneumoniae* and *E. coli* shared a common ancestral origin, with *E. coli* strains evolving earlier than *K. pneumoniae* strains. Among the *E. coli* strains, E1 and E3 exhibited the highest clonal relatedness, while K6 and K1 were the most genotypically identical among the *K. pneumoniae* strains. The observed evolutionary relationships and clonal relatedness were primarily driven by continuous nucleotide sequence changes resulting from various mutations. These mutations led to speciation and genotypic divergence from the ancestral genomes of the investigated *K. pneumoniae* and *E. coli* strains.

Keywords: Evolutionary Relationship, Clonal Relatedness, *Klebsiella pneumoniae*, *Escherichia coli*

Introduction

In Nigeria, livestock is a cornerstone of the agricultural system, particularly in the northern regions (Lawal, 2012; Ayoola et al., 2025). Ruminants, including sheep, goats, and cattle, dominate livestock rearing (Lawal, 2012; Kimeli et al., 2025). Enhancing livestock production is critical for leveraging Nigeria's abundant natural resources and supporting economic growth (Shittu et al., 2012). However, achieving this potential requires protecting animals from diseases such as mastitis, which significantly hampers productivity (Shittu et al., 2012).

Mastitis, characterised by mammary gland inflammation, is a complex and costly disease affecting dairy herds globally (Beheshi et al., 2010; Hussain et al., 2012). It involves chemical, physical, and bacteriological changes in milk, alongside pathological alterations in udder tissue (Radostis et al., 2000; Sharma et al., 2011). The disease results from interactions between infectious agents, environmental factors, and host resistance (Gera and Guha, 2011). Mastitis adversely impacts animal health, milk quality, and the economics of milk production, causing substantial financial losses worldwide, including in developed nations (Sharma, 2007; Halasa et al., 2007; Tiwari et al., 2010; Shittu et al., 2012; Lamey et al., 2013; Suleiman et al., 2013; Mbuk et al., 2016).

Coliform bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae*, are prevalent in bovine environments, including bedding materials, soil, manure, and other organic matter (Hogan and Smith, 2003). Poor hygiene during rearing and milking increases the risk of coliform mastitis (Shittu et al., 2013). These bacteria produce virulence factors upon invading mammary tissues, leading to inflammation, reduced milk production, compromised milk quality, increased veterinary and labour costs, and decreased reproductive efficiency (Friedman and Ezra, 2004; Santos et al., 2004). *E. coli* and *K. pneumoniae* are particularly associated with subclinical mastitis in dairy cattle and exhibit adaptability through antibiotic resistance mechanisms, including genetic mutations (Hogan and Smith, 2003; Radostis et al., 2007; Suleiman et al., 2013; Mbuk et al., 2016). While contagious bovine mastitis caused by *Staphylococcus* species has declined, coliform mastitis has become increasingly significant, especially in pastoral herds in Nigeria, where poor hygienic practices exacerbate vulnerability (Lamey et al., 2013).

Microbial evolution, driven by genetic variation, is fundamental to understanding bacterial adaptation and pathogenesis (Reid and Buckley, 2023; Teng et al., 2023). Genetic variation, fuelled by mutations such as point mutations, insertions, deletions, and recombination, is essential for the development of antibiotic resistance and virulence (Kachabi et al., 2025). Speciation, the emergence of new species, occurs in bacteria through mechanisms like genetic drift, gene flow, and natural selection (Usai et al., 2024). These processes shape the diversity and adaptability of pathogens like *E. coli* and *K. pneumoniae* (Lenski, 2023).

Despite the significant role of *E. coli* and *K. pneumoniae* in subclinical mastitis, there is a notable research gap regarding the impact of mutations on genetic variation and speciation in these bacteria within Nigerian pastoral herds (Kaluba, 2024). This knowledge gap impedes the development of effective mastitis control and prevention strategies. Therefore, this study aims to assess the role of mutation in genetic variations and speciation among strains of *Klebsiella pneumoniae* and *Escherichia coli* associated with subclinical mastitis in pastoral herds in Nigeria.

Methodology

Study Area

This study was conducted on pastoral herds in Kaduna State, Nigeria, located in the Northwest Geopolitical Zone of the country. Kaduna State is situated at 608 metres above sea level, between latitudes 6° and 11° North and longitudes 7° and 44° East. The region encompasses parts of the Guinea Savannah and Sudan Savannah, characterised by distinct wet and dry seasons. Covering approximately 48,473.25 square kilometres, Kaduna State comprises 23 Local Government Areas (LGAs) (Ladan et al., 2021) (Figure 1).

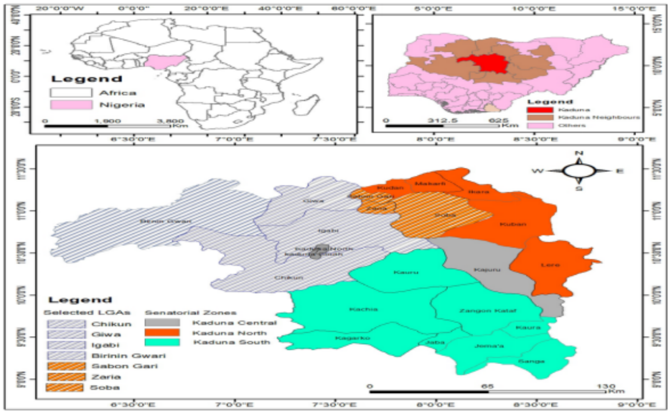

Figure 1 Map of Africa, Nigeria, and Kaduna State illustrating the research area (Makolo et al., 2019).

Bacterial Strains

Strains of *Klebsiella pneumoniae* and *Escherichia coli* used in this study were isolated from cases of subclinical mastitis. These strains were genotypically identified at the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria, and the Bioscience Centre, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Makolo et al., 2019).

Phylogenetic Analysis

Pairwise alignments of the gene sequences of the investigated microorganisms were performed using ClustalW to compare the sequences. Multiple Sequence Alignment Analysis was conducted to evaluate genetic events in the gene sequences. Phylogenetic analysis was carried out to assess the evolutionary relatedness among the isolated coliform strains using Bioedit and FigTree software, following the manufacturers' instructions.

Results

The Multiple Sequence Alignment of gene sequences from *Klebsiella pneumoniae* and *Escherichia coli* strains revealed genetic events responsible for speciation, leading to genotypic variance from their ancestral genomes (Figure 2).

The dendrogram constructed for all coliform isolates in this study indicated that all isolates shared a common ancestral origin and were evolutionarily related. However, speciation occurred over time due to genetic events, resulting in a daughter lineage at the root of 6, which has diverged to form a distinct species (Figure 3). Notably, strain E4 emerged from the ancestral root of 7 and remains genotypically identical to the ancestral genome. In contrast, the daughter lineage at the root of 6 diverged, giving rise to P1, which retains the same genotypic properties as it has not undergone speciation. Strains E3 and E1 share the same ancestral root with P1 at the root of 6 but are no longer genotypically identical due to speciation. However, E3 and E1 are currently genotypically identical and significantly similar to each other.

For *Klebsiella pneumoniae*, strain K4 originated from the root of 4 and retains an identical gene sequence to the ancestral lineage. Root 4 gave rise to root 3, from which K7 evolved. *Klebsiella pneumoniae* K7 has not undergone speciation and remains significantly identical to the ancestral genome. *Klebsiella pneumoniae* K2 diverged from the root of 3, also avoiding speciation and maintaining identity with the ancestral gene sequence at the root of 2. *Klebsiella pneumoniae* strains K1, K6, and K2 share the same ancestral lineage at root 2 but have been separated by speciation at the root of 1. Consequently, K1 and K6 have identical gene sequences but differ from the gene sequence of K2 (Figure 2).

Discussion

The phylogenetic analysis conducted in this study, based on the nucleotide sequences of isolated coliform bacteria, revealed that all strains share a common ancestral lineage. However, over time, these strains evolved from this common ancestry into their current evolutionary states. This evolution was primarily driven by continuous changes in nucleotide sequences resulting from various forms of mutation. These mutations led to speciation, resulting in genotypic variance from the ancestral genome, as evidenced by the Multiple Sequence Alignment results obtained in this study.

The phylogenetic analysis established relationships among the strains of *Klebsiella pneumoniae* and *Escherichia coli*. Strains P1, K4, K7, and K2 were identified as paralogous, while E3, E1, K1, and K6 were orthologous. Orthologous strains exhibit homology due to significant sequence similarity, indicating a shared ancestor, similar structure, and comparable biological functions. In contrast, paralogous strains retain the same gene sequence as

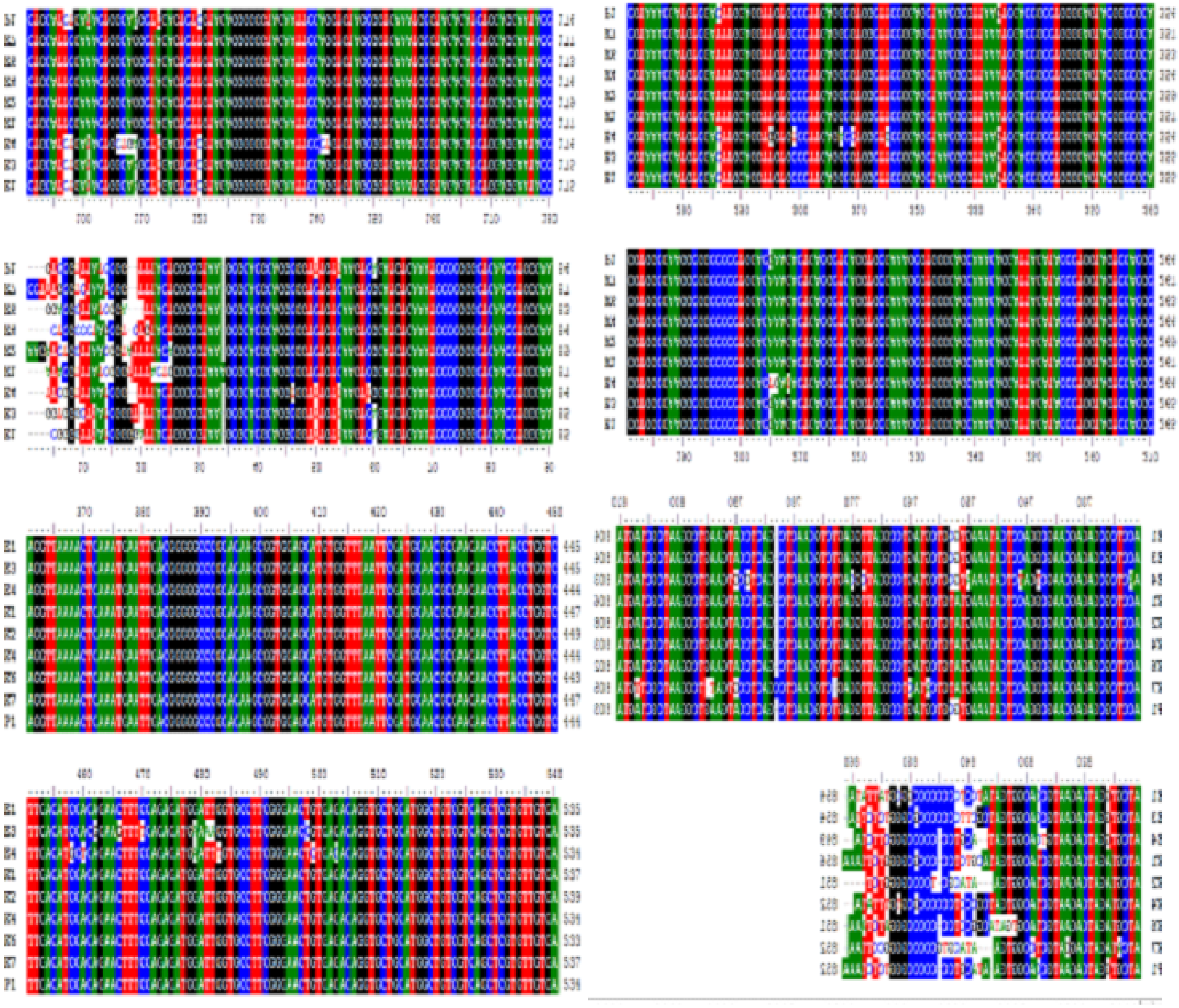

Figure 2 Multiple Alignments of the gene sequences showing gene events.

the ancestor due to gene duplication but differ in structure and biological functions (Teng et al., 2023).

Furthermore, the phylogenetic analysis indicated that *E. coli* strains evolved earlier than *K. pneumoniae* strains. Among the *E. coli* strains, E3 and E1 were the most clonally related, while K1 and K6 were the most closely related among the *K. pneumoniae* strains. These findings align with previous reports by Mbuk et al. (2016) and Makolo et al. (2021), which documented similar evolutionary relationships among coliform strains isolated from bovine mastitis cases in Kaduna State, Nigeria. The observed patterns are likely attributable to various mutations in the nucleotide sequences, as demonstrated in this study (Makolo et al., 2020).

Conclusion

This study has established the evolutionary relationships among strains of *Klebsiella pneumoniae* and *Escherichia coli* isolated from subclinical mastitis cases in pastoral herds. Phylogenetic analysis revealed that all strains share a common ancestry but have undergone genotypic changes due to mutations in their nucleotide sequences over time. These mutations have driven speciation and divergence from the ancestral genomes of the investigated *Klebsiella pneumoniae* and *Escherichia coli* strains. It is evident that mutation plays a significant role in speciation and genome modification of bacterial strains.

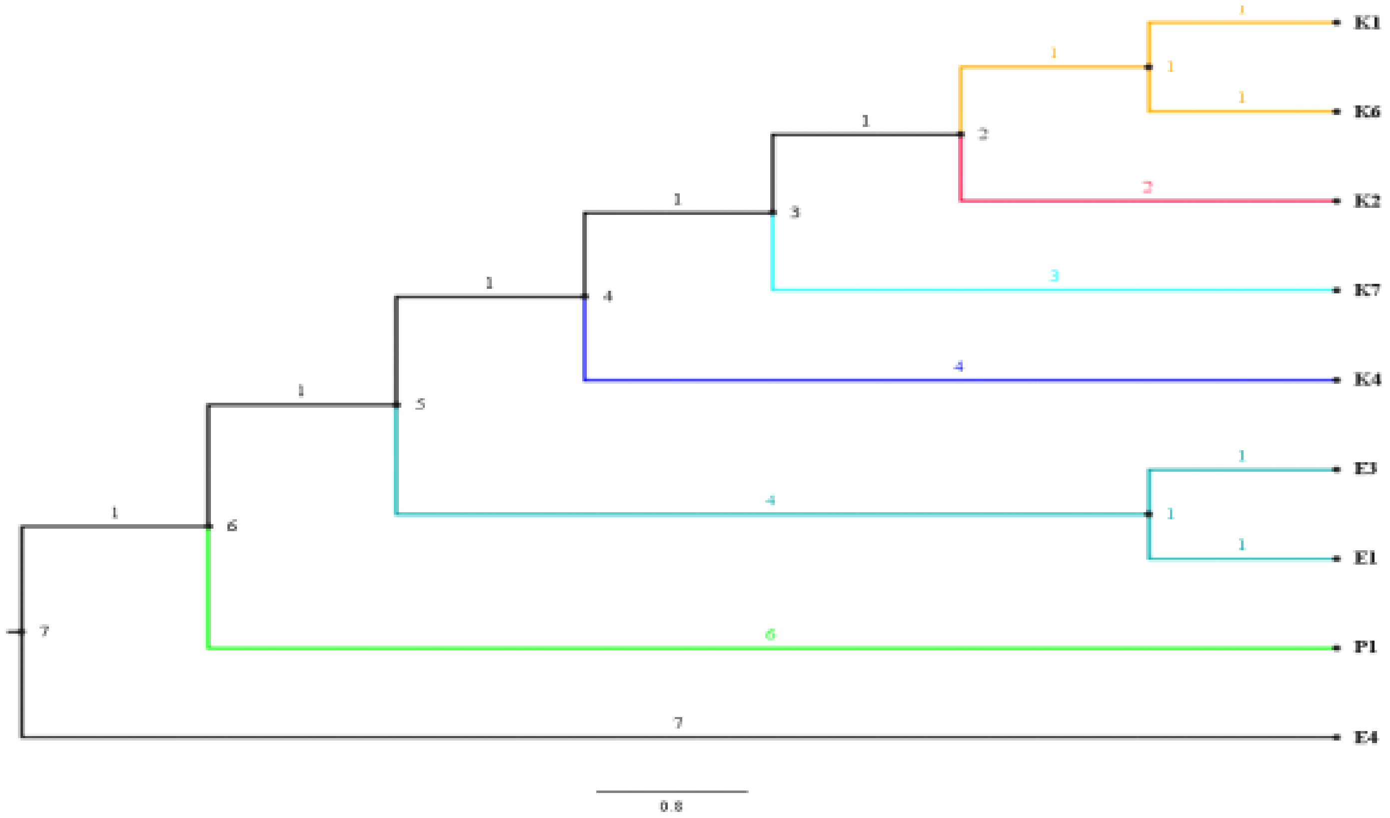

Figure 3 Phylogenetic dendrogram derived from 16S rRNA gene sequences showing the evolutionary relationship and clonal relatedness among the strains of *Klebsiella pneumoniae* and *Escherichia coli* isolated from cases of subclinical mastitis from pastoral herds.

Key:

- **K1, K6, K2, K7, K4** – Strains of *Klebsiella pneumoniae* isolated from mastitis-positive milk samples from pastoral herds.
- **E3, E1, P1, E4** – Strains of *Escherichia coli* isolated from mastitis-positive milk samples from pastoral herds.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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