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Early View

Original Research Article

Antibiotic Sensitivity Patterns in *Escherichia coli* isolated from Donkey Milk in association with Molecular Detection of Antibiotic Resistance Genes

Key words

antibiotic sensitivity;
donkey milk;
Escherichia coli;
resistance genes

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Abstract: The study investigates the prevalence and antibiotic resistance with molecular detection of antibiotic resistance genes of *Escherichia coli* (*E. coli*) in donkey milk. A total of 100 milk samples were collected from March to May 2023, with mastitis prevalence assessed using the California Mastitis Test (CMT) during the seasonal lactation period. Milk samples were transported to the laboratory for microbiological tests. *E. coli* detection involved initial cultivation on MacConkey agar and subsequent subculturing on Eosin-Methylene Blue agar. Biochemical identification was performed using IMViC, Urea, and TSI reactions. Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing. DNA extraction and PCR analysis were performed on phenotypically resistant isolates to detect specific resistance genes using established primers.

The results show that 7% of samples tested positive for clinical mastitis, while 14% had subclinical mastitis. Notably, Kisana Mori exhibited the highest rate of subclinical mastitis at 20%, whereas Rahooki had the highest clinical mastitis prevalence at 10%. Month-wise analysis revealed the highest occurrence of *E. coli* in April (23.5%), with an overall *E. coli* prevalence of 21%. Clinical and subclinical mastitis were most prevalent in March 10%, and 20%, respectively, statistical analysis indicated no significant variations in the prevalence of *E. coli*, clinical, or subclinical mastitis across the studied months and villages ($P<0.05$). Antimicrobial susceptibility testing revealed that *E. coli* was sensitive to enrofloxacin, tetracycline, and trimethoprim/sulfamethoxazole, while showing resistance to ciprofloxacin, erythromycin, and streptomycin. Molecular analysis identified significant resistance genes, particularly those for aminoglycosides (strA, 86%), tetracyclines (tetD, 71%; tetC, 57%), and chloramphenicol (cat1, 34%; cmlA, 23%). These findings highlight the critical need for effective mastitis management practices in donkey milk production to mitigate the risks associated with *E. coli* contamination and improve the quality and safety of donkey milk. The absence of significant differences in prevalence rates suggests a uniform spread of mastitis and *E. coli* across the regions and seasons studied.

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Introduction

Antibiotics are widely used not only to treat bacterial infections in humans and animals (during common veterinary practice) but also in animal production. Since the 1990s, antibiotics have been considered one of the most important discoveries of the century. The global consumption of antimicrobial drugs will increase by 67 % between 2010 and 2030 (1). The rise of antibiotic-resistant *E. coli* strains is multifactorial, influenced by factors such as the widespread and indiscriminate use of antibiotics in healthcare, agriculture, and aquaculture. This phenomenon jeopardizes the efficacy of commonly prescribed antibiotics, rendering once-reliable treatments ineffective and limiting options for infection control (2). Consequently, the study of antibiotic sensitivity patterns in E. coli is pivotal for the proactive identification of resistance trends, enabling healthcare providers to make informed decisions about

the selection and administration of antibiotics. 80 % of antibiotics sold in the world are widely used as supplements in livestock to promote healthy growth, prevent infections, and obtain a high-quality product (3).

In the past, the Miranda donkey (*Equus asinus*) played a significant role in agricultural practices and transportation in the rural regions of Sindh, (southern part of Pakistan) (4). However, due to the advent of agricultural mechanization, rural migration, and a substantial proportion (35–50%) of females giving birth to infertile foals (resulting from equine x asinine or asinine x equine hybrids), this once vital species is now facing the threat of endangerment. In response to the demanding landscape of mountainous rural regions with sparse populations and subpar soil quality, the Miranda donkey breed faces a current risk. Therefore, this breed offers a valuable model for investigating the behaviors of resistant microbes and the presence of resistance genes.

As a result, this breed serves as a valuable model for understanding the behavior of resistant microorganisms and the prevalence of resistance genes (5). There are significant risks to public health associated with the development and spread of *E. coli* strains resistant to antibiotics. To protect the quality and safety of these innovative dairy products, it is imperative to evaluate the antibiotic susceptibility profiles of the E. coli isolates obtained from donkey milk (6). The pathogen's susceptibility to particular drugs is typically taken into consideration when selecting antibiotics to treat bacterial infections. Determining the effectiveness of different antibiotics against a particular strain of bacteria requires the use of antibiotic sensitivity testing. Evaluating the antibiotic sensitivity profiles of *E. coli* in the context of donkey milk is essential for developing recommendations for sensible antibiotic use and stopping the formation and spread of antibiotic-resistant strains (7).

Moreover, the identification of antibiotic resistance genes using molecular tools in *E. coli* isolates offers a significant understanding of the genetic processes underlying resistance. The identification of genes that give resistance to particular antibiotics can help guide focused approaches aimed at preventing the spread of resistance. Our knowledge of the changing patterns of antibiotic resistance in microbial populations is improved by this technique, which also helps in identifying potential environmental reservoirs of antibiotic resistance genes (8). Both human and animal gastrointestinal tracts frequently include the gram-negative bacteria *Escherichia coli*. Antibiotic-resistant genes have the potential to spread to other harmful microbes through these strains, serving as repositories for such genes. Antimicrobial resistance (AMR) is a major public health hazard that affects the entire world.

Escherichia coli, a gram-negative bacterium, is commonly found in the human and animal gastrointestinal tracts. These strains can serve as reservoirs of antibiotic-resistant genes that may be transmitted to other pathogenic bacteria. Antimicrobial resistance (AMR) is a global concern and a significant public health issue (9, 10). Antimicrobial usage will be increasing by 99% in some countries, including South Africa, China, and India, which could be up to seven times higher than the predicted rate of population growth. Antibiotic resistance among *E. coli* strains has emerged and is spreading quickly, which presents a serious challenge to current medicine and complicates the effective management of *E. coli* infections (11).

This research endeavors to bridge the gap between the microbial safety of donkey milk and the potential risk posed by antibiotic-resistant *E. coli* strains. By combining traditional antibiotic sensitivity testing with molecular techniques, we aim to provide a comprehensive analysis of the antibiotic resistance profiles of *E. coli* isolates from donkey milk. This information is essential for both the dairy industry and public health authorities to establish effective measures for ensuring the safety of donkey milk products and mitigating the risk of antibiotic resistance transmission through the food chain.

Materials and methods

Sampling and detection of Escherichia coli

Overall, 100 milk samples were collected from the donkey milk samples were collected from six villages in the vicinity of Tandojam, Hyderabad, from March 2023 to May 2023. The lactating periods of donkeys, in Pakistan, are seasonal (from March through May), and donkey, milk samples were only available through these months within the aforementioned time frame. At each site, sampling of milk was performed according to the International Dairy Federation Guidelines. Samples (50 mL, in sterile glass containers) were transported to the laboratory at 4◦C within a maximum of 6–12 h after sampling. The subclinical mastitis was determined using the California Mastitis Test.

The initial cultivation of the samples was performed on MacConkey (MC) agar (Quelab, Canada). After the incubation period at 37 °C for 24-36 h, a pink colony from each plate suspicious to *E. coli* was subcultured on MC agar and Eosin-Methylene blue (EMB) agar (Quelab, Canada). Biochemical identification of the isolates as was determined based on IMViC, Urea, and TSI reactions. The isolates were cultured in Luria-Bertani broth (Merck, Germany), and after adding 15% glycerol, they were stored at -20 °C until further molecular analysis.

Antimicrobial analysis

Bacterial isolates were tested for antimicrobial susceptibility as recommended by the Clinical and Laboratory Standards Institute (CLSI), by the Kirby-Bauer method. The antimicrobial sensitivity test was performed on Mueller-Hinton agar (Sigma-Aldrich, USA), according to the previously published procedures (12). The antimicrobial agents tested were amoxicillin (10 mg), ampicillin (10 mg), streptomycin (10 mg), gentamicin (10 mg), erythromycin (15 mg), ciprofloxacin (5 mg), oxytetracycline (30 mg), trimethoprim/sulfamethoxazole (25 mg), tetracycline (30 mg), chloramphenicol (30 mg) and enrofloxacin (5 mg). Plates were incubated at 37°C for 24 h, and the sensitivity patterns were read and reported according to the diameter of the inhibition zone as sensitive, intermediate, or resistant.

DNA extraction and PCR amplification

DNA extraction will be performed on all *E. coli* isolates that were phenotypically resistant on the disk diffusion sensitivity test. Bacterial DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit (Life Technologies, USA), according to the manufacturer's instructions. The quality and quantity of isolated DNA were verified using NanoDrop 2000c (Thermo Fischer Scientific, USA) and stored at -20°C until further use.

PCR analysis was performed to detect 10 resistance using previously published primers (Table 1) (13). Briefly, each PCR reaction was performed in a volume of 25 µL that contained the following substrates: 1.5 µL MgCl2, 2.5 µL PCR buffer, 200 µM dNTPs, 1 µM each primer, 5 U of Taq DNA polymerase, and 1 µL (50–200 ng/µL) of test DNA. The PCR machine was set using the following program: Denaturation for 5 min at 95°C, then 30 cycles of 1 min at 94°C, 60 s at 52–58°C, and 1 min at 72°C. The final extension step was set to 10 min at 72°C. The resultant PCR products were viewed using 1.5% agarose gel using electrophoresis (13).

Table 1: Oligonucleotide primers used to detect antimicrobial resistance genes

Group of antibiotics	Target genes	Primer sequence	Size	Ref
Tetracycline	*tetC*	F: TGCTCAACGGCCTCAACC R: AGCAAGACGTAGCCCAGCG	397	(13)
	tetD	F: GGATATCTCACCGCATCTGC R: CATCCATCCGGAAGTGATAGC	436	(13)
Chloramphenicol	*cat1*	F: AGTTGCTCAATGTACCTATAACC R: TTGTAATTCATTAAGCATTCTGCC	547	(14)
	cmlA	F: CCGCCACGGTGTTGTTGTTATC R: CACCTTGCCTGCCCATCATTAG	698	(14)
Fluoroquinolone	*qnrA*	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTAGGTCA	516	(13)
	qnr	F: GGGTATGGATATTATTGATAAAG R: CTAATCCGGCAGCACTATTTA	670	(15)
Aminoglycosides	*strA*	F: CTTGGTGATAACGGCAATTC R: CCAATCGCAGATAGAAGGC	548	(13)
	strB	F: ATCGTCAAGGGATTGAAACC R: GGATCGTAGAACATATTGGC	509	(13)
Beta-lactams	*bla1*	F: TCGCCTGTGTATTATCTCCC R: CGCAGATAAATCACCACAATG	768	(13)
	bla2	F: GGCCAGAACTGACAGGCAAA R: TTTCTCCTGAACGTGGCTGGC	462	(13)

Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences version 8.6**.** Descriptive statistics were calculated to determine the prevalence (%) of clinical and subclinical mastitis, as well as the proportion of *E. coli-positive* samples across different villages and months. The Chi-square (χ^2) test was used to compare the prevalence of clinical and subclinical mastitis and *E. coli* infections among different villages and months. A p-value < 0.05 was considered statistically significant.

Results

Prevalence of clinical and subclinical mastitis in different villages

Table 2 shows the prevalence of clinical and subclinical mastitis in donkey milk samples collected from villages near Tandojam. The percentage of subclinical mastitis-positive samples was determined by the California Mastitis Test (CMT). Out of the total 100 samples collected, 7% were positive for clinical mastitis, and 14% were positive for subclinical mastitis. Kisana Mori had the highest subclinical mastitis prevalence at 20%, while Tando Qaisar and Detha also showed notable subclinical mastitis rates of 18.1% and 16.6%, respectively. Rahooki had the highest clinical mastitis prevalence at 10%, followed by Tando Qaisar at 9%. Kisana Mori reported no clinical mastitis-positive samples, indicating potentially better clinical management in this village or the absence of overt mastitis symptoms in sampled donkeys. The statistical analysis using the Chi-square test for independence indicates that there is no significant (P<0.05) difference in the prevalence of both clinical and subclinical mastitis among the six villages near Tandojam.

Month wise prevalence of E. coli, clinical mastitis, and subclinical mastitis in donkey milk

Table 3 shows the prevalence data of *E. coli*, clinical mastitis, and subclinical mastitis in donkey milk samples collected over three months (March, April, and May). The highest percentage of *E. coli* positive samples was observed in April (23.5%), followed by March (20%) and May (19.44%). Overall, *E. coli* was detected in 21% of the total samples. The prevalence of clinical mastitis was highest in March (10%), indicating a peak in visible mastitis symptoms early in the lactation season. The prevalence decreased in April (5.8%) and May (5.5%), with an overall prevalence of 7%. Subclinical mastitis was also most prevalent in March (20%), suggesting that both clinical and subclinical mastitis were more common at the start of the lactation season. The prevalence decreased in April (11.7%) and May (11.1%), with an overall prevalence of 14%. Statistically, there are no statistically significant (P<0.05) differences in the prevalence of *E. coli,* clinical mastitis, and subclinical mastitis across March, April, and May.

Table 2: Prevalence by village for clinical and subclinical mastitis in donkey milk

Villages	Total samples collected	Clinical mastitis-positive samples	Clinical mastitis-positive samples (%)	Subclinical mastitis-positive samples	Subclinical mastitis occurrence (%)
Tando Qaisar	22	2	9%	4	18.1%
Moosa Khatian	16	1	6.2%	2	12.5%
Detha	18	1	5.5%	3	16.6%
Kisana Mori	10	0	0%	2	20%
Rahooki	20	2	10	1	5%
Kunnar Plant	14	1	7.1%	2	14.2%
Total	100	7	7%	14	14%

Analysis	Chi-square (χ^2) value	p-value	Interpretation
Clinical mastitis occurrence	χ^2=2.284	0.809	No significant difference among villages
Subclinical mastitis occurrence	χ^2= 2.743	0.739	No significant difference among villages

Table 3: Prevalence by month for *E. coli*-positive clinical and subclinical mastitis

Months	Total samples collected	*E. coli* positive sample and (%)	Clinical mastitis-positive samples and (%)	Subclinical mastitis occurrence (%)
March	30	6 (20%)	3 (10%)	6 (20%)
April	34	8 (23.5%)	2 (5.8%)	4 (11.7%)
May	36	7 (19.44)	2 (5.5%)	4 (11.1%)
Total	100	21 (21%)	7 (7%)	14 (14%)

Analysis	Chi-square (χ^2) value	p-value	Interpretation
E. coli occurrence	χ^2= 0.293	0.863	not statistically significant differences were found in months
Clinical mastitis occurrence	χ^2=1.666	0.434	
Subclinical mastitis occurrence	χ^2= 1.029	0.597	

Antimicrobial susceptibility against E. coli isolated from donkey milk

Table 4 shows the results of antimicrobial susceptibility testing for various antibiotics against *E. coli* isolates from donkeys. Enrofloxacin, Tetracycline, and Trimethoprim/Sulfamethoxazole exhibited significant effectiveness, with clear zones of inhibition indicating sensitivity. Gentamicin and Ampicillin showed intermediate effectiveness, suggesting a possible need for higher doses or combination therapy for effective treatment. Ciprofloxacin, Erythromycin, Oxytetracycline, and Streptomycin were largely ineffective against the isolates, as indicated by the small zones of inhibition and resistance interpretation.

Molecular characterization of resistance genes in E. coli isolates

Table 5 shows the presence of specific antimicrobial resistance genes in *E. coli* isolates obtained from donkey milk samples. The data includes the group of antibiotics, target genes, the number of *E. coli* isolates carrying each gene, and the corresponding percentage of isolates among the total samples tested (n = 35). The highest prevalence of resistance genes was observed for aminoglycosides (strA, 86%), indicating significant resistance among *E. coli* isolates to this antibiotic group. Both tetC (57%) and tetD (71%) were present in a considerable number of isolates, suggesting moderate resistance to tetracycline. The resistance genes cat1 (34%) and cmlA (23%) indicate variable levels of resistance to chloramphenicol among the isolates. Resistance genes for beta-lactams (bla1 and bla2) were present at lower frequencies (17% and 12%, respectively), and no isolates carried fluoroquinolone resistance genes (qnrA and qnr), indicating low to no resistance to these antibiotics.

Table 4: Antimicrobial susceptibility against *E. coli* isolated from donkey milk

Antimicrobial Agents	Potency	Zone of inhibition (mm)	Interpretations
Amoxicillin	10 mg	15	Sensitive
Ampicillin	10 mg	10	Intermediate
Chloramphenicol	30 mg	16	Sensitive
Ciprofloxacin	5 mg	8	Resistant
Erythromycin	15 mg	6	Resistant
Enrofloxacin	5 mg	22	Sensitive
Gentamicin	10 mg	20	Intermediate
Oxytetracycline	30 mg	12	Resistant
Streptomycin	10 mg	5	Resistant
Tetracycline	30 mg	20	Sensitive
Trimethoprim/ sulfamethoxazole	25 mg	17	Sensitive

Discussion

The prevalence rates of clinical and subclinical mastitis in donkey milk from villages near Tandojam present notable differences when compared to previous research. While the present study identified a clinical mastitis prevalence of 7% and a subclinical mastitis prevalence of 14%, these figures show some variance with data from other regions and studies. The 7% prevalence rate for clinical mastitis in the current study is relatively moderate. This study agreed with (16) that Saudi Arabia reported a higher prevalence of clinical mastitis (12%) in donkeys, indicating potential differences in environmental or management factors between regions. Similarly, as reported by (17) in Italy revealed a clinical mastitis prevalence of approximately 10% in donkeys, which aligns more closely with the findings from the current study in Rahooki (10%). The relatively lower clinical mastitis rate observed in our study may indicate better overall management practices or reduced environmental exposure to pathogens. The prevalence of subclinical mastitis, which was 14% in this study, closely aligns with data reported in a previous study (18), A study in Brazil reported a subclinical mastitis rate of 15% in donkeys, which is considerably lower than the 24% prevalence reported by another source (19) in Egypt. The variability in subclinical mastitis rates can be attributed to differences in detection methods, management practices, and environmental factors. The higher prevalence of subclinical mastitis in Kisana Mori (20%) compared to other villages may indicate specific local factors or practices that contribute to increased risk, such as hygiene issues or variations in handling and milking practices. The Chi-square test results show no significant differences in mastitis prevalence among the six villages near Tandojam, suggesting a relatively uniform risk across the region. Similar findings were seen in the South Indian study by (20), which showed no discernible regional variations in the prevalence of mastitis across different villages. This suggests that the observed uniformity is probably the result of similar environmental and management conditions.

Table 5: Identification of antimicrobial resistance genes in *E. coli* isolates

Group of antibiotics	Target genes	Number of *E. coli* isolates carrying the gene (n = 35)	Percentage (%)
Tetracycline	*tetC*	20	57%
	tetD	25	71%
Chloramphenicol	*cat1*	12	34%
	cmlA	08	23%
Fluoroquinolone	*qnrA*	00	0%
	qnr	0	0%
Aminoglycosides	*strA*	30	86%
	strB	13	38%
Beta-lactams	*bla1*	06	17%
	bla2	04	12%

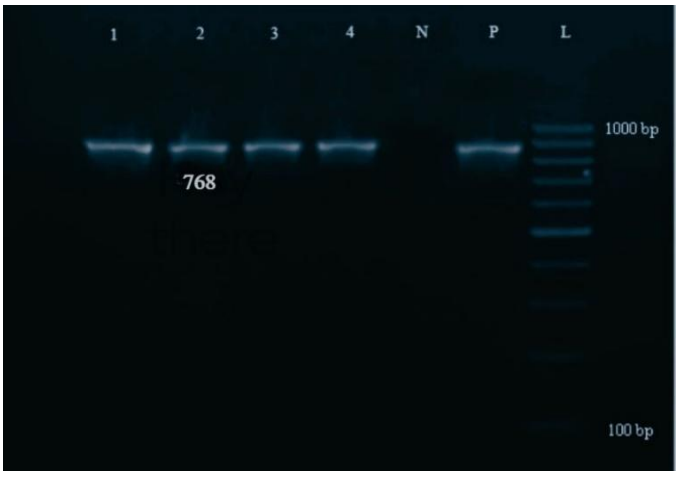

Figure 1: Agarose gel showing PCR amplification of E. coli *bla1* gene product (768 bp) P: Positive control, N: Negative control, L: DNA Ladder, Lane 1 to 4: Positive samples

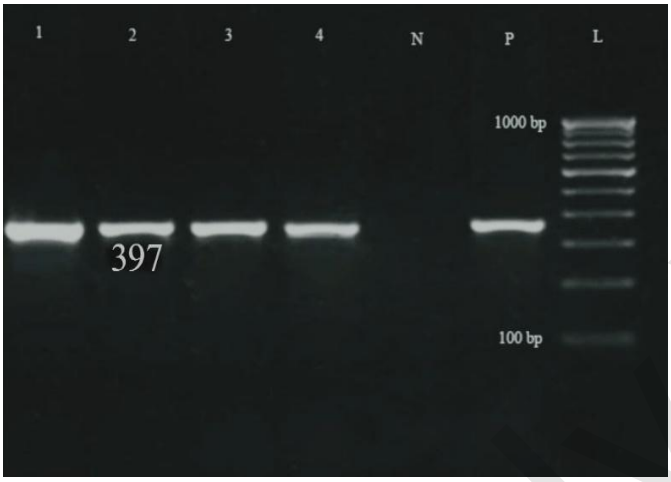

Figure 2: Agarose gel showing PCR amplification of E. coli *tetC* gene product (397 bp) P: Positive control, N: Negative control, L: DNA Ladder, Lane 1 to 4: Positive samples

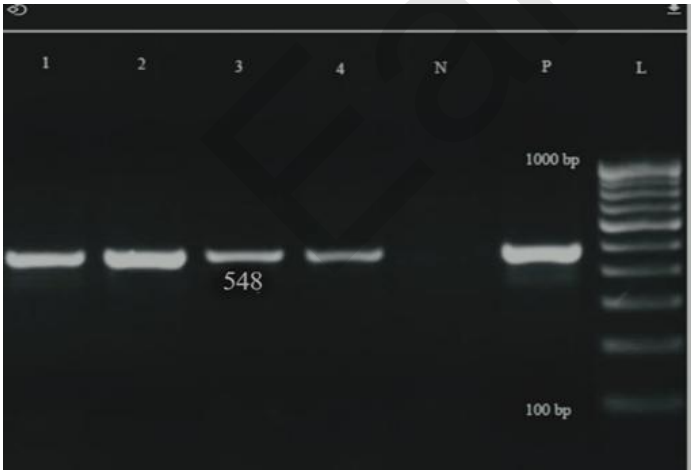

Figure 3: Agarose gel showing PCR amplification of *E. coli strA* gene product (548 bp) P: Positive control, N: Negative control, L: DNA Ladder, Lane 1

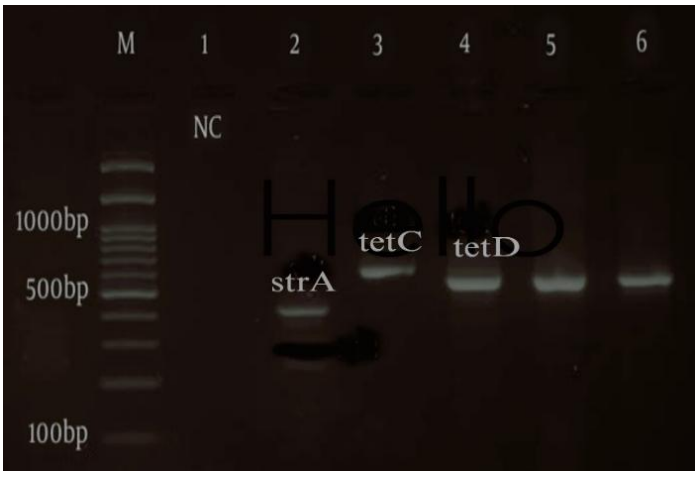

Figure 4: Agarose gel showing PCR amplification of *E. coli strA, tetC and tetD* gene product (548 bp, 397 bp and 436 bp) P: Positive control, N: Negative

In donkey milk samples, *E. coli* was found in 21% of samples overall. April had the highest detection rate (23.5%), followed by March (20%) and May (19.44%). The observed pattern suggests a potential seasonal impact on bacterial contamination, which is probably related to environmental variables that impact bacterial growth and transmission, like humidity and temperature. There are seasonal and regional variations in the incidence of *E. coli* in donkey milk. This study highlights geographical variations in hygiene methods and environmental conditions in contrast to the findings of (21) in Morocco, where a lower prevalence of *E. coli* (about 10%) was identified in donkey milk samples. Conversely, (22) observed a higher percentage of *E. coli* (28%) in samples of donkey milk, which is in line with the present results concerning variability and increased prevalence. Remarkably, March had the largest percentage of cases of clinical mastitis (10%), suggesting an early lactation season peak. After that, the percentage decreased in April and May (5.8% and 5.5%, respectively), yielding an overall prevalence of 7%. Other investigations have shown similar seasonal behaviors. For example, studies carried out in Spain by (23) found that the start of the lactation season is associated with a rise in clinical mastitis, which they attributed to stress and physiological udder changes.

According to a study conducted in Egypt by (24), there is a correlation between greater exposure to pathogens and variations in temperature in the environment, which might lead to a raised incidence of clinical mastitis early in the nursing process phase. March had the highest frequency of subclinical mastitis (20%), with a decrease in April (11.7%) and May (11.1%) leading to an overall prevalence of 14%. This implies that as the lactation season carried on, subclinical mastitis decreased. Notably, the study's findings on prevalence are consistent with those from other areas. Consider the 15% subclinical mastitis rate in donkey milk reported by (25) in Israel, where comparable seasonal decreases were seen as lactation developed.

According to studies conducted on other animals, the early season increase in subclinical mastitis could be related to physiological stress and greater susceptibility to infections after parturition

(26). There could be some reasons affecting the prevalence patterns of *E. coli*, clinical mastitis, and subclinical mastitis that were seen in March, April, and May. The increased frequency of *E. coli* in April raises the possibility that the month's climate, which includes higher humidity and warmer temperatures may encourage the growth of bacteria. Previous research has also shown similar patterns, with rising temperatures leading to higher contamination rates and surrounding bacterial populations.

Early in the lactation season in March, there was a peak incidence of both clinical and subclinical mastitis. This finding is consistent with previous studies indicating higher rates of mastitis immediately after pregnancy as a result of udder stress and immune system changes. The following fall in occurrence in April and May could be interpreted as evidence of good nursing adaption and early stressor recovery. Over time, the prevalence of both clinical and subclinical mastitis can be decreased with good management techniques and prompt management of early cases.

The high effectiveness of Enrofloxacin against *E. coli* isolates in donkey milk aligns with findings from various studies. The study reported by (27) that *E. coli* isolates in dairy cattle exhibited a high susceptibility rate to Enrofloxacin, which aligns with its known efficacy against Gram-negative bacteria. Enrofloxacin functions by inhibiting bacterial DNA gyrase and topoisomerase IV. The effectiveness observed in our current study suggests that Enrofloxacin remains a viable treatment option for *E. coli* infections in donkeys. The sensitivity recorded of *E. coli* isolates to Tetracycline and Trimethoprim/Sulfamethoxazole are corroborated by (28), reported similar susceptibility patterns in *E. coli* isolated from bovine mastitis cases. The mechanisms of action for Tetracycline (protein synthesis inhibition) and Trimethoprim/Sulfamethoxazole (folic acid synthesis inhibition) are effective against a broad spectrum of bacterial strains, including *E. coli*. However, the emergence of resistance in certain regions underscores the importance of cautious and targeted antibiotic use. The intermediate effectiveness of Gentamicin and Ampicillin suggests that while these antibiotics can inhibit *E. coli* growth, their efficacy may vary based on factors such as dosage and bacterial load. This study agreed with (29) has reported similar results, demonstrating that Gentamicin exhibits moderate effectiveness against *E. coli* in dairy animals. Notably, the moderate sensitivity of Ampicillin, a beta-lactam antibiotic, is significant, considering that resistance mechanisms such as beta-lactamase production can reduce its efficacy. This aligns with findings by (30) observed a growing resistance trend to Ampicillin in *E. coli* from livestock The fact that *E. coli* isolates are resistant to Ciprofloxacin, Erythromycin, Oxytetracycline, and Streptomycin is worrisome, although not entirely surprising. Specifically, Ciprofloxacin resistance has been on the rise as reported by (31) that *E. coli* from various animal sources discovered notable levels of resistance. The inefficacy of Erythromycin aligns with its limited activity against Gram-negative bacteria, primarily due to poor penetration of the outer membrane. This observation is supported by (32) that resistance observed in *E. coli* isolates to Oxytetracycline and Streptomycin, both of which have been extensively used in veterinary medicine, underscores the critical issue of antimicrobial resistance (AMR). Different published data highlighted the significance of AMR (33). The presence of the aminoglycoside resistance gene strA in 86% of the *E. coli* isolates indicates a high level of resistance to this class of antibiotics. The widespread prevalence of aminoglycoside resistance genes, such as strA, aligns with findings from various studies across different animal hosts. A study by (34) on dairy cattle in China reported a prevalence of aminoglycoside resistance genes in *E. coli* isolates at 82%, closely mirroring the current findings. Similarly, reported by (35) that 89% of *E. coli* isolates from swine harbored aminoglycoside resistance genes, highlighting the widespread problem of aminoglycoside resistance in livestock. Notably, the resistance genes tetC and tetD were present in 57% and 71% of the isolates, respectively, indicating a moderate to high level of resistance to tetracycline. These findings align with global trends in tetracycline resistance prevalence. A study by (36) reported that 65% of *E. coli* isolates from poultry in Malaysia carried tetracycline resistance genes, with tetA and tetB being the most common. Another study by (37) conducted in China revealed that 70% of *E. coli* isolates from cattle carried tetracycline resistance genes, which is similar to the prevalence of tetD observed in our current study. Additionally, the chloramphenicol resistance genes cat1 and cmlA were detected in 34% and 23% of the isolates, respectively, indicating varying levels of resistance to chloramphenicol. Although chloramphenicol resistance is less common than resistance to other antibiotic classes due to its restricted use in food-producing animals, it has still been reported in various studies. For instance, a study by (12) found chloramphenicol resistance genes in 28% of *E. coli* isolates from dairy cattle in the USA, comparable to the prevalence found in this study. The study by (38) in South Korea, reported that 30% of *E. coli* isolates from pigs carried chloramphenicol resistance genes, suggesting consistent resistance patterns across various livestock. Additionally, beta-lactam resistance genes, specifically bla1 and bla2, were detected in 17% and 12% of the isolates, respectively, indicating moderate resistance levels to beta-lactam antibiotics. Notably, the prevalence of beta-lactam resistance genes in *E. coli* appears to be increasing, as reported by various studies. This study contrasts with [35] found bla genes in 25% of *E. coli* isolates from dairy cows in Canada, slightly higher than the prevalence found in the current study. Another study by (39) reported that 20% of *E. coli* isolates from poultry in Spain carried beta-lactam resistance genes. The absence of fluoroquinolone resistance genes is a positive finding, contrasting with the increasing resistance trends reported in other studies. The study (40) reported qnr genes in 12% of *E. coli* isolates from dairy cattle in France, reflecting growing concerns over fluoroquinolone resistance in livestock.

Conclusion

This study highlights the substantial occurrence of clinical and subclinical mastitis in donkey milk. Subclinical mastitis is particularly prevalent in Kisana Mori. *Escherichia coli* was frequently detected, displaying high resistance to multiple antibiotics. The findings emphasize the need for better mastitis management and prudent antibiotic usage in donkey milk production. No sig-

nificant seasonal or regional variations in mastitis or *E. coli* prevalence were observed. Regular surveillance and improved hygiene practices are crucial to ensure the safety of donkey milk and reduce the risk of antibiotic-resistant bacteria.

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Ethical approval

This study was carried out after approval by the following ethical guidelines for animal research, and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Sind Agriculture University Tandojam, Approval No DAS-125-2023. Appropriate donkey owner consents were obtained in written forms before the samples were obtained from an affected donkey.

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Conflict of interest

The authors have no conflicts of interest to declare.

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Ključne besede: