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# Molecular Characterization and Antimicrobial Profiling of *Escherichia coli* isolates from Indian *Camelus dromedarius*

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## ABSTRACT

**Background:** *Escherichia coli* is frequently associated with multiple antimicrobial resistances and a major cause of bacterial extraintestinal infections in livestock and humans. *Escherichia coli* resides in the lower digestive tract as harmless commensals but a subset of *E. coli* strains has acquired the ability due to acquisition of virulence and antibiotic genes, cause intestinal or extraintestinal diseases.

**Methods:** In this field-laboratory investigation during 2020-2021, samples were taken from different localities of Bikaner district and surrounding are of Rajasthan. A total no. of 70 fecal samples were collected and immediately transferred to the Department of Veterinary Microbiology. In the laboratory, the collected samples were further processed for isolation and identification of *E. coli* bacteria.

**Result:** Confirmation of *E. coli* was done using primary and biochemical test which are screened for hemolysin property, biofilm formation, antibiogram study and antibiotic resistance gene. All the isolates of *E. coli* used to show characteristic metallic sheen on EMB agar plate and excellent identification was done with VITEK 2 system. All the camel isolates shown partial hemolysis on sheep blood agarand 45.71% camel isolates were positive for biofilm formation. All these 70 *E. coli* isolates from camel were resistant to Penicillin (94.38%) which was followed by amoxicillin+sulbactam (85.71%), erythromycin (71.14%), cefixime+clavulanic acid (71.43%). Highest sensitivity to chloramphenicol (81.28%) followed by sulphadiazine (48.57%) and cotrimoxazole (48.28%). All these 70 isolates were screened for antibiotic resistance genes. On the basis of molecular screening of the antibiotic resistance genes, majority of the isolates carried *Bla*TEM gene in camel (56/70; 80%), followed by *Str*A (29/70; 41.40%), *Sul*-3 in (22/70; 31.42%), *Sul*-2 (18/70; 25.71%), *aad*A (28/70; 40%), *tet*(B) (22/70; 31.42%) isolates.

**Key words:** Antimicrobial resistance, Camel, *E. coli*, Gene.

## INTRODUCTION

The intensity of bacterial infection and antimicrobial resistance (AMR) across human and animal populations presents a considerable and growing threat to global health and economic development (Muloi *et al*., 2022). *Escherichia coli* are an important cause of disease worldwide and occur in most mammalian species, including humans and in animals. Camels are known to harbor multidrug-resistant gram-negative bacteria and could be involved in the transmission of various microorganisms to humans (Carvalho *et al*., 2020). In the arid zone camels having, diarrhea and other infectious diseases are considered to be the most causes of economic loss associated with poor growth, medication costs and animal death (Bessalah *et al*., 2016).

Antibiotics are compounds produced by bacteria and fungi which are capable of killing, or inhibiting, competing for microbial species by mechanisms of the remarkable genetic plasticity of bacteria allows them to respond to environmental threats in wide array of action; this includes the presence of antibiotic molecules that can jeopardize their presence. All the bacteria shared the same ecological niche with antimicrobial-producing organisms have evolved ancient mechanisms to withstand the effect of the harmful antibiotic molecule. These bacteria use two major genetic strategies to adapt to the antibiotic “attack”, i) mutations in gene and ii) acquisition of foreign DNA coding for resistance determinants (HGT) through horizontal gene transfer and resistance with in the multidrug-resistant phenotypes could also be acquired where they will be related to extra chromosomal elements acquired from other bacteria in the environment like different types of mobile DNA segments,

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like plasmids, transposons and integrons or adaptive like efflux pumps and other cell surface modifications created by the stress of low-level antibiotics, etc. (Munita *et al.*, 2016).

The *Camelus dromedarius* (Indian camel) is an even-toed ungulate with one hump on its back and camels have been declared as the state animal of Rajasthan in 2014, as they supported 85% of the India's camel population. Rajasthan had the highest camel population across India about 213 thousand in 2019 (20$^{th}$ livestock census 2019, Animal Husbandry Statistics).

A major problem in camel productivity is that the high mortality in camel calves in the first 3 months of life (Tibary *et al.*, 2006). The causes of this high mortality are mainly poor management practice and infectious diseases (Kamber *et al.*, 2001). The diseases and losses in camel population can have devastating effects on the economic success of camel production (Abbas and Omer, 2005). Diarrhea and other infectious diseases are considered to be the main causes of economic loss related to poor growth, medication costs and animal death (Mohammed *et al.*, 2003).

## MATERIALS AND METHODS

### Collection of samples

A total of 70 fecal samples were collected from camels at different villages and also from the clinical complex of CVAS, Bikaner during 2021-22. The samples were collected using a Sterile Hi-Culture Collecting Device (Hi-Media) from rectal swabs. After that, swabs were immediately placed in cooled boxes and then transported to the Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, Bikaner (RAJUVAS), in ice-cooled containers for *E. coli* isolation.

### Isolation and Identification of *E. coli*

Fecal samples were first enriched by inoculating them into nutrient broth and incubating at 37°C for 18-24 hours. Loopful inocula from the nutrient broth were plated on MacConkey agar and incubated at 37°C for 18-24 hours. Lactose-fermenting colonies, appearing as pink-colored, were selected and transferred to Eosin Methylene Blue agar (EMB) plates for further incubation at 37°C for 18-24 hours (Kumar *et al.*, 2022). Colonies exhibiting a green metallic sheen were sub-cultured to obtain pure isolated colonies. These pure colonies were subjected to identification using biochemical tests through the VITEK-2 automated system.

### Hemolytic properties

*Escherichia coli* isolates were propagated on blood agar base supplemented with 5% washed sheep erythrocytes. Blood agar plates were incubated at 37°C for 24 hours and colonies producing clear/partial zones of hemolysis were then recorded as hemolysin-positive. After 24h incubation at 37°C, plates were examined for signs of β-hemolysis (clearing zones around colonies), α-hemolysis (a green-hued zone around colonies), or γ-hemolysis (no halo around colonies).

### Slime production test activity

Biofilm physiology is characterized by increased tolerance to stress, biocides (including antibiotics) and host immunological defenses. Slime production in *E. coli* was determined by cultivation on modified Congo Red Agar plates (CRA) (Mariana *et al.*, 2009). Cultured CRA plates were incubated at 37°C for 24 hours. The production of rough black colonies by slime-producing isolates was used to differentiate them from non-slime-producing *E. coli* isolates (Kagane *et al.*, 2021).

### Antibiogram of the *E. coli* isolates

Antibiotic susceptibility testing was done as per the disc diffusion method (Bauer *et al.*, 1966) following the guidelines of Clinical Laboratory Standard Institute (CLSI) against 20 antibiotics of different classes.

### Antibiotic resistance gene profiles of *E. coli* from fecal samples

PCR method was used for amplification of antibiotic resistance genes in *E. coli* using specific primer sequences. The primer pairs used for the PCR are given in Table 1.

**Table 1:** Details of the primers used for the antibiotic resistance genes of *E. coli* isolates (Dallenne *et al.*, 2010).

| Target gene | Primer | Sequence (5'- 3') | Amplicon size (bp) |
|---|---|---|---|
| *bla*TEM | *blaTEM*-F | TTAACTGGCGAACTACTTAC | 221 |
| | *blaTEM*-R | GTCTATTTCGTTCATCCATA | |
| *Str*A | *StrA*-F | ATGGTGGACCCTAAAACTCT | 893 |
| | *StrA*-R | CGTCTAGGATCGAGACAAAG | |
| *Sul-2* | *Sul-2*-F | CGGCATCGTCAACATAACCT | 721 |
| | *Sul-2*-R | TGTGCGGATGAAGTCAGCTC | |
| *Sul-3* | *Sul-3* F | GAGCAAGATTTTTGGAATCG | 799 |
| | *Sul-3* R | CATCTGCAGCTAACCTAGGGCTTTGG | |
| *aad*A | *aadA*-F | GTGGATGGCGGCCTGAAGCC | 525 |
| | *aadA*-R | AATGCCCAGTCGGCAGCG | |
| *tet*(A) | *tet(A)*-F | GTAATTCTGAGCACTGTCGC | 956 |
| | *tet(A)*-R | TGCCTGGACAACATTGCTT | |
| *tet*(B) | *tet(B)*-F | CTCAGTATTCCAAGCCTTTG | 414 |
| | *tet(B)*-R | ACTCCCCTGAGCTTGAGGGG | |

## RESULTS AND DISCUSSION

A total of 70 fecal samples were collected from camels in the Bikaner region for *E. coli* isolation. The samples were cultured on MacConkey agar and colonies from MacConkey agar were further streaked on Eosin Methylene Blue (EMB) agar for *E. coli* confirmation. All isolates exhibited pink colonies on MacConkey agar, with the characteristic green metallic sheen observed in the growth of cultures on EMB agar plates.

### Biochemical identification of *E. coli* isolates by VITEK-2 system

Biochemical identification of *E. coli* isolates using the VITEK-2 system was conducted of 35 samples (one from every two) with pure cultures at a concentration equal to 0.5 McFarland standards. The VITEK-2 Compact system positively identified *E. coli*, showing excellent confidence levels with an overall probability range of 96-98% (Fig 1).

These findings align with reports by Putra *et al.* (2020), which achieved 100% *E. coli* identification using the automatic VITEK-2 Compact method. Similar confirmation rates were observed by Voidarou *et al.* (2011) and Al-Marri *et al.* (2021). Automation of biochemical tests has significantly reduced identification time, from 5-10 hours to 3 hours for Gram-negative rods, improving reliability and efficiency with minimal manual sample preparation compared to manual miniaturized biochemical tests (Funke *et al.*, 1998).

The VITEK-2 Compact system, known for its widespread use and automation in bacterial identification based on biochemical profiles, utilizes fluorescence and/or colorimetry. Compact plastic cards containing selective or differentiated media or reagents enable bacterial identification in a shorter time than conventional methods (Barry *et al.*, 2003). Ueda *et al.* (2015), Fadlelmula *et al.* (2016); Putra *et al.* (2020) and El-Ghareeb *et al.* (2020) have collectively concluded that the VITEK-2 automated system is accepted, convenient and rapid for the correct identification of bacteria.

Moawad *et al.* (2018) applied the VITEK-2 system for phenotypical confirmation of extended-spectrum β-lactamase-producing isolates. Enterobacteriaceae were identified, with 87.5% being *E. coli*, 6.9% Enterobacter cloacae, 2.8% Klebsiella pneumoniae and 2.8% Citrobacter spp. Other Gram-negative and Gram-positive bacteria were also correctly identified by the VITEK-2 system.

Hara and Miller (2003) reported a correct identification rate of 93% for enteric strains, with 85.9% of gram-negative

RAJUVAS, BIKANER

bioMérieux Customer:
System #:

Laboratory Report

Printed Sep 7, 2021 11:00 IST
Printed by: Labadmin
Report Version: 1 of 1

Isolate: c12-1 (Approved)

Card Type: GN Bar Code: 2411552103327260 Testing Instrument: 00001899E593 (16347)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0405610550527610
Organism Quantity:

**Selected Organism: Escherichia coli**

| Comments: | |
|---|---|

| Identification Information | Card: | GN | Lot Number: | 2411552103 | Expires: | Feb 25, 2022 12:00 IST |
|---|---|---|---|---|---|---|
| | Completed: | Sep 6, 2021 17:32 IST | Status: | Final | Analysis Time: | 4.07 hours |
| Organism Origin | VITEK 2 | | | | | |
| Selected Organism | 99% Probability | | Escherichia coli | | | |
| | Bionumber: | 0405610550527610 | | | Confidence: | Excellent identification |
| SRF Organism | | | | | | |
| Analysis Organisms and Tests to Separate: | | | | | | |
| Analysis Messages: | | | | | | |
| Contraindicating Typical Biopattern(s) | | | | | | |

Biochemical Details

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | - | 4 | PyrA | - | 5 | IARL | - | 7 | dCEL | - | 9 | BGAL | + |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTp | - | 13 | dGLU | + | 14 | GGT | - | 15 | OFF | + |
| 17 | BGLU | - | 18 | dMAL | + | 19 | dMAN | + | 20 | dMNE | + | 21 | BXYL | - | 22 | BAlap | - |
| 23 | ProA | - | 26 | LIP | - | 27 | PLE | - | 29 | TyrA | + | 31 | URE | - | 32 | dSOR | + |
| 33 | SAC | + | 34 | dTAG | - | 35 | dTRE | + | 36 | CIT | - | 37 | MNT | - | 39 | 5KG | - |
| 40 | ILATk | + | 41 | AGLU | - | 42 | SUCT | + | 43 | NAGA | - | 44 | AGAL | + | 45 | PHOS | - |
| 46 | GlyA | + | 47 | ODC | + | 48 | LDC | + | 53 | IHISa | - | 56 | CMT | + | 57 | BGUR | + |
| 58 | O129R | + | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | - | | | |

Installed VITEK 2 Systems Version: 08.02
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

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**Fig 1:** Biochemical test identification report of *E. coli* by vitek2 system.

enteric strains identified at probability levels ranging from excellent to good.

**Hemolysin property of *E. coli* isolates**

In the present study 32 out of 70 camel *E. coli* isolates (45.71%) produces partially hemolysis on sheep blood agar (Fig 2). Red blood cell of the host organism is lysed due to the presence of hemolysin gene which in turn helps in the spread of the pathogen in the host blood (Bashar *et al.*, 2011). The hemolytic activity of *E. coli* is related to the presence of hemolysin genes. Dadheech *et al.* (2016) and Osman *et al.* (2018) had 100% recovery of *E. coli* isolates producing β-hemolysis on blood agar.

The mechanism by which *E. coli* causes diarrhea does not rely on the hemolytic nature of *E. coli* isolates but is due to toxin produced by its strains. Roy *et al.* (2006) was found 45.16% *E. coli* isolates produced hemolysis on sheep blood agar. Similarly, Shittu *et al.* (2010) *E. coli* isolates produces (45%) both α and β-hemolysis. Hemolysin production was determined to differentiate between the virulent hemolytic isolates and the avirulent non-hemolytic isolates. According to Osman *et al.* (2018) 96.66% *E. coli* isolates producing α-hemolysin; 3.33% isolate produced β-hemolysin instead of α–hemolysin.

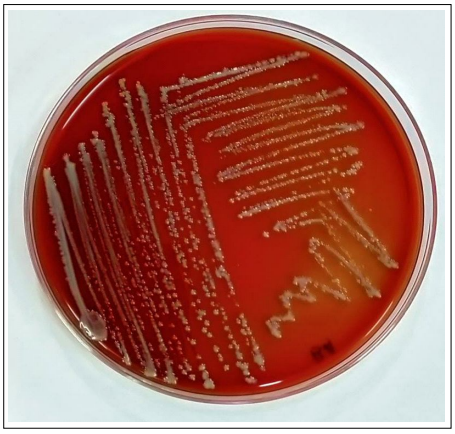


**Fig 2:** Isolation of *E. coli* on blood agar plate.

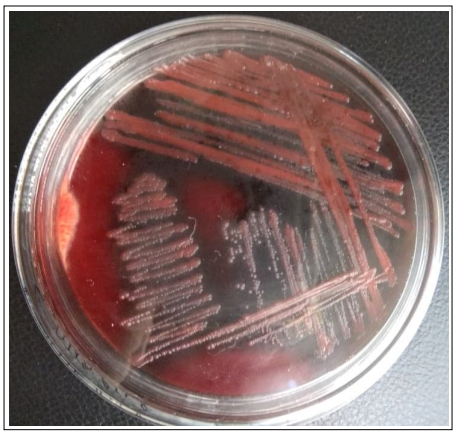


**Fig 3:** Biofilm producing *E. coli* on congo red plate.

Hemolytic strains are more virulent than non-hemolytic strains (Vaish *et al.*, 2016). α-Hemolysin, also known as cytotoxic necrotizing factor, is produced by invasive strains of *E. coli*, which sets the pace for the pathogenesis of renal disease and enhances virulence in a number of clinical infections (Herlax *et al.*, 2010). In another cross-sectional study Singh *et al.* (2021), Koutsianos *et al.* (2021); Grakh *et al.* (2021) found none of the *E. coli* isolates as hemolytic while as Adam *et al.* (2022) found one isolate produce hemolysis on sheep blood agar. Al Humam (2016) reported only (9.6%) camel *E. coli* isolates showed β-hemolysis on blood agar plates.

**Slime production test of *E. coli* isolates**

Biofilm formation was measured to determine the ability of isolates to colonize surfaces for environmental survival and persistence and a virulence factor. The ability to form biofilm as determined by slime production (assessed by Congo red uptake and an adherence assay in glass tubes) revealed a heterogeneity among the isolates, ranging from weak and moderate to strong biofilm formation. 32 out of 70 *E. coli* isolates of camel (45.71%) were positive as biofilm formation (Fig 3). Bacteria within biofilms can withstand host immune responses and are less susceptible to antimicrobials and disinfectants (Jenkins, 2018).

Ahmad *et al.* (2009) reported a result of 76.92% in the growth of brick-red black-colored colonies, indicative of pathogenic *E. coli*. The Congo red binding ability serves as a phenotypic marker for distinguishing between *E. coli* strains associated with septicaemia (invasive) and those that are not. It is also an epidemiological marker useful for discriminating pathogenic strains from commensals.

In a study by Yadav *et al.* (2014), results indicated a Congo red binding ability of 92.86% in *E. coli* isolates. Similarly, Adam *et al.* (2022) found that all isolates, accounting for 100%, exhibited positive results in the Congo red (CR) binding test. The Congo red agar test (CRA test) is considered an essential parameter for monitoring virulence characteristics of *E. coli* in both human and animal communities. In line with these findings, Kagane *et al.* (2021) observed 100% positivity in the Congo red binding test among their *E. coli* isolates.

**Antibiogram study for *E. coli* isolates**

In the present investigation, all 70 *E. coli* isolates were subjected to antibiotic sensitivity test using 20 different antibiotics. The response of organisms was interpreted as sensitive, intermediate and resistant based on the manufacturer guidelines (Himedia). The antibiotics tested belonged to various groups *i.e.* β-lactam antibiotics, aminoglycosides, glycopeptides, phenicoles, quinolones, tetracyclines, sulphonamide, RNA synthesis inhibitor, polypeptides, macrolides and lincosamides. β-lactam antibiotics included penicillins, cephalosporins, monobactums and carbapenems.

In β-lactum group (cell wall sysnthesis inhibitors) all the isolates were resistant to Penicillin-G. In the present study,

94% isolates from were resistant to penicillin. *E. coli* isolates were more resistant to ampicillinsulbactam, amoxycillin-sulbactam 76.15% and 85.71%. *E. coli* isolates were least resistant with 51.42% and 54.14% resistivity to third and fourth generation cephalosporins respectively. 22.57% *E. coli* isolates were sensitive and 62.42%isolates showed higher resistance to gentamicin (Table 2).

Nuesch-Inderbinen *et al.* (2020) reported resistance to tetracyclinefrom African camel was detected most frequently (11.7%), followed by ampicillin and streptomycin (both 10.5%) and sulfamethoxazole/trimethoprim (9.9%) and one isolatesshowed intermediate resistance to streptomycin, remaining were sensitive to amoxycillin/clavulanic acid, ciprofloxacin and kanamycin. Bessalah *et al.* (2016) observed that all *E. coli* isolates were sensitive to amikacin, chloramphenicol, ciprofloxacin, gentamicin and ceftiofur. The highest frequency of resistance was observed against tetracycline, ampicillin and streptomycin (52.8%, 37.1% and 21.4%, respectively). Resistance to sulfisoxazole and trimethoprim–sulfamethoxazole was noted in almost 20% and 18.5% of *E. coli* isolates, respectively. A lower percentage of resistance was identified against amoxicillin/clavulanic acid (2.8%), ceftriaxone (1.4%) and cefoxitin (2.8%).

Momtaz *et al.* (2013) reported higher resistance rates to gentamicin and streptomycin (62.42% and 67.32%, respectively) in *E. coli* isolates, which aligns with our findings. Adelaide *et al.* (2008) detected elevated resistance levels for tetracycline (75.9%) and cotrimoxazole (72.4%). Subedi *et al.* (2018) found that the maximum *E. coli* strains were resistant to ampicillin (98%), followed by co-trimoxazole (90%), with intermediate resistance to colistin (50%) and the highest sensitivity observed against gentamicin (84%).

The frequency of sensitivity of most susceptible antimicrobial agents observed chloramphenicol (71.14%), followed by enrofloxacin (60%), sulphadiazine (48.575%) and co-trimoxazole (48.28%) in camel *E. coli* isolates. Less susceptibleor intermediate antibiotics areoxytetracycline (37.49%), ciprofloxacin (31.42%) and gentamicin (22.57%). This finding is similar to Bessalah *et al.* (2016) who also reported chloramphenicol as most sensitive antimicrobial agent. This finding is also similar with those of previous reports on isolates associated with genital and mastitis infections of camels (Mshelia *et al.*, 2014). Azad *et al.* (2017) reported 36% sensitivity to gentamicin and 100% to erythromycin. Bhave *et al.* (2019) revealed high degree of resistance to commonly used antimicrobials, namely tetracycline (95.89%), trimethoprim (89.04%), colistin (82.88%) and ciprofloxacin (54.11%). However, further studies would be required in order to correlate the use of antimicrobials with the fecal carriage of AMR in camels.

### Profiling of antibiotic resistance associated genes in *E. coli* isolates

The present study was conducted to investigate detection of some genes responsible for imparting antibiotic resistance to the *E. coli* isolates obtained from the camel fecal samples. The outcome of this study resulted as presence of $bla_{TEM}$, *sul*2, *sul*3, *str*A, *aad*A, *tet*A, *tet*B in *E. coli* isolates. The *bla*TEM gene imparts resistance against various β-lactam antibiotic like penicillin and ampicillin, *Sul2* gene imparts resistance against sulfamethoxazole antibiotics, *str*A against

**Table 2:** Antibiotic sensitivity for *E. coli* isolates from camel.

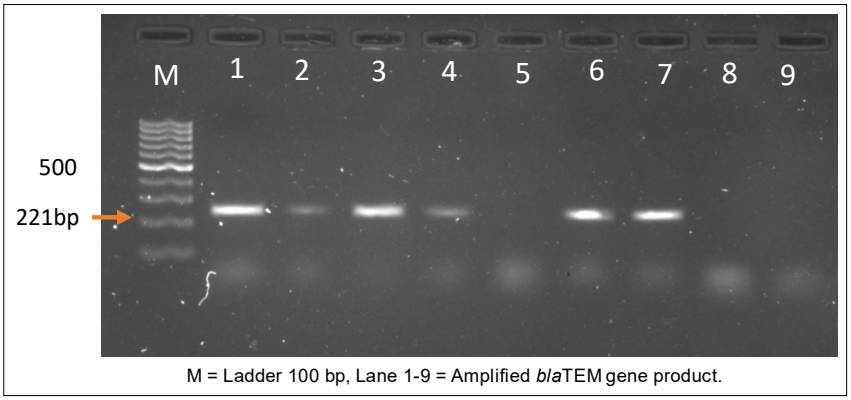
| Antibiotic name (30 mcg) | Sensitive | Intermediate | Resistance |
|---|---|---|---|
| E (Erythromycin) | 0% | 17.32% | 82.68% |
| C (Chloramphenicol) | 71.14% | 8.51% | 20.35% |
| OF (Ofloxacin) | 28.72% | 18.71% | 52.57% |
| CL (Colistin) | 11.43% | 48.57% | 40.00% |
| P (Penicillin) | 0% | 5.62% | 94.38% |
| CTX (Cefotaxime) | 14.28% | 25.72 | 60.00% |
| CIP (Ciprofloxacin) | 31.42% | 22.87% | 45.71% |
| TE (Tetracycline) | 31.42% | 1.73% | 66.85% |
| GEN (Gentamycin) | 22.57% | 15.01% | 62.42% |
| CTR (Ceftriaxone) | 42.85% | 31.42% | 51.42% |
| COT (Co-trimoxazole) | 48.28% | 11.72% | 40.00% |
| MO (Moxifloxacin) | 17.14% | 31.42% | 51.42% |
| S (Streptomycin) | 8.57% | 24.11% | 67.32% |
| CMC (Cefixime clavulanic acid) | 0% | 28.57% | 71.43% |
| SZ Sulphadiazine | 48.57% | 18.71% | 32.72% |
| A/S (Ampicillin sulbactam) | 17.36% | 6.49% | 76.15% |
| Oxytetracycline | 37.49% | 9.66% | 52.85% |
| AMS (Amoxicillin sulbactam) | 8.57% | 5.72% | 85.71% |
| CPS (Cefpirome clavulanic acid) | 8.57% | 37.29% | 54.14% |
| Enrofloxacin | 60% | 0% | 40% |

streptomycin, *tet*A and *tet*B for tetracycline, *aad*A gene encodes for aminoglycosides adenyl transferase enzyme which imparts resistance to amino glycosides antibiotics such as streptomycin.

In the present study the *bla*TEM gene was detected in fifty six out of seventy (80%) camel isolates (Fig 4), for *sul*2 gene eighteen out of seventy (25.71%) *E. coli* isolates (Fig 5), *Sul*3 gene was obtained as twenty two out of seventy (31.42%) isolates, twenty nine out of seventy (42.85%) camel isolates for *strA* gene (Fig 6), twenty eight of seventy (40%) isolates carried the *aadA* gene (Fig 7) and twenty two out of seventy (31.42%) were found positive for *tetB* gene (Fig 8). *tet*A, *tet*C, *tet*D, *tet*E, gene was not found in any isolate (Table 3).

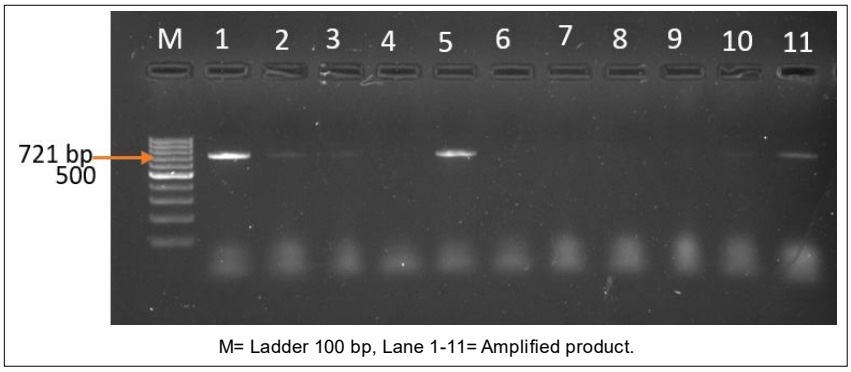
Bhave *et al.* (2019) observed *bla*TEM (20%), *bla*CTX-M (40%) and *bla*OXA (6.66%) in *E. coli* isolates respectively. Abd El Tawab *et al.* (2016) detected *bla*TEM gene in the genomic DNA (100%) in all isolates and 56.25% in plasmid DNA. While *bla*SHV gene was detected in the genomic DNA of (37.50%) and in plasmid DNA (28.12%) isolates. Carvalho *et al.* (2020) and Nuesch-Inderbinen *et al.* (2020) isolated *bla*CTX-M-1 producing *E. coli* from camel. Saidani *et al.* (2019) reported *bla*TEM gene in 18% isolates and the occurrence of CTX-M-15- and CTX-M-1-producing Enterobacteriaceae in camel.

Resistance to sulfonamides was due to the horizontal spread of resistance genes, expressing drug-insensitive variants of the target enzymes dihydropteroate synthase and



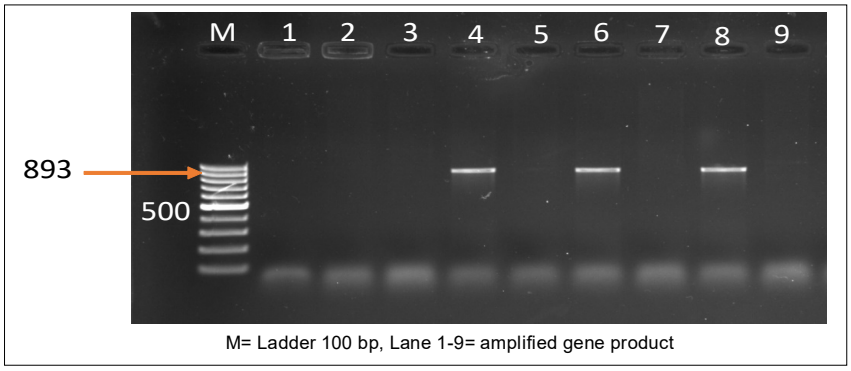

M = Ladder 100 bp, Lane 1-9 = Amplified *bla*TEM gene product.

**Fig 4:** Amplification of *bla*TEM gene from camel isolates.



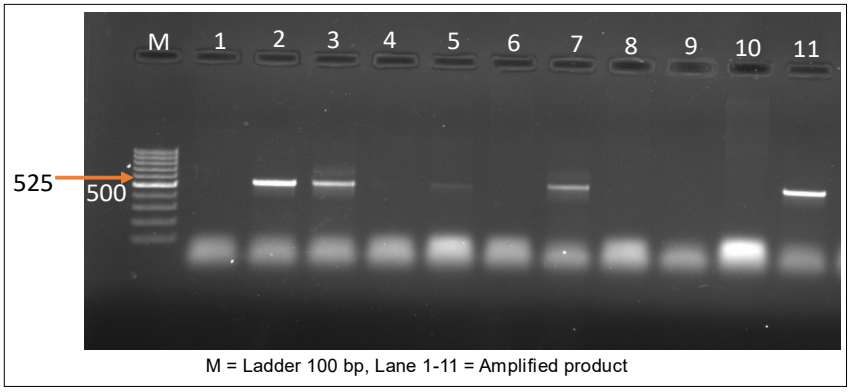

M= Ladder 100 bp, Lane 1-11= Amplified product.

**Fig 5:** Amplification of *sul*2 gene from camel isolates.



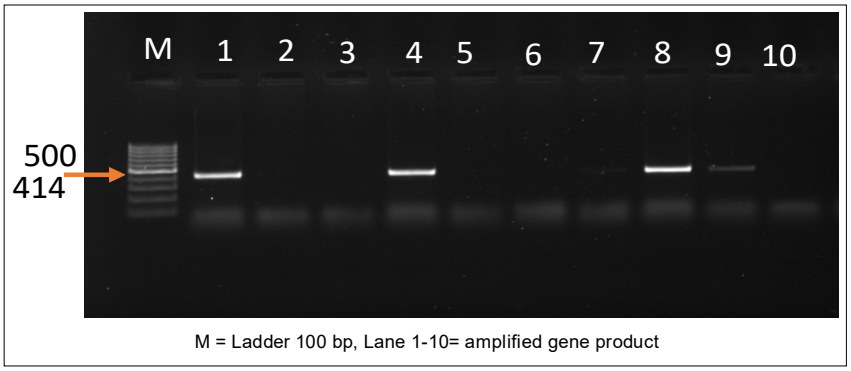

M= Ladder 100 bp, Lane 1-9= amplified gene product

**Fig 6:** Amplification of *str*Agene from camel isolates.




M = Ladder 100 bp, Lane 1-11 = Amplified product

**Fig 7:** Amplification of *aad*A gene from camel isolates.




M = Ladder 100 bp, Lane 1-10= amplified gene product

**Fig 8:** Amplification of *tet*Bgene from camel isolates.

**Table 3:** Prevalence of antibiotic resistance genes in *E. coli* isolates.

| Class of antibiotics | Antibiotic resistance gene | Total no. of *E. coli* isolates camel | Prevalence of antibiotic resistance gene (%) camel |
|---|---|---|---|
| Beta - Lactams | *Bla*TEM | 56 | 80% |
| Sulfonamides | *Sul*-2 | 18 | 25.71% |
| | *Sul*-3 | 22 | 31.42% |
| Aminoglycosides | *str*A | 29 | 42.85% |
| | *aad*A | 28 | 40% |
| Tetracyclines | *tet*(A) | - | - |
| | *tet*(B) | 22 | 31.42% |

dihydrofolate reductase, for sulfonamide and trimethoprim, respectively.In present study, a low resistance was observed in camel isolates for *sul*2 and *sul*3 gene. Ngbede *et al.* (2021) detected *Sul*2 and *Sul*3 in 71.42% in camel *E. coli* isolates from Nigeria. Ben Sallem *et al.* (2012) isolated *Escherichia coli* with 100% prevalence of *sul*2 gene from healthy food-producing animals in Tunisia. Rawat *et al.* (2022) observed sulfonamide resistance gene *sul3* (44%), *sul2* (28%) among the poultry isolates from North India. 36.9%; *sul*1 (1 isolate), *sul*2 (4 isolates).

Lanz *et al.* (2003) highest tetracycline resistance phenotypes observed in the *E. coli* isolates were linked to the presence of the *tet*A gene (63.2%) and considered to be the gene commonly identified followed by *tet*B (34.5%) in the *E. coli* isolates. They are among the widest spread *tet* genes found in Enterobacteriaceae and their occurrence was within the range reported by other investigators.

## CONCLUSION

The results of our study underscore the significance of multidrug-resistant *E. coli* in causing diarrhea and other diseases in camels, potentially impacting their health and growth rates. *E. coli* poses a global challenge concerning antimicrobial multidrug resistance, affecting both humans and animals. Our study revealed a high prevalence of antibiotic resistance among *E. coli* strains, both phenotypically and genotypically.

The identified virulence factors, hemolysin and biofilm adherence, further emphasize the pathogenic potential of *E. coli*. Our findings suggest that dromedary camels in

Bikaner may serve as reservoirs for *E. coli* strains exhibiting resistance to antimicrobials commonly used in human and veterinary medicine. Additionally, our study indicates the potential presence of ESBL-producing *E. coli* in camels, which could pose a risk of transmission to humans through direct contact or the food chain.

Therefore, implementing improved management practices, coupled with rapid and accurate diagnostics, is crucial. Judicious use of selected drugs based on antimicrobial susceptibility testing is essential to mitigate the spread of antimicrobial resistance and minimize the potential transmission of resistant strains from camels to humans.

**Conflict of interest**

All authors declared that there is no conflict of interest.

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