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Phylogenetic Grouping and Antibiogram of ESBL-Producing *Escherichia coli* Isolated from Bovine Mastitis

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ABSTRACT

Escherichia coli* (*E. coli*) is a major cause of mastitis in dairy cattle. Multidrug resistant *E. coli* poses an important public health threat due to the widespread antimicrobial resistance genes that can transmit to human pathogenic *E. coli* through consuming contaminated milk. This study aimed to determine the phylogenetic groups and antimicrobial resistance profile of *E. coli* isolates from bovine clinical mastitis cases. A total of 380 milk samples were collected from dairy farms in Jordan. *E. coli* was cultured using routine bacterial culture methods and identified initially based on morphological and biochemical characteristics. Suspected bacteria were then confirmed by detecting 16s rRNA gene using traditional PCR methods. The phylogenetic grouping was performed using triplex PCR. The antimicrobial resistance profile was determined using a disc diffusion test followed by the double disc diffusion test to detect ESβLs-producing strains and the syndromic multiplex PCR (mPCR) to detect ESβLs genes. A total of 74 (19.4%) of *E. coli* strains were isolated from the collected milk samples. The majority of the isolates (52 or 70%) belonged to phylogenetic group A, followed by 13 (17.5%), 7 (9.5%), and 2 (2.7%) isolates that belonged to phylogenetic groups D, B1, and B2, respectively. Sixty-three (85.1%) isolates showed resistance to at least 2 antimicrobial agents, with the highest resistance rates detected against amoxicillin (94.6%), tetracycline (75.7%), and streptomycin (66.2%). Sixty-three (85.1%) isolates showed resistance to at least 2 antimicrobial agents and 23 (31%) isolates showed resistance to at least 2 beta lactam antimicrobial agents. Twenty-two out of 23 (95.6%) of multi-beta lactam resistant isolates were ESβL positive, 22 isolates (100%) carried $bla_{CTX\text{-}M}$ gene, and 20 isolates (86.9%) carried the bla_{TEM} gene, while none of the isolates carried the bla_{SHV} gene. The results of this study demonstrate a high prevalence of multidrug resistant, ESβLs-producing *E. coli* in bovine mastitis, which may represent a serious threat to public health due to the high risk of dissemination of antimicrobial resistance genes through consumption of contaminated milk.

Keywords: antimicrobial resistance; bovine mastitis; ESβLs; Escherichia coli; phylogenetic analysis

INTRODUCTION

Mastitis is inflammation of the mammary tissue due to the invasion of the mammary glands with pathogenic microorganisms (Hota *et al.*, 2020). The disease is associated with significant economic losses due to the reduction of milk production, costs incurred by discarded milk, treatment, veterinary consultation, prevention efforts, and, in extreme situations, the loss of affected animals due to premature culling, euthanasia, or death. Clinically, mastitis can be presented as clinical or subclinical based on the appearance and severity of clinical signs (Hota *et al.*, 2020). While in subclinical mastitis, no signs can be detected, clinical mastitis is characterized by the appearance of local signs such as swollen, painful, and worm quarters. These abnormal milk secretions may contain clumps that become watery, bloody, or yellowish (Antanaitis *et al.*, 2022).

In severe cases, systemic clinical signs such as fever, depression, anorexia, and death are due to septicemia or endotoxemia (Antanaitis *et al.*, 2022). The majority of mastitis cases are caused by *Staphylococcus aureus*, *Streptococcus agalactiae*, Coliforms including *Escherichia coli* and *Klebsiella*, as well as coagulase-negative *Staphylococci* (Blum & Leitner, 2013). *E. coli* is a widespread gut commensal bacterium of humans and warm-blooded animals (Blum & Leitner, 2013). *E. coli* species include pathogenic and non-pathogenic strains (Blum & Leitner, 2013). Environmental contamination with fecal materials in dairy farms is the main source of mastitis caused by *E. coli* (Blum & Leitner, 2013).

Imprudent use of antimicrobials in dairy farms has raised concerns about the potential emergence of multidrug antimicrobial resistant *E. coli* that could spread to human populations via cross-contamination or consumption of contaminated milk (Lei *et al.*,

2010; Aworh *et al.*, 2021). Extended spectrum beta lactamases (ESβLs) are a group of enzymes that exhibit resistance to most beta-lactam antimicrobials (penicillin, cephalosporin, and oxyimino-monobactam aztreonam) (Castanheira *et al.*, 2021). Several recent studies have shown the existence of ESβL genes in *E. coli* strains isolated from bovine mastitis in several parts of the world (Ohnishi *et al.*, 2013; Timofte *et al.*, 2014; Su *et al.*, 2016). However, to the best of our knowledge, no studies documented the isolation of ESβL producing *E. coli* from mastitis cases in Jordan. Therefore, the objectives of this study were to isolate *E. coli* from bovine clinical mastitis cases and to determine the phylogenetic groups, antimicrobial resistance profile, and the prevalence of extended spectrum beta lactamase (ESβLs) genes in the isolated strains.

MATERIALS AND METHODS

Ethical Approval

This study was reviewed and approved by the Research Scientific Committees at the Basic Veterinary Medical Department, Faculty of Veterinary Medicine and Jordan University of Science and Technology and granted through Project number (56/2017). The study was also approved by the Institutional Animal Care and Use Committee of Jordan University of Science and Technology (approval number: 16/4/12/289).

Sample Collection

A total of 380 milk samples were collected from lactating cows affected with clinical mastitis from January to December 2016. Cows belonged to several dairy farms in central and northern Jordan (Al Muwaqqar area, Al Dulial valley, and Irbid). Farms were selected based on their willingness to participate in the study. Milk samples were collected from cows with new intrauterine infections that developed systemic or local signs of infection or both. To be included in the study, affected cows must not have had any systemic or local application of antibiotics within the last 2 weeks. Cows with signs of chronic mastitis, such as udder fibrosis, cows with repeated episodes of mastitis within the last 6 weeks, and cows with a persistent increase in milk somatic cells above 250,000 cells/mL (based on farm records), were excluded. Before sample collection, cows were subjected to general physical examination (rectal temperature, heart, and respiration rates), palpation of the udder and teats (for swelling, pain, heat, and redness) and inspection of milk secretion (for any change in milk color, consistency, or abnormal contents such as clumps or blood). Quarter milk samples were collected aseptically (Gautam *et al.*, 2019; Mbindyo *et al.*, 2020). Briefly, the teats were cleaned and dried with a single towel, and the teats were then disinfected with a cotton pad moistened with 70% alcohol. The first 3 squirts of milk were discarded, followed by collecting 10 mL of milk into a previously labeled, sterile glass, and plain test tubes. Samples were kept cooled in an ice box during transportation to the laboratory, where bacteriological examination was performed within 2-4 hours after sample collection.

Bacterial Isolation and Identification

A loopful of milk (~10 μL) from each sample was streaked on a blood agar media (5% sheep's blood; Oxoid, England) and MacConkey agar plate (Oxoid, England). After the plates were incubated at 37 °C for 18–24 hours, suspected colonies were streaked on Eosin Methylene Blue agar (DIFCO, France) and incubated overnight at 37 °C. *E. coli* colonies were then characterized by Gram stain and biochemical reactions (Bonnet *et al.*, 2019). Confirmation of *E. coli* isolates was carried out by detection of 16SrRNA gene using traditional PCR methods and commercially available primers (Fahim *et al.*, 2019) (Table 1). Colonies from the confirmed *E. coli* isolates were incubated in cryostat vials containing Muller Hinton Broth media (MHB, Biolab, Hungary) with 20% glycerol and preserved at – 70 °C for further analysis.

Preparation of DNA Template

Bacterial DNA was isolated by boiling freezing method as described previously (Liu *et al.*, 2014). Briefly, bacterial isolates were grown overnight on tryptic soya agar at 37 °C, then 3 well-isolated colonies were picked and suspended in 100 μL nuclease free water. The suspension was lysed by 15 minutes of boiling in a heat block, followed by freezing for 5 minutes, then centrifuged at 14,000 rpm for 15 minutes to separate the debris of the bacterial cells. The supernatant was collected and kept at -20 °C for amplification reactions as a template.

Phylogenetic Grouping of *E. coli* Isolates

Phylogenetic grouping of *E. coli* strains was determined using triplex PCR following the protocol described by Clermont *et al*. (2000). The methodology

Table 1. Target genes, primer sequences, and fragment size (bp) used in the characterization of *Escherichia coli* isolates from bovine mastitis

Target genes	Primer sequence 5'-3'	Size (bp)
16_S rRNA	GACCTCGGTTTAGTTCACAGA CACACGCTGACGCTGACCA	585
ChuA	GACGAACCAACGGTCAGGAT	279
	TGCCGCCAGTACCAAAGACA	
YjaA	TGAAGTGTCAGGAGACGCTG	211
	ATGGAGAATGCGTTCCTCAAC	
TspE4.C2	GAGTAATGTCGGGGCATTCA	152
	CGCGCCAACAAAGTATTACG	
bla_{TEM}	CATTTCCGTGTCGCCCTTATTC	800
	CGTTCATCCATAGTTGCCTGAC	
bla_{SHV}	AGCCGCTTGAGCAAATTAAAC	713
	ATCCCGCAGATAAATCACCAC	
bla_{CTX-M}	CGCTTTGCGATGTGCAG	550
	ACCGCGATATCGTTGGT	

involved the amplification of two specific genes (*chuA* and *yjaA*) and an unidentified DNA fragment (TSPE4.C2), enabling the classification of *E. coli* strains into phylogenetic groups (A, B1, B2, or D). The PCR reaction was conducted using a 25-µL reaction mixture, consisting of 4 µL of ready-to-load 5X Hot FIREPol® Blend Master Mix, which contains Hot FIREPol® DNA Polymerase, a proofreading enzyme, 5X Blend Master Mix Buffer, 12.5 mM $MgCl_2$, and 2 mM dNTPs. Additionally, 20 pmol of each primer listed in Table 1 and 5 µL of genomic DNA were added. The final volume was adjusted to 25 µL using nuclease-free water. The PCR amplification was performed using a BIO-RAD thermocycler with the following cycling conditions: initial denaturation at 94 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds. A final extension step was conducted at 72 °C for 7 minutes. To determine the size of the amplified products, a 50 bp DNA ladder (New England Biolabs, USA) was utilized as a molecular size marker.

Detection of Resistance Genes

The following resistance genes bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ were targeted in confirmed *E. coli* isolates using commercially available primers (Table 1). The PCR reaction was performed using a 25 µL mixture containing 4 µL of ready 5X Hot FIREPol® Blend Master Mix (Thistle Scientific, England), 12.5 mM $MgCl_2$, 2 mM dNTPs, 20 pmol of each primer (Table 1), 5 µLof genomic DNA, and nuclease free water to a total volume of 25 µL. The PCR reaction was carried out using a thermocycler (BIO-RAD, USA) according to the following settings: denaturation at 94 °C for 5 minutes; then 30 cycles of 30 seconds at 94 °C, 30 seconds at 55 °C, and at 72 °C for 30 seconds; and the final extension at 72 °C for 7 minutes. A 50 bp DNA ladder (BioLabs, USA) was used as a size marker.

Antimicrobial Susceptibility Test

Fourteen different antimicrobial agents (Bioanalyse, Turkey) were used in the antimicrobial susceptibility test as follows: amoxicillin (AX; 25 µg), gentamicin (CN; 10 µg), trimethoprim-Sulphamethoxazole (SXT; 1.25/23.75 µg), tetracycline (TE; 15 µg), enrofloxacin (ENO; 5 µg), streptomycin (S; 10 µg), kanamycin (K; 30 µg), neomycin (N; 30 µg), chloramphenicol (C; 30 µg), ciprofloxacin (CIP; 5 µg), cefoperazone (CEP; 75 µg), cefepime (FEP; 30 µg), cefotaxime (CTX; 30 µg), and ceftazidime (CAZ; 30 µg).

The antimicrobial susceptibility testing was performed using the modified Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Merck, Germany) according to the Clinical and Laboratory Standards Institute guidelines (Weinstein & Lewis, 2020). Briefly, 0.5 McFarland (10^8 cfu/mL) aliquot of bacterial broth culture was streaked on Mueller Hinton Agar medium. The spreading was performed in 3 perpendicular and 3 oblique directions to ensure confluent growth. The plates were covered and left on the counter for 10 minutes at room temperature (25 °C); then, the antimicrobial discs were applied firmly on the surface of the medium approximately 0.5 inch apart. The plates were incubated at 37 °C for 24 hours. Susceptibility was assessed by measuring the diameter of the inhibition zone around the discs using a ruler and reported as susceptible, intermediate, and resistant.

Detection of ESβLs
Double Disc Diffusion Test

ESβLs detection was performed using the double disc diffusion test according to previously reported methods (Weinstein & Lewis, 2020). Briefly, colonies from *E. coli* isolates were inoculated on Muller Hinton agar with the application of ceftazidime (30 µg) and cefotaxime (30 µg) antibiotic discs alone and in combination with clavulanic acid (10 µg). The inhibition zones of ceftazidime (30 µg) and cefotaxime (30 µg) antibiotic discs alone and in combination with clavulanic acid (10 µg) were then measured and compared after incubation at 37 °C for 24 hours. An increase of ≥ 5 mm of the inhibition zone in the case of clavulanic acid addition was recorded as a positive result.

Detection of ESβLs Encoding Genes

ESβLs producing strains were subjected to PCR targeting ESβLs encoding genes bla_{TEM}, $bla_{SHV,}$ and $bla_{CTX\text{-}M}$ using commercially available primers (Table 1) and previously published methods (Bubpamala *et al.*, 2018). Briefly, for detection of bla_{TEM} and bla_{SHV} genes, a multiplex PCR was carried out using the following settings: initial denaturation at 94 °C for 10 minutes, followed by 30 cycles of 94 °C for 40 seconds, 60 °C for 40 seconds and 72 °C for 1 minute; and a final elongation step at 72 °C for 7 minutes. For the detection of $bla_{CTX\text{-}M}$, the PCR conditions were as follows: initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, 45 °C for 1 minute, and 72 °C for 1 minute; and a final elongation step at 72 °C for 10 minutes. The amplified PCR products were loaded into electrophoresis (2% agarose) gel in 0.5X TBE buffer containing Ethidium bromide (0.5 µg/mL) and finally visualized under UV light. A 100 bp DNA ladder (BioLabs, USA) was used as a size marker.

Data Analysis

Descriptive analysis was used using Microsoft Excel sheets (Windows 10). Data were presented as percentages. Pearson's chi-square test was used to compare different phylogenetic groups for distribution of the antimicrobial resistance and presence of ESβL genes of the isolates. The statistical significance level was set at 0.05.

RESULTS

Bacterial Isolation

A total of 380 milk samples from bovine clinical mastitis cases were tested. From this, 74 (19.4%)

samples revealed *E. coli* isolates (Figure 1). Distributions of the isolates according to geographic regions of the study were: 48 isolates were from Al Dulial Valley, 15 isolates were from Irbid, and 11 isolates were from Al Muwaqqar.

Phylogenetic Grouping of *Escherichia coli* Isolates

Analysis of PCR results for determination of phylogenetic groups showed that out of 74 *E. coli* isolates, the majority 52 (70.3%) isolates were found belong to phylogenetic group A, followed by 13 (17.5%) isolates belonged to phylogenetic group D, 7 (9.5%) isolates belonged to phylogenetic group B, and 2 (2.7%) isolates belonged to phylogenetic group B2 (Table 2 and Figure 2-5).

Antimicrobial Susceptibility Profile, ESβL Production, and Resistance Genes

The antimicrobial susceptibility profiles of 74 *E. coli* isolates from bovine clinical mastitis cases are presented in Figure 6. Amoxicillin, tetracycline, and streptomycin had the highest resistance rate among *E. coli* isolates (94.6%, 75.7%, and 66.2%, respectively), while ciprofloxacin, enrofloxacin, and cefepime showed the lowest resistance rates (24.3%, 24.3%, and 28.4%, respectively). Sixty-three (85.1%) isolates showed resistance to at least 2 antimicrobial agents. Twenty-three (31%) isolates showed resistance to at least 2 beta lactam antimicrobial agents. Twenty-two out of 23 (95.6%) of multi-beta lactam resistant isolates were ESβL positive. All ESβL positive isolates carried the $bla_{CTX\text{-}M}$ gene (Figure 7), 20 isolates (86.9%) carried the bla_{TEM} gene (Figure 8), while none of the isolates carried the bla_{SHV} gene.

DISCUSSION

E. coli is commonly isolated from bovine clinical mastitis cases in Jordan. In this study, *E. coli* was isolated from 19.47% (74/380) of clinical mastitis cases. These results are in agreement with the results reported by Alekish *et al*. (2013) (16%) but higher than those reported by Ismail & Abutarbush (2020) (6.48%). The variation in the prevalence of *E. coli* as a cause of mastitis is most likely due to differences in farm management practices, environmental factors, and differences attributed to seasonality between these studies.

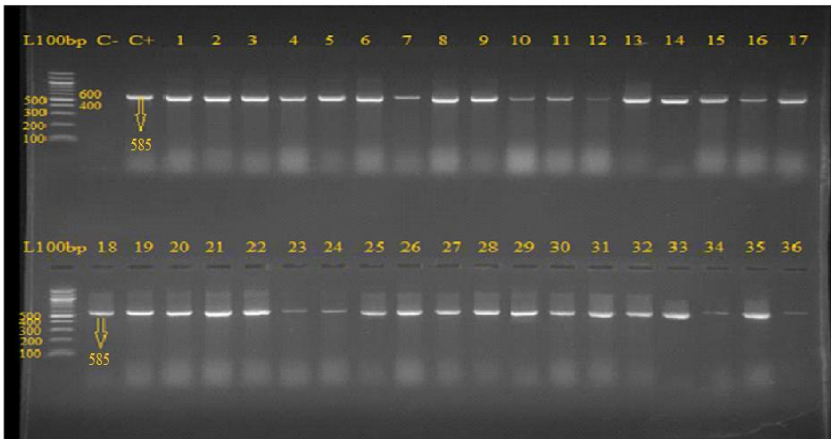

Figure 1. Agarose gel electrophoresis of PCR products targeting 16s rRNA gene (585 bp) of *Escherichia coli* isolates. Lane L= DNA molecular ladder (100 bp); Lane C- = negative control (nuclease free water), Lane C+ = *E. coli* reference strain (ATCC 22925). Lanes 1-36 are *E. coli* isolates from bovine clinical mastitis milk samples from different geographical regions; Lanes 1-23 are from Al Dulial valley, Lanes 24-30 are from Irbid, and Lanes 31-36 are from Al Muwaqqar.

Table 2. Distribution of *Escherichia coli* isolates among different phylogenetic groups in different geographical regions (N=74)

Phylogenetic group	Genomic characteristics of the phylogenetic group	Number of *E. coli* isolates in different geographical areas	Total number and (%) of phylogenetic group
A	*chuA* -, TSPE4.C2 -	Al Muwaqqar (8 isolates) Al Dulial (35 isolates) Irbid (9 isolates)	52 isolates (70.3)
B1	*chuA* -, TSPE4.C2 +	Al Muwaqqar (1 isolate) Al Dulial (4 isolates) Irbid (2 isolates)	7 isolates (9.5)
B2	*chuA* +, *yjaA* +	Al Muwaqqar (0 isolate) Al Dulial (2 isolates) Irbid (0 isolate)	2 isolates (2.7)
D	*chuA* +, *yjaA* -	Al Muwaqqar (2 isolates) Al Dulial (7 isolates) Irbid (4 isolates)	13 isolates (17.5)

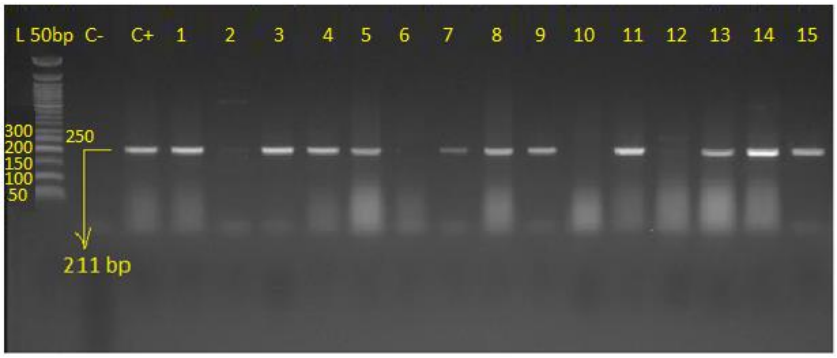

Figure 2. Agarose gel electrophoresis of PCR products for phylogenetic group A of *Escherichia coli* isolates. Lane L= DNA molecular ladder (50 bp); Lane C- = negative control (nuclease free water); Lane C+ = *E. coli* reference strain (ATCC 35344). Lanes 1-15 are *E. coli* isolates belonged to phylogenetic group (A) characterized by *chuA* -, TSPE4.C2 – regardless of *yjaA* present or not. *YjaA* (211bp) present in lanes 1, 3, 4, 5, 7, 8, 9, 11, 13, 14, 15 *E. coli* isolates and not present in lanes 2, 6, 10, and 12.

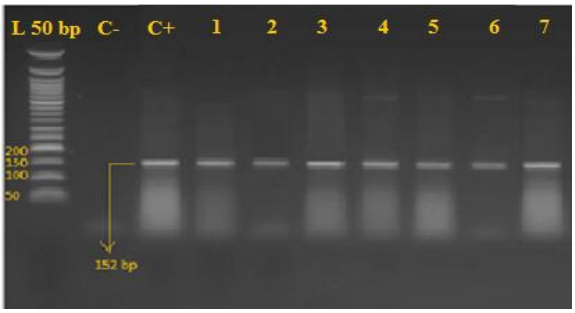

Figure 3. Agarose gel electrophoresis of PCR products for phylogenetic group B1 of *Escherichia coli* isolates. Lane L= DNA molecular ladder (50 bp); Lane C-= negative control (nuclease free water); Lane C+= *E. coli* reference strain (ATCC 35346). Lanes 1-7 are *E. coli* isolates belonged to phylogenetic group B1 characterized by *chuA* -, TSPE4.C2 + regardless of *yjaA* present or not. Lanes 1-7 have only TSPE4.C2 (152 bp).

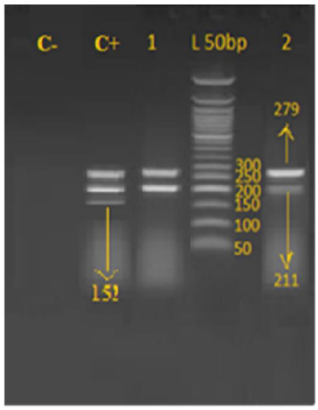

Figure 4. Agarose gel electrophoresis of PCR products for phylogenetic group B2 of *Escherichia coli* isolates. Lane L= DNA molecular ladder (50 bp); Lane C-= negative control (nuclease free water); Lane C+= *E. coli* reference strain (ATCC 35381). Lanes 1 and 2 are *E. coli* isolates belonged to phylogenetic group B2 characterized by *chuA* +, *yjaA* + regardless of TSPE4.C2 present or not. Lanes 1 and 2 have both *chuA* (279 bp) and *yjaA* (211 bp).

The development of multidrug antimicrobial resistance in bacteria is becoming a major threat to human health and wellbeing. Injudicious use of antimicrobial agents in farm animals contributes to the spread and emergence of such resistant bacteria. This study detected high resistance rates ranging between 24.3% to 94.6% to various beta lactam and non-beta lactam antimicrobials in *E. coli* from bovine clinical mastitis. Furthermore, 85% of the *E. coli* isolates were resistant to at least 2 antimicrobial agents. These results are similar to previously reported data from lactating cattle in Jordan (Alekish *et al.*, 2013; Obaidat *et al.*, 2018; Ismail & Abutarbush, 2020).

Phylogenetic grouping provides valuable insights into the evolutionary relationships and characteristics of *E. coli* strains, shedding light on their pathogenic potential and ecological niche (Valat *et al.*, 2012). In this study, 74 *E. coli* isolates were analyzed to determine their phylogenetic groups. Most of the isolates, comprising 52 (70%), were classified into phylogenetic group A. Phylogenetic group A primarily consists of commensal *E. coli* strains, commonly found as part of the normal microbial flora in the intestines of humans and animals (Mwambete & Kamuhabwa, 2014). These commensal strains are generally considered to have a low virulence potential and are not typically associated with the development of diseases.

In contrast, a smaller proportion of the isolates belonged to phylogenetic groups D, B1, and B2. Specifically, 13 isolates (17.5%) were classified into group D, 7 isolates (9.5%) into group B1, and only 2 isolates (2.7%) into group B2. Phylogenetic group D includes strains with a higher pathogenic potential than commensal strains and these strains often possess virulence factors and are associated with a range of clinical infections (Yılmaz & Aslantaş, 2020). Phylogenetic groups B1 and B2 are also associated with pathogenic strains, albeit to a lesser extent than group D.

Our findings regarding the distribution of isolates across the phylogenetic groups align with previous studies that have demonstrated the prevalence of commensal strains (groups A and B1) in *E. coli* populations from the environment (Ju & Willing, 2018). It is noteworthy that several studies focusing on *E. coli* isolates from bovine mastitis have consistently reported

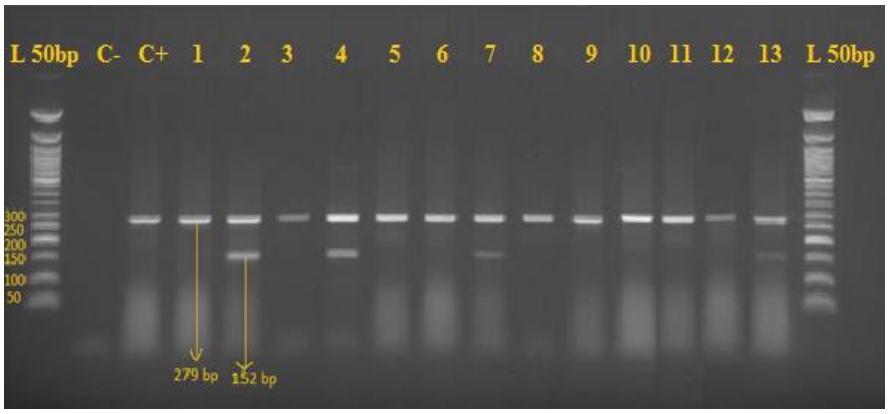

Figure 5. Agarose gel electrophoresis of PCR products for phylogenetic group D of *Escherichia coli* isolates. Lane L= DNA molecular ladder (50 bp); Lane C- = negative control (nuclease free water); Lane C+ = *Escherichia coli* reference strain (ATCC 35363). Lanes 1-13 are *E. coli* isolates belonged to phylogenetic group D characterized by *chuA* +, *yjaA* - regardless of TSPE4.C2 present or not. Lanes 1, 3, 5, 6, 8, 9, 10, 11, and 12 have only *chuA* (279 bp) gene. Lanes 2, 4, 7, and 13 have *chuA* (279 bp) and TSPE4.C2 (152 bp).

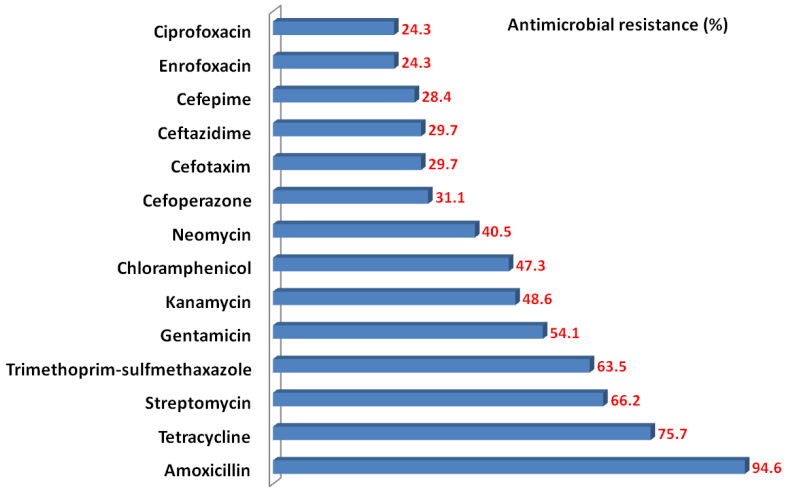

Figure 6. Antimicrobial susceptibility profiles of *Escherichia coli* isolates (n= 74) from bovine clinical mastitis.

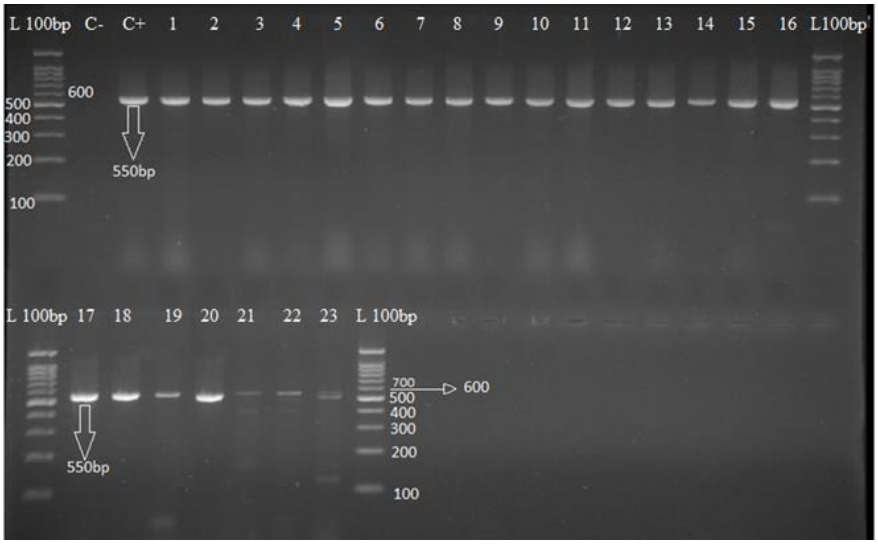

Figure 7. Agarose gel electrophoresis image of the blaCTX-M gene from *Escherichia coli* isolates from bovine mastitis. Lane L= DNA molecular ladder (100 bp). Lane C- = negative control (nuclease free water); Lane C+ = *E. coli* reference strain (ATCC BAA-2326); Lanes 1-23= *E. coli* isolates that produce blaCTX-M gene (550bp).

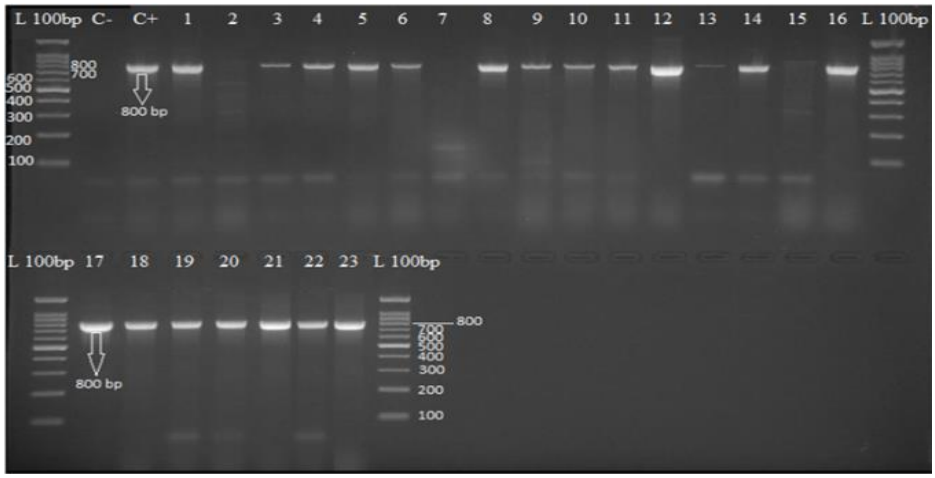

Figure 8. Agarose gel electrophoresis image of the ESβLs-producing *Escherichia coli* isolates from bovine mastitis. Lane L= DNA marker ladder (100bp); Lane C- = negative control (nuclease free water); Lane C+ = *E. coli* reference strain (ATCC BAA-196). Lanes (1-23) except lanes 2, 7, 15= *E. coli* isolates that produce blaTEM gene (800bp).

a predominance of isolates belonging to the commensal groups A and B1 (Kempf *et al.,* 2016; Fazel et a*l.,* 2019; Ismail & Abutarbush, 2020; Yoon & Lee, 2022). This further supports the notion that commensal *E. coli* strains are prevalent in certain infection sites and suggests their potential role in bovine mastitis.

Using the triplex PCR method for phylogenetic grouping in our study offers a rapid and efficient approach for classifying *E. coli* isolates into major phylogenetic groups. However, it is important to note that further investigations incorporating additional molecular and phenotypic characterization are necessary to gain a comprehensive understanding of the genetic diversity and functional attributes within and between these phylogenetic groups. This knowledge would provide valuable insights into the ecology, pathogenicity, and epidemiology of *E. coli* strains in various contexts.

In Jordan, ESβL- producing *E. coli* have been isolated from human hospitalized patients (Batchoun *et al.,* 2009; Salah *et al.,* 2013). In one study that covered 3 major hospitals in northern Jordan, 10.8% of *E. coli* isolates were ESβL positive (Batchoun *et al.,* 2009). Another study reported ESβL resistance in 30.6% of *E. coli* isolates from infants (Salah *et al.,* 2013). To the best of our knowledge, this is the first study in Jordan to investigate the presence of ESβL in *E. coli* isolates from bovine clinical mastitis. Twenty-three out of 74 (31.1%) *E. coli* isolates were ESβL producers, all of them were found to carry the $bla_{CTX\text{-}M}$ gene, and 20 isolates (86.9%) carried the bla_{TEM} gene, while none of the isolates carried the bla_{SHV} gene. Similar results were reported previously in Germany (Freitag *et al.,* 2017), while in a study from China, only 6 out of 153 *E. coli* isolates from mastitic milk were found to possess the bla_{SHV} gene (Ali *et al.,* 2016). These findings indicate that when *E. coli* isolates produce $bla_{\text{CTX-M}}$ and/ or bla_{TEM}, the predicted outcomes of antimicrobial treatment will be poor and the expression of these genes indicates resistance toward beta lactams antibiotics (Batchoun *et al.,* 2009).

CONCLUSION

The results of this study demonstrate a high prevalence of multidrug resistant, ESβLs-producing *E. coli* in bovine mastitis, which may represent a serious threat to public health due to the high risk of dissemination of antimicrobial resistance genes through the consumption of contaminated milk.

CONFLICT OF INTEREST

None of the authors have any potential conflict of interest to declare.

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