pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 39, No. 3, pp. 181~190 (2024)
https://doi.org/10.13103/JFHS.2024.39.3.181

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Review

Bovine mastitis-associated *Escherichia coli*

Hong Qui Le[1], Se Kye Kim[1], Jang Won Yoon[1,2]*

[1]*College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon, Korea*
[2]*The YOON Healthtech Co.,Ltd., Chuncheon, Korea*

(Received June 19, 2024/Revised June 21, 2024/Accepted June 21, 2024)

ABSTRACT - Bovine mastitis-associated *Escherichia coli* (BMEC) is considered the main causative agent of significant financial losses in the dairy industry worldwide, as it alters both the quantity and quality of milk produced and increases the rate of culling. This creates a variety of challenges for researchers, veterinarians, and farmers in understanding and determining the most effective therapies and diagnostic techniques. Subclinical mastitis is particularly concerning, as infected bovines exhibit no obvious symptoms and continue to secrete apparently normal milk over an extended period, allowing the causative pathogen, *E. coli*, to spread within the herd. For effective prevention, understanding the pathogenesis of mastitis through three stages invasion, infection, and inflammation is essential. To date, no clear correlation has been found between virulence factors and pathogenicity contributing to the clinical severity of BMEC. Multidrug-resistant *E. coli* and the evolution of novel resistance mechanisms have become concerns owing to the extensive use of antibiotics to treat mastitis. Therefore, it is vital to explore alternative controls to enhance the efficacy of BMEC treatment. Over the past 30 years, various genetic typing techniques have been used to examine the subspecies-level epidemiology of bovine mastitis. These studies have advanced our understanding of the origin, transmission pathway, population structure, and evolutionary relatedness of BMEC strains. In this review we provide an overview of BMEC, including insights into its etiology, genetic relationship, pathogenesis, and management of the disease, as well as new therapy options.

Key words: Bovine mastitis, *Escherichia coli*, Pathogenesis, Molecular epidemiology, Alternative therapy

Mastitis is considered one of the typical infectious illnesses in cattle, causing inflammation in the mammary gland and diminishing the quality of milk[1]. This infection in cattle can have a direct or indirect impact on farmers' livelihoods and, eventually, the national economy, through costs associated with veterinary care, diagnostic testing, milk loss, and labor increases[2]. The economic impact of seven provinces' worth of large dairy farms in China varied from $15,000 to $76,000 per farm each month[3]. It is estimated that the annual economic losses in the United States due to mastitis will be USD 2 billion[4], 800,000 USD in Colombia[5] and USD 180 million in New Zealand[6].

Based on the level of inflammation, there are two classes of bovine mastitis: clinical and subclinical. Visible abnormalities consisting of a red, swollen udder and fever in dairy cows are clear signs of clinical. There are flakes and clots in the milk, giving the impression that it is thin[7]. Depending on the severity of the inflammation, clinical mastitis can be further classified as per-acute, acute, or subacute[8]. Additionally, severe clinical mastitis cases can be lethal[7]. In contract, subclinical is the second form, this type is characterized by a significant increase in the number of somatic cells (>200,000 cells/mL) but lacks systemic symptoms and visible changes in the milk[9].

Numerous risk factors, encompassing pathogens (microorganisms), host characteristics (breed, age, udder structure), and environmental conditions (poor hygiene, wet bedding, hot and humid climate) are recognized as influential contributors to the occurrence of bovine mastitis. However, it is believed that microbes are the main culprit behind this disease[10]. Bacterial infections can be divided into two categories: contagious and environmental. The term "contagious mastitis" describes mastitis that can spread from cow to cow, in particular during milking. Contagious pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae*, along with less prevalent species like *Mycoplama bovis* and *Corynebacterium*, inhabit the cow's udder and teat skin, proliferating within the teat canal[10]. Besides, environmental

*Correspondence to: Jang Won Yoon, College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon, Kangwon 24341, Republic of Korea
Tel: +82-33-250-8791; Fax: +82-33-259-5625
E-mail: jwy706@kangwon.ac.kr

pathogens are found within the bedding and housing of the herd. The most accurate description of them is that they are opportunistic pathogens that hunt for chances to infect. Microorganisms in this group include *E. coli, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis,* and other coliforms[11]. Significantly, *E. coli* stands as a predominant cause of acute clinical mastitis in dairy cattle globally[12,13]. Due to the flexibility of genome, carrying various virulence genes and high adaptation capacity. Over time, this organism has transformed into pathogenic strains that can infect humans and cause other animal diseases, such as bovine mastitis. BMEC has been suggested as a novel pathotype that causes dairy cow mastitis[14]. Furthermore, antibiotic abuse in bovine inflammation treatment has led to an increase in the prevalence of MDR *E. coli*[15]. Crucially, these strains carry the potential to spread zoonotic pathogens to humans through contaminated milk consumption or direct contact with diseased cattle[16].

Given the aforementioned concerns, this review article aims to highlight an overview of the etiology, molecular epidemiology, pathogenesis, as well as potential alternative treatments. Such insights will contribute to identifying suitable treatment options for effectively managing diseases caused by this pathogen.

Etiological *Escherichia coli* mastitis

E. coli can be divided into pathogenic and non-pathogenic groups with the pathogenic strains causing various diseases in different human and animals, especially mastitis. These bacteria can then be further subdivided into different types based on the associated pathogenic mechanisms[12,17]. Moreover, the cow's udder provides an ideal environment for microbial growth. Under optimal udder conditions, including temperature, nutrition, and minimal external influences have created an extremely favorable condition for pathogenic *E. coli* existing in environment surrounding invade, multiply rapidly and initiate inflammatory response in dairy cow. This proliferation is the primary cause of udder damage and triggers the response recognized as mastitis[18]. Thus, it was determined that *E. coli* is an opportunistic pathogen with a variety of virulence factors (VFs)[19]. Besides, an innate immune response can be triggered by as little as 50 cfu of the initial inoculum after 8 hours post-infection[20]. Additionally, an investigation demonstrated that from four hours and up until the end of incubation, the growth in milk and lactose fermentation ability of the mastitis isolates was significantly higher compared with environment strains[21]. In the event of mild inflammation, overall signs typically disappear within 48 hours. But, if the inflammation is severe, the cow may not survive due to endotoxin from pathogenic *E. coli*[22].

Molecular epidemiology

Molecular epidemiological investigations of BMEC contributed to our understanding of various aspects, including host adaptation mechanisms, disease origins, transmission routes, pathogen evolution, potential targets for treatment or vaccine development, and the risks associated with pathogen or genetic element exchange between bacterial and host species. Bovine mastitis-causing pathogens are characterized by a variety of genotyping techniques, including PCR-based methods, multi locus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), microarrays or whole genome sequencing (WGS). To date, MLST and PFGE are considered gold standard techniques for the deeper discrimination of microorganisms in large-scale surveys. By measuring the nucleotide sequences of four to eight housekeeping gene, MLST can accurately records the variations in bacterial gene level. Meanwhile, PFGE is often used in investigation of epidemiology outbreaks based on electrophoretic banding patterns[23].

E. coli is recognized for its great intraspecific variability and has been assigned into phylogenetic groups (A, B1, B2, D, C, E, F, F, G, or Escherichia clades) based on the presence of particular genes[24]. Though groups A and B1 were primarily linked to commensal and diarrheagenic strains, whereas phylogenetic groups B2 and D were primarily detected in extra-intestinal illnesses and invasive strains[25]. In accordance with early strain typing research and epidemiological evidence, the majority of BMEC isolates belong to phylogenetic group A or B1. (Table 1). Hence, these findings confirm the hypothesis that environmental *E. coli* plays a part in such mastitis cases.

One the other hand, apart from the differences in phenotype between mastitis and environmental isolate groups, genetic relatedness using PFGE was also observed. Nevertheless, the relationship between isolates from the two groups was unclear[21]. It is challenging to compare PFGE data with findings from other studies and to deduce phylogeny from the data[23].

One of the earliest surveys of MLST for bovine mastitis was conducted by Blum and his colleages in 2013[26]. Additionally, this study demonstrated that some frequent STs were found in milk bovine mastitis as well as environmental sources. However, it is possible that the environment contains fewer strain that cause mastitis. Moreover, distribution of frequency STs like ST10, ST58 has been observed among worldwide, encompassing countries such as Canada, Brazil, China, Japan, Switzerland, Germany and Ireland (Table 1). It is suggested that these STs have a greater propensity to invade and effect the mammary gland than others[27]. What is esspecially concerning is that the majority of STs in these isolates carried the critical antimicrobial resistant determinant, such as *mcr-1, blaCTX-M-14 and blaCTX-M-*

Table 1. Molecular epidemiology studies of BMEC in the world

Continent	Country	Major phylogenetic group	Strain typing	Frequency ST	Reference
Africa	Algeria		MLST	162, 317, 949	75)
	Tunisia	A, B1	MLST	617, 167	76)
Asia	Afghanistan	A			77)
	China	A, B1	PFGE and MLST	58, 410	8)
	China	A, D	MLST	410	78)
	China	A, B1			42)
	China	A, B1			79)
	Japan		PFGE and MLST	10, 58, 167	80)
	Korea	A, B1, D	PFGE		50)
America	Brazil	A, B1	PFGE and MLST	10, 993	39)
	Brazil	A, B1	PFGE		81)
	Canada		WGS	10, 58, 1125	82)
Europe	France	A, B1			83)
	Ireland	A, B1, D	MLST	10, 58	84)
	Germany	A, B1, D	MLST	10	85)
	Switzerland	A, B1	MLST	10, 58, 1125	27)
Oceania	Australia		WGS	10, 4429	86)

28[8]. This might also be a part of the failure to treat persistent mastitis by antimicrobial therapy. Furthermore, a previous study employing WGS discovered distinct genes in BMEC compared with commercial isolates and offering promising prospects for future advancements in diagnosis and treatment[28].

Pathogenesis

Physical barriers at the teat tip and substances like complement, natural kill cells, neutrophils, macrophages, cytokines, and lactoferrin, which primarily function in the early stages of *E. coli* infections, are components of bovine innate immunity. Among them, phagocytosis and neutrophils are believed to be the first line of cellular defense, typically reacting quickly to the inflammatory process[29,30]. Lactoferrin is another essential element of the host's innate immunity, serving as an iron-tropic glycoprotein with bacteriostatic properties synthesized by both leukocytes and epithelial cells[31]. It has also been demonstrated to bind to iron ions in ruminants, preventing the growth of *E. coli*[32]. Moreover, lipopolysaccharides have been suggested to be the primary virulence factor of *E. coli* because they have the ability to induce apoptosis and endotoxic shock in mastitic tissues[33]. Additionally, they are believed to be immune stimulants capable of producing cytokines and chemokines both in the presence and absence of live *E. coli*[34]. Furthermore, the mammary gland's innate defense relies significantly on essential cytokines, such as interleukins (IL) (IL-B, IL-1, IL-6, IL-8), colony-stimulating factor (CSF), interferon (IFN), and tumor necrosis factor (TNF-a). Specific (or acquired) immunity, which is mediated by lymphocytes, arises when innate immunity is ineffective in combating the infection. Lymphocytes create antibodies that go from the bloodstream into the milk with the goal of the inflammatory response to mastitis is to destroy the pathogen's microbe, neutralize its poisons, and then repair damaged udder tissue to swiftly restore the amount of milk that would usually be produced[1].

On the other side, *E. Coli* adheres to the surface of the epithelial cells after overcoming the physical barriers and the mucosal membrane is thought to be the initial stage of host colonization[35]. Particularly, fimbrial adhesins such as P, S, F17 and AFA families are used to attach to receptors that contain Glc-NAc (N-acetyl-D-glucosamine) on the epithelial cells of cows[36]. In additional, genes encoding adhesion proteins, invasins, hemagglutinin, aerobactin, P-fimbria, toxins, hemolysins, intimins, capsule formation, biofilm formation, resistance to serum complement, and the capacity to scavenge iron are among the primary VFs found in *E. Coli* isolated from bovine mastitis cases[37-39]. Besides, different pathotypes of this pathogenic strains can be distinguished, and each pathotype results in a unique disease. They can be divided into two group: intestinal pathogenic *E. coli* (IPEC) and extraintestinal pathogenic *E. coli* (ExPEC) depending on infected area, illness signs, as well as VFs[27]. In cattle, ExPEC is considered as a brand-new pathotype termed as

the mammary pathogenic *E. coli* (MPEC) that has been linked to mastitis[14]. However, years of research have not yet provided a detailed description of the characteristics of this new group[40]. While the pathogenesis mechanisms of many *E. coli* genotypes are well understood for other epithelial systems, no precise relationship has been reported between the pathogenicity and the VFs known to contribute to the clinical severity of BMEC[41-43]. Some studies proposed that the persistence of infections may be attributed more to host factors rather than bacterial factors[44]. Apart from this, *E. coli* pathogens are protected by biofilm formation from host defenses and antibiotic activity, which allows them to persist in the host tissues and is thought to be a significant factor in the disease's transmission[45]. Then, endotoxin toxins from pathogenic *E. coli* trains such as hemolysins that are associated with cytotoxic properties in the udder, the cytotoxic necrotizing factor (CNF) toxins and shiga toxin could be released and damaged epithelial cells[35,46].

Escherichia coli mastitis treatment and control

Antimicrobial therapy

One of the most common strategies for treating infectious diseases in dairy farmers, such as intramammary infections, particularly in cases of clinical mastitis, is therapy using antimicrobial agents[47]. The over use or misuse of antibiotics in both human and animal populations contributed to the evolution of antimicrobial resistant bacteria through plasmid-mediated horizontal gene transfer or gene mutation[48]. Over the course of numerous years, the incessant emergence of antimicrobial-resistant genes in *E. coli* can be attributed to the protracted abuse and unwarranted utilization of antimicrobials[49]. Penicillines, tetracyclines, macrolides, sulfonamides, aminoglycosides, fluoroquinolones, cephalosporins, and β-lactams are among the antimicrobials that have been approved for the treatment of bovine mastitis. Crucially, one of the main concerns regarding the antibiotic resistance mechanism of *E. coli* mastitis is the extended-spectrum β-lactamase enzymes (ESBLs)[50]. Penicillins, first-, second-, third-, and fourth-generation cephalosporins, as well as monobactams like aztreonam, can all be hydrolyzed by extended spectrum β-lactamases, excluding cephamycins or carbapenems[51]. Furthermore, these enzymes' genes are rarely integrated into bacterial chromosomes; instead, they are linked to integrons, transposons like Tn2, insertion sequences including IS*Ecp1*, IS*CR1*, or IS*26*[52]. Additionally, MDR bacteria are directly linked to high human motility rates, therefore, the determination of isolates that produce ESBL genes is concerning[53]. As demonstrated in Table 2, ESBL-producing *E. coli* has been found in cattle with mastitis in several of countries. Their easy distribution via plasmids and other mobile genetic elements is most likely the reason for their success[54]. Particularly, conjugative I1-Ir plasmids play an important role as epidemic replicons, highly efficient in contributing to the emergence and spread of the *blaCMY* gene in dairy farms[50,55]. Additionally, the risk of consuming unpasteurized milk and dairy products is raised by the presence of these bacteria in milk, as it may result in the spread of ESBL-producing *E. coli* to people[56].

Another research suggests that antimicrobial therapy is not required for non-severe clinical cases caused by gram-negative pathogens[57], as it does not improve outcomes like SCC or the ability to produce milk[58]. Regardless of the expense, the dairy industry has experienced certain issues due to the overuse and misuse of antibiotics in the treatment of bovine mastitis. The presence of antibiotic residues in milk has resulted in some risks of allergies, especially antimicrobial resistance. As a result, there will be heavy fines when antibiotic residues are detected in milk[59].

Plant-based substances

These days, a lot of attention is paid to plant-derived antibacterial compounds because they are readily available and reasonably priced. Various plants have been proved to exhibit antimicrobial properties induced by pathogens or endotoxin by inactivating different inhibiting pathways[60]. Previous evidences have demonstrated that several plant-derived compound such as *Terminalia* chebula (Ethyl acetate extracts) *Cinnamomum* verum (Trans-cinnamaldehyde) and *Linum usitatissimum* (linolenic acid) were successful in lowering the *E. coli* levels of mammary gland[61-63]. Additionally, baicalin has minimal antibacterial effect, but it influences the drug resistance genes of *E. coli*, which increases the coli's susceptibility to antibiotics such ampicillin, penicillin, streptomycin, and ciprofloxacin[64].

Animal-based substances

Using immunomodulators-like lactoferrin, which is naturally produced by mammals-as possible non-antibiotic antimicrobial agents for the treatment and prevention of cow mastitis has been suggested. A glycoprotein called lactoferrin is present in a variety of bodily secretions, including milk, tears, saliva, and bronqueal mucus. Their antibacterial activity against a number of important mastitis-causing pathogens, including *K. pneumonia*, coagulase-negative *Staphylococci, E. coli*, and *S. aureus*[65]. Lactoferrin has shown a considerable inhibitory effect against the tested isolates, being more effective against *E. coli* and less effective against *S. aureus*[10].

Table 2. The distribution of antimicrobial resistance genes and replicon types among BMEC in the world

Continent	Country	ESBL genes	Other AMGs	Replicon types	Reference
Africa	Egypt	CTX-M-1, 9, 15; TEM-1	aa6-aph2; aadA1, A4; aphA; catA1; cmlA1; dfrA1, A12, A14, A17; erm(B); floR; mphA; msrC; qnrA1, S; tetA, K, L; sul1, 2, 3		87)
Asia	China	CTX-M-14, 15, 28, 55, 66, 69, 148, 177; CMY; SHV; TEM	mcr-1	FIB, FIC, FrepB HI2, N, P, X4, Y	8)
	China	CTX-M-1, 3, 14, 15, 55; SHV-12; TEM-1		FIA, B, C; Frep; FIIs, H1, 2; I1; K/B; N; L/M; Y	78)
	China		strA; strB; aadA; ant (3'');aph(3'');aph(6)-1d; aph(6)-1c and ant(6)		42)
	Japan	CTX-M-2, 14, 15; TEM-1			80)
	Korea	CTX-M-1, 3, 15; CMY-2; TEM-1		F; FIB; I1-Ir	50)
America	Canada	CARB-3; CMY-59; TEM-0	aadA2; acrA, B, D; aphh(3')-Ia; aph(3'')-Ib; aph(6)-Id; baeR; dfrA1, A5, A12, A16; emrA, B, Y, K; floR; kdpE; mdfA; tetA, B, C; sul1, 2; tolC		82)
	United States		aadA; sulA; strA, B; tet A, B, C		72)
Europe	France	CTX-M-1, 14		F2:A-:B- FII; I1/ST3	88)
	Germany	CTX-M-1, 2, 14, 15, 32; TEM-1	aac(6')-lb-cr		85)
	Switzerland	CTX-M-14; TEM-1			89)
Oceania	Australia	TEM-1	aphh(3')-Ia; aph(3'')-Ib; aph(6)-Id; dfrA5; qacE; tetA; sul2		86)

Bateriophage therapy

Bacteriophages are viruses that exclusively target bacteria, posing no harm to humans, animals, or plants. They are considered as an alternative weapon with high potential in reducing antimicrobial consumption, increasing livestock productivity, and protecting the environment[66]. For instance, T4 virus vB_EcoM-UFV13 phage treatment into the mammary gland resulted in a ten-fold decrease in *E. coli* concentration, a decrease in IL-10 expression, and an increase in TNF-α and IL-6 expression[67]. Furthermore, a bacteriophage cocktail, including 4 phages, was discovered by Porter et al. in 2016[68], showing a significant decrease in invasion and adhesion activity of this strain at mammary epithelial cells.

Nano-materials

Nanotechnology have demonstrated efficacy on some major mastitis associated-pathogens[59]. Zinc oxide and copper oxide nanoparticles (ZnO-NPs) doped with garlic and ginger extracts were synthesized by Ali et al., 2021[69] and proved antibacterial activity against *E. coli*. Besides, the structure of *E. coli* biofilms was found to be destroyed upon exposure to synthesized quercetin nanoparticles (QANPs) wrapped with silver[70].

Prevention

Dry cow therapy (DCT)

The DCT is an effective method for controlling and preventing mastitis progression during the dry period, a crucial stage in the lactation cycle. It's vital to ensure cow health before the next milking cycle, as any infections during this period can impact the following lactation. Before drying off the cows, they undergo a thorough check for mastitis signs, including chronic cases detected via the California mastitis test. Following the final milking, intra-mammary antibiotic injection and teat sealant application are administered to prevent bacterial invasion and milk leakage. DCT effectively eliminates existing intramammary infections and prevents new infections, with long-persisting antibiotics offering improved cure rates[71].

Milking system and hygiene

It is believed that effective control of BMEC infections can be achieved through appropriate management practices. These practices may include daily waste removal, minimizing moisture and organic matter in the environment before and

after milking along with providing food after milking to reduce the risk of teat contamination with feces that might accumulate on the farm and equipment facilities[72].

Vaccination

While vaccines have proven effective in controlling other bacterial diseases in dairy cows, the complexity of mastitis presents significant challenges to their success. This is due to the diverse array of evolving bacterial pathogens, variations in strains across farms and over time, as well as differences in virulence characteristics and immunogenic capabilities among these pathogens[9]. Commercial preparations have employed a mutant strain of *E. coli* O111:B4 (J5) to immunize cows against coliform mastitis. The bacterial cell wall's “O” antigen capsular section is absent from this mutant's expression of LPS, but it contains several core antigens and lipid A on its surface. A comprehensive explanation of its mechanism of action is still unclear. However, upon exposure to this antigen, it triggers the synthesis of immunoglobulins (such as IgM, IgG1, IgG2) that cross-react with core antigens of various bacteria, offering immunity and protection against a broad spectrum of bacterial strains[73]. Moreover, the J5 vaccination increases the levels of specific antibodies against *E. coli* LPS in both blood and milk, thereby enhancing its opsonization[74]. Since various bacterial pathogens can cause mastitis, as previously mentioned, it is essential to develop a vaccine that can guard against a broad spectrum of strains, as different strains can coexist within a herd and within a single cow.

Conclusion

BMEC is believed as an opportunistic pathogen that cause gland infections ranging from mild to severe. Although several specific characteristics that differentiate BMEC from other environmental *E. coli* have been demonstrated, ExPEC is also proposed as a new pathotype of this disease. However, the correlation between VFs and pathogenicity that influence the severity of mastitis has not been found to be well correlated. Through molecular epidemiology studies have proved that some clonal strains of BMEC have evolved their defense mechanisms that allow them to exceed other co-infecting bacteria and the host’s defense system. While antibiotics remain a primary strategy for treating bovine mastitis, it is undeniable that they also present significant public health and food security concerns. These include the emergence of antibiotic-resistant bacteria, the presence of antibiotic residues in milk and the food chain as well as environmental effects. Consequently, a broad range of compound substitutes should be promoted and popularized in managing udder inflammation, particularly bacteriophages, vaccines, nanoparticles, and natural compounds derived from plants and animal.

Acknowledgments

This study was supported by a grant from Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry (IPET) through Technology Commercialization Support Program, Ministry of Agriculture, Food and Rural Affairs (RS-2022-IP322055), Republic of Korea.

국문 요약

소 유방염 관련 대장균(BMEC)은 생산되는 우유의 양과 품질을 변화시키고 도태율을 높임으로써 전 세계 낙농 산업에 심각한 재정적 손실을 초래할 수 있는 주요 원인 물질로 간주된다. 연구자, 수의사, 농부가 가장 효과적인 치료법과 진단 기술을 이해하고 결정하는 것은 젖소 유방염을 극복하는데 중요하다. 특히 무증상 혹은 준임상형 유방염의 경우, 소는 뚜렷한 증상을 보이지 않고, 장기간에 걸쳐 겉보기에 정상적인 우유를 계속 분비하여 원인 병원체인 대장균이 무리 내에서 감염을 퍼뜨릴 수 있다. 유방염 예방을 위해서는, 병원균의 유방 내 침입, 감염 확립, 유방의 염증의 3단계 병인 과정에 대한 이해가 필수적이다. 지금까지 대장균 유방염의 임상적 중증도에 기여하는 독성 인자와 병원성 사이에 명확한 상관관계가 발견되지 않았다. 다제내성 대장균과 새로운 내성 기전의 진화는 유방염 치료에 항생제를 광범위하게 사용하고 있기 때문에 문제시 되고 있는 실정이다. 따라서 BMEC 치료의 효능을 향상시키기 위해서는 대체제 발굴이 중요하다. 지난 30년 동안 소 유방염의 역학 조사를 위해 다양한 유전자형 분석 기술이 사용되었다. 이러한 연구는 BMEC 계통 간의 진화 관련성 뿐 아니라 기원, 전염 경로, 개체군 구조에 대한 이해를 크게 향상시켰다. 따라서 본 리뷰에서는 BMEC의 전반적 개요를 제공하여 병인, 유전적 관계, 발병 기전, 관리 및 질병 통제를 위한 새로운 치료 옵션에 대한 통찰력을 제공하고자 한다.

Conflict of interests

The authors declare no potential conflict of interest.

ORCID

Hong Qui Le https://orcid.org/0009-0004-7152-8125
Se Kye Kim https://orcid.org/0000-0002-9161-2533
Jang Won Yoon https://orcid.org/0000-0002-6874-5290

References

1. Goulart, D.B., Mellata, M., *Escherichia coli* Mastitis in dairy cattle: etiology, diagnosis, and treatment challenges. *Front. Microbiol.*, **13**, 928346 (2022).
2. Azooz, M.F., El-Wakeel, S.A., Yousef, H.M., Financial and economic analyses of the impact of cattle mastitis on the profitability of Egyptian dairy farms. *Vet. World*, **13**, 1750-1759 (2020).
3. He, W., Ma, S., Lei, L., He, J., Li, X., Tao, J., Wang, X., Song, S., Wang, Y., Wang, Y., Shen, J., Cai, C., Wu, C., Prevalence, etiology, and economic impact of clinical mastitis on large dairy farms in China. *Vet. Microbiol.*, **242**, 108570 (2020).
4. Dalanezi, F.M., Joaquim, S.F., Guimaraes, F.F., Guerra, S.T., Lopes, B.C., Schmidt, E.M.S., Cerri, R.L.A., Langoni, H., Influence of pathogens causing clinical mastitis on reproductive variables of dairy cows. *J. Dairy Sci.*, **103**, 3648-3655 (2020).
5. Romero, J., Benavides, E., Meza, C., Assessing financial impacts of subclinical mastitis on colombian dairy farms. *Front. Vet. Sci.*, **5**, 273 (2018).
6. Denis, M., Wedlock, D.N., Lacy-Hulbert, S.J., Hillerton, J.E., Buddle, B.M., Vaccines against bovine mastitis in the New Zealand context: what is the best way forward? *N Z Vet. J.*, **57**, 132-140 (2009).
7. Gruet, P., Maincent, P., Berthelot, X., Kaltsatos, V., Bovine, mastitis and intramammary drug delivery: review and perspectives. *Adv. Drug Deliv. Rev.*, **50**, 245-259 (2001).
8. Shafiq, M., Huang, J., Shah, J.M., Wang, X., Rahman, S.U., Ali, I., Chen, L., Wang, L., Characterization and virulence factors distribution of bla(CTX-M) and mcr-1carrying *Escherichia coli* isolates from bovine mastitis. *J. Appl. Microbiol.*, **131**, 634-646 (2021).
9. Ruegg, P.L., A 100-Year Review: Mastitis detection, management, and prevention. *J. Dairy Sci.*, **100**, 10381-10397 (2017).
10. Cheng, W.N., Han, S.G., Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - a review. *Asian-Australas J. Anim. Sci.*, **33**, 1699-1713 (2020).
11. Sztachanska, M., Baranski, W., Janowski, T., Pogorzelska, J., Zdunczyk, S., Prevalence and etiological agents of subclinical mastitis at the end of lactation in nine dairy herds in North-East Poland. *Pol. J. Vet. Sci.*, **19**, 119-124 (2016).
12. Blum, S.E., Heller, E.D., Jacoby, S., Krifucks, O., Leitner, G., Comparison of the immune responses associated with experimental bovine mastitis caused by different strains of *Escherichia coli*. *J. Dairy Res.*, **84**, 190-197 (2017).
13. Gao, J., Barkema, H.W., Zhang, L., Liu, G., Deng, Z., Cai, L., Shan, R., Zhang, S., Zou, J., Kastelic, J.P., Han, B., Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms. *J. Dairy Sci.*, **100**, 4797-4806 (2017).
14. Shpigel, N.Y., Elazar, S., Rosenshine, I., Mammary pathogenic *Escherichia coli*. *Curr. Opin. Microbiol.*, **11**, 60-65 (2008).
15. Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A., Laxminarayan, R., Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci. U S A*, **112**, 5649-5654 (2015).
16. Maity, S., Ambatipudi, K., Mammary microbial dysbiosis leads to the zoonosis of bovine mastitis: a One-Health perspective. *FEMS Microbiol. Ecol.*, **97**, 1-17 (2020).
17. Kaper, J.B., Nataro, J.P., Mobley, H.L., Pathogenic *Escherichia coli*. *Nat. Rev. Microbiol.*, **2**, 123-140 (2004).
18. Smith, K.L., Hogan, J.S., Environmental mastitis. *Vet. Clin. North Am. Food Anim. Pract.*, **9**, 489-498 (1993).
19. Fernandes, J.B., Zanardo, L.G., Galvao, N.N., Carvalho, I.A., Nero, L.A., Moreira, M.A., *Escherichia coli* from clinical mastitis: serotypes and virulence factors. *J. Vet. Diagn. Invest.*, **23**, 1146-1152 (2011).
20. Frost, A.J., Hill, A.W., Brooker, B.E., Pathogenesis of experimental bovine mastitis following a small inoculum of *Escherichia coli*. *Res. Vet. Sci.*, **33**, 105-112 (1982).
21. Blum, S., Heller, E.D., Krifucks, O., Sela, S., Hammer-Muntz, O., Leitner, G., Identification of a bovine mastitis *Escherichia coli* subset. *Vet. Microbiol.*, **132**, 135-148 (2008).
22. Burvenich, C., Van Merris, V., Mehrzad, J., Diez-Fraile, A., Duchateau, L., Severity of *E. coli* mastitis is mainly determined by cow factors. *Vet. Res.*, **34**, 521-564 (2003).
23. Zadoks, R.N., Middleton, J.R., McDougall, S., Katholm, J., Schukken, Y.H., Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. *J. Mammary Gland Biol. Neoplasia*, **16**, 357-372 (2011).
24. Clermont, O., Christenson, J.K., Denamur, E., Gordon, D. M., The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ. Microbiol. Rep.*, **5**, 58-65 (2013).
25. Clermont, O., Bonacorsi, S., Bingen, E., Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl. Environ. Microbiol.*, **66**, 4555-4558 (2000).
26. Blum, S.E., Leitner, G., Genotyping and virulence factors assessment of bovine mastitis *Escherichia coli*. *Vet. Microbiol.*, **163**, 305-312 (2013).
27. Nuesch-Inderbinen, M., Kappeli, N., Morach, M., Eicher, C., Corti, S., Stephan, R., Molecular types, virulence profiles and antimicrobial resistance of *Escherichia coli* causing bovine mastitis. *Vet. Rec. Open*, **6**, e000369 (2019).
28. Jung, D., Park, S., Ruffini, J., Dussault, F., Dufour, S., Ronholm, J., Comparative genomic analysis of *Escherichia coli* isolates from cases of bovine clinical mastitis identifies nine specific pathotype marker genes. *Microb. Genom.*, **7**, 000597 (2021).
29. Bassel, L.L., Caswell, J.L., Bovine neutrophils in health and disease. *Cell. Tissue Res.*, **371**, 617-637 (2018).
30. Ingvartsen, K.L., Moyes, K.M., Factors contributing to immunosuppression in the dairy cow during the periparturient period. *Jpn. J. Vet. Res.*, **63 Suppl 1**, S15-24 (2015).
31. Shimazaki, K.I., Kawai, K., Advances in lactoferrin research concerning bovine mastitis. *Biochem. Cell Biol.*, **95**, 69-75

(2017).
32. Rybarczyk, J., Kieckens, E., Vanrompay, D., Cox, E., *In vitro* and *in vivo* studies on the antimicrobial effect of lactoferrin against *Escherichia coli* O157:H7. *Vet. Microbiol.*, **202**, 23-28 (2017).
33. Long, E., Capuco, A.V., Wood, D.L., Sonstegard, T., Tomita, G., Paape, M.J., Zhao, X., *Escherichia coli* induces apoptosis and proliferation of mammary cells. *Cell Death Differ.*, **8**, 808-816 (2001).
34. Bannerman, D.D., Paape, M.J., Hare, W.R., Sohn, E.J., Increased levels of LPS-binding protein in bovine blood and milk following bacterial lipopolysaccharide challenge. *J. Dairy Sci.*, **86**, 3128-3137 (2003).
35. Ghanbarpour, R., Oswald, E., Phylogenetic distribution of virulence genes in *Escherichia coli* isolated from bovine mastitis in Iran. *Res. Vet. Sci.*, **88**, 6-10 (2010).
36. Zaatout, N., An overview on mastitis-associated *Escherichia coli*: Pathogenicity, host immunity and the use of alternative therapies. *Microbiol. Res.*, **256**, 126960 (2022).
37. Fairbrother, J.H., Dufour, S., Fairbrother, J.M., Francoz, D., Nadeau, E., Messier, S., Characterization of persistent and transient *Escherichia coli* isolates recovered from clinical mastitis episodes in dairy cows. *Vet. Microbiol.*, **176**, 126-133 (2015).
38. Guerra, S.T., Orsi, H., Joaquim, S.F., Guimaraes, F.F., Lopes, B.C., Dalanezi, F.M., Leite, D.S., Langoni, H., Pantoja, J.C. F., Rall, V.L.M., Hernandes, R.T., Lucheis, S.B., Ribeiro, M.G., Short communication: Investigation of extra-intestinal pathogenic *Escherichia coli* virulence genes, bacterial motility, and multidrug resistance pattern of strains isolated from dairy cows with different severity scores of clinical mastitis. *J. Dairy Sci.*, **103**, 3606-3614 (2020).
39. Orsi, H., Guimaraes, F.F., Leite, D.S., Guerra, S.T., Joaquim, S.F., Pantoja, J.C.F., Hernandes, R.T., Lucheis, S.B., Ribeiro, M.G., Langoni, H., Rall, V.L.M., Characterization of mammary pathogenic *Escherichia coli* reveals the diversity of *Escherichia coli* isolates associated with bovine clinical mastitis in Brazil. *J. Dairy Sci.*, **106**, 1403-1413 (2023).
40. Leimbach, A., Poehlein, A., Witten, A., Wellnitz, O., Shpigel, N., Petzl, W., Zerbe, H., Daniel, R., Dobrindt, U., Whole-genome draft sequences of six commensal fecal and six mastitis-associated *Escherichia coli* strains of bovine origin. *Genome. Announc.*, **4**, e00753-16 (2016).
41. Dopfer, D., Almeida, R.A., Lam, T.J., Nederbragt, H., Oliver, S.P., Gaastra, W., Adhesion and invasion of *Escherichia coli* from single and recurrent clinical cases of bovine mastitis in vitro. *Vet. Microbiol.*, **74**, 331-343 (2000).
42. Liu, Y., Liu, G., Liu, W., Liu, Y., Ali, T., Chen, W., Yin, J., Han, B., Phylogenetic group, virulence factors and antimicrobial resistance of *Escherichia coli* associated with bovine mastitis. *Res. Microbiol.*, **165**, 273-277 (2014).
43. Sarowska, J., Futoma-Koloch, B., Jama-Kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-Ploskonska, G., Choroszy-Krol, I., Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. *Gut. Pathog.*, **11**, 10 (2019).
44. White, L.J., Schukken, Y.H., Dogan, B., Green, L., Dopfer, D., Chappell, M.J., Medley, G.F., Modelling the dynamics of intramammary *E. coli* infections in dairy cows: understanding mechanisms that distinguish transient from persistent infections. *Vet. Res.*, **41**, 13 (2010).
45. Silva, V.O., Soares, L.O., Silva Junior, A., Mantovani, H.C., Chang, Y.F., Moreira, M.A., Biofilm formation on biotic and abiotic surfaces in the presence of antimicrobials by *Escherichia coli* Isolates from cases of bovine mastitis. *Appl. Environ. Microbiol.*, **80**, 6136-6145 (2014).
46. Blum, S.E., Heller, E.D., Sela, S., Elad, D., Edery, N., Leitner, G., Genomic and phenomic study of mammary pathogenic *Escherichia coli*. *PLoS One*, **10**, e0136387 (2015).
47. Saini, V., McClure, J.T., Léger, D., Keefe, G.P., Scholl, D.T., Morck, D.W., Barkema, H.W., Antimicrobial resistance profiles of common mastitis pathogens on Canadian dairy farms. *J. Dairy Sci.*, **95**, 4319-4332 (2012).
48. Tseng, C.H., Liu, C.W., Liu, P.Y., Extended-spectrum β-lactamases (ESBL) producing bacteria in animals. *Antibiotics (Basel)*, **12**, 661 (2023).
49. Klaas, I.C., Zadoks, R.N., An update on environmental mastitis: Challenging perceptions. *Transbound. Emerg. Dis.*, **65 Suppl 1**, 166-185 (2018).
50. Tark, D.S., Moon, D.C., Kang, H.Y., Kim, S.R., Nam, H.M., Lee, H.S., Jung, S.C., Lim, S.K., Antimicrobial susceptibility and characterization of extended-spectrum β-lactamases in *Escherichia coli* isolated from bovine mastitic milk in South Korea from 2012 to 2015. *J. Dairy Sci.*, **100**, 3463-3469 (2017).
51. Naranjo-Lucena, A., Slowey, R., Invited review: antimicrobial resistance in bovine mastitis pathogens: a review of genetic determinants and prevalence of resistance in European countries. *J. Dairy Sci.*, **106**, 1-23 (2023).
52. Poirel, L., Madec, J.Y., Lupo, A., Schink, A.K., Kieffer, N., Nordmann, P., Schwarz, S., Antimicrobial resistance in *Escherichia coli*. *Microbiol. Spectr.*, **6**, 1-27 (2018).
53. Bengtsson, B., Unnerstad, H.E., Ekman, T., Artursson, K., Nilsson-Ost, M., Waller, K.P., Antimicrobial susceptibility of udder pathogens from cases of acute clinical mastitis in dairy cows. *Vet. Microbiol.*, **136**, 142-149 (2009).
54. Tooke, C.L., Hinchliffe, P., Bragginton, E.C., Colenso, C.K., Hirvonen, V.H.A., Takebayashi, Y., Spencer, J., β-lactamases and β-lactamase inhibitors in the 21st century. *J. Mol. Biol.*, **431**, 3472-3500 (2019).
55. Tamang, M.D., Nam, H.M., Gurung, M., Jang, G.C., Kim, S.R., Jung, S.C., Park, Y. H., Lim, S.K., Molecular characterization of CTX-M β-lactamase and associated addiction systems in *Escherichia coli* circulating among cattle, farm workers, and the farm environment. *Appl. Environ. Microbiol.*, **79**, 3898-3905 (2013).
56. Freitag, C., Michael, G.B., Kadlec, K., Hassel, M., Schwarz, S., Detection of plasmid-borne extended-spectrum β-lactamase (ESBL) genes in *Escherichia coli* isolates from

bovine mastitis. *Vet. Microbiol.*, **200**, 151-156 (2017).
57. Oliveira, L., Ruegg, P.L., Treatments of clinical mastitis occurring in cows on 51 large dairy herds in Wisconsin. *J. Dairy Sci.*, **97**, 5426-5436 (2014).
58. Fuenzalida, M.J., Ruegg, P.L., Negatively controlled, randomized clinical trial to evaluate use of intramammary ceftiofur for treatment of nonsevere culture-negative clinical mastitis. *J. Dairy Sci.*, **102**, 3321-3338 (2019).
59. Gomes, F., Henriques, M., Control of bovine mastitis: old and recent therapeutic approaches. *Curr. Microbiol.*, **72**, 377-382 (2016).
60. Pasca, C., Mărghitaş , L., Dezmirean, D., Bobiş, O., Bonta, V., Chirilă, F., Matei, I., Fiţ , N., Medicinal plants based products tested on pathogens isolated from mastitis milk. *Molecules*, **22**, 1473 (2017).
61. Ananda Baskaran, S., Kazmer, G.W., Hinckley, L., Andrew, S.M., Venkitanarayanan, K., Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens in vitro. *J. Dairy Sci.*, **92**, 1423-1429 (2009).
62. Kaithwas, G., Mukerjee, A., Kumar, P., Majumdar, D.K., *Linum usitatissimum* (linseed/flaxseed) fixed oil: antimicrobial activity and efficacy in bovine mastitis. *Inflammopharmacology*, **19**, 45-52 (2011).
63. Kher, M.N., Sheth, N.R., Bhatt, V.D., *In vitro* antibacterial evaluation of *Terminalia* chebula as an alternative of antibiotics against bovine subclinical mastitis. *Anim. Biotechnol.*, **30**, 151-158 (2019).
64. Zhao, Q.Y., Yuan, F.W., Liang, T., Liang, X.C., Luo, Y.R., Jiang, M., Qing, S.Z., Zhang, W.M., Baicalin inhibits *Escherichia coli* isolates in bovine mastitic milk and reduces antimicrobial resistance. *J. Dairy Sci.*, **101**, 2415-2422 (2018).
65. Kutila, T., Suojala, L., Lehtolainen, T., Saloniemi, H., Kaartinen, L., Tähti, M., Seppälä, K., Pyorala, S., The efficacy of bovine lactoferrin in the treatment of cows with experimentally induced *Escherichia coli* mastitis. *J. Vet. Pharmacol. Ther.*, **27**, 197-202 (2004).
66. Carvalho, C., Costa, A.R., Silva, F., Oliveira, A., Bacteriophages and their derivatives for the treatment and control of food-producing animal infections. *Crit. Rev. Microbiol.*, **43**, 583-601 (2017).
67. da Silva Duarte, V., Dias, R.S., Kropinski, A.M., Campanaro, S., Treu, L., Siqueira, C., Vieira, M.S., da Silva Paes, I., Santana, G.R., Martins, F., Crispim, J.S., da Silva Xavier, A., Ferro, C.G., Vidigal, P.M.P., da Silva, C.C., de Paula, S.O., Genomic analysis and immune response in a murine mastitis model of vB_EcoM-UFV13, a potential biocontrol agent for use in dairy cows. *Sci. Rep.*, **8**, 6845 (2018).
68. Porter, J., Anderson, J., Carter, L., Donjacour, E., Paros, M., In vitro evaluation of a novel bacteriophage cocktail as a preventative for bovine coliform mastitis. *J. Dairy Sci.*, **99**, 2053-2062 (2016).
69. Ali, M., Ijaz, M., Ikram, M., Ul-Hamid, A., Avais, M., Anjum, A.A., Biogenic synthesis, characterization and antibacterial potential evaluation of copper oxide nanoparticles against *Escherichia coli*. *Nanoscale Res. Lett.*, **16**, 148 (2021).
70. Yu, L., Shang, F., Chen, X., Ni, J., Yu, L., Zhang, M., Sun, D., Xue, T., The anti-biofilm effect of silver-nanoparticle-decorated quercetin nanoparticles on a multi-drug resistant *Escherichia coli* strain isolated from a dairy cow with mastitis. *PeerJ*, **6**, e5711 (2018).
71. Vilar, M.J., Rajala-Schultz, P.J., Dry-off and dairy cow udder health and welfare: Effects of different milk cessation methods. *Vet. J.*, **262**, 105503 (2020).
72. Hogan, J., Smith, K.L., Managing environmental mastitis. *Vet. Clin. North Am. Food Anim. Pract.*, **28**, 217-224 (2012).
73. Rainard, P., Gilbert, F.B., Germon, P., Foucras, G., Invited review: a critical appraisal of mastitis vaccines for dairy cows. *J. Dairy Sci.*, **104**, 10427-10448 (2021).
74. Brade, L., Hensen, S., Brade, H., Evaluation of a LPS-based glycoconjugate vaccine against bovine *Escherichia coli* mastitis: formation of LPS Abs in cows after immunization with *E. coli* core oligosaccharides conjugated to hemocyanine. *Innate Immun.*, **19**, 368-377 (2013).
75. Tahar, S., Nabil, M.M., Safia, T., Ngaiganam, E.P., Omar, A., Hafidha, C., Hanane, Z., Rolain, J.M., Diene, S.M., Molecular characterization of multidrug-resistant *Escherichia coli* isolated from milk of dairy cows with cinical mastitis in algeria. *J. Food Prot.*, **83**, 2173-2178 (2020).
76. Saidani, M., Messadi, L., Soudani, A., Daaloul-Jedidi, M., Chatre, P., Ben Chehida, F., Mamlouk, A., Mahjoub, W., Madec, J.Y., Haenni, M., Epidemiology, antimicrobial resistance, and extended-spectrum β-lactamase-producing enterobacteriaceae in clinical bovine mastitis in Tunisia. *Microb. Drug Resist.*, **24**, 1242-1248 (2018).
77. Aslam, N., Khan, S.U., Usman, T., Ali, T., Phylogenetic genotyping, virulence genes and antimicrobial susceptibility of *Escherichia coli* isolates from cases of bovine mastitis. *J. Dairy Res.*, **88**, 78-79 (2021).
78. Ali, T., Rahman, S.U., Zhang, L., Shahid, M., Han, D., Gao, J., Zhang, S., Ruegg, P. L., Saddique, U., Han, B., Characteristics and genetic diversity of multi-drug resistant extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from bovine mastitis. *Oncotarget*, **8**, 90144-90163 (2017).
79. Zhang, D., Zhang, Z., Huang, C., Gao, X., Wang, Z., Liu, Y., Tian, C., Hong, W., Niu, S., Liu, M., The phylogenetic group, antimicrobial susceptibility, and virulence genes of *Escherichia coli* from clinical bovine mastitis. *J. Dairy Sci.*, **101**, 572-580 (2018).
80. Ohnishi, M., Okatani, A.T., Harada, K., Sawada, T., Marumo, K., Murakami, M., Sato, R., Esaki, H., Shimura, K., Kato, H., Uchida, N., Takahashi, T., Genetic characteristics of CTX-M-type extended-spectrum-β-lactamase (ESBL)-producing enterobacteriaceae involved in mastitis cases on Japanese dairy farms, 2007 to 2011. *J. Clin. Microbiol.*, **51**, 3117-3122 (2013).
81. Campos, F.C., Castilho, I.G., Rossi, B.F., Bonsaglia, E.C.R., Dantas, S.T.A., Dias, R.C.B., Fernandes Júnior, A., Hernandes, R.T., Camargo, C.H., Ribeiro, M.G., Pantoja, J.C.F.,

Langoni, H., Rall, V.L.M., Genetic and antimicrobial resistance profiles of mammary pathogenic *E. coli* (MPEC) isolates from bovine clinical mastitis. *Pathogens*, **11**, 1435 (2022).
82. Majumder, S., Jung, D., Ronholm, J., George, S., Prevalence and mechanisms of antibiotic resistance in *Escherichia coli* isolated from mastitic dairy cattle in Canada. *BMC Microbiol.*, **21**, 222 (2021).
83. Tomazi, T., Coura, F.M., Goncalves, J.L., Heinemann, M.B., Santos, M.V., Antimicrobial susceptibility patterns of *Escherichia coli* phylogenetic groups isolated from bovine clinical mastitis. *J. Dairy Sci.*, **101**, 9406-9418 (2018).
84. Keane, O.M., Genetic diversity, the virulence gene profile and antimicrobial resistance of clinical mastitis-associated *Escherichia coli*. *Res. Microbiol.*, **167**, 678-684 (2016).
85. Eisenberger, D., Carl, A., Balsliemke, J., Kampf, P., Nickel, S., Schulze, G., Valenza, G., Molecular characterization of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from milk samples of dairy cows with mastitis in Bavaria, Germany. *Microb. Drug Resist.*, **24**, 505-510 (2018).
86. Alawneh, J.I., Vezina, B., Ramay, H.R., Al-Harbi, H., James, A.S., Soust, M., Moore, R.J., Olchowy, T.W.J., Survey and sequence characterization of bovine mastitis-associated *Escherichia coli* in dairy herds. *Front. Vet. Sci.*, **7**, 582297 (2020).
87. Ahmed, W., Neubauer, H., Tomaso, H., El Hofy, F.I., Monecke, S., Abd El-Tawab, A.A., Hotzel, H., Characterization of enterococci- and ESBL-producing *Escherichia coli* isolated from milk of bovides with mastitis in egypt. *Pathogens*, **10**, 97 (2021).
88. Dahmen, S., Métayer, V., Gay, E., Madec, J.Y., Haenni, M., Characterization of extended-spectrum β-lactamase (ESBL)-carrying plasmids and clones of Enterobacteriaceae causing cattle mastitis in France. *Vet. Microbiol.*, **162**, 793-799 (2013).
89. Geser, N., Stephan, R., Hächler, H., Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. *BMC Vet. Res.*, **8**, 21 (2012).