"Isolation, Characterization, and Antibiotic Resistance Profiling of Bovine Milk- Derived Pathogenic Bacteria"

[1]Nibha Agarwal ,

[1]M.Sc. in Applied Microbiology and Biotechnology ,
[1]Department of Environmental Microbiology and Immunology,
[1]ICMR- National Institute of Research in Environmental Health, Bhopal, India

Abstract : This study aimed to enumerate the isolation of bacterial pathogens, their biochemical and molecular characterization and antibiotic resistance profiling of pathogenic bacteria from bovine milk. Raw bovine milk samples (n=2) were collected from Barkheda Salam in Bhopal in falcon tubes, which were cultured in desired media plates shows the presence of *Staphylococcus aureus, E. coli* and *Acinetobacter baumanii* in a milk sample, whereas strains of *Pseudomonas and Salmonella* spp. were not confirmed. DNA band showing **ECO** gene for *E. coli,* **FemA** gene and **Nu C** gene for *Staphylococcus aureus*, and **blaOXA_51** gene for *Acinetobacter baumanii*, of product size **585 bp, 132 bp, 395 bp, 353 bp** were detected, whereas **Stn, Inv A, St_iroB** (*Salmonella spp*.) and **Opr L** for *Pseudomonas* spp. were not detected. **4** isolates of *Staphylococcus aureus*, **5** isolates of *E. coli*, and **1** isolate of *Acinetobacter baumanii* were detected, showing their high rate of resistance against **Kanamycin, Streptomycin, Imipenem, Augmentin, Ceftriaxone, Ticarcillin, Ciprofloxacin**. This high prevalence of bovine milk contamination with antimicrobial resistance necessitates the precise control on antibiotic prescription in medicines and drugs.

IndexTerms - **Bovine milk, Bacterial pathogens, Biochemical test, Antibiotic resistance, Drugs.**

INTRODUCTION

Nowadays, one of the biggest issues to be discussed in terms of global mortality, public health issues, and economic burden is bacteria that are resistant to antibiotics. (Taneja and Sharma 2019) It typically occurs following the emergence of novel microbial strains in response to certain antimicrobial strains—which may also have antiviral, antifungal, or even antimalarial properties. One of the worldwide concerns that needs to be addressed for patients who are at risk of developing serious illnesses is the steady development in antibiotic-resistant bacteria.

Bovine milk is a common dairy product that contains complex lipids, including a unique combination of fatty acids, lipids, proteins, amino acids, vitamins and minerals, as well as other protein-based receptors such as immunoglobulins, growth factors, cytokines, enzymes, hormones, peptides, lactose, and prolactin enzyme, which provide nourishment and nutrition to humans (Msalya 2017). It also promotes the formation of a diverse microbial population, which can then be used to produce prebiotic supplements. Milk protein is separated into two fractions: caseins and whey proteins, with an 80:20 ratio (Daniloski, McCarthy, and Vasiljevic 2021). In 1864, Pasteurization of milk was first established by Louis Pasteur in order to promote sterility. Some of the desired pathogenic bacteria found in bovine or raw milk include *Staphylococcus aureus, Acinetobacter baumanii, Pseudomonas aeruginosa, and Salmonella enterica*, which are responsible for the transmission of some life-threatening nosocomial disorders in humans and colonize on various biofilm surfaces, increasing the likelihood of antibiotic-resistant bacterial development through food consumption. For example, some harmful organisms, such as *Salmonella typhimurium* DT104 and several E. coli isolates, are always active against many antibiotics. Some bacteria have been found that are responsible for spreading unpleasant illnesses in our human bodies, such as *Staphylococcus* *aureus*, which is gram positive in nature and causes skin infections, pneumonia, endocarditis, septic arthritis, osteomyelitis, and is responsible for food poisoning.

Pseudomonas aeruginosa, which is gram negative, mucoid and colourless, causes septicaemia, pneumonia, eye infections, skin infections or cellulitis, osteomyelitis, endocarditis, and meningitis, and is known to thrive in a variety of environments. *Acinetobacter baumanii* is a gram-negative, strictly aerobic, non-fermentative bacterium that is widely distributed in nature. It is the primary cause of health-care-associated infections and community-acquired infections such as gastrointestinal, urinary, and respiratory tract infections. *Salmonella spp.* are gram-negative organisms that belong to the Enterobacteriaceae family. They are non-acid fast, non-capsulated, non-sporing, aerogenic, and have peritrichous motility. *S. enterica* serovar Typhimurium is also meant to cause food poisoning, as well as the transmission of zoonotic diarrhoea, and it's major enteric pathogen.

Ingestion of polluted water and/or food spreads a bacterium that causes gastrointestinal disorders in the intestinal section. As a result, the impact of antibiotic resistance bacteria on humans via bovine milk emphasizes the importance of conducting experiments involving comprehensive surveillance and characterization of antibiotic resistant or sensitive bacteria in milk products, in order to understand their prevalence, resistance mechanisms, and specific risks to be addressed. This also elucidates the specific research done to discover the antibiotic resistant bacterial pattern, therefore expanding our knowledge on antibiotic resistance in the dairy business, which acts as a precaution for animal welfare, ensuring the quality of dairy products, and protecting public health from the threats of antibiotic-resistant bacteria.

The main objective of this study is to isolate, identify and characterize these bacterial pathogens based on their antibiotic resistance profiles, by mixture of microbiological, biochemical, and molecular techniques like PCR and agarose gel electrophoresis.

LITERATURE REVIEW

2.1 Bovine milk sample

Essentially, milk is one of the ancient sources, containing some macro and micronutrients, and is usually obtained from extracted soya or almonds. According to 10,000-year-old history, farmers and pastoralists were the first humans to stay with cows and other domestic animals for dairy and agricultural purposes. All milk sources contain four different forms of casein, which when combined to create clusters of casein micelles, result in high calcium concentrations through the creation of colloidal calcium phosphate. Other than milk, various traditional cheeses play an important role in the development of microbial richness, including flavour, texture, and population density (Montel et al. 2014), by demonstrating its potential to battle pathogenic activity, which is connected with native antipathogenic or antimicrobial strains that protect against Listeria monocytogenes, making it well equipped for sale (Montel et al. 2014). The most significant advantage is the ability to store it in batches, which addresses some issues during the lactation cycle and ensures a consistent supply of milk to humans for the long term (Guo et al. 2024)

Based on the given milk content, which contains high fat, protein, and calcium contents (Cremonesi et al. 2021), it is quite easy to detect these pathogens going to resistant and sensitive action, and analyse the intensity of causing malfunctions in the human body system (Malik 2021; Mancuso et al. 2021). The rationale for collecting milk from a cow udder is the presence of microorganisms in the intramammary ecosystem that code for udder homeostasis and mastitis susceptibility, hence maintaining the quality of milk and other dairy products (Cremonesi et al. 2021). The most important dairy milk product, bovine milk, contains key elements such as proteins, carbs, lipids, vitamins, and minerals that are required for human health and nutrition. Furthermore, the supplied bovine milk can be distinguished by several physicochemical parameters such as mineral content, particle size, and the type of electrostatic charge created on the skimmed milk surface that is independent of temperature variations (Daniloski, McCarthy, and Vasiljevic 2021).

2.2 Pathogenic bacteria targeted from milk

Certain opportunistic based bacterial pathogens, such as *Enterobacter spp., Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella, and Acinetobacter baumanii*, (Mohamed et al. 2022), affected species growth and development as well as income requirements (Sahoo et al. 2023). Even the quality of pasteurized milk can suffer greatly due to the presence of spoilage bacteria such as Bacillus cereus, which can produce proteinase and phospholipase enzymes, altering the texture, taste, and flavour. (Palmeri et al. 2019)

There is no doubt that these types of pathogenic microorganisms causes udder infections, such as bovine mastitis, which are genuinely contagious and harmful in nature, causing methicillin-based virulent and multidrug resistant, nosocomial and specific in nature, which clearly depends on the utilization of pasteurized and raw milk samples as well as other food-borne illness (Souza et al. 2019).

One of the bacterial isolates named *Acinetobacter baumanii*, are gram negative, non-fermenting, and aerobic in nature, and belong to the Moraxellaceae family (Kyriakidis et al. 2021). It is spread through personal contact, water, and food contamination (Saad, Amin, and MOSTAFA 2018). They are mostly isolated from residual water or fluids of milk present in milk pipelines or containers that have been poorly sanitized, lack of storage, and improper transportation facilities (Mohamed et al. 2022). The term Acinetobacter is derived from the Greek word "Akinetos," which means "not mobile," and was first used in the twentieth century by Dutch microbiologist Beijerinck to describe the calcium acetate-rich soil bacterium Micrococcus calcoaceticus. It is responsible for causing community-acquired illnesses.

Most *A. baumanii* -based strains can be developed to convert nitrates to nitrites at a given optimum temperature and are resistant to dryness, which are some of the necessary factors for beneficial microbe survival in certain processed milk and milk-based products such as kareish and traditional artisan raw cheddar cheese. Antibiotic resistance strains can be conferred on existing pathogens through a variety of mechanisms, including control of antibiotic transport through membrane porins due to reduced permeability or increased efflux activity, antibiotic target modification, and enzymatically inactivated antibiotics. For example, it intrinsically exhibits its resistance to penicillin and cephalosporin medicines, which are the kind of beta-lactams with beta-lactamases enzymes, which leads to hydrolytic inactivation, variations of efflux activity, and antibiotic action.

One of the most important pathogenic bacteria to be highlighted is *Staphylococcus aureus*, which is gram-positive, non-sporing forming, non-motile, and facultative anaerobic in nature., a cocci-shaped clustered form of colonies, facultative anaerobic, solitary bacterium found in pairs or in short chains in the human digestive tract. It is regarded as the primary cause of infectious breast illnesses and mammary gland-based infections in bovine herds and cattle (Souza et al. 2019). Other infectious diseases include skin and soft tissue infections such as impetigo, cellulitis, and skin-based syndromes, which can cause endocarditis, meningitis, osteomyelitis, and pneumonia when medical equipment, such as intravenous fluid catheters or surgical instruments, is contaminated (Tălăpan, Sandu, and Rafila 2023).

The further isolation of *S. aureus* produces changes in milk and other dairy products due to improper storage, handling, and use of filthy vessels and tanks during transportation (Gebremedhin et al. 2022), which has been identified as a serious issue in the food and dairy industries, in addition to staphylococcus contamination in raw milk samples. *S. aureus* is further distinguished by its ability to produce antibiotic-resistant genes, which can be gained through spontaneous genetic mutations and horizontal gene transfer of antibacterial resistance genes via bacteriophages. Some antibiotics acting on Staphylococcus aureus infections, such as vancomycin, bind with the dipeptide D-Ala4-D-Ala5 of lipid II, limiting the PBP2 (penicillin-binding protein 2) catalysed trans glycosylation and transpeptidation activity, which is necessary for bacterial cell wall production and the inhibition of peptidoglycan remodelling (Tălăpan, Sandu, and Rafila 2023). Additionally, several macrolides, such as clarithromycin, azithromycin, and erythromycin, and other beta lactam antibiotics, are used therapeutically to treat staphylococci-based infections caused by methicillin-resistant strains (Tălăpan, Sandu, and Rafila 2023; Souza et al. 2019)

E.coli, been gram negative, pink-coloured in EMB plate, small rod-shaped colonies, facultative anaerobic in nature, and highly motile in nature, thereby creating single and short chains when going for gram staining, consisting of several markers like that of STEC (coding for Shiga toxin production) (Perelle et al. 2007), VTEC (for encoding Verotoxigenic E.coli for Vero toxin production) (Sandhu et al. 1996), and the most important serotype of *E.coli* O157:H7, codes for phenotypically delayed fermentation of D-sorbitol, certainly requiring enrichment and nutrition while growing in desired media, which is mostly associated with more food poisoning like producing toxigenicity and other gastrointestinal diseases that affect both children and adults.

The following *E.coli*-related illnesses include haemolytic uraemic syndrome, diarrhoea, and one of the most prevalent is acute renal failure caused by the intake of poor quality vegetables, effluent, fruits, and fresh cheese from diseased animals such as bovine mastitic cow. It also operates as a source of faecal contamination generated by pathogenic or toxic organisms.

Salmonella spp. are facultative anaerobic bacteria that belong to the Enterobacteriaceae family. They are non-acid fast, non-capsulated, non-sporing, aerogenic, and exhibit peritrichous motility. The colonies generated are circular, low convex, smooth, and more translucent than coliform colonies, measuring 2-3 mm in diameter, 0.7-1.5 um in diameter, and 2-5 um in length, respectively. They are also widely transmitted by domestic and wild animals such as chickens, pigs, companion animals such as cats, dogs, birds, and reptiles, contaminating the human body system, among other things.

Salmonella serovars have genome sizes ranging from 4460 kb to 4857 kb, with a total of 2,579 serovars participating in the salmonella group and possessing a greater variety of host, which are responsible for being employed as multi-drug resistance phenotypic, developed by forming extremely virulent clones, which code for changes in bacterial fitness and pathogenic activity over multiple host ranges (Foley et al. 2013). They are often identified by O,H, and VI antigens (Andino and Hanning 2015). Some *Salmonella* strains can cause infections such as Salmonellosis when ingested through contaminated water or food. Even *Salmonella*-based virulence plasmids contain several virulent factors, such as spv ABCD and its regulator spv R, which bind with it, making it essential for causing systemic virulence, when exposed to nutritionally based conditions provided to host cells, resulting in the formation of systemic virulent strains with lower G+C content than the normal core plasmid of host cells. This demonstrates the in vivo transfer of Salmonella virulent plasmids of certain species in host cell genomes as recipients.

Furthermore, some *Salmonella*-based isolates have class 1 integrons that are transferred to bacterial plasmids or chromosomal regions using molecular cloning techniques such as SNPs, which use S. Typhimurium 's DT9 and DT135 genes to code for host phage types. provides resistance to antimicrobial agents, which is also required for the production of antimicrobial drugs to treat multidrug resistant genes (Foley et al. 2013). Human disease like Salmonellosis occurs by direct and indirect contact with numerous species evolve due to contaminated meat, drugs, cattle, chemicals, poultry, raw milk and dairy products and sewage water etc (Castañeda-Salazar et al. 2021). *S. enterica* serovar Typhimurium is meant for causing food poisoning and is one of major zoonotic disease for diarrhoea, and it's a primary enteric pathogen which can be caused by ingestion of contaminated water and /or food which is then reached towards intestinal epithelium which triggers the response towards gastrointestinal disease which threatens the human body, causing respiratory diseases, gastroenteritis, fever, abdominal pain, nausea and vomiting, and diarrhoea, thereby weakening the immune system (Fàbrega and Vila 2013). Pathogenesis can be caused by *Salmonella spp.* in the form of clusters like that of SPI's T3SS receptors which are evenly distributed around it's genome and meant for colonizing over the host organism present. First line antibiotics such as ampicillin, chloramphenicol, trimethoprim- sulphathiazole act as multidrug resistance in *Salmonella enterica* based spp. Majority of infections caused by *Salmonella* from chicken eggs, can be made resistant to bacitracin, colistin, polymyxin-B (Wang et al. 2019).

Other gram-negative based pathogens like *Pseudomonas aeruginosa and Klebsiella pneumonia* of Enterobacteriaceae family, been non-sporulating, non-ciliated and showing mucoid based colonies, are one of the opportunistic pathogens, found in a solid state through in vitro culturing method (Kowalczyk et al. 2022).

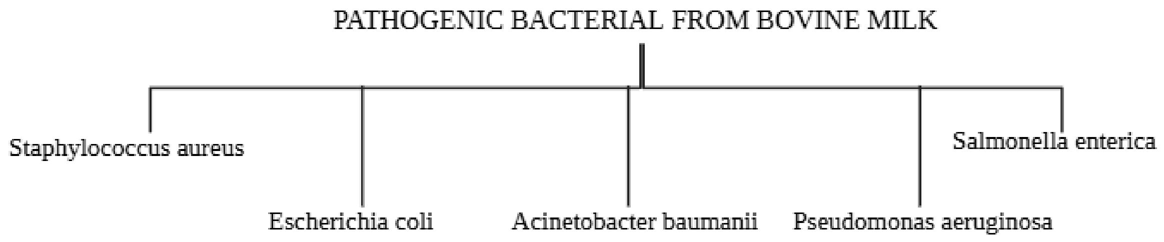

Figure showing the classification of pathogenic bacterial isolates enumerated from bovine milk

As a result, milk and milk-based products have longer shelf lives and higher quality. Furthermore, some of the isolates also show beta-galactosidase and phospholipase activity, including *Pseudomonas, Aeromonas, and Sphingobacterium* (Yuan et al. 2018), thereby determining the production and potentiality of desired bacterial strains present in raw milk sample, giving a bitter taste certain natural based products, including plant species containing an array of secondary metabolites like phytochemicals, piperine, daptomycin, thymol, and caffeic acid, etc. (Yuan et al. 2018)

2.3 Mechanisms involved in bacterial pathogens acquiring resistance

Most processes necessary for antimicrobial resistance development include modulation of antibiotic transport through cell membrane pores, changes in antibiotic target sites, and enzymatic action in antibiotic neutralization (Kyriakidis et al. 2021). There are numerous molecular mechanisms that contribute to the gradual or quick rise of AMRs in the human body, including cell wall production of proteins, nucleic acids, and other metabolic activities. Mutations generated by gene activation and regulation have also been passed down to offspring via horizontal and vertical gene transfer. (Singh 2018; Yuan et al. 2018; Mancuso et al. 2021)

For example, a variety of dairy-based products such as cheese, yogurt, butter, and cream contain suitable concentrations of harmed and unharmed isolated microbes such as *E. coli, Salmonella, Staphylococcus aureus, Klebsiella, Pseudomonas aeruginosa, and Acinetobacter baumanii*, which are gram positive and negative in nature, and have been responsible for the cause of other leading nosocomial infections like enteric illness, gastroenteritis, salmonellosis, nausea, vomiting. These are primarily spread by direct contact through milk carriers such as air, water, and soil.

When it comes to the evolution of antibiotic resistance and its importance in daily life, microbes can develop resistance to antibiotic drugs through point mutation and horizontal gene transfer methods such as conjugation, transduction, and transformation, among others (Shlaes et al. 1997), which can then be passed down to future generations via vertical transmission (Walsh 2000). The greatest approach for transferring mutant strains to subsequent generations is transduction by DNA packed bacteriophage mating. This optimizes the ecological niche of antimicrobial species, demonstrating their potential as antibiotic medications.
Even insect vectors such as flies and arachnids contribute to disease transmission by sucking animal blood or biting an insect, resulting in diarrhea, murine, and scrub typhus (Doron and Gorbach 2008).

Different microbial strains have evolved through different causes, such as the propensity to transmit illnesses and the amount of bacterial toxicity (Doron and Gorbach 2008), that help them adapt to drug action, including as gene mutation and the acquisition of foreign DNA from mutant bacteria via horizontal gene transfer (HGT), resulting in resistance to pharmacological supplements taken (Munita and Arias 2016).
The most essential mechanisms were adequate dose absorption, drug target change and deactivation, and drug efflux pumps, which are obviously dependent on the affinity and type of bacterial structural agents (Mancuso et al. 2021).

Furthermore, many mechanisms in species cell composition, such as enzymatic modification and biofilm growth, provide pressure on them to produce resistant mutant genes, posing a substantial risk to human and animal health (Ahmed et al. 2024). A biofilm is a bacterial community that forms its own extracellular matrix. It helps to block antibiotic penetration and prevent the creation of bacterial resistance concentrations in gaseous and nutritive flow (Uruén et al. 2020).

2.4 Antimicrobial resistance

AMR is one of the most important and main concern to be described in terms of mortality, public health problem and economic burden across the world due to spread of pathogens through food spoilage, waste water, and other source of contaminants present across the surroundings (Taneja and Sharma 2019). Even due to excessive use of antibiotics causes certain side effects in our body like severe illness, genetic mutations and sudden death (Sijbom et al. 2021). Even other bacterial isolates were evaluated by standard techniques including antimicrobial susceptibility test. In fact the concept of antimicrobial therapy includes the single or combined use of antibiotics with other medicinal drugs which remains effective against exposure of pathogenic species (Mulani et al. 2019).

Antibiotics have benefits and drawbacks based on dosage. To combat drug-related infections, novel therapeutic agents such as adjuvants, bacteriophages, nanoparticles, and photodynamic light therapy have been developed which are important for several clinical investigations (Mulani et al. 2019). The concept of antibiotic resistance and susceptibility is applied for enhancing of agricultural and dairy practices result in higher yields and high-quality products suitable for both humans and animals (Golkar, Bagasra, and Pace 2014; He et al. 2014).

2.5 Different Antibiotics applied for milk production

AMR is nowadays one of the most active points in the current scenario, whether in terms of mortality, public health problems, or economic burden around the world as a result of pathogen spread through food spoilage, waste water, and other sources of contaminants that are present in the environment (Taneja and Sharma 2019; K. Brown et al. 2020). Certain antibiotic drugs such as penicillin, amoxicillin, erythromycin, fluoroquinolones, cephalosporins, and others are used in the production of milk and other dairy products derived from infected animals, particularly mastitic cows.

Further employed in the form of metabolic components found in poultry-based goods, which are edible for everybody to ingest (Anika et al. 2019). This aids in the treatment and detection of clinical and subclinical disorders, reduces the spread of hazardous toxins, and promotes cattle rearing (Virto et al. 2022; K. Brown et al. 2020; Bitas and Samanidou 2018). However, excessive antibiotic use can create adverse effects in our bodies such as severe disease, genetic mutations, and sudden death; in reality, the greatly unleashed toxicity has resulted in the radical evolution of superbugs, which is a scourge on human life (Sijbom et al. 2021; Rahman, Hassan, and Chowdhury 2021).

As other milk-based goods have become a beneficial reason for the growth of the food-based business, it is critical to discuss their impact on bovine milk samples (Kumar, Panda, and Sharma 2022). Antimicrobial therapy is defined as the use of antibiotics alone or in combination with other therapeutic medications to prevent pathogenic species exposure (Mulani et al. 2019).

Pseudomonas aeruginosa and Salmonella spp. have been identified analytically from a given food source, including antimicrobial susceptibility testing, with *E.coli* being one of the main pathogens caused by changes in hygienic conditions such as poor milk quality and negligence during milk collection and processing. Furthermore, certain types of antibiotics are involved in the treatment of livestock respiratory diseases and urinary based infections, which include beta-lactams, tetracyclines, lincomycin, erythromycin, and sulfamethazine, which enhances the rate of antibiotic consumption, through milk products, reducing the risk of hyper allergenic reactions, carcinogenicity, hepatotoxicity, bone marrow toxicity, and certain reproductive disorders, which disrupts the gut microbial population (Virto et al. 2022).

Furthermore, certain types of antibiotics are involved in the treatment of livestock respiratory diseases and urinary based infections, which include beta-lactams, tetracyclines, lincomycin, erythromycin, and sulfamethazine , can be examined using some therapeutic-based methods enhancing the rate of antibiotic consumption, through milk products, reducing the risk of hyper allergenic reactions, carcinogenicity, hepatotoxicity, bone marrow toxicity, and certain reproductive disorders, which disrupts the gut microbial population (Virto et al. 2022)

Even if antibiotic strains have been detected in milk, they are advised for use as veterinary medicine, which is most common in India's northern and northeast regions (Kumar, Panda, and Sharma 2022).

Hence, the concept of antibiotic resistance and susceptibility is applied for enhancing of agricultural and dairy practices result in higher yields and high-quality products suitable for both humans and animals (Golkar, Bagasra, and Pace 2014; He et al. 2014).

DISCUSSION

Milk is one of the most nutritive sources, active for human growth and development (Lemma et al. 2021), but due to improper conditions, it detects the presence of bacterial pathogens including *E. coli, Salmonella spp., Bacillus spp.* which causes bacterial contamination (Martin, Evanowski, and Wiedmann 2023), which is the biggest challenge for human health and medication, and also responsible for excessive economic crisis. Different types of media has been used for microbial culturing like MSA, CET, EMB, Hektoen Enteric agar, and MAC, in which some are considered to be differential, selective or both, like for example, *Staphylococcus aureus* and *E.coli* shows highest growth in their respective media, thereby creating a risk factor for the cause of subclinical and bovine mastitis in animals (Birhanu et al. 2017), while that of *Pseudomonas, Acinetobacter and Salmonella* were less found, but sudden outbreak, through contaminated food and milk may lead to Salmonellosis, typhoid and other sort of diarrhoea associated infections, resulting in urinary tract issues, skin and wound infections, bacteremia, meningitis, and other bone-related diseases.

Biochemical tests are mostly performed to determine bacterial characteristics, which becomes a vital source for their identification in a given sample.

By gradient PCR method, we did molecular confirmation of all five bacterial pathogens, using virulent gene markers such as Fem A gene, Nu C gene, ECO gene, blaOXA_51, Stn, Inv A, St_iroB, and oprL. The PCR product sizes were 132 bp, 395 bp, 585 bp, 353 bp, showing their highly specific activity whereas the product sizes of genes of *Salmonella and Pseudomonas* were not detected.

We also performed antibiotic susceptibility test for determining the resistant and sensitive action of microbes against certain antimicrobial and therapeutic agents containing antimicrobial strains, and other mutants, necessary to produce new medicines and drugs for the progress of several pharmaceutical industries and some clinical based sectors(Mattar et al. 2020). It mostly focusses on defining the occurrence of AMR in the environment as well as development of analytical methodologies and mitigation strategies.

CONCLUSION

Our results show that raw milk has a great potential for transmission of antibiotic resistant pathogens such as E. coli, Staphylococcus aureus and Acinetobacter baumanii. In the present study, high levels of resistance were observed among the screened isolates towards Kanamycin, Streptomycin, Imipenem, Augmentin, Ceftriaxone, Ticarcillin, Ciprofloxacin, which provided evidence on high risk of food borne pathogens on humans through raw milk.

Since antibiotics have extensive applications in dairy cattle farms in developing countries, where the microbiota of raw milk may contain relatively high levels of antibiotic resistance bacteria. Therefore enhancing the safety of milk and implementing good manufacturing practices are extremely important for the health of consumers. Pasteurization of raw milk, prevention of cross-contamination, storage of raw milk in cold temperature, appropriate authority supervision, and regulatory monitoring on the use of antibiotics in dairy cattle farms are necessary to ensure the safety of milk products.

The main route for contamination of raw milk with resistant bacteria can be the subject of future studies to determine whether the bacteria get into the milk via cow's udder or mixed into the milk during or after milking.

The antibiotic resistance profiling of bovine derived pathogenic bacteria can be used to ensure the genetic variations of resistant bacterial isolates in near future.

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REFERENCES

1. Ahmed*, Omar B., and Anas S. Dablool. 2017. "Quality Improvement of the DNA Extracted by Boiling Method in Gram Negative Bacteria." *International Journal of Bioassays* 6 (04): 5347. https://doi.org/10.21746/ijbio.2017.04.004.
2. Ahmed, Sirwan Khalid, Safin Hussein, Karzan Qurbani, Radhwan Hussein Ibrahim, Abdulmalik Fareeq, Kochr Ali Mahmood, and Mona Gamal Mohamed. 2024. "Antimicrobial Resistance: Impacts, Challenges, and Future Prospects." *Journal of Medicine, Surgery, and Public Health* 2 (April):100081. https://doi.org/10.1016/j.glmedi.2024.100081.
3. Andino, A., and I. Hanning. 2015. "*Salmonella Enterica* : Survival, Colonization, and Virulence Differences among Serovars." *The Scientific World Journal* 2015:1–16. https://doi.org/10.1155/2015/520179.
4. Anika, Tasnia Tabassum, Zakaria Al Noman, Most Rifat Ara Ferdous, Sayekul Hasan Khan, Mufsana Akter Mukta, Md Shakil Islam, Md Tarek Hossain, and Kazi Rafiq. 2019. "Time Dependent Screening of Antibiotic Residues in Milk of Antibiotics Treated Cows." *Journal of Advanced Veterinary and Animal Research* 6 (4): 516–20. https://doi.org/10.5455/javar.2019.f376.
5. Birhanu, Misrak, Samson Leta, Gezahegne Mamo, and Shimelis Tesfaye. 2017. "Prevalence of Bovine Subclinical Mastitis and Isolation of Its Major Causes in Bishoftu Town, Ethiopia." *BMC Research Notes* 10 (1): 767. https://doi.org/10.1186/s13104-017-3100-0.
6. Bitas, Dimitrios, and Victoria Samanidou. 2018. "Molecularly Imprinted Polymers as Extracting Media for the Chromatographic Determination of Antibiotics in Milk." *Molecules (Basel, Switzerland)* 23 (2): 316. https://doi.org/10.3390/molecules23020316.

7. Brown, Kelsey, Maina Mugoh, Douglas R. Call, and Sylvia Omulo. 2020. “Antibiotic Residues and Antibiotic-Resistant Bacteria Detected in Milk Marketed for Human Consumption in Kibera, Nairobi.” Edited by James E. Wells. *PLOS ONE* 15 (5): e0233413. https://doi.org/10.1371/journal.pone.0233413.

8. Brown, S., H. K. Young, and S. G. B. Amyes. 2005. “Characterisation of OXA-51, a Novel Class D Carbapenemase Found in Genetically Unrelated Clinical Strains of Acinetobacter Baumannii from Argentina.” *Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases* 11 (1): 15–23. https://doi.org/10.1111/j.1469-0691.2004.01016.x.

9. Castañeda-Salazar, Rubiela, Adriana Del Pilar Pulido-Villamarín, Geraldine Lorena Ángel-Rodríguez, Camila Andrea Zafra-Alba, and Olimpo Juan Oliver-Espinosa. 2021. “Isolation and Identification of Salmonella Spp. in Raw Milk from Dairy Herds in Colombia.” *Brazilian Journal of Veterinary Research and Animal Science* 58 (June):e172805. https://doi.org/10.11606/issn.1678-4456.bjvras.2021.172805.

10. Cremonesi, Paola, Marco Severgnini, Alicia Romanò, Lorenza Sala, Mario Luini, and Bianca Castiglioni. 2021. “Bovine Milk Microbiota: Comparison among Three Different DNA Extraction Protocols To Identify a Better Approach for Bacterial Analysis.” Edited by Christina A. Cuomo. *Microbiology Spectrum* 9 (2): e00374-21. https://doi.org/10.1128/Spectrum.00374-21.

11. Daniloski, Davor, Noel A. McCarthy, and Todor Vasiljevic. 2021. “Bovine β-Casomorphins: Friends or Foes? A Comprehensive Assessment of Evidence from in Vitro and Ex Vivo Studies.” *Trends in Food Science & Technology* 116 (October):681–700. https://doi.org/10.1016/j.tifs.2021.08.003.

12. Doron, S., and S.L. Gorbach. 2008. “Bacterial Infections: Overview.” In *International Encyclopedia of Public Health*, 273–82. Elsevier. https://doi.org/10.1016/B978-012373960-5.00596-7.

13. Fàbrega, Anna, and Jordi Vila. 2013. “Salmonella Enterica Serovar Typhimurium Skills To Succeed in the Host: Virulence and Regulation.” *Clinical Microbiology Reviews* 26 (2): 308–41. https://doi.org/10.1128/CMR.00066-12.

14. Foley, Steven L., Timothy J. Johnson, Steven C. Ricke, Rajesh Nayak, and Jessica Danzeisen. 2013. “Salmonella Pathogenicity and Host Adaptation in Chicken-Associated Serovars.” *Microbiology and Molecular Biology Reviews* 77 (4): 582–607. https://doi.org/10.1128/MMBR.00015-13.

15. Gebremedhin, Endrias Zewdu, Addisu Bedashu Ararso, Bizunesh Mideksa Borana, Kebede Abdisa Kelbesa, Nega Desalegn Tadese, Lencho Megersa Marami, and Edilu Jorga Sarba. 2022. “Isolation and Identification of Staphylococcus Aureus from Milk and Milk Products, Associated Factors for Contamination, and Their Antibiogram in Holeta, Central Ethiopia.” Edited by Nora Mestorino. *Veterinary Medicine International* 2022 (May):1–13. https://doi.org/10.1155/2022/6544705.

16. Golkar, Zhabiz, Omar Bagasra, and Donald Gene Pace. 2014. “Bacteriophage Therapy: A Potential Solution for the Antibiotic Resistance Crisis.” *The Journal of Infection in Developing Countries* 8 (02): 129–36. https://doi.org/10.3855/jidc.3573.

17. Guo, Wenli, Shuai Liu, Muhammad Z. Khan, Jingjun Wang, Tianyu Chen, Gibson M. Alugongo, Shengli Li, and Zhijun Cao. 2024. "Bovine Milk Microbiota: Key Players, Origins, and Potential Contributions to Early-Life Gut Development." *Journal of Advanced Research* 59 (May):49–64. https://doi.org/10.1016/j.jare.2023.06.016.

18. He, Xuexiang, Stephen P. Mezyk, Irene Michael, Despo Fatta-Kassinos, and Dionysios D. Dionysiou. 2014. "Degradation Kinetics and Mechanism of β-Lactam Antibiotics by the Activation of H2O2 and Na2S2O8 under UV-254nm Irradiation." *Journal of Hazardous Materials* 279 (August):375–83. https://doi.org/10.1016/j.jhazmat.2014.07.008.

19. Hou, Chenggong, and Feng Yang. 2015. "Drug-Resistant Gene of blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58 in Acinetobacter Baumannii." *International Journal of Clinical and Experimental Medicine* 8 (8): 13859–63.

20. Karimzadeh, R., and R. Karimzadeh Ghassab. 2022. "Identification of Nuc Nuclease and Sea Enterotoxin Genes in Staphylococcus Aureus Isolates from Nasal Mucosa of Burn Hospital Staff: A Cross-Sectional Study." *New Microbes and New Infections* 47 (April):100992. https://doi.org/10.1016/j.nmni.2022.100992.

21. Kasa, Gezehagn, Betelihem Tegegne, and Belege Tadesse. 2020. "Isolation and Identification of Major Pathogenic Bacteria from Clinical Mastitic Cows in Asella Town, Ethiopia." Edited by Sumanta Nandi. *Veterinary Medicine International* 2020 (November):1–6. https://doi.org/10.1155/2020/6656755.

22. Kiedrowski, Megan R. 2012. "Characterization of Staphylococcus Aureus Extracellular Nuclease Activity." Doctor of Philosophy, University of Iowa. https://doi.org/10.17077/etd.jlcehkjn.

23. Kobayashi, N., H. Wu, K. Kojima, K. Taniguchi, S. Urasawa, N. Uehara, Y. Omizu, Y. Kishi, A. Yagihashi, and I. Kurokawa. 1994. "Detection of mecA, femA, and femB Genes in Clinical Strains of Staphylococci Using Polymerase Chain Reaction." *Epidemiology and Infection* 113 (2): 259–66. https://doi.org/10.1017/s0950268800051682.

24. Kowalczyk, Joanna, Ilona Czokajło, Marta Gańko, Marcin Śmiałek, and Andrzej Koncicki. 2022. "Identification and Antimicrobial Resistance in Klebsiella Spp. Isolates from Turkeys in Poland between 2019 and 2022." *Animals* 12 (22): 3157. https://doi.org/10.3390/ani12223157.

25. Kumar, Atul, Ashok Kumar Panda, and Neelam Sharma. 2022. "Determination of Antibiotic Residues in Bovine Milk by HPLC-DAD and Assessment of Human Health Risks in Northwestern Himalayan Region, India." *Journal of Food Science and Technology* 59 (1): 95–104. https://doi.org/10.1007/s13197-021-04988-8.

26. Kyriakidis, Ioannis, Eleni Vasileiou, Zoi Dorothea Pana, and Athanasios Tragiannidis. 2021. "Acinetobacter Baumannii Antibiotic Resistance Mechanisms." *Pathogens* 10 (3): 373. https://doi.org/10.3390/pathogens10030373.

27. Lemma, Fikirte, Haile Alemayehu, Andrew Stringer, and Tadesse Eguale. 2021. "Prevalence and Antimicrobial Susceptibility Profile of Staphylococcus Aureus in Milk and Traditionally Processed Dairy Products in Addis Ababa, Ethiopia." Edited by Bo Han. *BioMed Research International* 2021 (July):1–7. https://doi.org/10.1155/2021/5576873.

28. Malik, Taqdees. 2021. "Detection and Analysis of Pathogenic Bacteria from Raw and Pasteurized Milk." *Open Access Journal of Complementary & Alternative Medicine* 3 (1). https://doi.org/10.32474/OAJCAM.2021.03.000151.

International Research Journal
IJNRD
Research Through Innovation

29. Mancuso, Giuseppe, Angelina Midiri, Elisabetta Gerace, and Carmelo Biondo. 2021. "Bacterial Antibiotic Resistance: The Most Critical Pathogens." *Pathogens (Basel, Switzerland)* 10 (10): 1310. https://doi.org/10.3390/pathogens10101310.

30. Martin, N.H., R.L. Evanowski, and M. Wiedmann. 2023. "Invited Review: Redefining Raw Milk Quality—Evaluation of Raw Milk Microbiological Parameters to Ensure High-Quality Processed Dairy Products." *Journal of Dairy Science* 106 (3): 1502–17. https://doi.org/10.3168/jds.2022-22416.

31. Mattar, Caline, Suzanne Edwards, Enrico Baraldi, and Jennie Hood. 2020. "An Overview of the Global Antimicrobial Resistance Research and Development Hub and the Current Landscape." *Current Opinion in Microbiology* 57 (October):56–61. https://doi.org/10.1016/j.mib.2020.06.009.

32. Mohamed, Hams M. A., Hanan H. Abd-Elhafeez, Omar A. Al-Jabr, and Mona A. El-Zamkan. 2022. "Characterization of Acinetobacter Baumannii Isolated from Raw Milk." *Biology* 11 (12): 1845. https://doi.org/10.3390/biology11121845.

33. Montel, Marie-Christine, Solange Buchin, Adrien Mallet, Céline Delbes-Paus, Dominique A. Vuitton, Nathalie Desmasures, and Françoise Berthier. 2014. "Traditional Cheeses: Rich and Diverse Microbiota with Associated Benefits." *International Journal of Food Microbiology* 177 (May):136–54. https://doi.org/10.1016/j.ijfoodmicro.2014.02.019.

34. Msalya, G. 2017. "Contamination Levels and Identification of Bacteria in Milk Sampled from Three Regions of Tanzania: Evidence from Literature and Laboratory Analyses." *Veterinary Medicine International* 2017:1–10. https://doi.org/10.1155/2017/9096149.

35. Mulani, Mansura S., Ekta E. Kamble, Shital N. Kumkar, Madhumita S. Tawre, and Karishma R. Pardesi. 2019. "Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review." *Frontiers in Microbiology* 10 (April):539. https://doi.org/10.3389/fmicb.2019.00539.

36. Munita, Jose M., and Cesar A. Arias. 2016. "Mechanisms of Antibiotic Resistance." Edited by Indira T. Kudva and Qijing Zhang. *Microbiology Spectrum* 4 (2): 4.2.15. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015.

37. Nitz, Fabiana, Bruna Oliveira De Melo, Luís Cláudio Nascimento Da Silva, Andrea De Souza Monteiro, Sirlei Garcia Marques, Valério Monteiro-Neto, Rosimary De Jesus Gomes Turri, et al. 2021. "Molecular Detection of Drug-Resistance Genes of blaOXA-23-blaOXA-51 and Mcr-1 in Clinical Isolates of Pseudomonas Aeruginosa." *Microorganisms* 9 (4): 786. https://doi.org/10.3390/microorganisms9040786.

38. Palmeri, Parafati, Trippa, Siracusa, Arena, Restuccia, and Fallico. 2019. "Addition of Olive Leaf Extract (OLE) for Producing Fortified Fresh Pasteurized Milk with An Extended Shelf Life." *Antioxidants* 8 (8): 255. https://doi.org/10.3390/antiox8080255.

39. Perelle, Sylvie, Françoise Dilasser, Joël Grout, and Patrick Fach. 2007. "Screening Food Raw Materials for the Presence of the World's Most Frequent Clinical Cases of Shiga Toxin-Encoding Escherichia Coli O26, O103, O111, O145 and O157." *International Journal of Food Microbiology* 113 (3): 284–88. https://doi.org/10.1016/j.ijfoodmicro.2006.08.014.

40. Rahman, Md. Sahidur, Mohammad Mahmudul Hassan, and Sharmin Chowdhury. 2021. "Determination of Antibiotic Residues in Milk and Assessment of Human Health Risk in Bangladesh." *Heliyon* 7 (8): e07739. https://doi.org/10.1016/j.heliyon.2021.e07739.

41. Rashno Taee, Sedighe, Behzad Khansarinejad, Hamid Abtahi, Mohammad Najafimosleh, and Ehsanollah Ghaznavi-Rad. 2014. "Detection of algD, oprL and exoA Genes by New Specific Primers as an Efficient, Rapid and Accurate Procedure for Direct Diagnosis of Pseudomonas Aeruginosa Strains in Clinical Samples." *Jundishapur Journal of Microbiology* 7 (10): e13583. https://doi.org/10.5812/jjm.13583.

42. Saad, N.M, W.F. Amin, and S.M. MOSTAFA. 2018. "DETECTION OF ACINETOBACTER SPECIES IN MILK AND SOME DAIRY PRODUCTS." *Assiut Veterinary Medical Journal* 64 (156): 34–40. https://doi.org/10.21608/avmj.2018.168683.

43. Sahoo, Sonali, Manas Behera, Biswajit Mishra, Priyadarshini Sahoo, and Sonali Kar. 2023. "Antibiotic-Resistant Bacteria in Bovine Milk in India." *Journal of Advanced Veterinary and Animal Research* 10 (1): 21. https://doi.org/10.5455/javar.2023.j648.

44. Sandhu, K. S., R. C. Clarke, K. McFadden, A. Brouwer, M. Louie, J. Wilson, H. Lior, and C. L. Gyles. 1996. "Prevalence of the *Eae* A Gene in Verotoxigenic *Escherichia Coli* Strains from Dairy Cattle in Southwest Ontario." *Epidemiology and Infection* 116 (1): 1–7. https://doi.org/10.1017/S095026880005888X.

45. Shlaes, D. M., D. N. Gerding, J. F. John, W. A. Craig, D. L. Bornstein, R. A. Duncan, M. R. Eckman, et al. 1997. "Society for Healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the Prevention of Antimicrobial Resistance: Guidelines for the Prevention of Antimicrobial Resistance in Hospitals." *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 25 (3): 584–99. https://doi.org/10.1086/513766.

46. Sijbom, Martjin, Nicholas Saadah, Frederike Büchner, and Mattijs Numans. 2021. "Determinants of Inappropriate Antibiotic Prescription in Primary Care in Developed Countries with General Practitioners as Gatekeepers: A Systematic Review and Construction of a Framework." *Pathogens* 10 (10): 1310. https://doi.org/10.3390/pathogens10101310.

47. Singh, Bhoj R. 2018. "ESKAPE Pathogens in Animals and Their Antimicrobial Drug Resistance Pattern." *Journal of Dairy & Veterinary Sciences* 7 (3). https://doi.org/10.19080/JDVS.2018.07.555715.

48. Souza, Geziella Áurea Aparecida Damasceno, Anna Christina De Almeida, Mauro Aparecido De Sousa Xavier, Lívia Mara Vitorino Da Silva, Cintya Neves Sousa, Demerson Arruda Sanglard, and Alessandra Rejane Ericsson De Oliveira Xavier. 2019. "Characterization and Molecular Epidemiology of Staphylococcus Aureus Strains Resistant to Beta-Lactams Isolated from the Milk of Cows Diagnosed with Subclinical Mastitis." *Veterinary World* 12 (12): 1931–39. https://doi.org/10.14202/vetworld.2019.1931-1939.

49. Tălăpan, Daniela, Andreea-Mihaela Sandu, and Alexandru Rafila. 2023. "Antimicrobial Resistance of Staphylococcus Aureus Isolated between 2017 and 2022 from Infections at a Tertiary Care Hospital in Romania." *Antibiotics* 12 (6): 974. https://doi.org/10.3390/antibiotics12060974.

50. Taneja, Neelam, and Megha Sharma. 2019. "Antimicrobial Resistance in the Environment: The Indian Scenario." *The Indian Journal of Medical Research* 149 (2): 119–28. https://doi.org/10.4103/ijmr.IJMR_331_18.

International Research Journal
Research Through Innovation

51. Turton, Jane F., Neil Woodford, Judith Glover, Susannah Yarde, Mary E. Kaufmann, and Tyrone L. Pitt. 2006. "Identification of Acinetobacter Baumannii by Detection of the blaOXA-51-like Carbapenemase Gene Intrinsic to This Species." *Journal of Clinical Microbiology* 44 (8): 2974–76. https://doi.org/10.1128/JCM.01021-06.

52. Uruén, Cristina, Gema Chopo-Escuin, Jan Tommassen, Raúl C. Mainar-Jaime, and Jesús Arenas. 2020. "Biofilms as Promoters of Bacterial Antibiotic Resistance and Tolerance." *Antibiotics* 10 (1): 3. https://doi.org/10.3390/antibiotics10010003.

53. Virto, Mailo, Gorka Santamarina-García, Gustavo Amores, and Igor Hernández. 2022. "Antibiotics in Dairy Production: Where Is the Problem?" *Dairy* 3 (3): 541–64. https://doi.org/10.3390/dairy3030039.

54. Walsh, Christopher. 2000. "Molecular Mechanisms That Confer Antibacterial Drug Resistance." *Nature* 406 (6797): 775–81. https://doi.org/10.1038/35021219.

55. Wang, Xuchu, Silpak Biswas, Narayan Paudyal, Hang Pan, Xiaoliang Li, Weihuan Fang, and Min Yue. 2019. "Antibiotic Resistance in Salmonella Typhimurium Isolates Recovered From the Food Chain Through National Antimicrobial Resistance Monitoring System Between 1996 and 2016." *Frontiers in Microbiology* 10:985. https://doi.org/10.3389/fmicb.2019.00985.

56. Yuan, Lei, Faizan A. Sadiq, Tong-jie Liu, Yang Li, and Huan Yang. 2018. "Spoilage Potential of Psychrotrophic Bacteria Isolated from Raw Milk and the Thermo-Stability of Their Enzymes*." *Springerlink.Com* 14 (August):1114424. https://doi.org/10.1631/jzus.B1700352.

57. Zhou, Jindan, and Kenneth E. Rudd. 2013. "EcoGene 3.0." *Nucleic Acids Research* 41 (Database issue): D613-624. https://doi.org/10.1093/nar/gks1235.

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