INT. J. BIOL. BIOTECH., 20 (3): 479-492, 2023.

ANTIBIOTIC RESISTANCE OF BACTERIAL ISOLATES FROM MILK AND MILK BYPRODUCTS FROM TANDOJAM

Nabeela Yousaf[1], Dildar Hussain Kalhoro[1], Shahid Hussain Abro[1], Hubdar Ali Kolachi[2], Ali Raza Nizamani[1] Muhammad Saleem Kalhoro[1], Azizullah Memon[1], Sindhu Akbar Abro[1], Mehkar Hussain[3], Ghulam Mustafa Solangi[2], Muhammad Mohsen Rahimoon[2] and Hasina Baloch[1]

[1]*Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, Sindh Pakistan*
[2]*Shaheed Benazir Bhutto University of Veterinary & Animal Sciences, Sindh, Pakistan*
[3]*University of Karachi, Sindh, Pakistan*
Corresponding author: drdildarkalhoro@gmail.com

ABSTRACT

The study comprised of milk samples of goat (n=50), sheep (n=50), cow (n=50) and buffalo (n=50) from different dairy farms. This study isolated and characterized bacterial species in milk and its by-products, including goat, sheep, cow, and buffalo milk samples, as well as yogurt and butter samples. The samples were identified using Gram staining and biochemical tests. The results showed that the environment was the major source of contamination. Among the milk samples, *E. coli*, *Salmonella typhi, Staphylococcus aureus, Klebsiella pneumonia,* and *Shigella sonnei* were prevalent. The percentages of positive samples varied among the different milk types. Antimicrobial resistance was observed against several drugs, with different resistance patterns for each bacterial species. For example, *E. coli* from sheep milk showed high resistance to Oxytetracycline, Methicillin, Vancomycin, and Amoxicillin, while *K. pneumonia* from the same source displayed resistance to Oxytetracycline, Bacitracin, Methicillin, and Amoxicillin. Similar resistance patterns were observed in other milk types. In conclusion, the study found the prevalence of *E. coli, S. typhi, S. aureus, K. pneumonia, and S. sonnei* in milk and its by-products. Butter had the highest contamination rate of *E. coli* compared to other bacterial species. *S. aureus* exhibited high resistance to Amoxicillin, Tetracycline, and Methicillin.

Key words: Antibiotic resistance, milk, milk byproducts, Tandojam, Pakistan

INTRODUCTION

Milk and milk byproducts are consumed since ancient times and have an important role in daily human diet (Pal, 2014). Raw milk and several dairy products contain several nutrients that are essentially needed. The milk and milk by products are rich in vitamins, proteins and minerals that are necessarily required for healthy human life (Das *et al.*, 2015). Several products are produced by milk like, cheese, butter, yoghurt, ice cream and dried milk or milk powder etc. are consumed worldwide (Pal and Jadhav, 2013). There is difference in chemical composition of milk from ruminants and produce essential components for human diet. There is different chemical, biological and physical qualities of milk, its freezing point, specific gravity, pH, consistency, taste, odor and color of milk characteristics remain constant (Fadaei, 2014). Raw milk and their products are consumed directly by large numbers of people and raw milk is the excellent medium for microbial growth causing serve food borne diseases. This could be considered as one of the risks factor of disease outbreaks that may be transmitted through milk and its products, the consumption of contaminated milk not only a threat to human health but its nutritional quality is severely affected (Fadaei, 2014).

Common transmittable diseases are brucellosis, Q-fever, diphtheria, tuberculosis, scarlet fever and gastroenteritis from milk and byproducts. However, salmonellosis, campylobacteriosis, listeriosis and yersiniosis also considered as milk borne pathogens. Moreover, new pathogens are emerging which mainly include *Campylobacter jejuni, Listeria monocytogenes* and *Escherichia coli* (Prasad, 1998). Continuous use and resistance of antibiotics to pathogens is considered as an alarming issue in last two decades. Apart from the spreading resistance of food borne pathogens to antibiotics, it may be possible that commensal bacteria linked to food may carry resistance genes (Sharma *et al.*, 2014). Milk is considered as a complete diet because it is rich in all vital nutrients needed by humans (Chatterjee *et al.*, 2006). There are above 200 food-borne bacterial, fungal and viral diseases (Prasad, 1998). Among the coliform bacteria, mostly the *E. coli* are causing foodborne diseases (Chatterjee *et al.*, 2006)).

For the preparation of milk byproducts conventional protocol should be applied in relation to raw

material to ensure the hygienic quality of milk byproducts (Grewal, 1990). For improving the quality of milk and milk byproducts, precautionary measures should be followed against the contaminants (Roy *et al.*, 1998). About 50 % milk is utilized in fresh or boiled form, while 16-17 % are used in making yoghurt, butter, ice cream, cheese, khoya, rabri, kheer etc. Moreover, around 90 % diseases are transmitting to humans through unhygienic milk products (Ryser, 1998). Therefore, it is necessary to pasteurize the milk for human protection (Anjum *et al.*, 1989).

The prevalence and microbial loads of food borne pathogens (such as *C. jejuni*, *L. monocytogenes* and *Salmonella*) in milk varies considerably. The prevalence of these pathogens in milk are related to the factors such as farm size, sex, no of animals, hygienic status, farm management, sampling variation and samples type, difference in detection method, geographic distribution, and season. By analyzing these factors, it is concluded that milk is the major cause of producing foodborne illness in humans Jayarao (Jayarao and Henning, 2001). (Rohrbach *et al.*, 1992) analysed 292 samples from milk bulk tanks and reported 12.3% prevalence of *Camphylobacteria. Jejuni* 8.9% *Salmonella* spp. 4.1% *Listeria monocytogenes* and 15.1 % *Yersinia enterocolitis.* (Rohrbach *et al.*, 1992) observed that hat out of 84 dairy farms, 35% contamination were recorded at hygienic milking farms where teat sterilization was done along with the antimicrobial therapy of dry cow, while 35.00% contamination were recorded from dairy farmers those who consumed raw milk.

Types of spoilage microbes: Microorganisms are present everywhere, including air, water, soil, spring and ocean. Microbes may reach to the milk and dairy products by various sources and cause spoilages. Therefore, milk and milk by products contamination has become challenge to the producer Singh (Singh *et al.*, 2011). The contamination of milk byproducts may occur after the pasteurization due to the unhygienic filling machines hence the biofilm formation occurs after packing Dogan (Dogan and Boor, 2003). Formation of biofilm after the pasteurization shows the poor hygienic environment that implants a bad effect on milk processing efficiencies (Pal and Jadhav, 2013). This study shows that the bacterial count could be develop up to 108 CFU cmG^2; and their elimination is challenging under unhygienic environment (Sarkar, 2015).

1. Psychographs

Psychotropic microbes are cold loving bacteria as it grows on low temperature and correspond to a significant bacterial proportion in raw milk. The optimum temperature required for its growth is 3-7^oC and pH ranges between 4.5-4.7 but in cottage cheese psychotropics bacteria grows rapidly due to the low salt concentration which reduce shelf life cottage-cheese (Ledenbach and Marshall, 2009). Psychotropic bacteria including *Flavobacterium* spp. and *Pseudomonas* spp., *Achromobacter* spp., *Alcaligenes* spp., are so far concerned with the quality of cheese Fernandes (Fernandes, 2009).

2. *Listeria monocytogenes*

Listeria monocytogenes is a zoonotic food-borne pathogen in relation to public health concern. Recently (Pal, 2014) have published a review on contamination of milk and milk by products by *L. monocytogenes*. *L. monocytogenes* are present either in soft or semi soft cheese prepared from low heat-treated or raw cow milk. This organism grows more rapidly in pasteurized milk as compared to raw milk at 4-7°C, while in butter (prepared from contaminated cream) the organism grows slowly at 4 or 13°C and may survive for months in frozen butter without decreasing in numbers (Fernandes, 2009).

3. Spore-forming bacteria

The spore forming bacteria are generally grows in raw milk that could be transmitted to the dairy products. The number of bacterial loads may rarely exceed up to 5,000/ml before pasteurization which can contaminate the milk after processing as well. *Bacillus cereus, B. mycoides, B. megaterium, B. licheniformis* and *B. subtilis* are most common spore-forming bacteria isolated from dairy products (Ledenbach and Marshall, 2009). The bacterial spores are resistant to heat and remains active in raw and treated milk whereas, spores of *B. cereus* generally form biofilm (Vidal *et al.*, 2016).

Antimicrobial resistance

Antimicrobial resistance is a serious problem of public health concern in animals, farmer and environment (Garipcin and Seker, 2015). The resistance of *Staphylococcus aureus* is pathogenic to man as it transfers the resistance gene to other foodborne pathogens, humans and animals (Teuber, 2001). The investigation regarding the dairy production and antimicrobial resistance generally focuses on udder pathogens and resistance. Therefore, it is necessary to evaluate the presence of resistant bacteria by collecting

raw milk samples from diseased and healthy animals to assess the potential spread of resistant species (Teuber, 2001). The physicians in early 1970s stated that all the bacterial infections are treatable; this confidence ignored the development of antibiotic resistance mainly in *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Due to the multiple factors there is increase in resistance of bacterial species such as inappropriate and widespread use of antimicrobial drugs (Fuchs *et al.*, 2002). Among antimicrobial drugs quinupristin, dalfopristin and linezolid are new drugs that are active against resistant *staphylococci.* These drugs are active against G+ve bacteria which inhibits the bacterial protein synthesis. Quinupristin dalfopristin have bactericidal and anti-*Staphylococcal* activity. Cross resistance is not observed for linezolid, but one clinical isolate has evolved resistance during therapy, daptomycin is a novel bactericide is currently under clinical trials that damages cytoplasmic membrane of bacteria (Fuchs *et al.*, 2002).

The present study was carried out to isolate bacterial organisms from milk and milk by products. Additionally, antimicrobial resistance against bacterial isolates from milk and milk by- products were also investigated.

MATERIALS AND METHODS

Research description:

Present study was carried out at CVDL (Central Veterinary Diagnostic Lab), Tandojam to isolate and identify bacterial species in milk and milk by products as well as the antibiotic resistance against bacterial isolates. A total of two hundred milk samples cow (n=50), buffalo (n=50), sheep (n=50) and goat (n=50) from different dairy farms and one hundred dairy products (yogurt, n=50), butter, n=50) from retail shops were collected in sterilized glass bottles and brought to the Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, SAU, Tandojam and CVDL, Tandojam. The milk samples were kept at 4ºC in refrigerator until examine. The isolated bacteria species were identified by morphological and biochemical properties (Cruickshank, 1973).

Isolation of bacterial species:

Samples were cultured on different media i.e., Blood agar, MacConkey agar, *Salmonella shigella* agar, Brilliant Green agar, Nutrient agar and Mannitol salt agar and incubated at 37◦C for 24-48 hours. Grams staining method was used to identify bacterial species. Bacterial isolates were further confirmed by biochemical tests such as catalase, coagulase, indole, oxidase, urease, simmon citrate triple sugar test. The reagents and media used for isolation and identification of bacterial species.

Antimicrobial resistance bacterial isolates:

The antimicrobial resistance of bacterial isolates from milk and milk by products were determined according to the Guidelines from the clinical and Laboratory Standards Institute (CLSI) for disk diffusion methods (CLSI, 2018). A Total of fourteen antibiotics were tested such as Methicillin, Vancomycin, Norfloxacin, Tetracycline, Ampicillin Oxytetracycline Penicillin-G, Streptomycin, Gentamycin, Erythromycin, Doxycycline, Bacitracin, Amoxicillin, and Neomycin.

Muller Hinton agar used as a growth medium. Media and antibiotic accumulated discs (Detroit, USA) were obtained from (Defco Lab, USA) Inhibition zones were measured to determine sensitivity or resistance. A pure colony was picked and poured into normal saline solution mix well no solid material formed Took a cotton swab and spread into broth culture took a sterilized Muller Hinton plate and swab on it leave it 5 minutes. The desired antibiotics were placed on the agar surface and lightly press with a sterile forcep. The plates were closed and packed in aluminum foil and incubated overnight at 37^{o}C on the next day zones of inhibition clearly observed inhibition zones are measured by metric ruler according to Clinically Laboratory and Standard Institution (CLSI) protocol.

RESULTS

Isolation and identification of bacterial species in milk and milk by products were carried out during the month of (April to August) 2019 in Tando jam district Hyderabad. A total of 200 milk samples of goat (n=50), sheep (n=50), cow (n=50), and buffalo (n=50) were collected from local dairy farms and commercial dairy shops in Tando jam district Hyderabad. Besides that, 100 samples of milk by products such as yogurt (n=50) and butter (n=50) were also collected for isolation and identification of bacterial species. The

biochemical tests performed were Coagulase, Catalase, Oxidase, Urease, TSI (Triple Sugar Iron), MR (Methyl Red), VP (Voges-Proskauer), Indole and Simmon citrate tests. The antimicrobial tests of isolated bacterial species against various drugs included Oxytetracycline, Ampicillin, Bacitracin, Streptomycin, Neomycin, Norfloxacin, Gentamycin, Vancomycin, Methicillin, Amoxicillin, Doxycycline, Penicillin, Tetracycline, Erythromycin were also performed.

Milk samples

***Escherichia coli*:** Out of 200 milk samples examined in this investigation, 31 samples (15. 5 %) were found positive for E. coli in marketed milk. Present study revealed that goat and cow milk is highly contaminated with E. coli, followed by buffalo milk and least contamination was seen in sheep milk samples Table 1).

Table 1. Isolation of *E. coli* from the milk of various small and large ruminants

Milk Samples	No. of Samples	Positive	%
Goat	50	9	18
Sheep	50	5	10
Cattle	50	9	18
Buffalo	50	8	16
Total	200	31	15.5

***Salmonella typhi*:** Overall, out of 200 milk samples examined, 15 samples were found positive S. typhi, suggesting 7.5 % S. typhi prevalence in milk. It was observed that occurrence of S. typhi was more in cow milk, followed by goat and sheep milk while least contamination was observed in buffalo milk samples (Table 2).

Table 2. Isolation of Salmonella typhi from the milk of various small and large Ruminants

Milk Samples	No. of Samples	Positive	%
Goat	50	4	8
Sheep	50	4	8
Cattle	50	5	10
Buffalo	50	2	4
Total	200	15	7.5

Staphylococcus aureus: Out of 200 milk sample, 33 samples were found positive for *Staph. aureus*, suggesting 16.5% Staph. aureus prevalence (Table 3).

Table 3. Isolation of *Staphylococcus aureus* from the milk of various small and large ruminants

Milk Samples	**No. of Samples**	**Positive**	**%**
Sheep	50	9	18
Goat	50	8	16
Cattle	50	7	14
Buffalo	50	9	18
Total	200	33	16.5

Klebsiella pneumonia: The data showed that out of 200 samples 14 samples were found positive for *K. pneumonia* suggesting 7% prevalence. Higher K. pneumonia prevalence was detected in sheep and buffalo milk as compared to goat and cow milk in local dairy farm Tandojam (Table 4).

Table 4. Isolation of Klebsiella pneumonia from the milk of various small and large ruminants

Milk Samples	No. of Samples	Positive	%
Sheep	50	4	8
Goat	50	3	6
Cattle	50	3	6
Buffalo	50	4	8
Total	200	14	7.00

***Shigella sonnei*:** Overall, out of 200 samples 14 samples were found positive for *S. sonnei*, suggesting 7. % milk contamination. Higher *S. sonnei* prevalence was isolated in buffalo milk as compared to the milk samples of other animals (Table 5).

Table 5. Isolation of *Shigella sonnei* from the milk of various small and large ruminants

Milk Samples	No. of Samples	Positive	%
Goat	50	2	4
Sheep	50	3	6
Cattle	50	2	4
Buffalo	50	7	14
Total	200	14	7

Milk by products (Yogurt and Butter)

The milk by products (yogurt n=50, butter n=50) that are commonly consumed were also examined for microbial contamination (Table 6). *Escherichia coli* prevalence was 24% and 26 %, *Salmonella typhi* 10% and 8 % samples, *Staph. aureus* 20% and 18 %, Klebsiella *pneumonia* 8% and 6 %, whereas *Shigella sonnei* was isolated from 6% and 4 % yoghurt and butter samples respectively. The prevalence of *E. coli* was found remarkably higher in yoghurt and butter samples followed by *Staph. aureus* and *Salmonella typhi* while the prevalence of *Klebsiella pneumonia* and *Shigella sonnei* was comparatively lower in yoghurt and butter samples examined. The *E. coli* prevalence was relatively higher in butter samples as compared to yoghurt, while *Salmonella typhi*, *Staph. aureus*, *Klebsiella pneumonia* and *Shigella sonnei* prevalence was more in butter as compared to yoghurt (Table 6).

Table 6: Isolation of bacterial species from different milk by products available in the retail market Tando jam.

Bacterial species	Milk by-products	No. sample	Positive samples	Individual %
E. coli	Yoghurt	50	12	24
	Butter	50	13	26
Salmonella typhi	Yoghurt	50	5	10
	Butter	50	4	8
Staph. Aureus	Yoghurt	50	10	20
	Butter	50	9	18
Klebsiella pneumonia	Yoghurt	50	4	8
	Butter	50	3	6
Shigella sonnei	Yoghurt	50	3	6
	Butter	50	2	4

Biochemical examination

Biochemical tests such as Coagulase, Catalase, Oxidase, Urease, Triple Sugar Iron, Methyl Red, Voges-Proskauer, Indole and Simmon citrate were performed using standard protocols (Table 7).

Table 7. Biochemical properties of bacterial isolates.

S.no	Biochemical tests	Bacterial isolates				
		Staphylococcus	*Salmonella typhi*	*Escherichia coli*	*Shigella sonnei*	*Klebsiella pneumonia*
1	Coagulase	+ve	–ve	–ve	–ve	–ve
2	Catalase	+ve	+ve	+ve	+ve	+ve
3	Oxidase	–ve	–ve	–ve	–ve	–ve
4	Urease	+ve	–ve	–ve	–ve	+ve
5	Triple Sugar Iron	+ve	+ve	–ve	–ve	+ve
6	Methyl Red	+ve	+ve	+ve	+ve	–ve
7	Voges-Proskauer	+ve	–ve	–ve	+ve	+ve
8	Indole	–ve	–ve	+ve	–ve	–ve
9	Simmon citrate	–ve	+ve	–ve	–ve	+ve

Antimicrobial resistance of various pathogens isolated from sheep milk: *E. coli* isolates from sheep milk exhibited a high degree of resistance to antibiotics Oxytetracycline, Ampicillin, Streptomycin, Vancomycin, Methicillin, Amoxicillin, Penicillin and Erythromycin. Intermediate, Bacitracin, Doxycycline Neomycin and Tetracycline (Table 8).

Table 8. Antimicrobial resistance of various pathogens isolated from sheep milk.

Bacterial species	Antibiotic disc used	Inhibitory zone around disc	Resistance %	Interpretive Categories
E.coli	Oxytetracycline-30	0 mm	0 %	Resistant
	Ampicillin-10	0 mm	0 %	Resistant
	Bacitracin-10	18mm	90%	Intermediate
	Streptomycin-10	7 mm	70 %	Resistant
	Neomycin-10	20mm	100%	Intermediate
	Noroflaxacin-10	22mm	88%	Sensitive
	Gentamycin-30	25mm	100%	Sensitive
	Vancomycin-30	0 mm	0 %	Resistant
	Methicillin-10	0 mm	0 %	Resistant
	Amoxicillin-10	0 mm	0 %	Resistant
	Doxycycline-30	17mm	85%	Intermediate
	Penicillin-10	0 mm	0 %	Resistant
	Tetracycline-30	20mm	100%	Intermediate
	Erythromycin-15	10 mm	100 %	Resistant
Klebseilla Pneumonia	Oxytetracycline-30	0 mm	0 %	Resistant
	Ampicillin-10	17mm	94%	Intermediate
	Bacitracin-10	0 mm	0 %	Resistant
	Streptomycin-10	13 mm	100 %	Resistant
	Neomycin-10	12 mm	92 %	Resistant
	Noroflaxacin-10	25mm	89%	Sensitive
	Gentamycin-30	28mm	100%	Sensitive
	Vancomycin-30	10 mm	76%	Resistant
	Methicillin-10	12 mm	92%	Resistant

	Amoxicillin-10	0 mm	0%	Resistant
	Doxycycline-30	18mm	100%	Intermediate
	Penicillin-10	10 mm	76 %	Resistant
	Tetracycline-30	10 mm	76 %	Resistant
	Erythromycin-15	8 mm	61%	Resistant
Shigella Sonnei	Oxytetracycline-30	0mm	0%	Resistant
	Ampicillin-10	18mm	94%	Intermediate
	Bacitracin-10	21mm	84%	Sensitive
	Streptomycin-10	25mm	100%	Sensitive
	Neomycin-10	17mm	89%	Intermediate
	Noroflaxacin-10	22mm	88%	Sensitive
	Gentamycin-30	8mm	80%	Resistant
	Vancomycin-30	0mm	0%	Resistant
	Methicillin-10	10mm	100%	Resistant
	Amoxicillin-10	4mm	40%	Resistant
	Doxycyclin-30	10mm	100%	Resistant
	Penicillin-10	0mm	0%	Resistant
	Tetracycline-30	22mm	88%	Sensitive
	Erythromycin-15	19mm	100%	Intermediate
Staphylococcus Aureus	Oxytetracycline-30	22mm	78%	Sensitive
	Ampicillin-10	0 mm	0 %	Resistant
	Bacitracin-10	18mm	90%	Intermediate
	Streptomycin-10	20mm	100%	Intermediate
	Neomycin-10	25mm	89%	Sensitive
	Noroflaxacin-10	17mm	85%	Intermediate
	Gentamycin-30	28mm	100%	Sensitive
	Vancomycin-30	13 mm	100 %	Resistant
	Methicillin-10	0 mm	0 %	Resistant
	Amoxicillin-10	0 mm	0 %	Resistant
	Doxycycline-30	4mm	30 %	Resistant
	Penicillin-10	0 mm	0 %	Resistant
	Tetracycline-30	0 mm	0 %	Resistant
	Erythromycin-15	11 mm	84 %	Resistant
Salmonella typhiurium	Oxytetracycline-30	0 mm	0 %	Resistant
	Ampicillin-10	17mm	85%	Intermeditae
	Bacitracin-10	23mm	92%	Sensitive
	Streptomycin-10	10 mm	83 %	Resistant
	Neomycin-10	18mm	90%	Intermediate
	Noroflaxacin-10	20mm	100%	Intermediate
	Gentamycin-30	25mm	100%	Sensitive
	Vancomycin-30	10 mm	83 %	Resistant
	Methicillin-10	12 mm	100 %	Resistant
	Amoxicillin-10	0 mm	0%	Resistant
	Doxycycline-30	8 mm	66%	Resistant
	Penicillin-10	17mm	85%	Intermediate
	Tetracyclin-30	0 mm	0 %	Resistant
	Erythromycin-15	0 mm	0%	Resistant

Criteria: Sensitivity ≥21mm: Intermediate 17-20mm: Resistance ≤ 16mm (CLSI, 2018).

Antimicrobial resistance of various pathogens isolated from Goat milk: *E. coli* isolated from goat milk proved highly resistant to Oxytetracycline, Ampicillin, Methicillin, Penicillin Amoxicillin, Bacitracin and Erythromycin. Intermediate Streptomycin, Neomycin, Gentamycin, Vancomycin (Table 9).

Table 9. Antimicrobial resistance of various pathogens isolated from Goat milk.

Bacterial species	Antibiotic disc used	Inhibitory zone around disc	Resistance %	Interpretive categories
E. coli	Oxytetracyclin-30	0mm	0%	Resistant
	Ampicillin-10	10mm	83%	Resistant
	Bacitracin-10	10mm	83%	Resistant
	Streptomycin-10	17mm	85%	Intermediate
	Neomycin-10	19mm	95%	Intermediate
	Noroflaxacin-10	22mm	88%	Sensitive
	Gentamycin-30	20mm	100%	Intermediate
	Vancomycin-30	18mm	90%	Intermediate
	Methicillin-10	0mm	0%	Resistant
	Amoxicillin-10	8mm	66%	Resistant
	Doxycyclin-30	25mm	100%	Sensitive
	Penicillin-10	2mm	16%	Resistant
	Tetracycline-30	0mm	0%	Resistant
	Erythromycin-15	12mm	100%	Resistant
Klebseilla pneumonia	Oxytetracycline-30	0mm	0%	Resistant
	Ampicillin-10	10mm	100%	Resistant
	Bacitracin-10	0mm	0%	Resistant
	Streptomycin-10	0mm	0%	Resistant
	Neomycin-10	18mm	94%	Intermediate
	Noroflaxacin-10	25mm	100%	Sensitive
	Gentamycin-30	19mm	100%	Intermediate
	Vancomycin-30	4mm	40%	Resistant
	Methicillin-10	8mm	80%	Resistant
	Amoxicillin-10	10mm	100%	Resistant
	Doxycycline-30	0mm	0%	Resistant
	Penicillin-10	0mm	0%	Resistant
	Tetracycline-30	8mm	80%	Resistant
	Erythromycin-15	24mm	96%	Sensitive
Shigella Sonnei	Oxytetracycline-30	0mm	0%	Resistant
	Ampicillin-10	4mm	33%	Resistant
	Bacitracin-10	8mm	66%	Resistant
	Streptomycin-10	10mm	83%	Resistant
	Neomycin-10	12mm	100%	Resistant
	Noroflaxacin-10	20mm	100%	Intermediate
	Gentamycin-30	22mm	100%	Sensitive
	Vancomycin-30	21mm	95%	Sensitive
	Methicillin-10	0mm	0%	Resistant
	Amoxicillin-10	6mm	50%	Resistant
	Doxycyclin-30	10mm	83%	Resistant
	Penicillin-10	0mm	0%	Resistant
	Tetracycline-30	4mm	33%	Resistant
	Erythromycin-15	17mm	85%	Intermediate

Staphylococcus auerus	Oxytetracycline-30	10mm	66%	Resistant
	Ampicillin-10	8mm	53%	Resistant
	Bacitracin-10	22mm	95%	Sensitive
	Streptomycin-10	13mm	86%	Resistant
	Neomycin-10	20mm	100%	Intermediate
	Noroflaxacin-10	18mm	90%	Intermediate
	Gentamycin-30	23mm	100%	Sensitive
	Vancomycin-30	13mm	86%	Resistant
	Methicillin-10	0mm	0%	Resistant
	Amoxicillin-10	7mm	46%	Resistant
	Doxycycline-30	0mm	0%	Resistant
	Penicillin-10	0mm	0%	Resistant
	Tetracycline-30	0mm	0%	Resistant
	Erythromycin-15	15mm	100%	Resistant
Salmonella typhiurium	Oxytetracycline-30	0mm	0%	Resistant
	Ampicillin-10	20mm	100%	Intermediate
	Bacitracin-10	0mm	0%	Resistant
	Streptomycin-10	0mm	0%	Resistant
	Neomycin-10	24mm	96%	Sensitive
	Noroflaxacin-10	25mm	100%	Sensitive
	Gentamycin-30	10mm	83%	Resistant
	Vancomycin-30	0mm	0%	Resistant
	Methicillin-10	8mm	66%	Resistant
	Amoxicillin-10	0mm	0%	Resistant
	Doxycycline-30	0mm	0%	Resistant
	Penicillin-10	10mm	83%	Resistant
	Tetracycline-30	12mm	100%	Resistant
	Erythromycin-15	17mm	85%	Intermediate

Antimicrobial resistance of various pathogens isolated from Cow milk: *E. coli* isolated from cow milk showed highly degree of resistance to Oxytetracycline, Ampicillin, Bacitracin, Streptomycin, Gentamycin, Vancomycin, Amoxicillin, Methicillin, Penicillin and Tetracycline Intermediate Neomycin and Noroflaxcine while showed sensitivity to Doxycycline and Erythromycin (Table 10).

Table 10. Antimicrobial resistance of various pathogens isolated from Cow milk.

Bacterial species	Antibiotic discs Used	Inhibitory zone around disc	Resistance %	Interpretive Categories
E.coli	Oxytetracycline-30	0 mm	0 %	Resistant
	Ampicillin-10	10 mm	76%	Resistant
	Bacitracin-10	13mm	100%	Resistant
	Streptomycin-10	7 mm	53%	Resistant
	Neomycin-10	18mm	90%	Intermediate
	Noroflaxacin-10	20mm	100%	Intermediate
	Gentamycin-30	8mm	61%	Resistant
	Vancomycin-30	10 mm	76%	Resistant
	Methicillin-10	4 mm	30%	Resistant
	Amoxicillin-10	0mm	0%	Resistant
	Doxycycline-30	27mm	100%	Sensitive
	Penicillin-10	0 mm	0%	Resistant

	Tetracycline-30	4 mm	30%	Resistant
	Erythromycin-15	25 mm	92%	Sensitive
Klebseilla Pneumonia	Oxytetracycline-30	22mm	88%	Sensitive
	Ampicillin-10	10 mm	76%	Resistant
	Bacitracin-10	25mm	100%	Sensitive
	Streptomycin-10	12 mm	92%	Resistant
	Neomycin-10	13 mm	100%	Resistant
	Noroflaxacin-10	18mm	90%	Intermediate
	Gentamycin-30	20mm	100%	Intermediate
	Vancomycin-30	10 mm	76%	Resistant
	Methicillin-10	12 mm	92%	Resistant
	Amoxicillin-10	20mm	100%	Intermediate
	Doxycycline-30	18mm	90%	Intermediate
	Penicillin-10	11 mm	84%	Resistant
	Tetracycline-30	8 mm	72%	Resistant
	Erythromycin-15	23mm	92%	Sensitive
Shigella sonnei	Oxytetracycline-30	25mm	100%	Sensitive
	Ampicillin-10	0 mm	0%	Resistant
	Bacitracin-10	20mm	100%	Intermediate
	Streptomycin-10	19mm	95%	Intermediate
	Neomycin-10	17mm	85%	Intermediate
	Noroflaxacin-10	21mm	84%	Sensitive
	Gentamycin-30	24mm	96%	Sensitive
	Vancomycin-30	0 mm	0%	Resistant
	Methicillin-10	0 mm	0%	Resistant
	Amoxicillin-10	22mm	88%	Sensitive
	Doxycycline-30	4 mm	50%	Resistant
	Penicillin-10	0 mm	0%	Resistant
	Tetracycline-30	8 mm	100%	Resistant
	Erythromycin-15	20mm	100%	Intermediate
Staphylococcus aureus	Oxytetracycline-30	18mm	100%	Intermediate
	Ampicillin-10	0 mm	0%	Resistant
	Bacitracin-10	5 mm	41%	Resistant
	Streptomycin-10	2 mm	16%	Resistant
	Neomycin-10	10 mm	83%	Resistant
	Noroflaxacin-10	12 mm	100%	Resistant
	Gentamycin-30	22mm	91%	Sensitive
	Vancomycin-30	4 mm	33.3%	Resistant
	Methicillin-10	0 mm	0%	Resistant
	Amoxicillin-10	0 mm	0%	Resistant
	Doxycycline-30	17 mm	94%	Intermediate
	Penicillin-10	0 mm	0%	Resistant
	Tetracycline-30	0 mm	0%	Resistant
	Erythromycin-15	24 mm	100%	Sensitive
Salmonella	Oxytetracycline-30	0mm	0%	Resistant
	Ampicillin-10	24mm	100%	Sensitive
	Bacitracin-10	8mm	61%	Resistant
	Streptomycin-10	22mm	91%	Sensitive
	Neomycin-10	20mm	100%	Intermediate
	Noroflaxacin-10	21mm	87%	Sensitive
	Gentamycin-30	18mm	90%	Intermediate

typhiurium	Vancomycin-30	10mm	76%	Resistant
	Methicillin-10	17mm	89%	Intermediate
	Amoxicillin-10	0mm	0%	Resistant
	Doxycycline-30	13mm	100%	Resistant
	Penicillin-10	19mm	100%	Intermediate
	Tetracycline-30	10mm	76%	Resistant
	Erythromycin-15	12mm	92%	Resistant

Criteria: Sensitivity ≥21mm: Intermediate 17-20mm: Resistance ≤ 16mm (CLSI, 2018).

Antimicrobial resistance of various pathogens isolated from Buffalo milk: *E. coli* detected from buffalo milk samples showed high level of resistance to Oxytetracycline, Ampicillin, Bacitracin, Vancomycin, Methicillin, Amoxicillin, Penicillin and Tetracycline Intermediate to Streptomycin, Neomycin whereas, *E.coli* showed sensitivity to Gentamycin, Norfloxacin, Doxycycline and Erythromycin. Similarly, *Klebsiella pneumonia* showed high degree of resistance to Ampicillin, Bacitracin, Streptomycin, Neomycin, Vancomycin, Methicillin, Amoxicillin, Doxycycline, Penicillin and Tetracycline (Table 11).

Table 11. Antimicrobial resistance of various pathogens isolated from Buffalo milk.

Bacterial species	Antibiotic discs Used	Inhibitory zone around disc	Resistance %	Interpretive categories
E.coli	Oxytetracycline-30	8mm	80%	Resistant
	Ampicillin-10	2mm	20%	Resistant
	Bacitracin-10	10mm	100%	Resistant
	Streptomycin-10	20mm	100%	Intermediate
	Neomycin-10	18mm	90%	Intermediate
	Noroflaxacin-10	21mm	77%	Sensitive
	Gentamycin-30	25mm	92%	Sensitive
	Vancomycin-30	4mm	40%	Resistant
	Methicillin-10	0mm	0%	Resistant
	Amoxicillin-10	0mm	0%	Resistant
	Doxycycline-30	22mm	81%	Sensitive
	Penicillin-10	5mm	50%	Resistant
	Tetracycline-30	0mm	0%	Resistant
	Erythromycin-15	27mm	100%	Sensitive
Klebseilla Pneumonia	Oxytetracycline-30	22mm	81%	Sensitive
	Ampicillin-10	10mm	66%	Resistant
	Bacitracin-10	0mm	0%	Resistant
	Streptomycin-10	13mm	86%	Resistant
	Neomycin-10	12mm	80%	Resistant
	Noroflaxacin-10	17mm	94%	Intermediate
	Gentamycin-30	18mm	100%	Intermediate
	Vancomycin-30	10mm	66%	Resistant
	Methicillin-10	0mm	0%	Resistant
	Amoxicillin-10	14mm	93%	Resistant
	Doxycycline-30	8mm	53%	Resistant
	Penicillin-10	15mm	100%	Resistant
	Tetracycline-30	10mm	66%	Resistant
	Erythromycin-15	27mm	100%	Sensitive
	Oxytetracycline-30	18mm	90%	Intermediate
	Ampicillin-10	2mm	15%	Resistant
	Bacitracin-10	22mm	81%	Sensitive

Shigella sonnei	Streptomycin-10	23mm	85%	Sensitive
	Neomycin-10	13mm	100%	Resistant
	Noroflaxacin-10	25mm	92%	Sensitive
	Gentamycin-30	27mm	100%	Sensitive
	Vancomycin-30	0mm	0%	Resistant
	Methicillin-10	0mm	0%	Resistant
	Amoxicillin-10	17mm	85%	Intermediate
	Doxycycline-30	20mm	100%	Intermediate
	Penicillin-10	8mm	61%	Resistant
	Tetracycline-30	4mm	30%	Resistant
	Erythromycin-15	20mm	100%	Intermediate
Staphylococcus aureus	Oxytetracycline-30	18mm	90%	Intermediate
	Ampicillin-10	4mm	30%	Resistant
	Bacitracin-10	8mm	61%	Resistant
	Streptomycin-10	20mm	100%	Intermediate
	Neomycin-10	12mm	92%	Resistant
	Noroflaxacin-10	22mm	88%	Sensitive
	Gentamycin-30	25mm	100%	Sensitive
	Vancomycin-30	13mm	100%	Resistant
	Methicillin-10	10mm	76%	Resistant
	Amoxicillin-10	0mm	0%	Resistant
	Doxycycline-30	24mm	96%	Sensitive
	Penicillin-10	8mm	61%	Resistant
	Tetracycline-30	17mm	85%	Intermediate
	Erythromycin-15	19mm	95%	Intermediate
Salmonella typhiurium	Oxytetracycline-30	0mm	0%	Resistant
	Ampicillin-10	25mm	96%	Sensitive
	Bacitracin-10	22mm	84%	Sensitive
	Streptomycin-10	18mm	90%	Intermediate
	Neomycin-10	17mm	85%	Intermediate
	Noroflaxacin-10	25mm	96%	Sensitive
	Gentamycin-30	13mm	100%	Resistant
	Vancomycin-30	4mm	30%	Resistant
	Methicillin-10	8mm	61%	Resistant
	Amoxicillin-10	0mm	0%	Resistant
	Doxycycline-30	20mm	100%	Intermediate
	Penicillin-10	10mm	76%	Resistant
	Tetracycline-30	26mm	100%	Sensitive
	Erythromycin-15	22mm	84%	Sensitive

Criteria: Sensitivity ≥21mm: Intermediate 17-20mm: Resistance ≤ 16mm (CLSI, 2018).

DISCUSSION

Pathogenic bacteria in milk have been a matter of public health concern and many diseases are transmissible via milk by products. The outbreaks of bacterial infection demand attention to work on milk borne pathogens, especially the emerging pathogens. The highly nutritious nature of dairy products makes them especially good media for the growth of microorganisms (Ledenbach and Marshall, 2009). The spoilage of milk or milk by products occurs when physicochemical properties of milk are degraded by the microorganisms (Das *et al.*, 2015). Hence, this study was performed to isolate and characterize bacterial species in milk and milk byproducts.

The present study was planned to isolate bacterial pathogens from goat, sheep, cow and buffalo milk. The milk samples were mostly contaminated by *E. coli* 15.5%. *S. typhi* 7.5 %, *Staph. aureus* 16.5%, *K.*

pneumonia S. sonnei. 7% prevalence. Out of 50 yogurt and butter samples the *E. coli* was isolated from 24% and 26 % samples of yoghurt and butter respectively, S. *typhi* from 10 % and 8 % in samples, *Staph. aureus* from 20 % and 18 %, *K. pneumonia* from 8% and 6 %, whereas, S. *sonnei* was isolated from 6% and 4 % yoghurt and butter samples. Goat and cow milk samples were more contaminated with *E. coli*, followed by buffalo milk while the least contamination was seen in sheep milk samples. *S. typhi* contamination was higher in cow milk followed by goat and sheep milk while least *S. typhi* prevalence was seen in buffalo milk samples. Higher *Staph. aureus* prevalence was observed in sheep and buffalo milk than goat and cow milk. Higher *K. pneumonia* prevalence was observed in sheep and buffalo milk as compared to goat and cow milk. Higher prevelance of *S. sonnei* was observed in buffalo milk as compared to the milk samples of other animals. The prevalence of *E. coli* was higher in yoghurt and butter samples, followed by *Staph. aureus* and *Salmonella typhi,* while the prevalence of *K. pneumonia* and *S. sonnei* was relatively lower in yoghurt and butter samples. These results are in agreement with past workers (Akineden *et al.,* 2008) who isolated *Staphylococcus aureus* from goat milk and cheese while Bonfoh *et al.* (2003) found that microbiological quality of cow milk was deteriorated by contamination of milk with *Klebsiella pneumonia, Salmonella typhi* and *Staphylococcus aureus.* Chambers (2001) reported that *Staphylococcus aureus* was the major organism that cause spoilage of milk. Chapaval *et al.* (2010) found that *E. coli* has been the major microbial organism that caused milk spoilage. Chatterjee *et al.* (2006) reported that the *E. coli, Shigella* and *Salmonella* were general contaminants of buffalo milk.

Antimicrobial resistance profile of bacterial isolates from goat, sheep, cow and buffalo milk showed higher resistance to different antimicrobial drugs. Our results are in agreement with other authors who reported similar results. Barber *et al.,* (2003) and Cocconcelli *et al.* (2003) detected microbial resistance to vancomycin and tetracycline; while Das *et al.* (2015) examined microbial load quality of milk and milk based dairy products. It was notice that most of the microbial species showed resistance to antimicrobial agents. DeBuyser *et al.* (2001) tested milk and milk by products in food-borne diseases in France and reported greater microbial resistance to drugs.

Conclusion

E. coli, S. typhi, Staph. aureus, K. pneumonia and S. sonnei were recorded as microbial species isolated from milk and milk by products. Goat and cow milk were mostly contaminated by *E. coli*, followed by buffalo milk while the least contamination was seen in sheep milk samples. Occurrence of *S. typhi* was more in cow milk, followed by goat and sheep milk while least *S. typhi* contamination was seen in buffalo milk samples. Sheep and buffalo milk were under higher contamination of *Staph. aureus* as compared to goat milk and cow milk. Higher *K. pneumonia* prevalence was detected in sheep and buffalo milk as compared to goat milk and cow milk in Tando jam district Hyderabad. Higher *S. sonnei* prevalence was isolated in buffalo milk as compared to the milk samples of other ruminants. The prevalence of *E. coli* was higher in yogurt and butter samples, followed by *Staph. aureus* and *Salmonella typhi*; while the prevalence of *K. pneumonia* and *S. sonnei* was relatively lower. The *E. coli* prevalence was relatively higher in butter samples as compared to yoghurt while *S. typhi*, *Staph. aureus, K. pneumonia* and *S. sonnei* prevalence was more in butter as compared to yoghurt. *E. coli, Salomella typhi, Shigella sonnei, Klebseilla pneumonia, Staphylococcus aureus* highly resistance to Amoxcilline, Tetracycline and Methicillin.

REFERENCES

Akineden, Ö., A. A. Hassan, E. Schneider and E. Usleber (2008). Enterotoxigenic properties of staphylococcus aureus isolated from goats' milk cheese. *Int. J. Food. Microbiol.,* 124(2): 211-216.

Anjum, M. S., K. Lodhi, A. A. Raza, F. Walters and S. Krause (1989). Pakistan's dairy industry: Issues and policy alternatives. The economic analysis network project/USAID/394-0491-C00-5035 Islamabad Pakistan.

Barber, D. A., G. Y. Miller and P. E. McNamara (2003). Models of antimicrobial resistance and foodborne illness: Examining assumptions and practical applications. *J. Food. Protec.,* 66(4): 700-709.

Bonfoh, B., A. Wasem, A. Traore, A. Fane, H. Spillmann, C. Simbé, I. Alfaroukh, J. Nicolet, Z. Farah and J. Zinsstag (2003). Microbiological quality of cows' milk taken at different intervals from the udder to the selling point in bamako (Mali). *Food Control,* 14(7): 495-500.

Chambers, H. F. (2001). The changing epidemiology of staphylococcus aureus? *Emerg. Infect. Dis.,* 7(2): 178.

Chapaval, L., D. H. Moon, J. E. Gomes, F. R. Duarte, S. M. Tsai and R. T. Nassu (2010). Efeito da

temperatura sobre a produção de enterotoxina estafilocócica em leite. *Hig. Aliment*: 162-167.
Chatterjee, S., I. Bhattacharjee, S. Chatterjee and G. Chandra (2006). Microbiological examination of milk in Tarakeswar, India with special reference to coliforms. *Afri. J. Biotech.,* 5(15): 1383-1385.
CLSI, (2018). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. *CLSI* supplement VET08,4th ed, Clinical and Laboratory Standards Institute: Wanyne, NJ, USA;ISBN 978-1-68440-008-4.
Cruickshank R,, J. P. Dugaid, B P Marmion and R. H. Swain (1973). *Medical Microbiology,* Vol.2,12th Edn., Churchill Livingstone Publishing, Edingurgh.
Das, S., G. A. Hasan and S. Parveen (2015). Evaluation of microbial load and quality of milk & milk based dairy products. *Octa. J. Biosci.,* 3(1): 1-4.
De Buyser, M.-L., B. Dufour, M. Maire and V. Lafarge (2001). Implication of milk and milk products in food-borne diseases in France and in different industrialized countries. *Intr. J. Food Microbiol*. 67(1-2): 1-17.
Dogan, B. and K. J. Boor (2003). Genetic diversity and spoilage potentials among pseudomonas spp. Isolated from fluid milk products and dairy processing plants. *Applied. Environ. Microbiol.,* 69(1): 130-138.
Fadaei, A. (2014). Bacteriological quality of raw cow milk in Shahrekord, Iran. *Vet. World*. 7(4): 240-243.
Fernandes, R. (2009). *Microbiol. Handbook: Dairy products*. Royal Society of Chemistry.
Fuchs, P. C., A. L. Barry and S. D. Brown (2002). *In vitro* bactericidal activity of daptomycin against staphylococci. *J. Antimicro. Chemo*. 49(3): 467-470.
Garipcin, M. and E. Seker (2015). Nasal carriage of methicillin-resistant staphylococcus aureus in cattle and farm workers in turkey. *Veterinarski arhiv,* 85(2): 117-129.
Grewal, J. S. and R. P. Tiwari (1990). Microbiological quality of rasmalai. *J. Food Sci. Tech*., Mysore, 27: 178-179.
Jamro, M. R., S. Tunio, U. Buriro and Q. Chachar (2015). Effect of planting dates on growth and yield of true potato seed (tps) in nursery raising approach. *J. Basic. Appl. Sci*., 11: 318-322.
Jayarao, B. and D. Henning (2001). Prevalence of foodborne pathogens in bulk tank milk. *J. Dairy. Sci*., 84(10): 2157-2162.
Lalitha, M.K. (2004). Manual on antimicrobial suspectibility testing. Performance strandard for antimicrobial testing*:* Twelfth informational Supplement, 56238, pp.454-456
Ledenbach, L. H. and R. T. Marshall (2009). Microbiological spoilage of dairy products. *Compendium of the microbiol spoilage. Foods and Beverages*: 41-67.
Vidal, A.M.C., A., O. Durival Rossi Junior, I. Leite de Abreu, K. Paes Bürger, M. Vedovelli Cardoso, A. Carolina Siqueira Gonçalves and G. Augusto Marques Rossi (2016). Detection of *Bacillus cereus* isolated during ultra-high temperature milk production flowchart through random amplified polymorphic DNA polymerase chain reaction. Ci. Rural. 46(2): 286-292.
Pal, M. (2014). Spoilage of dairy products due to fungi. *Beverage. Food World*. 41(7): 37-38.
Pal, M. and V. Jadhav (2013). Microbial contamination of various India milk products. *Beverage, Food World*, 40(12): 43-44.
Prasad, D. (1998). Hygienic and safety aspects of dairy products. *Indian Dairyman.* 50: 112-118.
Rohrbach, B. W., F. A. Draughon, P. M. Davidson and S. P. Oliver (1992). Prevalence of listeria monocytogenes, campylobacter jejuni, yersinia enterocolitica, and salmonella in bulk tank milk: Risk factors and risk of human exposure. *J. Food. Protec*, 55(2): 93-97.
Roy, R., J. Chakrabarti and R. Gandhi (1998). Microbiological quality-assurance of milk sweets (sandesh and kalakand) by haccp. *Indian Dairyman*, 50: 67-71.
Ryser, E. (1998). *Public health concerns applied dairy microbiology.* New York: Dekker.
Sarkar, S. (2015). Microbiological considerations: Pasteurized milk. *Intr. J. Dairy Sci.,* 10(5): 206-218.
Sharma, P., S. K. Tomar, P. Goswami, V. Sangwan and R. Singh (2014). Antibiotic resistance among commercially available probiotics. *Food. Res. Intr.,* 57: 176-195.
Singh, V., S. Kaushal, A. Tyagi and P. Sharma (2011). Screening of bacteria responsible for the spoilage of milk. *J. Chemi. Pharma. Res.,* 3(4): 348-350.
Teuber, M. (2001). Veterinary use and antibiotic resistance. *Current Opinion in Microbiology*, 4(5): 493-499.

(*Accepted for publication June 2023*)