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Research Article

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Quarantine and Antibiotic Susceptibility of *Enterobacteriaceae* Strains and other Gram-Negative Bacteria in Dairy Sweetmeat Milk (Doodh) Peda

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Abstract

The present study aimed to assess the prevalence and isolation of Enterobacteriaceae strains and other gram-negative bacteria of milk (doodh) peda-a traditional milk product of India. Bacteria of the family Enterobacteriaceae are the indicator organisms that provide evidence of poor hygiene, inadequate processing or post-process contamination of foods. A total of 25 peda samples were collected from different places in the Mysuru district to evaluate the prevalence of Enterobacteriaceae. Seventeen gram-negative fermentative bacteria of clinical significance were detected and isolated. API 20E biochemical identification syatem and other associated biochemical tests are used as a supportive tool to identify gram-positive and gram-negative bacteria. Our outcomes indicate that milk peda contaminated with the Enterobacteriaceae like Enterobacter cloacae, Yersinia bercovieri, Yersinia rohdei, Raoutlella terigena, Acinetobacter lwoffii, Pantoea agglomerans PA2, Klebsiella pneumoniae subsp pneumoniae, Leclericia adecarboxylata, Photorhabdus nematophilus, Enterobacter gergoviae, Acinetobacter baumannii/calcoacetius, Cronobacter sakazaki (Enterobacter sakazaki). Further, the antimicrobial resistance of these Enterobacteriaceae groups was also investigated against 20 antibiotics by the disc diffusion assay method. All isolates revealed susceptibility to the fluoroquinolones and aminoglycosides. But resistance to the nitrofurantoin (70.59 %), augmentin (52.94%), and cefpodoxime (47.06%) antibiotics. Our finding was the first report of the prevalence and detection of E. gergoviae in a food sample (milk peda) and examined for antibiotic resistance.

Keywords: Peda or Dairy Confectionary, *Enterobacteriaceae*, Antibiotic Resistance

Introduction

Among the Indian indigenous dairy products, khoa and khoa based milk confectionaries provide an excellent means of preserving surplus milk solids. Milk (doodh) peda or peda, a traditional milk product of India, is prepared from khoa mixed with sugar and flavors. Khoa has been obtained by thermal evaporation of cow or buffalo milk to get 65-70% of solids. The quantity of peda produced in India exceeds any other indigenous milk-based sweet using khoa as the raw material. Milk products, including doodh peda or milk peda, have been implicated in outbreaks of enteritis and food poisoning in India. Food can also be contaminated by infected handlers, cross-contamination due to poor hygiene, and from feces from an infected animal or a person [1].

The manufacture of these products has been based on traditional methods with less concern for the raw materials used and the hygienic quality of the products. Under such conditions, many microorganisms can find access to the milk products [2]. The unhygienic conditions at the production units lead to contamination of products with different types of microorganisms [3]. Microorganisms may entry food at any stage of processing stage, like packing, transport, storage, etc. So it becomes imperative to take all kinds of preventive measures and evaluate them at every stage subsequently influencing the microbiological quality [4].

Enterobacteriaceae families are responsible for food spoilage and also causing foodborne disease, which therefore contribute to substantial economic losses and food wastage. During the last decade, a rapid upswing in resistance among *Enterobacteriaceae* has markedly deteriorated health conditions worldwide. *Enterobacteriaceae* are gram-negative facultative anaerobe in human and animal intestine as their natural host. *Enterobacteriaceae* like *Escherichia coli, Klebsiella* spp., *Proteus* spp., *Morganell*a spp., *Providentia* spp., *Enterobacter* spp., *Serrati*a spp. are the primaru pathogens

of the urinary tract, respiratory tract, bloodstream and wounds. Other *Enterobacteriaceae* like *Salmonella, Shigella, Yersinia enterocolitica* and *Vibrio* are foodborne pathogens responsible for the most frequently occurring foodborne diseases worldwide [5,6]. *Raoultella terrigena* (*Klebsiella terrigena*) is a rarely encountered gram-negative bacterium. The first case of human infection in a liver transplant recipient who developed fatal endocarditis due to *R. terrigena* was reported in 2007 [7]. The association between *R. terrigena* and sepsis was reported in 2011, representing the second human infection case [8].

Antibiotics can be cytotoxic or cytostatic to the microorganisms, allowing the body's immune system to eliminate them. The membrane disorganizing agents or other specific actions of antibiotics inhibits the bacterial cell synthesis, synthesis of proteins, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) [9]. The antibacterial activity of amoxicillin, ampicillin, piperacillin, ticarcillin inhibits the synthesis of cell walls against enteric bacteria. Aminoglycoside modifying enzymes catalyze the modification at -OH or -NH_2 groups of the 2-deoxystreptamine nucleus or the sugar moieties. Amikacin, gentamicin, netilmicin and tobramycin block the protein synthesis of 30s ribosomal subunit. Nitrofurantoin, trimethoprim and trimethoprim-sulfamethoxazole damage the DNA and inhibit the folic acid synthesis, respectively having considerable antibacterial activity against enteric bacteria. For decades, multiple varieties of antibiotics have not only been used for therapeutic purposes but accomplished prophylactically across other industries like agriculture and animal husbandry. Some drugs are excreted quickly from the animal; others are not readily metabolized or excreted. If these residues persists in the animal tissues, they enter the human food chain, creating health risks for consumers [10,11].

Many *Enterobacteriaceae* family members are increasingly resistant to currently available antimicrobials [12]. *Enterobacteriaceae* strains can develop resistance to polymyxins due to the modification of lipopolysacharide (LPS) molecule [13].

Pathogens have developed a high level of resistance to antibiotics which facilitates the disease-causing bacteria to establish effective means of resistance to these drugs [14]. Antimicrobial resistance severely threatens public health worldwide, leading to increased health care costs, treatment failures, and deaths. Antibiotic resistance among foodborne microorganisms is an ongoing public health threat. The bacterial isolates with highest percentage of antimicrobial resistance may have significant implications for human and animal health with adverse economic implications.

Nowadays, multi-drug resistant strains have developed, which possess several resistant mechanisms against different antibiotic groups. Human pathogenic or opportunistic bacteria such as *Campylobacter sp*., *Klebsiella pneumoniae, Salmonella sp., Pseudomonas aeruginosa* and fish pathogens have developed a wide range of multiple antibiotic resistances [15]. Consequently, the main goal of the present study was to investigate the prevalence and isolation of antibiotic-resistant *Enterobacteriaceae* isolates from milk peda.

From a public health standpoint, evaluating the prevalence of antibiotic resistance to gram-negative Enterobacteriaceae in milk peda samples is essential.

Materials and Methods

Sampling

Peda samples were obtained from different local vendors (20), private manufacturers (4) and organized dairies (1) (Mysore Milk Dairy (Mysore and Chamarajanagar District Co-operative Milk Producers Societies Union Ltd., Siddartha Nagar, T. Narsipura road, Mysuru- 570011). The samples were packed in polyethylene bags to prevent gain loss or moisture. To maintain the privacy of sample sources, the samples have been designated as S1, S2, S3 to S25. Twenty-five peda samples (100 g each) were collected from different shops to enumerate *E. coli, coliform,* yeast and mold. *Salmonella* counts were also screened for the presence of other gram-negative *Enterobacteriaceae*. Isolation and identification of presumptive bacteria (*Enterobacteriaceae*) were performed in all peda samples.

Determination of Microbiological Quality of the Milk Peda

Peda samples were analyzed to enumerate *E. coli and coliform, Enterobacteriaceae* and other gram- negative bacteria. Each sample (25g) was placed into sterile stomacher bags under aseptic conditions, pooled with 225 ml of peptone water (0.1%), and then was homogenized in a laboratory stomacher for 2 min. The samples were serially diluted in 9 ml of sterile peptone water (0.1%). Volumes of 1 ml, 0.1 ml and 0.01 ml of suitable dilutions were transferred to double strength Lauryl Tryptose MUG (LST MUG, double concentration broth) tubes and incubated for 48 h at 35° C to enrich *E. coli* and *coliform*.

After incubation, a loopful of inoculum in each positive test tube was streaked onto an EMB (Eosin Methylene Blue Agar) plate and incubated at 35° C for 24 h for the appearance of E. coli colonies. Simultaneously 1 ml, 0.1 ml and 0.01 ml of the homogenate of suitable dilutions were plated in duplicate on Violet Red Bile agar (VRBA) and Violet Red Bile Glucose agar (VRBGA) by pour plate method to enrich *coliform* and *Enterobacteriaceae*, respectively. The overlaid plates were incubated aerobically at 37° C for 24 h. Suspected colonies (pink, red, or purple with or without precipitation halos) that developed on VRBGA plates which gave reactions suggestive of *Enterobacteriaceae* were isolated and stored at 4° C on Nutrient agar (NA) slants for further biochemical characterization. All bacteriological media and antibiotic discs were purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India. API 20 E was purchased from Biomerieux India Pvt. Ltd.

Biochemical Identification of Enterobacteriaceae

Further, to reveal *Enterobacteriaceae* members and other non-fastidious gram-negative rods on peda samples, different morphological colonies were grown on Violet Red Bile Glucose agar (VRBGA) and subsequently identified via the API E (bioM´erieux,

USA) bacterial identification system containing 21 miniaturized biochemical tests according to the manufacturer's instructions [16]. All isolates were gram-stained and tested for oxidase, catalase activity, and fermentation of glucose, by standard methods. Glucose-fermenting, gram-negative, oxidase-negative, catalase-positive strains were considered to belong to the family *Enterobacteriaceae*, and these isolates were stored for further study. In addition, tests such as yellow pigmentation on tryptone soy yeast extract agar, phenylalanine deaminase test, esculin hydrolysis, mucate test, utilization of acetate, motility, nitrate reduction, malonate utilization, hemolysis on blood agar, methyl red test and fermentation of adonitol, cellobiose, salicin were implied to confirm the isolates as per Bergey's manual [17].

Antibiotic Susceptibility Testing of Enteric Isolates

Antimicrobial susceptibility was determined for all the isolates by the disk diffusion method on Mueller-Hinton agar using 20 different antibiotics belonging to 9 different classes [18]. Bacterial suspensions (0.85% sterile saline) with optical density equivalent to 0.5 McFarland standards corresponding to 108 CFU/ml were inoculated onto Muller-Hinton agar (Difco, Le Pont de Claix, France). The zone of inhibition was observed after incubation at 35° C for 24 h. Inhibition diameters were measured and interpreted as resistant, intermediate, or susceptible according to the "Performance standards for antimicrobial susceptibility testing" recommended by the Clinical and Laboratory Standard Institute (CLSI) of USA [19].

Antibiotic discs designed for gram-negative organisms were purchased from (HiMedia Icosa-G-II-Minus). The antibiotics tested were imipenem (IPM) 10 µg/disc, tobramycin (TOB) 10 µg/disc, ofloxacin (OF) 5 µg/disc, levofloxacin (LE) 5 µg/disc, nalidixic acid (NA) 30 µg/disc, cefoxitin (CX) 30 µg/disc, gentamicin (GEN) 10 µg/disc, aztreonam (AT) 30 µg/disc, cefpodoxime (CPD) 10 µg/disc, trimethoprim or co-trimoxazole (COT) 25 µg/disc, ciprofloxacin (CIP) 5 µg/disc, moxifloxacin (MO) 5 µg/disc, ceftazidime (CAZ) 30 µg/disc, norfloxacin (NX) 10 µg/disc, colistin (CL) 10 µg/disc, augmentin (amoxicillin and clavulanate potassium) (AMC) 30 µg/disc, gatifloxacin (GAT) 5 µg/disc, amikacin (AK) 30 µg/disc, ceftriaxone (CTR) 30 µg/disc, nitrofurantoin (NIT) 300 µg/disc. The multiple antibiotic resistances (MAR) index was calculated by employing the following formula:

MAR index = (Number of resistance antibiotics per isolate)/ (total number of antibiotics tested).

Isolates classified as intermediate on the basis of the inhibition zone were considered as sensitive for the MAR index [20].

Results

Prevalence of E. Coli, Coliforms and other Enterobacteriaceae

A total of 25 peda samples collected from different shops were analyzed for the presence of *Enterobacteraceae* and other Gram-negative bacteria. Out of 25 samples, none of the samples were positive for *E. coli* and *coliforms*. No gas and no turbidity were seen in any of the tubes. However, many of the opportunistic pathogens belonging to the *Enterobacteriaceae* had been detected in the peda samples. Nearly 50 different colonies were isolated from peda samples. The colonies with similar morphological features were grouped together and further confirmed with biochemical tests.

Out of 25 samples, 15 peda samples (S2, S3, S4, S8, S9, S10, S11, S13, S15, S16, S17, S18, S19, S22, and S24) showed positive results for *Enterobacteriaceae*. The isolates were identified as members of the *Enterobacter cloacae* (6 isolates), *Yersinia bercovieri* (2 isolates), *Yersinia rohdei* (1 isolates), *Raoutlella terigena* (3 isolates), *Acinetobacter lwoffii* (1 isolate), *Pantoea agglomerans* PA_2 (1 isolate), *Klebsiella pneumoniae subsp. pneumoniae* (1 isolate), *Leclericia adecarboxylata* (1 isolate), *Photorhabdus nematophilus* (1 isolate), *Enterobacter gergoviae* (1 isolate), *Acinetobacter baumannii/calcoacetius* (1 isolate), *Cronobacter sakazaki* (*Enterobacter sakazaki*) (1 isolate). These isolates were identified and confirmed by API 20E and biochemical test as per Bergey's manual [17]. Their presence in the samples was significant as all sections of the society consume this food product. *E. cloacae* and *E. gergoviae* were the major Enterobacter species isolated from 4 of the 25 samples along with other gram negativegram-negative fermentative bacteria. The percentage distribution of *Enterobacteriaceae* and other gram-negative fermentative bacteria isolated from peda samples is shown in Figure 1. Among these isolates, *Enterobacter cloacae* showed the highest percentage of incidence (12%), followed by *Raoutlella terigena* (12%), and *Yersinia bercovieri* with 8% of incidence. The remaining isolates showed the most negligible percentage of incidences (4%). The total incidence rate is 68% (17 out of 25).

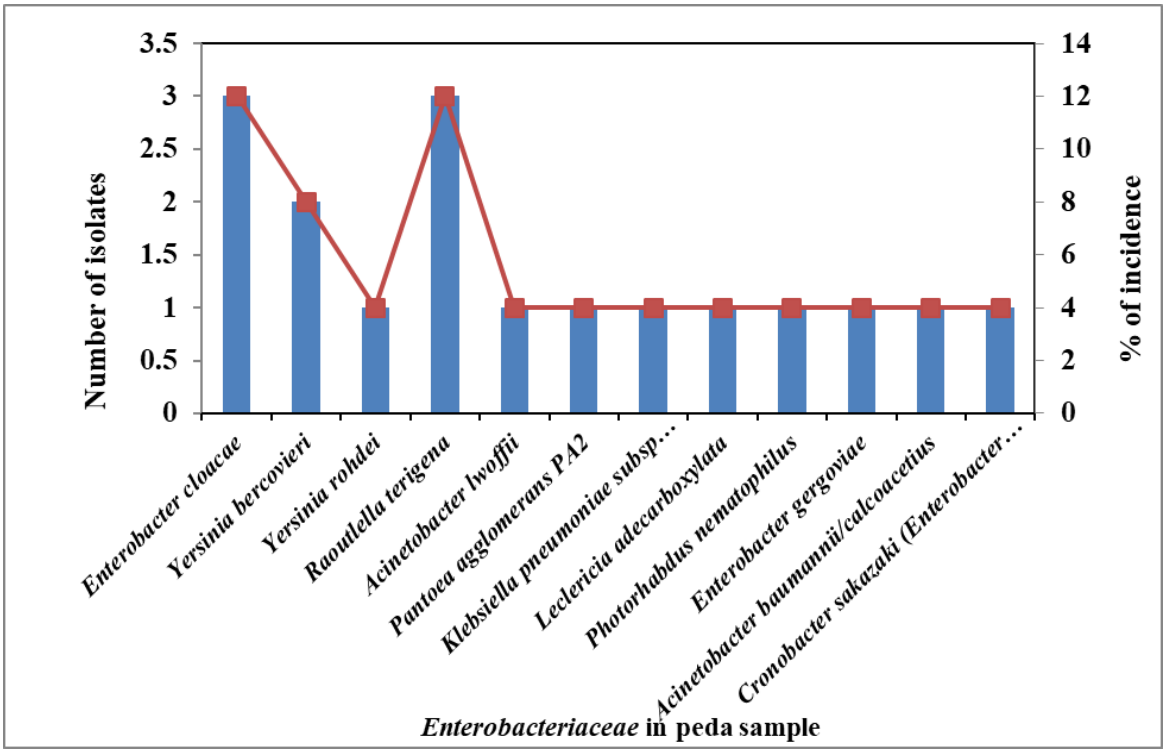

Figure 1: Frequency Enterobacteriaceae in peda samples

Antibiotic Sensitivity Pattern of the Isolates

Antibiotic sensitivity was tested for all 17 presumptive isolates with 20 different antibiotics. Table 1 shows the degree of sensitivity (resistant, intermediary and susceptible) of Enterobacteriaceae and other gram-negative bacteria against a different class of antibiotic tested. The susceptibility, intermediate and resistance range was 17.65 to 100%, 5.88 to 47.06 and 5.88 to 23.53, respectively. The highest resistance was to nitrofurantoin (70.59%), followed by augmentin (amoxicillin and clavulanate potassium) (52.94%), cefpodoxime (47.06%) third generation of class cephalosporins, cefoxitin (23.53%) second generation of class cephalosporins, nalidixic acid and ceftazidime (17.65%) first and third generation of class fluoroquinolones and cephalosporins, respectively. Ceftriaxone (11.75%), second generation of class cephalosporins, aztreonam, moxifloxacin (fourth generation of class fluoroquinones), and oflaxacin (second generation of class fluoroquinones) reported the lowest resistance level of 5.88 %. of the 17 isolates of *Enterobacteriaceae*, 16 (94.1%) were resistant to at least one antibiotic tested. Thirteen (81.25%) out of 17 isolates presented multiple antibiotic resistance patterns against 11 antibiotics (Table 2). There were significant differences in antimicrobial resistance among the identical isolates. The observation showed that *Acinetobacter baumannii/calcoacetius* showed resistance to 5 antibiotics in comparison to *Enterobacter cloacae* (A3), *Raoutlella terigena* D3, and *Enterobacter gergoviae*, which showed resistance to 4 antibiotics. The lowest number of antibiotic resistances was observed with *Yersinia bercovieri* B1, *Yersinia bercovieri* B2 and *Yersinia rohdei* C1 with only one antibiotic each (NIT and NAL), which belong to 1st generation fluoroquinolones and nitrofurantoin, respectively. *Leclericia adecarboxylata* was susceptible to all tested antibiotics. The variation in the susceptibility and resistance among the same species may be due to variation in their strain level. The maximum and average multiple antibiotic resistance (MAR) indices of isolates were 0.25 and 0.15, respectively, and the MAR index of other isolates ranged from 0.05 to 0.20.

Table 1: Enumeration of Enterobacteriaceae isolates from peda samples

Sample code	Yeast and mould CFU/g	*Salmonella* CFU/g	*Enterobacteriaceae* (No of isolates)	% of incidence
S1	ND	ND	absent	0
S2	ND	ND	*Enterobacter cloacae* (1)	12
S3	ND	ND	*Klebsiella pneumoniae subsp. pneumoniae* (1) and *Pantoea agglomerans* PA_2 (1)	4,4
S4	ND	ND	*Acinetobacter lwoffii* (1)	4

S5	ND	ND	absent	0
S6	ND	ND	absent	0
S7	ND	ND	absent	0
S8	ND	ND	*Cronobacter sakazaki* (Enterobacter sakazaki) (1)	4
S9	ND	ND	*Raoutlella terigena* (1)	4
S10	ND	ND	*Acinetobacter baumannii/calcoacetius* (1)	4
S11	ND	ND	*Leclericia adecarboxylata* (1)	4
S12	ND	ND	absent	0
S13	ND	ND	*Enterobacter gergoviae* (1)	4
S14	ND	ND	absent	0
S15	ND	ND	*Raoutlella terigena* (1)	12
S16	ND	ND	*Enterobacter cloacae* (1)	4
S17	ND	ND	*Raoutlella terigena* (1)	12
S18	ND	ND	*Yersinia rohdei* (1)	4
S19	ND	ND	*Yersinia bercovieri* (1)	8
S20	ND	ND	absent	0
S21	ND	ND	absent	0
S22	ND	ND	*Yersinia bercovieri* (1)	8
S23	ND	ND	absent	0
S24	ND	ND	*Enterobacter cloacae* (2)	12
S25	ND	ND	absent	0

Table 2: Sensitivity of *Enterobacteriaceae* isolates to different class of antibiotics

Class of antibiotics		**Enterobacteriaceae isolates (n=17)**		
		Number of Susceptible isolates (%)	**Number of Intermediary isolates (%)**	**Number of Resistant isolates (%)**
Fluoroquinones				
First generation	Nalidixic acid	10 (58.82%)	4 (23.53%)	3 (17.65%)
	Norfloxacin	16 (94.12%)	1 (5.88%)	0 (0.0%)
Second generation	Ciprofloxacin	14 (82.35%)	3 (17.65%)	0 (0.0%)
	Oflaxacin	16 (94.12%)	0 (0.0%)	1 (5.88%)
	Levofloxacin	17 (100%)	0 (0.0%)	0 (0.0%)
Third generation	Gatifloxacin	15 (88.24%)	2 (11.76%)	0 (0.0%)
Fourth generation	Moxifloxacin	14 (82.35%)	2 (11.76%)	1 (5.88%)
Aminoglycosides				
	Tobramycin	16 (94.12%)	1 (5.88%)	0 (0.0%)
	Gentamycin	15 (88.24%)	2 (11.76%)	0 (0.0%)
	Amikacin	10 (58.82%)	7 (41.18%)	0 (0.0%)
Cephalosporins				
Second generation	Cefoxitin	12 (70.59%)	1(5.88%)	4 (23.53%)
Third generation	Ceftriaxone	7 (41.18%)	8 (47.06%)	2 (11.76%)
	Ceftazidime	9 (52.94%)	5 (29.41%)	3 (17.65%)
	Cefpodoxime	3 (17.65%)	6 (35.29%)	8 (47.06%)

Others				
Carbapenem	Imipenem	12 (70.59%)	5(29.41%)	0 (0.00%)
co-trimoxazole	Trimethoprim	17 (100.00%)	0 (0.00%)	0 (0.0%)
Polymixin	Colistin	17 (100.00%)	0 (0.00%)	0 (0.00%)
Monobactams	Aztreonam	9 (52.94%)	7 (41.18%)	1 (5.88%)
Nitrofurantoin		3 (17.65%)	2 (11.76%)	12 (70.59%)
Pencillins	Augmentin (amoxicillin and clavulanate potassium)	5 (29.41%)	3 (17.65%)	9 (52. 94%)

IPM, Imipenem; TOB, Tobramycin; Ofloxacin; LE, Levofloxacin; NA, Nalidixic acid; CX, Cefoxitin; GEN, Gentamicin; AT, Aztreonam; CPD, Cefpodoxime; COT, Trimethoprim or Co-Trimoxazole; CIP, Ciprofloxacin; MO, Moxifloxacin; CAZ, Ceftazidime; NX, Norfloxacin; CL, Colistin; AMC, Augmentin (amoxicillin and clavulanate potassium); GAT, Gatifloxacin; AK, Amikacin; CTR, Ceftriaxone; NIT, Nitrofurantoin.

Table 3: *Enterobacteriaceae* strains identified with antibiotic resistance profile

Enterobacteriaceae isolates	Number of antibiotics	Multiple antibiotic resistance pattern	No. (%) of isolates	MAR index*
Leclericia adecarboxylata H1	0	Susceptible to all tested antibiotics	1 (5.88)	0
Yersinia bercovieri B1	1	NIT	3 (17.64)	0.05
Yersinia bercovieri B2		NA		
Yersinia rohdei C1		NA		
Enterobacter cloacae (A1)	2	NIT, AMC	4 (23.52)	0.10
Raoutlella terigena D1		NIT, AMC		
Acinetobacter lwoffii E1		CPD, NIT		
Cronobacter sakazaki (Enterobacter sakazaki) L1		CPD, NIT		
Raoutlella terigena D2	3	CPD, NIT, AMC	4 (23.52)	0.15
Pantoea agglomerans PA_2 F1		CX, CTR, AMC		
Klebsiella pneumoniae subsp pneumoniae G1		CX, NIT, AMC		
Photorhabdus nematophilus I1		CAZ, CPD, ATM		
Enterobacter cloacae (A2)	4	CX, CPD, NIT, AMC	3 (17.64)	3 (17.64)
Enterobacter cloacae (A3)		CX, CPD, NIT, AMC		
Raoutlella terigena D3		OF, MO, NIT, AMC		
Enterobacter gergoviae J1		NA,CAZ, CPD, NIT		
Acinetobacter baumannii/calcoacetius K1	5	CTR,CAZ, CPD, NIT, AMC	1 (5.88)	0.25

IPM, Imipenem; TOB, Tobramycin; Ofloxacin; LE, Levofloxacin; NA, Nalidixic acid; CX, Cefoxitin; GEN, Gentamicin; AT, Aztreonam; CPD, Cefpodoxime; COT, Trimethoprim or Co-Trimoxazole; CIP, Ciprofloxacin; MO, Moxifloxacin; CAZ, Ceftazidime; NX, Norfloxacin; CL, Colistin; AMC, Augmentin (amoxicillin and clavulanate potassium); GAT, Gatifloxacin; AK, Amikacin; CTR, Ceftriaxone; NIT, Nitrofurantoin.

MAR, multiple antibiotic resistance.

* According to Singh, Yadav, Singh, & Bharti, 2010

Discussion

Dairy products are potential vehicles for microorganisms from the *Enterobacteriaceae* family, which can exhibit multi-drug resistance to available antimicrobials, reduced susceptibility phenotypes to carbepenems, and produce biofilm, proteolytic enzymes, lipolytic enzymes, and antimicrobial substances, providing advantages for the bacteria in a competitive niche. All these factors represent potential risks to the health of consumers of dairy products, particularly immunocompromised consumers.

In the supply chain of dairy products, like production lines, transport, and storage, one must follow good manufacturing practices, hygiene, and best practices in commercialization, mainly for products consumed without any prior processing. Additionally, the absence of pathogens, like *Salmonella spp*. and *E. coli* does not indicate that the product is fit for consumption since other potentially pathogenic bacteria of the same family may be present in the food. Thus, testing for Enterobacteriaceae, including species not yet assessed according to regulator standards, may offer a better view of dairy foods quality, sanitary conditions, and safety.

Bacterial antibiotic resistance has been recognized as a global problem in medical and agricultural fields. There is a worldwide concern about the increased prevalence of antimicrobial resistance in bacteria. Resistant bacteria reach the human population through a variety of pathways. Most studies have focused on pathogenic microorganisms that present immediate risks to human health, but there is a growing interest in commensal components of the microbiota associated with food [21]. Observance of hygiene can essential in ensuring food safety and controlling the transmission of resistant bacteria from produce.

Our investigation shows neither *coliforms* nor the hygiene indicating bacteria, *E. coli* is present in any of the samples examined. Peda, a heat desiccated product, it will be free from gram-negative fermentative heat-sensitive bacteria when prepared. But subsequent handling and environmental factors result in a contaminated product. Literature reported the prevalence of *Salmonella* and other pathogens such as *E. coli*, Shigella, hemolytic Streptococci and *Pseudomonas aeruginosa* in peda samples [22]. Many of the opportunistic pathogens belonging to nterobacteriaceae were also detected in the peda samples. Their presence in the peda samples was significant as all age groups of the society consume this food product. Enterobacter species are now increasingly encountered as nosocomial infections causing urinary tract infections and bacteremia. *Enterobacter* species, namely, *E. cloacae* and *E. gergoviae* have been detected in 16% of peda samples. *E. gergoviae* has been reported in environmental, cosmetics, and clinical samples in France, Africa and USA [23,24]. Our finding was the first report of the prevalence and detection of *E. gergoviae* in a food sample. *Y. bercovieri* was isolated from 2 samples and *Y. rohdei* from one sample. *Y. bercovieri*, one among the eight new *Y. enterocolitica*-like species, was previously designated Y. enterocolitica biogroup 3B. It produces heat stable enterotoxin [25].

Acinetobacter lwoffii, A. baumannii/calcoacetius, Pantoea agglomerans PA2, K. Pneumoniae subsp. pneumonia, C. sakazaki (E. sakazaki), Leclericia adecarboxylata and P. nematophilus are also detected in the peda samples. *Acinetobacter* was considered a low-grade pathogen and reported the high pathogenicity and ability to cause invasive disease by the members of this genus [26]. *A. lwoffi*, an opportunistic pathogen, can survive for long periods under desiccated conditions [27]. *P. agglomeran*s, previously called as *E. agglomerans*, have been implicated in outbreaks of septicemia in the United States and Canada. Contamination of closures on bottles of infusion fluids was the source of the outbreaks [28].

K. pneumoniae sub sp. *pneumoniae* is an opportunistic pathogen that primarily attacks immunocompromised individuals and hospitalized patients [29]. *R. terigena* (formerly *K. terigena*) was isolated from 2 samples. *C. sakazakii* (*E. sakazakii*) was isolated from one sample. It has linked with severe infections in infants following the consumption of powdered infant formula [30-32]. The powdered infant formula has lowest aw. *E. sakazakii* infections have caused the preterm, deficient birth weight neonate with meningitis and infants with bacteraemia in India [33]. Leclericia adecarboxylata isolated from peda sample is an opportunistic human pathogen that phenotypically resembles *E. coli*. But the biochemical characteristics like lysine decarboxylase, malonate assimilation, acid production from arabitol and cellobiose, but not from adonitol and sorbitol, allowed definitive separation of L. adecarboxylata from *E. coli* [34].

Even though *E. coli* and *coliforms* were not detected in the surveillance, and Enterobacteriaceae forms were common. Antibiotic sensitivity studies on the isolates were conducted, keeping in mind the prevalence of horizontal gene transfer among closely related bacteria. Bacterial antibiotic resistance has been recognized as a global problem in medical and agricultural fields. Most antibiotic resistance studies have been focused on pathogenic microorganisms that present immediate risks to human health. Still, there is a growing interest in commensal components of the microbiota associated with food [21].

Most of the isolates were susceptible to the fluoroquinolones and aminoglycosides tested. Some isolated were resistant to nalidixic acid. This antibiotic was used to treaturinary tract infections caused by *E. coli*, *Proteus, Shigella*, *Enterobacter* and *Klebsiella*. It is no longer clinically used for this indication in the USA. Among the cephalosporins, 47% of the isolates were resistant to cefpodoxime (third generation cephaolosporin). In the recent past, third-generation cephalosporins have gained importance in treating enteric fever. Concerning other antibiotics tested, 64.7% of isolates to nitrofurantoin and 52.9% to augmentin were resistant. Thus, examining the specimens of apparently healthy dairy handlers is imperative to clarify their role in shedding bacterial pathogenic agents.

Uncleaned hands, poor quality of milk, unhygienic conditions of manufacturing unit, inferior quality of material used, water supplied for washing the utensils, and post processing contamination

might be the cause of the presence of *enterobacteriaceae* in peda samples.

The study reveals the absence of the top foodborne pathogens such as E. coli and coliforms from peda samples. Although *E. coli* and *coliforms* were not detected in any of the peda samples, the presence of other *Enterobacteriaceae* was confirmed by biochemical tests. Although *E. gergoviae* has been reported in environmental, cosmetics, and clinical samples, our finding was the first report of prevalence and detection in a food sample. ThusThus, focusing attention on the safety of food processing, microbiological evaluation of peda samples revealed the presence of other gram-negative bacteria of clinical significance. The isolates also showed varying resistance to antibiotics like nalidixic acid, cefoxitin, cefpodoxime, aztreonam, nitrofuratoin and augmentin. To the best of our knowledge, this is the first report evaluating antimicrobial resistance in *Enterobacteriaceae* isolated from milk peda.

It is advised to the local vendors to keep in view the public health prominence of consumer and should practice strict hygienic preventive measures during pre and post-preparation handling, storage, and marketing of the finished products. This practice will increase the quality of the product. It is concluded from the present findings that peda samples from organized dairy were superior to that of private and local vendors.

Authors' Contributions

HPMK conceptualized, planned and designed the experiments, executed biochemical tests, interpreted data, and wrote the paper. PV contributed to experimental planning and elucidation of conclusion during manuscript preparation. FA performed antibiotic screening assays. All authors have approved the contents of the manuscripts.

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Conflict of interests

All the authors declare that they have no conflict of interest.

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