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OLECULAR IDENTIFICATION AND CHARACTERIZATION OF ANTIBIOGRAM OF SOME FOOD BORNE BACTERIA FROM LOCAL DAIRY PRODUCTS IN KURDISTAN REGION-IRAQ

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ABSTRACT

The major objective of the study was to employ molecular approaches to detect antibiotic resistance and identify microorganisms from dairy products. The different dairy products might be contaminated with different germs, so the handlers are at high risk of diseases. Therefore, knowledge of the identification and antibiotic resistance profiles of these bacteria will be vital for food safety and for treatment of diseases caused by them. A total of 113, 69, 47 and 37 different bacteria isolated from four kinds of dairy products namely cheese, hard cheese, soft cheese and yogurt were Escherichia coli, Staphylococcus aureus, Bacillus cereus and Salmonella typhimurium correspondingly. The species were subsequently identified utilizing genotype (molecular) approaches including polymer chain reaction. In addition, the bacteria were studied for their susceptibility towards germicide chemicals utilizing disc diffusion and Vitek 2 system methods. Antibacterial susceptibility test findings demonstrated that various isolates had variable sensitivity levels against several drugs. For example, 55 of 69 S.aureus, 11 of 47 B.cereus, and 26 of 113 E. coli isolates are resistant to common antibiotics (various drugs: clindamycin, tetracycline, rifampicin, erythromycin, vancomycin, and ampicillin). Some isolates were resistant to various kinds of drugs, phenomena called multidrug resistance.

Key words: Antibiotics, Dairy products, foodborne disease, Resistance.

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INTRODUCTION

Foodborne pathogens Microorganisms (including bacteria, viruses, parasites and fungi) which can contaminate food products and cause foodborne diseases or food poisoning when consumed by people. These pathogens are a major global public health concern, as they can cause a spectrum of health problems, from mild digestive upset to serious and potentially fatal diseases. Some examples of food-borne pathogens are Salmonella, E. coli, Campylobacter, Listeria, Norovirus and different kinds of parasites and fungi. Contamination can happen anywhere in the food production chain, including processing and manufacturing, packaging, transportation, storage and cooking. Proper handling, storage, and cooking practices play an essential role in the safe handling of food and in preventing the growth of these microorganisms and reducing the chances of food-borne illness. These illnesses can cause nausea, vomiting, diarrhea, abdominal cramps, fever, and sometimes dehydration, organ failure or even death. In order to reduce these risks, consumers and regulators should focus on food safety by maintaining proper hygiene practices and proper food handling procedures through strict food safety standards throughout the food supply chain. Food can be contaminated at different stages of the food production processes, from the agricultural level to the moment it is finally consumed by people. Food contamination is largely due to improper handling, storage and preparation of food, as well as environmental contamination such as water or soil. Foods such as meats, poultry, eggs and dairy products are very susceptible to bacterial contamination because they contain a lot of organisms that can cause

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harm (Kamboj et al., 2020). The existence of dangerous micro-organisms, such as bacteria, in milk and other dairy products is a great problem for many people. Poor handling and processing procedures may contaminate these foods, impacting the health of people (Imre et al., 2022). The appearance of antibiotic-resistant bacteria has made the issue of public health globally a complicated one and is becoming a growing threat to the well-being of humanity (Kamboj et al., 2020). It has been demonstrated that antibiotic resistant bacteria in human food, especially dairy products, are contributing to the growing problem of drug resistance leading to chemotherapy failures. Multidrug-resistant bacteria are developing, causing deadly diseases and contributing to the deaths of thousands of individuals every year, making antimicrobial resistance a huge problem. Horizontal gene transfer has been identified as one of the pathways for the transfer of antimicrobial resistance genes (ARGs) from foodborne pathogens to the human population. When bacteria evolve in a way that they become immune to the effects of antibiotics, antibiotic resistance occurs. This process occurs naturally over time due to genetic mutations, but it is sped up when antibiotics are misused. Antibiotics expose bacteria to high doses of the medication, increasing the chance of bacteria developing resistance to them. (Nadi et al., 2020). One of the most serious issues to be solved urgently is antimicrobial resistance. Germs becoming resistant to antibiotics makes treating bacterial diseases more difficult, which can mean longer recoveries and greater risk of death, among other issues. In some cases, some infections cannot be treated with some antibiotics because the bacteria have developed resistance (Fymat, 2017).

MATERIALS AND METHODS

Sample collection and isolation method: Table 1. Collection of 300 dairy products from the Kurdistan region during October 2021 to January 2022. Each sample was collected in a sterile plastic bottle and transported to the lab under refrigerated conditions for further analyses. To begin the testing the pure peptone water was used in the amount of 225 ml, in each sample 25 grams. Then decimal dilutions were prepared and 1ml of various allowed dilutions were plated on the surface of several selective media. The separation of food pathogens was facilitated by this step (Chauhan & Jindal, 2020).

Table1. Sample collection

Place	Type of Samples	Number of Samples
Dohuk	Cheese	25
	Hard Cheese	25
	Soft Cheese	25
	Yogurt	25
Hawler	Cheese	40
	Hard Cheese	40
	Soft Cheese	40
	Yogurt	40
Sulaimany	Cheese	10
	Hard Cheese	10
	Soft Cheese	10
	Yogurt	10

Isolation of Salmonella spp.

The sample is subjected to a series of laboratory tests. First of all, pre-enrichment is done by adding the sample to the broth, for example, Buffered Peptone Water (BPW), allowing Salmonella contained in the sample to multiply without limiting the multiplication of other possible bacteria. After pre-enrichment, the selective enrichment stage takes place, whereby some portion of the pre-enriched sample is moved to selective broth. Commonly employed selective media include selenite cystine broth (SC) or tetrathionate broth, which contain inhibitors that suppress the growth of competing bacteria while promoting Salmonella growth. The selective enrichment is then subjected to specific incubation conditions to facilitate Salmonella proliferation. Subsequent to the enrichment process, a loopful or a precisely measured volume of the selective enrichment broth is streaked onto selective agar plates. Examples of selective agar media used for isolating Salmonella from dairy products include (XLD, BGA or MacConkey) agars supplemented with bile salts and crystal violet. These agar plates are then incubated at an appropriate temperature, typically around 37°C, for a duration of 24 to 48 hours (Javadi & Khatibi, 2017).

Isolation of Bacillus spp.

Heating phase samples activated after the first stage to obtaining Bacillus spp., spore

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activating done through heating the flask containing the sample with buffer peptone water for 10-20 minutes in 70-80 ° C and then transferred 0.1ml Inoculate the surface of a nutrient agar plate and place it in an incubator at 37°C for a period of 18 to 24 hrs. (Tabit, 2010).

Isolation of *Staphylococcus aureus*:

1ml. of the sample was utilized in combination with Cooked-Meat broth (Merck, Germany), to which 10% NaCl was added. The mixture was then subjected to an incubation process at a temperature of 37 degrees Celsius for a duration of 24 hours. Following this incubation, a small amount of the cultured material was inoculated onto Baird-Parker agar (Hi Media India). This agar was supplemented with egg yolk and a tellurite emulsion (50 ml per liter). The inoculated agar was subsequently incubated again at 37 degrees Celsius for 24 hours. Diagnostic are separate colonies with a dark, shiny, characteristic appearance with a transparent ring around them. Verification of the colonies was done with the help of various evaluations such as Gram staining, biochemical testing. Tests performed were catalase enzyme activity, hemolytic reaction on blood agar (Hi Media India), ability to ferment mannitol on Mannitol Salt agar (Hi Media, India), coagulase production and DNase enzyme activity. The last verification process for the bacteria was done by running the samples through the Vitek Compact System 2 system based on recommendations by (Makled et al., 2025)

Isolation of *E. coli* and *E. coli O157: H7*

Several steps are involved in the isolation and identification of E. coli and E. coli O157:H7. In the first place, a sample of the suspected dairy product should be taken. Then, in the enrichment step, the sample is treated with a culture medium which stimulates the growth of E. coli and E. coli O157:H7. This medium also inhibits the growth of other bacteria in the sample. After enrichment stage, a selective culture method is applied. To this end, Sorbitol MacConkey agar supplemented with potassium tellurite and cefixime is used. This medium only allows the development of the selected bacteria. This means that *E.coli O157:H7* develops but no other bacteria develop. After obtaining such colonies, biochemical tests should be performed to confirm that these colonies are E. coli. Serotyping is needed for identifying and characterizing *E. coli O157:H7* to detect O157 and H7 antigens. Molecular methods (PCR) can also be applied further. Further characterization of the isolated strains can also be performed to test their virulence factors and antibiotic resistance. These are all essential steps for the isolation and identification of E. coli and E. coli O157:H7 to ensure food safety and take appropriate actions in response to potential health concerns from these strains (Chekabab et al., 2013)

Identification of the bacteria by PCR Technique

Genomic DNA Extraction: DNA was taken out from separated bacteria in the following manner : a pure colony of each bacterium type was placed into 90 ml of (TSBYE), then left to incubate overnight at a temperature of 37 °C . The genomic DNA was then extracted from the cultured solution using the Mericon™ DNA Bacteria Kit (Qiagen, USA), following the guidelines on condition that by the constructer. The concentration of DNA was assessed using a method that was not specified in the text (Thermo Scientific NanoDrop 2000™)

Detection of 16srRNA of Bacteria Genes by PCR: The specific DNA fragment sequence from the extracted bacteria's gene is detailed in Table No. 2. To prepare the PCR reaction mix (25 µL), we utilized the Ready MixTM Taq PCR Reaction Mix with MgCl2 from Sigma, which included 12.5 µL of PCR premix, 0.4 M of each primer, and 2 µL of the DNA sample. DNA amplification was carried out using a thermal cycler (Mastercycler Personal, Eppendorf) with the following settings: an initial denaturation step for 2 minutes at 94°C, followed by 40 cycles consisting of denaturation at 94°C for 20 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds. The final extension was performed at 72°C for 5 minutes, with slight variations in annealing and extension temperatures for different bacterial varieties. Typically, agarose gel concentrations range

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from 0.5% to 2%, depending on the sizes of the DNA fragments to be separated. After cooling the gel solution to approximately 55°C, a casting tray serves as a mold, and a well-former template (commonly referred to as a comb) is placed along the edge of the casting tray to create wells when the gel solution solidifies.

Table 2. Primer's sequencing and its product size

Target gene 16srRNA	Nucleotide sequence	Product size	References
S. enterica (Typhimurium)	F 5 -CGG.,ACG,GGT,GAG,TAA,TGT,CT3- R 5 -GTT,AGC,CGG,TGC,TTC,TTC,.TG3-	406	Saeed et al., 2013
B. cereus	F 5 -CAAGTCAAGATAAGAGGCTTC3- R 5 -AAAGCTCTTGCCAAATAACC3-	470	Fangio et al., 2010
S. aureus	F 5 -GTAGGTGGCAAGCGTTATCC3- R 5 -CGCACATCAGCGTCAG3-	228	Awadalla et al., 2010
E.coli	F 5'-GGAAGAAGCTTGCTTCTTTGCTGAC-3' R 5'AGCCCGGGGATTTCACATCTGACTTA3'	594	Rady et al., 2014)

Antibacterial Susceptibility Test

Antibacterial susceptibility test is a very important laboratory test to evaluate the efficacy of antibiotics to the bacteria that cause infectious disease. The first step is to collect the sample containing the bacteria. After the specific bacteria causing the infection is isolated and identified, a standard number of bacteria is inoculated onto an agar plate. Next, we put antibiotics in a circle pattern called disks. The disks are placed on the surface of the plate and incubated. The diameter of the clear zone of inhibition around the antibiotic disks is measured. Then the size of this zone is compared to the interpretive standards which results in classifying the bacterial strain as susceptible, intermediate or resistant to particular antibiotics. According to the results, the type of treatment to be used is decided (Behroozian et al., 2023).

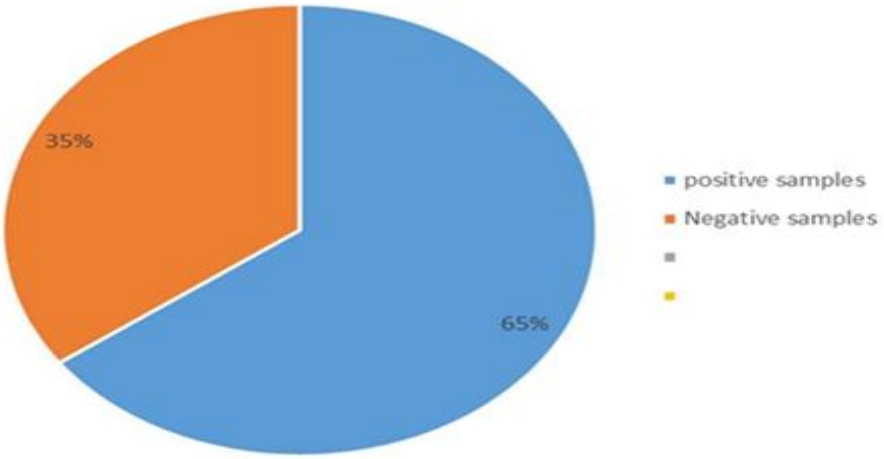

Figure 1. The positive and negative growth for tested samples

RESULTS AND DISCUSSION

The contamination rate of local dairy product samples: Mainly, the primary aim of this particular study is to extract and identify pathogens among bacterial species. In the Kurdistan area regions, which include Duhok, Hawler, and Sulaimania, three hundred samples of milk products were collected. The selected milk products included cheese, soft cheese (Lore and Zhazhi) with local Kurdish names, fermented cheese, and yogurt. For all samples, seventy-five for each of the product categories, were extracted. Of the total amount of samples, 195(65%) had contaminated with bacteria species, whereas 105(35%) did not, as illustrated in Fig 1. As illustrated in Figure 2, the highest contaminated dairy product samples were cheese (84%). Soft cheese (75%) and hard cheese (70%) were also contaminated, but only 52% of yogurt was contaminated with pathogenic bacteria.

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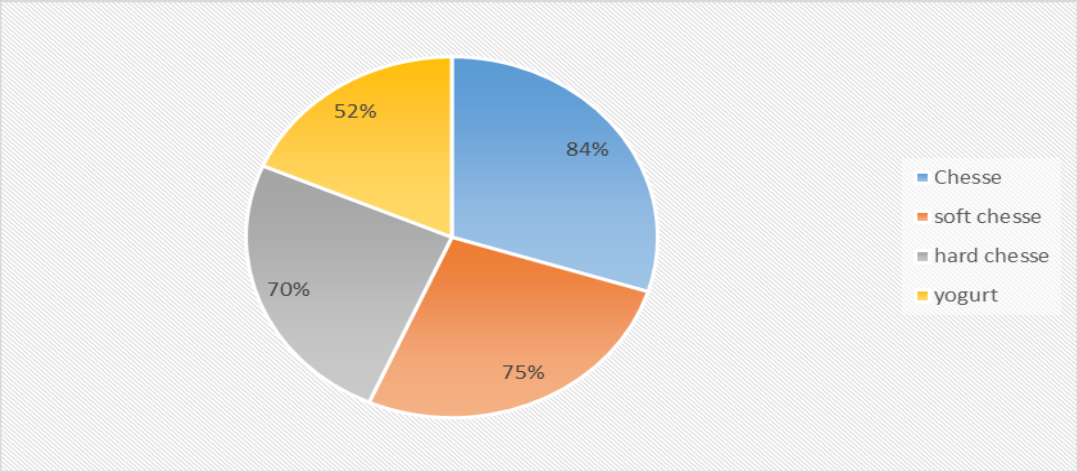

Figure 2. The Percentage of Contaminated Samples

emerged as the predominant bacterial source within the samples, a recurring observation in numerous studies involving Mediterranean cheeses (Gundogan & Avci, 2014). This correspondence has been ascribed to distinct sensory attributes of cheese, encompassing its aroma, taste, and texture, all of which contribute to its distinctive sensory profile. However, this prevalence exceeded the findings reported by Khani et al. (2025). In accordance with international regulations, dairy products derived from pasteurized milk with coliform counts surpassing 10 CFU/mL are deemed substandard. Hence, ensuring effective thermal treatment, averting recontamination, and instituting quality control protocols for fresh resources is of paramount importance. Dairy products are vulnerable to microbial contamination, which can arise from a range of sources including the atmosphere, soil, sewage, water, livestock feed, human contact, ingredients, equipment, packaging, and insects. The types and quantities of microorganisms introduced into dairy products from these origins can fluctuate considerably, contingent on the level of hygiene measures implemented during food processing and handling (Grudlewska-Buda, K., et al. (2023). The current study has gone beyond traditional and rapid procedures for the identification of E. coli, S. aureus, S. enterica (Typhimurium), and Bacillus spp. in local dairy products, by combining selective culture media and confirmed by the Vitec compact system 2, and by molecular identification followed by16S rRNA amplification gene through PCR technique. The results showed that 37 isolates were characterized as Salmonella enterica, 113 isolates were E. coli, 69 isolates of S. aureus, and 37 isolates as B. cereus. The prevalence of these bacteria in the tested dairy samples was recorded as follows: The highest number, 35 (30.9%), and 30 (26.5%), of E. coli were isolated from cheese and soft cheese, respectively, and 27 (23.8%) and 21 (18.5%) isolated from yogurt and hard cheese, respectively. While 22 (31.8%), 18 (26.03%), 17 (24.6%), and 12 (17.3%) of S. aureus bacteria were isolated from soft cheese, cheese, yogurt, and hard cheese, respectively. About Bacillus cereus, 19 (40.4%), 11 (23.4%), and 10 (21.2%) were isolated from soft cheese, cheese, and yogurt samples as follows: 12 (32.4%), 11 (29.7%), 8 (21.6%), and 6 (16.2%) from cheese, hard cheese, yogurt, and soft cheese, respectively. These numbers of bacteria were obtained using the effectiveness of several enrichment and selective media, as well as cultural characters and some biochemical tests that were chosen for initial identification of the four target bacteria in the tested dairy product samples. The confirmation of the detection by Vitec compact 2 systems and PCR was done. The results were shown in figure (3). This system is often used in clinical microbiology and food safety testing to quickly and accurately identify bacteria present in samples. The study's finding that *B. cereus*, *S. aureus*, *E. coli*, and *S. enterica Typhymurium* were present in dairy products is significant because these bacteria are known to cause foodborne illnesses.

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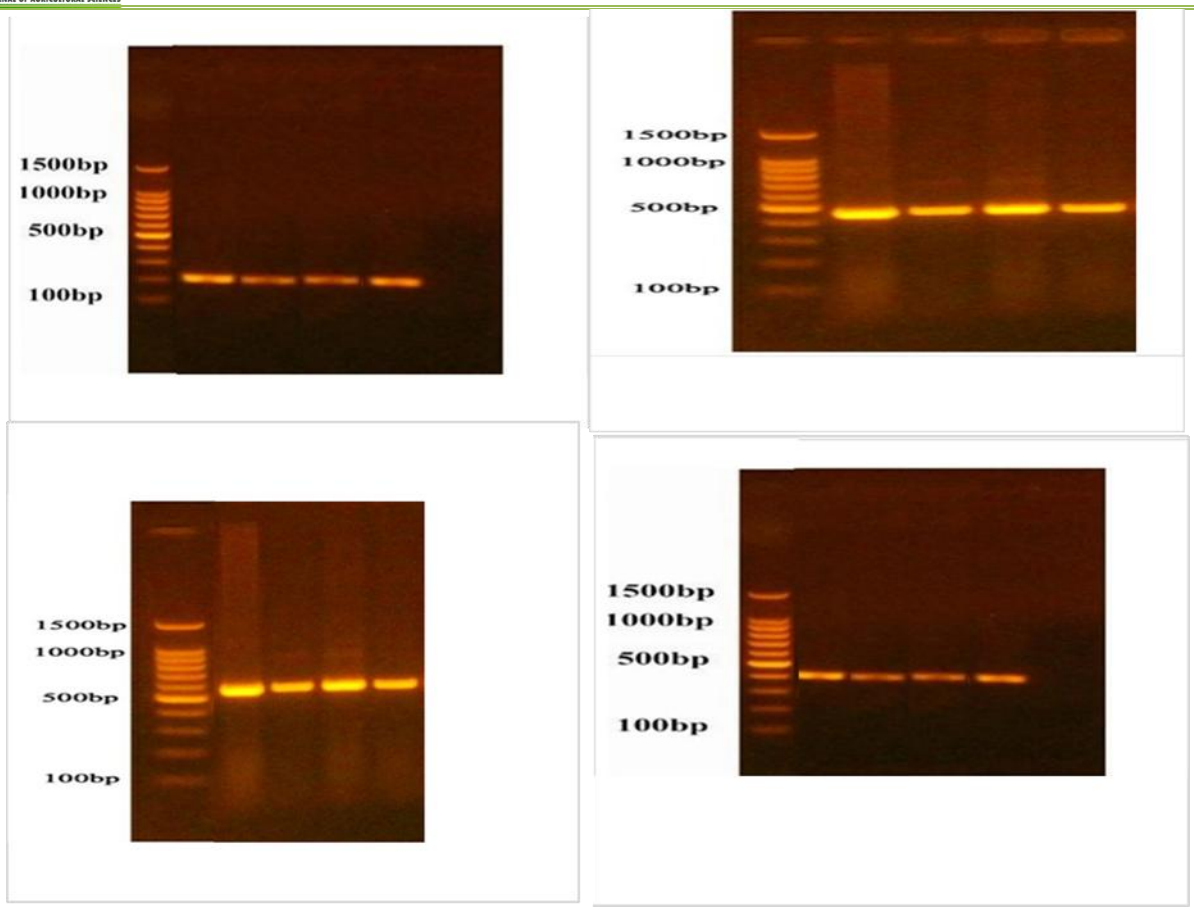

Figure 3 .(A) Identification of S. aureus 16 rRNA gene amplicon size 298 bp.(B) B. cereus amplicon size 470bp. (C) E. coli amplicon size 594bp.(D) S. enterica Typhimurium amplicon size 406 pb

Table 3. The prevalence of food borne pathogenic bacteria in local dairy product sources

Samples	B. cereus		S. aureus		E. coli		S. enterica Typhimurium	
	No.	%	No.	%	No.	%	No.	%
cheese	11	23.4	18	26.08	35	30.9	12	32.4
hard cheese	7	14.8	12	17.3	21	18.5	11	29.7
soft cheese	19	40.4	22	31.8	30	26.5	6	16.2
yogurt	10	21.2	17	24.6	27	23.8	8	21.6
Total	47	100	69	100	113	100	37	100

Antibiotic susceptibility tests

The Kirby Bauer technique, a manual approach for assessing antimicrobial susceptibility also recognized as the Antibiotic Disc Diffusion method, was utilized for the evaluation. A diverse array of antibiotics was administered against all isolated strains of *S. aureus, B. cereus, E. coli, and S. Typhimurium*. Furthermore, the Vitec system 2 instrument was employed to establish the minimum inhibitory concentration unique to each species, confirming their susceptibility profiles. This procedure was conducted to identify the patterns of multidrug resistance commonly linked with these bacterial strains and to determine optimal therapeutic strategies. The outcomes of this investigation are presented in Table 4.

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Table 4. The prevalence of food borne pathogenic bacteria in local dairy product sources

Type of bacteria	Antibiotics disk and concentration	MIC	No. of resist bacteria	% Resistance
S. aureus	Erythromycin	1	53	77%
	Clindamycin	>=8	65	94%
	Vancomycin	>=32	25	36%
	Tetracycline	>=16	62	90%
	Rifampicin	8	61	88%
	Ampicillin	>=32	69	100%
	Benzypencillin	>=0.5	11	26.8%
	Oxacillin	>=4	10	24.4%
B. cereus	Levofloxacin	1	13	32%
	Vancumycin	>=32	11	26.8%
	Nitrofurantion	128	11	26.8%
	Fisidic Acid	8	10	24.4%
	Teicoplanin	>=0.5	12	29.3%
E.coli	Tetracycline	>=16	26	23%
	Vancumycin	>=32	26	23%
	Ampicillin	>=32	26	23%
	Rifampicin	8	26	23%
	Clindamycin	>=8	26	23%
S.Typhimurium	Levofloxacin	1	2	05%
	Nitrofurantion	128	2	05%

Antimicrobial Susceptibility Test was performed by Vitec system 2 method and Kirby-Bauer method. In the experiment, a wide range of 12 antibacterials such as Amoxicillin, Tetracycline, Erythromycin, Vancomycin, Clindamycin, Rifampicin, Benzylpenicillin, Oxacillin, Levofloxacin, Nitrofurantoin, Fucidic Acid and Teicoplanin were used for testing. Using the Vitek kit technique allowing us to choose the (MIC), a total of 26 antibacterials including the above mentioned antibacterials remained in use against the isolated strain. 94 %, 90 %, 88 %, 77 %, and 36 % of the genus were found to be resistant to Clindamycin, Tetracycline, Rifampicin, Erythromycin and Vancomycin respectively. The resistance of *Salmonella typhimurium* to Levofloxacin and Nitrofurantoin was 0.5% respectively. 23% of *E. coli* were resistant to different antibacterials. particularly Amoxicillin, Tetracycline, Vancomycin, Clindamycin, and Rifampicin. Nevertheless, 32% of the Bacillus cereus strains were found to be resistant to Levofloxacin while 29.3% to Teicoplanin and 28.6% to Vancomycin, Nitrofurantoin, and Benzylpenicillin while 24.4% were resistant to Oxacillin and Fucidic Acid. There have been reports in literature highlighting a significant increase in the incidence of resistance to penicillins among all *S. aureus* isolates, specifically for those which are methicillin-resistant as stated in the research conducted by den Heijer et al. (2013). According to these reports, the rates of resistance to penicillins ranged from 97.5%, 87.8%, to 100% for the above-mentioned strains. One possible reason behind the high resistance to the penicillins among the *S. aureus* isolates (Baghbaderani et al., 2020).

Patterns of the Isolates' Multi-Drug Resistance: A substantial number of the isolates analyzed in this study were observed to be highly resistant to a number of antibiotics, which could be attributed to MDR or XDR. As indicated by the results given in Table 5, there were 55 cases, or 79.7% of the S. aureus isolates, which were found to have XDR characteristics, while 11 cases, or 23.4% of the B. cereus isolates, were noted to be antibiotic resistant. Also, there were 26 cases, which constituted 23% of the *E. coli* isolates, having MDR characteristics. Of the 37 *S. Typhimurium* isolates analyzed, only two were found to be resistant to Levofloxacin and Nitrofurantoin.

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Table 5. Resistance pattern for Isolated bacteria

Total No. of isolates	No. and (%) of resistance	No. of antibiotic resisted	Resistance type
S. aureus (69)	55(79.7%)	5	XDR
B. cereus (47)	11(23.4%)	7	XDR
E. coli (113)	26(23%)	5	XDR

In addition, some recent studies have shown that the studied bacteria are highly resistant to a number of antibiotics with the characteristics of MDR and XDR. This development of resistance may be due to genetic mutation and the bacteria acquiring plasmids which confer resistance to antibiotics. And again, the overprescription and over-reliance on broad-spectrum antibiotics have also contributed to the formation of E. coli strains that show resistance to multiple drugs, as pointed out in a study conducted by Kemp (2020). The high level of resistance to antibiotics is a serious concern to public health because Escherichia coli is responsible for UTI as reported by Mazzariol et al (2017). The World Health Organization (WHO) has issued a worrying warning about the rapidly growing antibiotic resistance labeled as an urgent issue and considered as one of the main health challenges of the 21st century. Bacteria have evolved many mechanisms to resist a single antibiotic and genes encoding for this resistance are horizontally transferred to other bacteria, including those found in the human gut microbiota. Toth et al. (2020), showed that antimicrobial resistance genes (ARGs) are present in raw milk. Furthermore, the use of antimicrobial agents in the agriculture sector is widespread and it contributes to the spread of resistance in milk microbiomes due to the favorable conditions provided by raw milk. The issue of antibacterial resistance in foodborne pathogens is widespread and can be attributed to the significant diversity among pathogenic strains (Grudlewska-Buda et al., 2023). A major challenge arises from strains that produce extended-spectrum β-lactamases and carbapenemases, as these not only confer resistance to cephalosporins but also extend to quinolones and aminoglycosides (Alegría et al., 2020; Findlay et al., 2022). For instance, Dembélé et al. (2020) reported that all clinical E. coli strains in their study exhibited resistance to amoxicillin as well as a combination of amoxicillin and clavulanic acid. Moreover, 60% of these strains displayed resistance to third- and fourth-generation cephalosporins. Canizalez-Roman et al. (2013) identified tetracycline resistance in Stx-producing strains, while Mohamed et al. found tetracycline resistance in strains isolated from ready-to-eat vegetables. Similar findings were observed by Canizalez-Roman et al. (2013). Canizalez-Roman et al. identified tetracycline resistance in Stx-producing strains, while Mohamed et al. found tetracycline resistance in strains isolated from ready-to-eat vegetables. Multiple investigations have confirmed the presence of multidrug-resistant (MDR) foodborne *E. coli* strains, with several studies documenting a significant prevalence of MDR isolates that are resistant to three or more classes of antimicrobials (Dembélé, R., et al. 2020.; Zhang, S., et al. 2021). It should be noted that these MDR strains have a considerable impact on a large number of described isolates, as reported in studies by Horcajada et al. (2019). Speaking about the problem of methicillin-resistant *S. aureus* strains it should be emphasized that the increasing prevalence of MRSA strains is a serious epidemiological problem. Lade et al. (2021) have shown that these MRSA strains are not only resistant to different types of beta-lactam antibiotics, but also highly insensitive to other antibiotic groups. Szczuka et al. (2022) also reported that a large proportion of *S. aureus* isolates from milk and fresh soft cheese showed characteristics of multidrug resistance (MDR), including MRSA. The percentage of multidrug resistance in *S. aureus* isolates from food sources varies between studies. Some publications report numbers ranging from few percent to several dozens percent, as shown in the study of Seow et al. (2021). Similarly, Masood et al. (2024) found the isolation of high proportion (75.2%) of multidrug resistant strains of *S. aureus* from beef burgers and hot dog sandwiches. As a result, they discovered a rather high percentage of vancomycin-resistant strains of

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S. aureus at 2.1%, which is quite alarming as vancomycin is usually used to treat MRSA infections. Furthermore, isolates of linezolid-resistant S. aureus (LRSA) have been recovered that complicate the management of infection with MRSA. Therefore, the risk of the presence of such bacteria in food production is quite obvious, if we take into account antibiotic resistance, toxin production by these strains and possible underestimation of the real percentage of diseases caused by them (especially MRSA and VRSA). Therefore, proper control measures must be taken. With respect to *B. cereus*, previous studies conducted by researchers have identified isolates from raw milk and dairy products that exhibited resistance to ampicillin and trimethoprim/ sulfamethoxazole (Gundogan & Avci, 2014; Chang et al., 2021). According to research carried out by Hu et al. (2021), they identified several Bacillus species in food samples, and the *B. cereus* strains they studied were resistant to various antibiotics. In our own work we have also identified *B. cereus* strains among these isolates and found them to be resistant to vancomycin. In the study of Kong et al. (2021), *B. cereus* bacteria were detected in meat and meat products. All strains studied by them were resistant to ampicillin, and a large proportion of the strains were resistant to rifampicin. However, most of the *B. cereus* strains were sensitive to gentamicin, chloramphenicol and trimethoprim-sulfamethoxazole. It has been also reported that multidrug-resistant *B. cereus* strains are found in different studies, but the frequency of their detection is different (Seow et al., 2021). They included the testing of ampicillin and gentamicin resistant bacterial isolates. However, it should be noted that the research carried out in our laboratory has shown a high sensitivity to ampicillin and gentamicin among the strains of *the B. subtilis* group. Although previous studies have shown that there may be *B. subtilis* contamination of dairy products which may cause some health risks for people consuming those products, it should be mentioned that this problem area requires further scientific exploration as pointed out by Pasvolsky et al. (2014). Antibiotic resistance of non-typhoidal Salmonella enteritidis and *S. Typhimurium* is a significant problem. This resistance is due to the acquisition of mobile elements by Salmonella such as plasmids with replicons which are responsible for multi-drug resistance of bacteria (Jacob et al., 2022). All tested *S. Typhimurium* strains were ampicillin resistant, as reported by Nadi et al. (2020). Still, *S. Typhimurium* serotypes showed susceptibility to ciprofloxacin and nalidixic acid. In conclusion, it can be said that resistance of various pathogenic bacteria such as *E. coli, S. aureus, B. cereus* and Salmonella strains to antibiotics has become a significant problem which needs to be solved since it poses significant health risks for consumers. The bacteria become resistant by obtaining antibiotic resistance genes and via horizontal gene transfer. Multidrug-resistant strains pose a special challenge because they are hard to treat. Their presence in the food chain increases the risk of transmission.

CONCLUSION

Antimicrobial resistance (AMR) remains a persistent problem impacting global populations and contributing to high rates of morbidity and mortality worldwide. Furthermore, regional differences exist concerning the regulation and management of the use of antibiotics. This includes differences in policies, procedures and technologies for tracking and detecting AMR. Food-borne diseases are the result of human activity and human decisions, and they are not always made rationally. Many pathogens identified in the food industry have been zoonotic pathogens, meaning they can transfer from animals to humans. The misuse and abuse of antibiotics in animal husbandry including misuse of antibiotic types and excessive dosage have been well recognized as factors leading to the emergence and spread of antibiotic resistant food-borne pathogens. Resistance in bacteria isolated from foods has been increasingly reducing the effectiveness of many antibiotics like beta-lactams, sulfonamides, tetracyclines and fluoroquinolones.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR/S DECLARATION

The authors declare that this manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. All authors have read and approved the final version of the manuscript and agree to its submission to Journal.

AUTHOR'S CONTRIBUTION STATEMENT

A.A. conceptualized the study and wrote the original draft; B.B. performed the methodology and data analysis; C.C. reviewed and edited the manuscript; D.D. supervised the project. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Awadalla, H., Khalil, I., Bassim, H. H., Ahmed, M. N., & Wahba, L. (2010). Molecular typing of methicilin-resistant Staphylococcus aureus isolates at Ain Shams University Hospital, Egypt. African Journal of Microbiology Research, 4(15), 1639-1646. http://www.academicjournals.org/ajmr

Baghbaderani, Z., Shakerian, A., & Rahimi, E. (2020). Phenotypic and genotypic assessment of antibiotic resistance of Staphylococcus aureus bacteria isolated from retail meat. Infection and drug resistance, 1339-1349. https://doi.org/10.2147/IDR.S241189

Behroozian, S., Zlosnik, J. E., Xu, W., Li, L. Y., & Davies, J. E. (2023). Antibacterial activity of a natural clay mineral against Burkholderia cepacia complex and other bacterial pathogens isolated from people with cystic fibrosis. Microorganisms, 11(1), 150. https://doi.org/10.3390/microorganisms11010150

Canizalez-Roman, A., Gonzalez-Nuñez, E., Vidal, J. E., Flores-Villaseñor, H., & León-Sicairos, N. (2013). Prevalence and antibiotic resistance profiles of diarrheagenic Escherichia coli strains isolated from food items in northwestern Mexico. International journal of food microbiology, 164(1), 36-45. https://doi.org/10.1016/j.ijfoodmicro.2013.03.020

Chang, Y., Xie, Q., Yang, J., Ma, L., & Feng, H. (2021). The prevalence and characterization of Bacillus cereus isolated from raw and pasteurized buffalo milk in southwestern China. Journal of dairy science, 104(4), 3980-3989. https://doi.org/10.3168/jds.2020-19432

Chauhan, A., & Jindal, T. (2020). Microbiological methods for food analysis. In Microbiological methods for environment, food and pharmaceutical analysis (pp. 197-302). Springer. https://link.springer.com/chapter/10.1007/978-3-030-52024-3_8

Chekabab, S. M., Paquin-Veillette, J., Dozois, C. M., & Harel, J. (2013). The ecological habitat and transmission of Escherichia coli O157: H7. FEMS microbiology letters, 341(1), 1-12. https://doi.org/10.1111/1574-6968.12078

den Heijer, C. D., van Bijnen, E. M., Paget, W. J., Pringle, M., Goossens, H., Bruggeman, C. A., ... & Stobberingh, E. E. (2013). Prevalence and resistance of commensal Staphylococcus aureus, including meticillin-resistant S aureus, in nine European countries: a cross-sectional study. The Lancet infectious diseases, 13(5), 409-415. https://doi.org/10.1016/S1473-3099(13)70036-7

Dembélé, R., Konaté, A., Traoré, O., Kaboré, W. A., Soulama, I., Kagambèga, A., ... & Barro, N. (2020). Extended spectrum beta-lactamase and fluoroquinolone resistance genes among Escherichia coli and Salmonella isolates from children with diarrhea, Burkina Faso. BMC pediatrics, 20(1), 459. https://doi.org/10.1186/s12887-020-02342-z

Fangio, M. F., Roura, S. I., & Fritz, R. (2010). Isolation and identification of Bacillus spp. and related genera from different starchy foods. Journal of food science, 75(4), M218-M221. https://doi.org/10.1111/j.1750-3841.2010.01566.x

Findlay, J., Perreten, V., Poirel, L., & Nordmann, P. (2022). Molecular analysis of OXA-48-producing Escherichia coli in Switzerland from 2019 to 2020. European

journal of clinical microbiology & infectious diseases, 41(11), 1355-1360. https://doi.org/10.1007/s10096-022-04493-6

Fymat, A. L. (2017). Antibiotics and antibiotic resistance. Biomed J Sci & Tech Res, 1(1), 1-16. DOI: 10.26717/BJSTR.2017.01.000117

Canizalez-Roman, A.; Gonzalez-Nuñez, E.; Vidal, J.E.; Flores-Villaseñor, H.; León-Sicairos, N. Prevalence and antibiotic resistance profiles of diarrheagenic Escherichia coli strains isolated from food items in northwestern Mexico. Int. J. Food Microbiol. 2013, 164, 36–45. DOI: 10.1016/j.ijfoodmicro.2013.03.020

Grudlewska-Buda, K., Bauza-Kaszewska, J., Wiktorczyk-Kapischke, N., Budzyńska, A., Gospodarek-Komkowska, E., & Skowron, K. (2023). Antibiotic Resistance in selected emerging bacterial foodborne Pathogens—An issue of concern?. Antibiotics, 12(5), 880. https://doi.org/10.3390/antibiotics12050880

Gundogan, N., & Avci, E. (2014). Occurrence and antibiotic resistance of E scherichia coli, S taphylococcus aureus and B acillus cereus in raw milk and dairy products in T urkey. International journal of dairy technology, 67(4), 562-569. https://doi.org/10.1111/1471-0307.12149

Horcajada, J. P., Montero, M., Oliver, A., Sorlí, L., Luque, S., Gómez-Zorrilla, S., Benito, N., & Grau, S. (2019). Epidemiology and treatment of multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa infections. Clinical microbiology reviews, 32(4), 10.1128/cmr. 00031-00019. https://doi.org/10.1128/cmr.00031-19.

Hu, Q., Fang, Y., Zhu, J., Xu, W., & Zhu, K. (2021). Characterization of Bacillus species from market foods in Beijing, China. Processes, 9(5), 866. https://doi.org/10.3390/pr9050866

Imre, K., Ban-Cucerzan, A., Herman, V., Sallam, K. I., Cristina, R. T., Abd-Elghany, S. M., ... & Morar, A. (2022). Occurrence, pathogenic potential and antimicrobial resistance of Escherichia coli isolated from raw milk cheese commercialized in Banat Region, Romania. Antibiotics, 11(6), 721. https://doi.org/10.3390/antibiotics11060721

Jacob, J. J., Solaimalai, D., Rachel, T., Pragasam, A. K., Sugumar, S., Jeslin, P., ... & Veeraraghavan, B. (2022). A secular trend in invasive non-typhoidal Salmonella in South India, 2000–2020: Identification challenges and antibiogram. Indian Journal of Medical Microbiology, 40(4), 536-540. https://doi.org/10.1016/j.ijmmb.2022.07.015

Javadi, A., & Khatibi, S. A. (2017). Effect of commercial probiotic (Protexin®) on growth, survival and microbial quality of shrimp (Litopenaeus vannamei). Nutrition & Food Science, 47(2), 204-216. https://doi.org/10.1108/NFS-07-2016-0085

Kamboj, S., Gupta, N., Bandral, J. D., Gandotra, G., & Anjum, N. (2020). Food safety and hygiene: A review. International journal of chemical studies, 8(2), 358-368. DOI: https://www.doi.org/10.22271/chemi.2020.v8.i2f.8794

Kemp, S. A. (2020). Patterns of Antimicrobial Resistant E. coli and Genetic Interplay Between Livestock, Humans and Their Shared Environment in a High-Density Livestock-Human Population in Western Kenya. The University of Liverpool (United Kingdom). DOI: 10.17638/03084779

Khani, N., Shakeri, A. H., Ashkezary, M. R., Aghapour, B., Soleimani, R. A., Hosseinzadeh, N., Rezaei-Savadkouhid, N., Khorrami, R., Shkouhian, S. M. J., & Homayouni-Rad, A. (2025). Potential of postbiotics in the biodegradation of antinutrients in foods. Probiotics and Antimicrobial Proteins, 1-12. doi: 10.1007/s12602-025-10649-5. Epub 2025 Jul 14.

Kong, L., Yu, S., Yuan, X., Li, C., Yu, P., Wang, J., Guo, H., Wu, S., Ye, Q., & Lei, T. (2021). An investigation on the occurrence and molecular characterization of Bacillus cereus in meat and meat products in China. Foodborne Pathogens and Disease, 18(5), 306-314. https://doi.org/10.1089/fpd.2020.2885

Lade, H., Joo, H.-S., & Kim, J.-S. (2022). Molecular basis of non-β-lactam antibiotics resistance in Staphylococcus aureus. Antibiotics, 11(10), 1378. https://doi.org/10.3390/antibiotics11101378

Makled, A. F., Labeeb, A. Z., Moaz, H. M., & Sleem, A. S. (2025). Chlorhexidine and benzalkonium chloride: promising adjuncts in combating multidrug resistant Klebsiella pneumoniae in healthcare settings. BMC Infectious Diseases, 25(1), 670. https://link.springer.com/article/10.1186/s12879-025-10980-w

Masood, E., Mohamed, F., Abo EL-Roos, N.A. and Sabike, I.I., 2024. Prevalence and critical antibiotic-resistance traits of Bacillus cereus and Staphylococcus aureus isolated from raw and ready-to-eat meat products. Benha Veterinary Medical Journal 46(2), 85-89. https://doi.org/10.21608/bvmj.2024.283334.1809

Mazzariol, A., Bazaj, A., & Cornaglia, G. (2017). Multi-drug-resistant Gram-negative bacteria causing urinary tract infections: a review. Journal of chemotherapy, 29(sup1), 2-9. https:/doi/full/10.1080/1120009X.2017.1380395

Nadi, Z. R., Salehi, T. Z., Tamai, I. A., Foroushani, A. R., Sillanpaa, M., & Dallal, M. M. S. (2020). Evaluation of antibiotic resistance and prevalence of common Salmonella enterica serovars isolated from foodborne outbreaks. *Microchemical Journal*, *155*, 104660. DOI: 10.1016/j.microc.2020.104660

Pasvolsky, R., Zakin, V., Ostrova, I., & Shemesh, M. (2014). Butyric acid released during milk lipolysis triggers biofilm formation of Bacillus species. International journal of food microbiology, 181, 19-27. https://doi.org/10.1016/j.ijfoodmicro.2014.04.013

Rady, M. H., Merdan, B., Essa, E., Salama, M., & El Sayes, S. (2014). The First Record of Escherichia coli O157: H7 Isolated from Wastes and Associated Houseflies in Egypt. World Applied Sciences Journal, 31(8), 1437-1447. DOI:10.5829/idosi.wasj.2014.31.08.83134

Saeed A.A., Taher, K. N ., Al-Nassrawi H. A.A.(2013). Detection of Salmonella typhimurium in chicken meat imported in the local markets of Diwaniya city By using PCR technique. AL-Qadisiya Journal of Vet.Med.Sci. Vol./12 No./2. DOI:10.29079/VOL12ISS2ART267

Seow, W.-L., Mahyudin, N. A., Amin-Nordin, S., Radu, S., & Abdul-Mutalib, N. A. (2021). Antimicrobial resistance of Staphylococcus aureus among cooked food and food handlers associated with their occupational information in Klang Valley, Malaysia. Food Control, 124, 107872. https://doi.org/10.1016/j.foodcont.2021.107872

Szczuka, E., Porada, K., Wesołowska, M., & Łęska, B. (2022). Occurrence and characteristics of Staphylococcus aureus isolated from dairy products. Molecules, 27(14), 4649. https://doi.org/10.3390/molecules27144649

Tabit, F.T., Incidence and characterisation of Bacillus sporothermodurans isolated from UHT milk. 2010, University of Pretoria. http://hdl.handle.net/2263/25568

Tóth, A.G.; Csabai, I.; Krikó, E.; Tőzsér, D.; Maróti, G.; Patai, Á.V.; Makrai, L.; Szita, G.; Solymosi, N. Antimicrobial resistance genes in raw milk for human consumption. Sci. Rep. 2020, 10, 7464. https://doi.org/10.1038/s41598-020-63675-4

Zhang, S., Huang, Y., Yang, G., Lei, T., Chen, M., Ye, Q., Wang, J., Gu, Q., Wei, X., & Zhang, J. (2021). High prevalence of multidrug-resistant Escherichia coli and first detection of IncHI2/IncX4-plasmid carrying mcr-1 E. coli in retail ready-to-eat foods in China. International journal of food microbiology, 355, 109349. https://doi.org/10.1016/j.ijfoodmicro.2021.109349

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التعريف الجزيئي وتوصيف المضاد الحيوي لبعض البكتيريا التي تنتقل عن طريق الغذاء من منتجات الألبان المحلية في إقليم كردستان العراق

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المستخلص

الهدف من هذه الدراسة هو إجراء التعرف الجزيئي وتوصيف المضاد الحيوي للبكتيريا المنقولة بالغذاء المعزولة من منتجات الألبان المحلية. منتجات الألبان معرضة بشدة للتلوث بمختلف مسببات الأمراض البكتيرية، مما يشكل خطرًا على صحة المستهلك. لذلك، فإن فهم هوية هذه البكتيريا ومقاومتها للمضادات الحيوية أمر بالغ الأهمية لضمان سلامة الأغذية والعلاج الفعال للعدوى ذات الصلة. تم الحصول على 113 و69 و47 و37 عزلة بكتيرية من E.coli و Staphylococcus aureus و Bacillus cereus و Salmonella typhimurium على التوالي من منتجات الألبان المختلفة، بما في ذلك الجبن والجبن الصلب والجبن الطري والزبادي. تم استخدام التقنيات الجزيئية، مثل تفاعل البلمرة المتسلسل (PCR) لتحديد الأنواع. تم تمييز العزلات البكتيرية لتحديد أنماط حساسيتها لمضادات الميكروبات باستخدام نظام الانتشار القرصي ونظام Vitek 2. أظهر اختبار الحساسية للمضادات الحيوية أنماط مقاومة متفاوتة بين العزلات. أظهر اختبار الحساسية للمضادات الحيوية أنماط مقاومة متفاوتة بين العزلات. لوحظت مقاومة للمضادات الحيوية شائعة الاستخدام، مثل [كليندامايسين، تتراسيكلين، ريفامبيسين، إريثروميسين، فانكومايسين وأمبيسيلين، .....] في 55 S. aureusالمعزولة55 عزله من 69، 11 عزلة من 47 من بكتيريا B. cereus و26 معزولة من 113 معزولة. من مقاومة الإشريكية القولونية للعديد من المضادات الحيوية المقاومة لفئات متعددة من المضادات الحيوية، والمعروفة باسم مقاومة الأدوية المتعددة، تم اكتشافها أيضًا في مجموعة فرعية من العزلات.

الكلمات المفتاحية: المضادات الحيوية، منتجات الألبان، الأمراض المنقولة بالغذاء، المقاومة.