

Detection of locally produced cheese with bacterial contaminants in Diyala province

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Abstract:

Food plays a vital role in our life, contamination of foodstuff is an important issue that affects millions of persons every year across the world. Bacterial contamination of foodstuff is affecting developed as well as undeveloped nations. This occurs when food is contaminated with disease-causing bacteria, viruses, parasites, or chemicals that can cause illness or even death. To investigate *Klebsiella* contamination of Cheese, Forty samples of local cheese were collected randomly from Diyala Governorate. All samples were cultured to obtain fermenting bacteria on MacConkey agar, and some of biochemical tests urease, motility, oxidase, indole, citrate and catalase test carried out to identify and confirm biochemical properties of bacterium. Next, a sensitivity test has been done for all samples using antibiotic disk to detect their susceptibility.

The result exposed that the positive percentage of isolate was 10 % gained from soft cheese in this study. The highest level of Antimicrobial activity was (19.66 mm) for Gentamycin, (17.33 mm) for Amikacin, whereas all isolates exhibited resistance against other antibiotics.

Key word: Soft Cheese, Antibiotic, Coliform, Dairy products contamination



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Introduction:

Dairy products are considered as one of the greatest important official food strategies of some countries. It is also classified as one of the best vital components and has been part of the human diet for more than 8,000 years. There are unlimited nutritional aspects such as proteins, carbohydrates, vitamins, In addition to minerals such as potassium, calcium and phosphorus. The importance of dairy products is due to the requirements of

nutritional references for dairy consumption in providing the recommended amounts of calcium and other nutrients that significant for human health (10).

Food can be spoiled by unwanted microbes during manufacture, storing and allocation. Due to their high nutritional importance, especially it contains high amounts of protein and fat, dairy products of all kinds supply an ideal environmental growth of a variety of harmful germs (12).

Various types of enteric pathogens have been detected in cooled milk and cheeses consumed without boiling. Contamination of cheese with pathogenic microorganisms may be caused by post-pasteurization processing, as well as manufacturing, processing, equipment, temperature changes during transportation and storage conditions (10). Food spoilage poses a threat to human health and causes huge economic losses. It has been found that about 15-25% of food products worldwide undergo spoilage and the type of microorganisms causing spoilage is mainly discovered by the nature of dairy product (12).

Enterobacteriaceae are known as macrobacilli, a heterogeneous group of Gram-negatives, which naturally live in the intestinal tract of both humans and animals, they contain many genuses (such as Salmonella, Serratia, Enterobacter, Shigella, Escherichia coli, Klebsiella, and Proteus). These species have been found to be common in the environment and can contaminate milk through feces, bedding, improperly cleaned cow udder teats, equipment of milk treatment, and tools contaminated with dirt or unclean water (4). Undesirable changes may occur that make the product poor quality, unmarketable and not suitable for human consumption due to the appearance of Klebsiella bacteria in dairy products.

Moreover, its presence indicates fecal contamination. Therefore, the quality of foods, especially dairy products, is routinely evaluated according to the presence of intestinal bacteria (14). One of the most important pathogens that affect food safety

and cause various human diseases around the world as a result of eating foodstuffs, especially dairy products that contaminated with pathogens or their toxins are food-borne microorganisms (7).

According to (20), the misuse and overuse of antibiotics and the increase in antimicrobial resistance are considered mostly serious human public health problems in the world. The aim of the study is to study bacterial contamination in locally white cheese that produced in Diyala Governorate.

Material and Methods:

Forty random samples of locally soft cheese were collected from different region (baqubah , al khalis and bani saad) at Diyala province. All samples were saved in lonely sterile laboratory bags and kept in a safe freeze box, directly transported to the public health research laboratory under sterile atmosphere and inspected as rapidly as probable without stoppage. According to (5), each sample was macerated well using a sterile spatula, the required amount of each sample was accurately weighed under sterile conditions.

General guidelines of detection, isolation and identification of Enterobacteriaceae from dairy products were applied in this study according to (9), twenty five grams (25gm) of each labelled cheese sample was diluted in 225 ml nutrient broth, a loopful of inoculum's from the enriched broth was streaked on M MacConkey agar for primary screening of coliforms based on colony morphology after overnight of incubation at 37 °C. All strains were negative on indole,

methyl red tests, H₂S, oxidase and urease tests. In contrast, completely isolated microbial strains showed positive on catalase and citrate, motility tests, voges-proskauer (VP) tests and gas production.

Antibiogram Assay:

One colony of the test organisms was taken using a one-use loop, mixed in 5 ml of Brain Heart Infusion Broth (Liofilchem Italy), and hatched overnight at 37°C to reach 0.5 McFarland standard turbidity. The suspension was distribute on the surface of Mueller Hinton Agar (Oxoid, England) with a cotton swab, suitable test antibiotic test

disks were prepared and distributed on the surface of the agar, balanced distance from each other via a sterile needle, dishes were hatched for 24 hr. at 37°C, finally inhibition zones was measured (18).

Antibiotics used in this study were, Gentamycin, Amikacin, Vancomycin, Methicillin, piperacillin, Clindamycin, Erythromycin Amoxicillin/clavulanic, Meropenem and Rifampicin (antimicrobial sensitivity disc from HIMEDIA® lab.).

Results

Isolation and identification of bacteria

In present study, all samples which looked ferment mucoid pink colonies on macConkey agar (figure 1).



Figure (1): Show phenotypic appearance of Klebsiella spp

Table (1): Antibiotics effect against Klebsiella isolated in this study (mean inhibition zone)

Antibiotic	Diameter inhibition zone in mm			Mean(mm)
Amikacin(30 µg)	17	17	18	17.33
Gentamycin(10 µg)	19	19	21	19.66
Clindamycin (2 µg)	0	0	0	0
Vancomycin(30 µg)	0	0	0	0
Methicillin(5µg)	0	0	0	0
Piperacillin 100 µg)	0	0	0	0
Erythromycin(15µg)	0	0	0	0
Amoxicillin/clavulanic 20/10 µg)	0	0	0	0
Meropenem(10 µg)	0	0	0	0

Results of Inhibition zone diameter according to antimicrobial disc types



Figure (2): show Antibacterial Sensitivity against Amikacin, Gentamycin, Clindamycin, Vancomycin, Methicillin, Methicillin, Piperacillin, Erythromycin, Amoxicillin and Meropenem antimicrobial sensitivity disc from HIMEDIA® lab.

Discussion:

Soft cheese is considered as an important global foodstuff. It is commonly expended within less than month after manufacturing. In countryside areas and distant hamlets traditional un ripen soft cheese is prepared from non-pasteurized fresh milk.

Cheese atmosphere might have a main effect on bacterial growth. Diverse elements, resembling pH, water activity and temperature generally controlled by cheese technology, can express the microbial ecosystem of cheese. As well, tap water rather than non-hygienic water commonly recycled in the washing of the instruments which used in cheese manufacture besides common uses everyday (8).

The coliform group of bacteria is opportunistic pathogens responsible for a wide range of infections, while the others are classified as part of the normal intestinal flora (11). Improper handling and/or unhygienic production of milk and milk utensils combined with poor sanitary conditions leads to the spread of these organisms in dairy products (6). There are two ways of raw milk contamination; the first is internal contamination, represented by infection of cows with the pathogen that will move into the blood circulation (systemic infection) to reach the udder tissue or through direct infection of the udder to produce contaminated raw milk (21). The other way is external contamination, as it is believed that contamination of raw milk with feces during or after the collection process, as well as the outer surface of the udder, nipples, and skin, are source of infections in addition environmental

contamination (21). Besides, raw milk contamination by pathogenic organisms can be affected by several aspects, including general health of dairy cows, the environment surrounding the animal, the hygienic milking process, farm management practices, storage conditions, location, and seasonal variation (15).

Antibiotics are frequently consumed widely in animals with the purposes of inhibition and management of clinical diseases besides increasing growth and development (17). This routine may affect several aspects of food processing, as it has been observed that antibiotic-resistant bacteria in animals can spread rapidly in various food products (19).

The results showed that Gram-negative bacteria are characterized by a significantly increased level of resistance against beta-lactam antibiotics due to the presence of special genes called extended-spectrum beta-lactamase (ESBL) which found in enteric bacteria, mainly in *Escherichia coli* and *Klebsiella pneumoniae*.

Klebsiella bacteria resistant as a result of antibiotics misuse on animal farms represent a serious problem for public health and livestock (3). The organic materials of bedding such as wood by-products and livestock feces are the most important sources of *Klebsiella* species on dairy farms (16). It was also found that to reduce this problem, microbial agents are added to the feed of dairy cows on pastures to reduce disease-causing bacteria (13).

In this study, by examining the sensitivity of a number of antibiotics towards

Klebsiella, it was found that all isolates exhibited sensitive toward Gentamycin and amikacin while resistance against other antibiotics (Methicillin, Clindamycin, Vancomycin, piperacillin, Erythromycin, Amoxicillin/clavulanic, Meropenem and Rifampicin).

Our findings are consistent with a study conducted by (1) which examined the microbial content of the local soft cheese and local cream which found that 10% of soft cheese and cream were contaminated with Klebsiella.

Previous study conducted by (2) that showed from 234 samples of milk and dairy products, about 17 (6.5%) of the isolates on agar media seem phenotypically suggested to be Klebsiella, Furthermore, no zone of inhibition was observed against the Ampicillin, Amoxicillin, Bacitracin, Penicillin, Clindamycin and Cloxacillin. In contrast, these isolates were sensitive to Levofloxacin, Ciprofloxacin, Amikacin, Lincomycin, Chloramphenicol, Ertapenem and Gentamycin. It was noted that all isolates were resistant to erythromycin, except one that was moderately. In Addition, several of these separates showed various levels of sensitivity to some antimicrobial disc, as: Oxytetracycline, Streptomycin, Tobramycin, Kanamycin, Nitrofurantoin, Tetracycline, Piperacillin, Cefoprazone/Sulbactin, Ceftriaxone, Cephoxitin, Sulfamethoxazole/ Trimethoprim, Ceftazidime and Cefotaxime.

Our result revealed that all isolate (100%) were resistant to methicillin this nearly similarity with the same previous study conducted by (2) which found that single

isolate was methicillin sensitive, whereas the other isolates showed resistant.

Furthermore, wholly isolates showed susceptible to imipenem and doxycycline apart from two of which that were resistant. additionally, altogether isolates were susceptible to Clavulanic acid / Amoxicillin, but three isolates showed intermediary, this disagreement with our result which the later antibiotic don't show activity against all isolate.

Conclusions:

1-The factors causing high contamination of soft white cheese samples with Klebsiella bacteria include poor general hygiene conditions during the production, manufacture and storage of milk and cheese, as well as the low refrigeration levels and the absence of treatment stages as milk pasteurization process to kill pathogenic organisms.

2-Gentamycin and Amikacin were the most effective antibiotics against Klebsiella while this bacterium was resistance to other antibiotic (Methicillin, Clindamycin, Vancomycin, piperacillin, Erythromycin, Amoxicillin/clavulanic, Meropenem, and Rifampicin).

Recommendations:

We recommend taking the necessary measures to monitor raw milk quality, environmental and hygienic conditions through production and marketing. It is needful to veterinary checks on the dairy cows repeatedly by veterinarian. Furthermore, fresh and pasteurized milk

must be used to make local soft white cheese and preserved in saltwater.

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