

Detection of pseudomonas Contamination in Milk and some dairy products in Diyala Province

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Abstract:

Background: Food putrefaction is a critical topic strongly influencing the global require to counteract food instability. Despite the costly use little temperatures, correct preservation for not old dairy yields is constantly endangered on various phases of manufacturing and commercialization via psychotrophic bacteria mostly according to the genus *Pseudomonas*

Aims: To investigate pseudomonas contamination of milk and dairy products

Methods: Sixty samples were collected randomly from Diyala province, 10 sample for each product. Samples were cultivated to attain non-fermented bacteria on MacConkey agar, and were then tested using the oxidase reagent for confirmation. After that, a sensitivity test was conducted using antibiotic disk to determine their susceptibility.

Results : The maximum percentage of isolate was 80% gained from raw milk, 40 % obtained from soft cheese then 20% from each pasteurized milk and salt cheese, whereas all cream sample not displayed any contamination with pseudomonas. The highest Antimicrobial activity was (30.5 mm) for Meropenem, (20.5 mm) for Amikacin, (18mm) for Gentamycin, finally 8mm for Erythromycin.

Conclusion : The high prevalence of *P. aeruginosa* was located in raw milk. 2-The highest sensitivity was against Meropenem while the less sensitivity was against Erythromycin. presence of heat stable enzymes produced by *Pseudomonas* spp., that effect on milk and its products like bad odor, bitter taste, and also affect texture represent another source of contamination additionally to presence viable cells of *Pseudomonas* spp. Contamination of milk with prevalence psychotrophic microbial contamination post pasteurization is consider a latent difficult.

Key word : pseudomonas; dairy products; spoilage



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Introduction

human mainly utilize cow milk ,but they also use camels milk, goats, ,sheeps, and buffaloes is furthermore consumed for the manufacturing of milk products. Around fifty percentage of the milk produced is spent as boiled or fresh ,1\6 as curd or yoghurt and residual is consumption for industrialized of numerous kinds of dairy products for instance butter, ice cream, milk powder , cheese, which are usually existing. ⁽¹⁾

Lafarge *et al* .,2004 ⁽²⁾ reported that important losses for the food manufacturing belong to Milk Decay because of existence of the psychrotrophic microbes and is a specific pattern of the dairy trade . Refregeneration of Milk is for less than week previous heating it . Diminish value of raw milk due growing psychrotrophic germs at storage it at little temperatures.

highest of Gram-negative bacteria are *Pseudomonas* species that can treat dairy products creating heat constant extracellular enzymes . These enzymes likewise can persist both UHT treatment and pasteurization .They have an influence taking place on the value of dairy foods resulting in rotten tastes ,bitter and altering milk features . Many reasons permit to *pseudomonas* to persist on milking machines, bulk tanks, pipelines such as ,these microbes have great inherited variety and metabolic capacity, they can survive in different environments, such as soil, water, and air. ⁽³⁾

The multiplication and growth of microbes in the cheese be determined by many issues including the pH ,water activity , storage temperature and availability of nutrients. Cheeses are favorable medium to the bacterial putrefaction as a result of low concentration of salt and neutral pH and high moisture content. ⁽⁴⁾

resistance of Bacteria to antibiotic is reflected a global difficult in the agricultural ,environmental, medical fields. Numerous investigators concentrated on pathogenic microorganisms conflict against antibiotic ,that attitude direct hazards for social well-being. Though, further and further studies are concentrating about microbes that living in close association related with nutriment . previous studies displayed widespread fighting of *Pseudomonas* spp. isolated from raw milk toward numerous antibiotics, also certain even show the maximum recognized stages of antibacterial conflict ⁽⁵⁾.

Material and Methods :

A total of 60 random samples of milk and its products as raw and pasteurized milk, locally soft and salted cheese and cream were collected from different region at Diyala province. All samples were kept in an isolated, sterile plastic bag and conserved in an protected freeze box, after that they were transported immediately to the workroom below hygienic environments and inspected as rapidly as probable without interruption. The isolation and identification of *pseudomonas* occur according to (Isenberg,

2007) ⁽⁶⁾ , also For further identification , purification colonies were carried out according to morphological appearance and biochemical tests (ISO, 2004) ⁽⁷⁾. All strains were negative on indole , methyl red tests, Voges Proskauer (VP) tests and urease tests. In contrast that wholly isolated bacterial strains displayed positive on catalase and motility tests.

Preparation of Bacterial Suspensions For Antimicrobial Sensitivity:

Antimicrobial sensitivity test was carried out, according to McFadden, 2004 ⁽⁸⁾ by the

Kirby-Bauer method, inoculate spreading over Muller- Hinton agar plates by using a sterilized cotton swab. Different Discs were employed moderately at a balanced distance from each other via a sterile needle, after that these plates were incubated for 24 hr at 37 °C , lastly result of inhibition zones were measured. Drug used in this study were, Erythromycin (15), Gentamycin (G10), Amikacin (30), Meropenem .

Results

Isolation and identification of bacteria

In present study, all samples which appeared non ferment colonies on MacConkey agar and display (dark purple at first 10 second) in Oxidase test confirm *Pseudomonas* bacteria ,as in figure (1)



Figure(1):shows positive oxidase test for *Pseudomonas*

Table(1):show source and percentage of isolate during this study

Source isolate	Frequency	Percentage
Raw milk	8 out of 10	80%
Pasturized milk	2 out of 10	20%
Soft cheese	4 out of 10	40%
Salt cheese	2 out of 10	20%
Imported cream	0 of 10	0%
Localy cream(gaimer)	0 of 10	0%

Table (2) :Antibacterial effect of some antibiotic against pseudomonas isolated in this study (mean inhibition zone)

Antibiotic	Diameter inhibition zone			Mean(mm)
Meropenem	30	30	31	30.5
Amikacin	20	21	20	20.5
Gentamycin	17	19	17	18
Erythromycin	8	8	8	8

Discussion:

Use of short temperatures have permitted delay of the spoilage and retailing of several newly milky yields. Conversely, obviously happening microbes, for example pseudomonads, have progressively turn into a actual anxiety toward refrigerated -kept fresh dairy foods since its capability to adapt themselves also grow at low temperatures. They are accountable for noticeable decay characters (rheology changes, discolorations, structure loss,) and non-observable faults (off-odours, off-flavours and protein

breakdown), which considerably diminish the excellence and shelf-life of dairy yields. At each phase of the industrial process of dairy yields, Pseudomonads contamination happens also they converts to extra persistent and unaffected to hygienic measures once microbial cells raise as biofilm. The greatest common decomposition microbes of milk and its products are gram-positive spore-forming bacteria, clostridium spp Bacillus, Streptococcus spp and lactic acid producing bacteria, and Gram-negative rod-shaped bacteria, Coliform and Pseudomonas spp., and as reported by IDF (1994)⁽⁹⁾. Bacterial

adulteration of cheese belong to many causes for example packaging material ,handler, and environment ⁽¹⁾.

⁽¹⁰⁾ reported that several factors lead to spoilage of the samples such as the environment in which the cow is housed and milked ,the health and hygiene of the cow, and the procedures used in cleaning and sanitizing the milking and temperature and length of storage ,storage equipment's, wholly encouragement bacterial amounts in raw milk . ⁽¹¹⁾ reported that the Cheeses are prepared-to-eat diet yields since they not need suffer additional application to certify its security before ingestion. Furthermore, impurity of cheeses can occur at numerous steps in the manufacture chain. Hence , all the evidence about bacterial features and susceptibility is essential to avoid contamination of dairy yields with pathogens. According to Dogan, and Boor , 2003 ⁽¹²⁾, Reported prior studies were interested of detection of raw milk and dairy goods contamination with *Pseudomonas* spp. , however *P. fluorescens* is the major commonly involved in milky yields through milk destruction .

Investigation carried out by Gennari and Dragotto ,1992 ⁽¹³⁾ described that frequency of *Pseudomonas* spp. Was less than 90 % from the environmental samples plus foodstuffs, greatest frequently isolated (38.3%) was *Pseudomonas fluorescens* biovar. Also, 54.5% of *Pseudomonas* spp. were exposed from raw milk cheeses ⁽¹⁴⁾, and fifty % of *Pseudomonas* spp. was gained starting from 1day time raw milk cheeses ⁽¹⁵⁾. At a prior study, the occurrence of *Pseudomonas* spp. was 30.5% from raw milk samples ⁽¹⁶⁾. Furthermore, sixty seven % of *Pseudomonas* spp. Present in UHT milk, *Pseudomonas* species characterize the

terribly common group of psychrotrophic bacteria associated to milk putrefaction. On the extra adjacent, *Pseudomonas* species are capable to develop at cooling temperatures (7 °C or less), producing fermentation, putrefaction, , bitter ,sweet tastes because they metabolize fat and protein. ⁽¹⁷⁾

Current result in this study documented that the percentage contamination of raw and pasteurized milk were (80%,20 %) respectively with *pseudomonas* in agreement with study conducted by Derakhshani et al.,2020 ⁽¹⁸⁾ whom revealed that Psychrotrophic bacteria, mainly genus *Pseudomonas*, governor the microbial society in stored ,refrigerated raw milk, and possibly too be a contaminant of pasteurized milk, Their incidence in refrigerated raw milk is greatest likely by reason of contamination during and/or after milking, with the milking ,milking apparatus usually responsible for the highest contamination ⁽¹⁹⁾. Moreover , after – heat treatment contamination of the milk with *Pseudomonas* spp. is an significant reason prompting the superiority deterioration of chilled pasteurized milk. During growth in raw milk, some bacteria particularly *pseudomonas* spp. yield out cellular lipolytic and proteolytic enzymes and these enzymes stay lively post pasteurization milk .

Earlier results definite the outcomes of many personnels such as Decimo et al 2016 ⁽⁵⁾ , Munsch-Alatossava, and Alatossava ,2007 ⁽²⁰⁾ whom described conflict outlines of Gram-negative psychrotrophic bacteria toward antibiotic, though, psychrotrophic isolates from pasteurized , raw milk have

been instituted to be unaffected to gentamicin⁽²¹⁾ this finding is disagreement with our result which revealed that inhibitory zone diameter for *pseudomonas* was (17mm) toward Gentamycin.

Conclusion

1-The high prevalence of *P. aeruginosa* was located in raw milk.

2-The highest sensitivity was against Meropenem while the less sensitivity was against Erythromycin

3- presence of heat stable enzymes produced by *Pseudomonas* spp., that effect on milk and its products like bad odor, bitter taste, and also affect texture represent another source of contamination additionally to presence viable cells of *Pseudomonas* spp.

4-Contamination of milk with prevalence psychrotrophic microbial contamination post pasteurization is consider a latent difficult.

Recommendations:

1. For assurance hygienic milk and Dairy product manufacture also supply, Regularly examination must be used.
2. Dairy product must be carried out in healthy ways.
3. Keep milk and dairy products in distinct cooler to avoid contamination and growth microorganism.

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