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# Isolation, Characterization, and Antimicrobial Sensitivity of *Enterobacter aerogenes* in Locally Produced Cheese from Diyala Province

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**Abstract:**

**Back ground:** Food safety has continued to receive significant attention from consumers, food manufacturers, and producers. Foodborne illness results from eating contaminated food.

**Aims:** to determine the frequency of contamination ***with Enterobacter aerogenes*** in locally produce cheese in Diyala province

**Methods:** sixty cheese samples were randomly collected from Diyala province. All samples were cultured to isolate fermented bacteria on MacConkey agar plates. Presumptive isolates were then subcultured on XLD agar and identified biochemically using tests for urease, motility, oxidase, indole production, citrate utilization and catalase. Identities were confirmed with the Vitek system to definitively identify isolates and verify biochemical properties. Antibiotic susceptibility testing was then conducted on confirmed isolates using antibiotic disks to determine susceptibility profiles.
**Results:** A total of 6/60,( 10%) was positive isolation rate for *E. aerogenes* from soft cheese samples .The higher rate of contamination was in AL-Khalis region , (2/15%13.33% followed by Buhriz and Khan Bani Saad ,(1/10,10%).while the lowest contaminated samples were from Al-Muqdadiyah(2/25,8%. Isolates exhibited the highest susceptibility to Gentamicin (21.6 mm zone) and Amikacin (19.6 mm zone), while all isolates were resistant to Methicillin, Clindamycin, Vancomycin, and Rifampicin.

**Key word**: Enterobacter Aerogenes; Soft Cheese, Antibiotic

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## Introduction:

The characteristic flavors and nutritional value of dairy products like cheese and butter have driven growing global consumer demand in recent years (1). Traditional artisanal cheeses are important sources of nutrients like protein, fat, calcium, iron, phosphorus, vitamins, and essential amino acids that are critical for health and growth needs (2).

Raw milk most probably contaminated with bacteria that compromise both quality and safety for the cheese (3). Dairy products normally contain species from Enterobacteriaceae family (4,5). These bacteria which is most probably come from fecal contaminates impairs cheese's qualities and cause early blowing (6,7).

Human illnesses have been connected to tainted dairy products, emphasizing the need for improved management of Enterobacteriaceae and other harmful microorganisms throughout the production process (8,9). Growing antimicrobial resistance complicates this by endangering the effectiveness of antibiotics in treating infections linked to dairy (10). Ensuring the quality and safety of cheese achieved by healthy monitoring and application of hygienic measures (11,12).

This study set out to determine how common Enterobacteriaceae, particularly Enterobacter species, were in raw milk cheeses. Analyze the Enterobacter isolates' patterns of antibiotic susceptibility from raw milk cheeses as well.

## Material and Methods:

### Ethical Agreement:

Current study was approved by scientific committee of college of veterinary medicine, university of Diyala, Iraq

### Sample Collection and Preparation

Sixty samples of locally produced soft cheese were randomly collected from various regions of Diyala province, Iraq. Samples were kept in sterile plastic bags stored in a cooled container and transported to the microbiology laboratory under hygienic conditions for immediate analysis. In the lab, 25g of each cheese sample was diluted in 225mL sterile broth, macerated thoroughly with a sterile spatula, and weighed under sterile conditions.

### Isolation and Identification of Bacteria

Standard techniques were used to detect, isolate and identify Enterobacteriaceae according to published guidelines (14). The diluted samples were enriched in broth, then inoculated on MacConkey agar plates to screen for coliforms based on colony morphology after 24hr incubation at 37°C. Isolates were negative for indole, methyl red, $H_2S$, oxidase and urease tests. All isolates displayed positive catalase, citrate utilization, motility, VP and gas production. Presumptive isolates were stored at -80°C in glycerol prior to identification using the Vitek system and antimicrobial susceptibility testing.

### Preparation of Bacterial Suspensions for Antimicrobial Sensitivity:

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, as described by

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McFadden (2004). Bacterial isolates were swabbed evenly across agar plates. Antimicrobial impregnated disks, including gentamicin, amikacin, vancomycin, methicillin, piperacillin and clindamycin, were gently applied at equal distances using a sterile needle. Plates were incubated at 37°C for 24 hours. Subsequently, zones of inhibition surrounding each disk were measured (Figure 2) to determine susceptibility or resistance to the antibiotics based on standard interpretive criteria.

**Statistical analysis:**

Statistical analysis depends on frequency analysis [16-18].Calculation down by the Statistical Package of the Social Sciences for windows version 17 (SPSS, Armonk,NY: IBM Corp) [19-21].

## Results

### Isolation and identification of bacteria

In the present study, all bacterial samples that displayed non-lactose fermenting colonies on MacConkey agar and yellow colonies on xylose lysine deoxycholate (XLD) agar underwent further biochemical testing (Figure 1). These additional tests listed in table (1). Based on the full battery of test results, the bacterial isolates were identified as *Enterobacter aerogenes* using the Vitek automated microbial identification system (Table 1). By employing selective growth media and confirmatory biochemical assays, this methodology allowed for the isolation and confirmation of Enterobacter Aerogenes from the clinical samples analyzed in this study. As shown in table (1) ,A total of 6/60,( 10%) was positive isolation rate for *E. aerogenes* from soft cheese samples .The higher rate of contamination was in AL-Khalis region , (2/15%13.33% followed by Buhriz and Khan Bani Saad ,(1/10,10%).while the lowest contaminated samples were from Al-Muqdadiyah(2/25,8%)

**Table (1):Frequency Of Enterobacter aerogenes Isolated From Cheese Samples According To The Region**

| Region | Total Number Cheese samples | Enterobacter aerogenes positive samples | Percentage% |
|---|---|---|---|
| Muqdadiyah | 25 | 2 | 8% |
| Buhriz | 10 | 1 | 10% |
| AL-Khalis | 15 | 2 | 13.33% |
| Khan Bani Saad | 10 | 1 | 10% |
| Total | 60 | 6 | 10% |

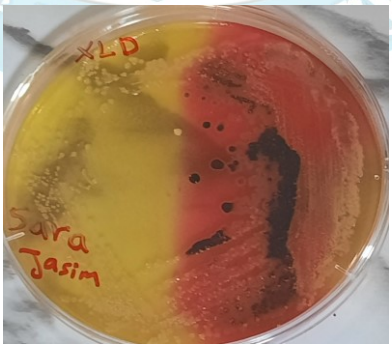


**Figure 1. Yellow Phenotypic appearance of Enterobacter aerogenes isolates on (XLD) agar.**

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**Table 2. Key Biochemical Properties of *Enterobacter aerogenes***

| Biochemical Details | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | + | 4 | PyrA | + | 5 | lARL | - | 7 | dCEL | + | 9 | BGAL | + |
| 10 | H2S | - | 11 | BNAG | + | 12 | AGLTp | - | 13 | dGLU | + | 14 | GGT | + | 15 | OFF | + |
| 17 | BGLU | + | 18 | dMAL | + | 19 | dMAN | + | 20 | dMNE | + | 21 | BXYL | + | 22 | BAlap | - |
| 23 | ProA | + | 26 | LIP | - | 27 | PLE | + | 29 | TyrA | + | 31 | URE | - | 32 | dSOR | + |
| 33 | SAC | + | 34 | dTAG | + | 35 | dTRE | + | 36 | CIT | + | 37 | MNT | + | 39 | 5KG | + |
| 40 | lLATk | + | 41 | AGLU | - | 42 | SUCT | + | 43 | NAGA | + | 44 | AGAL | + | 45 | PHOS | + |
| 46 | GlyA | + | 47 | ODC | + | 48 | LDC | + | 53 | lHISa | - | 56 | CMT | + | 57 | BGUR | - |
| 58 | O129R | + | 59 | GGAA | - | 61 | lMLTa | - | 62 | ELLM | - | 64 | lLATa | - | | | |

**Table 3. Antibacterial Activity of Antibiotics Against Enterobacter aerogenes Isolates (Zone of Inhibition in mm)**.

| Antibiotic | Diameter inhibition zone | | | Mean(mm) |
|---|---|---|---|---|
| **Amikacin** | **20** | **20** | **19** | **19.6** |
| **Gentamycin** | **22** | **22** | **21** | **21.6** |
| **Clindamycin** | **0** | **0** | **0** | **0** |
| **Vancomycin** | **0** | **0** | **0** | **0** |
| **Methicillin** | **0** | **0** | **0** | **0** |
| **piperacillin** | **0** | **0** | **0** | **0** |

**Antibiotic Susceptibility Testing**

Antibiotic susceptibility testing was performed on bacterial isolates using the Kirby-Bauer disc diffusion method. Inhibition zone diameters were measured and interpreted as sensitive, intermediate, or resistant according to CLSI guidelines.

The bacteria displayed in vitro susceptibility to the aminoglycoside antibiotics amikacin and gentamicin. Mean inhibition zone diameters were 19.6 mm for amikacin and 21.6 mm for gentamicin, indicating susceptibility.

However, the organisms tested were resistant in vitro to a variety of other antibiotic classes. No inhibition zones were observed around discs containing the antibiotics clindamycin, vancomycin, methicillin, or piperacillin. The lack of zones indicates complete resistance.

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**Figure 2. Antibacterial susceptibility of Enterobacter aerogenes isolates. Zone of inhibition diameters for E. aerogenes isolates tested against a panel of antibiotics on Mueller-Hinton agar.**

**Discussion:**

Soft cheeses are an important food worldwide. In Iraq, traditional un ripened soft cheeses made from unpasteurized milk are generally consumed within 3-4 weeks of production (16). These cheeses are produced in rural areas and remote villages. The cheese environment impacts microbial growth based on factors like temperature, pH, and water activity controlled by cheesemaking (22,23,24).

Enterobacteriaceae are of technological interest in cheesemaking because some ferment lactose, producing gas and alterations at high levels in curd/cheese (20). Raw milk is thus a primary contamination source (25). Additionally, poor worker hygiene, packaging, transportation, marketing, and non-potable water use for cleaning can secondarily contaminate soft cheeses (29). A previous Iraqi study revealed a 13.1% prevalence of Klebsiella, Enterobacter aerogenes and Proteus vulgaris contamination in dairy products (20), indicating raw milk is unsafe for consumption (24) unless pasteurized, which eliminates Enterobacteriaceae (29). Thus, pasteurization should be required.Similarly, 66.7% of farm milk cheeses showed Enterobacteriaceae contamination, including 46.7% with *Citrobacter freundii,* 20% Escherichia coli, 6.6% *Enterobacter aerogenes*, 3% Pseudomonas aeruginosa, 16.7% Proteus mirabilis, and 10% Salmonella spp. (26). Higher coliforms were also reported in soft cheeses versus hard types (29,30,31). Indiscriminate antibiotic use in dairy animals for disease/growth promotion (27,28,29) may disseminate antibiotic resistance into foods (30). Indeed, examined Enterobacter aerogenes isolates exhibited multidrug resistance, consistent with intrinsic and acquired resistance reported for Enterobacter spp. (36,37). Increasing resistance complicates treatment and raises mortality, hospitalization rates, and costs (39,40,41).

**Conclusion:**

This study found a high rate of contamination with *Enterobacter aerogenes* in raw milk cheeses. indicating widespread contamination and poor hygiene practices during manufacturing. Additionally, the Enterobacter isolates displayed multidrug resistance patterns. Most Enterobacter aerogenes isolates were resistant to several clinically important antibiotics like methicillin, clindamycin, vancomycin, and piperacillin. However, they remained largely susceptible to gentamycin and amikacin.

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