

## 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<sub>2</sub>S, 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

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

## 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

of inhibition surrounding each disk were measured (Figure 2) to determine susceptibility or resistance to the antibiotics based on standard interpretive criteria.

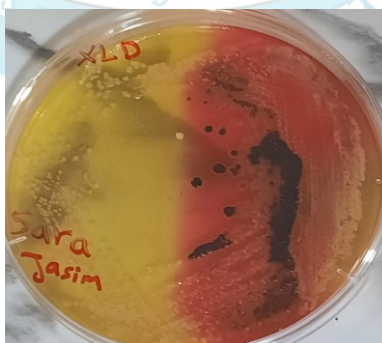
### Statistical analysis:

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].

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.**



**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.



**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 world-wide. 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 cheese-making (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.

## References:

- [1] FAO, 1990. The technology of traditional milk contaminates from middle belt and southwestern products in developing countries. FAO, United Nigeria. Afr. J. Microbiol. Res., 2: 332-339. nation. Rome, 43: 163.
- [2] Pazakova, J., M. Pipova, P. Turek and J. Nagy, 2001 Changes in some microbiological and chemical parameters during the ripening of sheep cheese at different temperature. Czech. J. Food Sci., 19: 121-124
- [3] Montel, M.-C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D.A., Desmasures, N., Berthier, F., 2014. Traditional cheeses: Rich and diverse microbiota with associated benefits. International Journal of Food Microbiology 177, 136–154. doi:10.1016/j.ijfoodmicro.2014.02.019
- [4] Morales, P., Fernández-García, E., Nuñez, M., 2003. Caseinolysis in cheese by Enterobacteriaceae strains of dairy origin. Letters in Applied Microbiology 37, 410–414.
- [5] Guggisberg, D., Schuetz, P., Winkler, H., Amrein, R., Jakob, E., Fröhlich-Wyder, M.T., Wechsler, D., 2015. Mechanism and control of the eye formation in cheese. International Dairy Journal 47, 118–127. doi:10.1016/j.idairyj.2015.03.001
- [6] Sheehan J.J., 2007. 57 What causes the development of gas during ripening? in: McSweeney P.L.H. (Ed), Cheese problems solved, Woodhead Publishing Limited, Abington, pp. 131–132.
- [7] Cohen I, Powderly WG and Opal SM (2017): Infectious diseases, 4 thEd. El Sevier Ltd.
- [8] Martin RM and Bachman MA (2018): Colonization, infection, and the Accessory Genome of Klebsiella pneumoniae. J. Front. cell. Infec. Microbial., 8(4).
- [9] Abebe E, Gugsu G and Ahmed M (2020): Review on Major Foodborne Zoonotic Bacterial Pathogens. J.Trop.Med. vol. 2020, <https://doi.org/10.1155/2020/4674235>.
- [10] Davin-Regli, A.; Pagès, J.M. Enterobacter aerogenes and Enterobacter cloacae; versatile bacterial pathogens confronting antibiotic treatment. Front. Microbiol. 2015, 6, 392.
- [11] Annavajhala, M.K.; Gomez-Simmonds, A.; Uhlemann, A.C. Multidrug-Resistant Enterobacter cloacae Complex Emerging as a Global, Diversifying Threat. Front. Microbiol. 2019, 10, 44. [CrossRef] [PubMed]
- [12] Rossolini, G.M.; Arena, F.; Pecile, P.; Pollini, S. Update on the antibiotic resistance crisis. Curr. Opin. Pharmacol. 2014, 18, 56–60.
- [13] Duncan, S. E.; B. R. Yaun; S. S. Sumner and Bruhn, J. (2004), 'Chapter 9, Microbiological Methods for Dairy Products', in J. Bruhn (ed.), Standard Methods for the Examination of Dairy Products (American Public Health Association), 249–68
- [14] ISO 21528, 2004. General guidance for the detection of Enterobacteriaceae with pre-enrichment, pp: 43-45.
- [15] Georgescu et al., (2014). Macfaddin, J.F. Biochemical tests for Identifications of medical bacteria. 3rd ed. Lippincott Williams and Wilkins. 1979. Philadelphia, USA
- [16] García, L. A. and Díaz, M. (2011), '2.72 - Cleaning in Place', in Moo-Young Editor-in-Chief Murray (ed.), Comprehensive Biotechnology (Second Edition) (Burlington: Academic Press), 983-97.
- [17] Al-Ezzy AIA, Jameel GH, Minnat TR. Isolation of Malassezia Furfur and Evaluation of Ivermectin and Calvatia Craniiformis as A Novel Antifungal Agents for Pityriasis Versicolor with Special Refer to Risk Factors in Iraqi Patients. International Journal of



- Current Pharmaceutical Review and Research. 2017;8(4):311-9.
- [18] Hassan Al-Khalidi AA, Hameed MS, Ali Al-Ezzy AI, Ibrahim SN. Effects Of *Saccharomyces Cerevisiae* As Probiotic On Blood Indices, Humoral Immunity And Performance Of Isa Brown Laying Hens In Diyala Province, Iraq. *Biochemical & Cellular Archives*. 2020;20(1).
- [19] AL-Ezzy AIA, Kadhim AT. Comprehensive Evaluation For The Life Style And Zoonotic Risk Factors Associated With *Cryptosporidium Parvum* Infection In Children Under Five Years. *Diyala Journal For Veterinary Sciences*. 2021;1(2):77-92.
- [20] AL-Ezzy AIA. Chromotrope Gram Hot And Giemsa Staining Techniques As Alternatives For Ziehl-Neelsen Hot Stains For Detection Of *C. Parvum* Infection In Children And Calves. *Diyala Journal for Veterinary Sciences*. 2021;1(3):100-11.
- [21] Hameed MS, Al-Ezzy AIA, Jalil WI, Al Khalidi AAH. Impact of Stress Factors on Physiological Level of Interleukin 10 in Healthy Calves in Diyala Province-Iraq. *International Journal of Pharmaceutical Research* (09752366). 2020;12(2).
- [22] Abdul-Ratha HA, Mohammad AJ. The occurrence of urinary tract infection caused bacteria in human and animals in Baghdad city and it's susceptibility to antibiotics. *Journal of Genetic and Environmental Resources Conservation*. 2013;1(3):204-8.
- [23] Swai, E. S. and Schoonman, L. (2011), 'Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania', *Asian Pacific Journal of Tropical Biomedicine*, 1 (3), 217-22
- [24] Hill, B.; B. Smythe; D. Lindsay and Shepherd, J. (2012), 'Microbiology of raw milk in New Zealand', *International Journal of Food Microbiology*, 157 (2), 305-08.
- [25] Uyttendaele, M.; L-A. Jaykus; P. Amoah; A. Chiodini; D. Cunliffe; L. Jaxsens; K. Holvoet; L. Coveney, H.M., G.F. Fitzgerald and C. Daly, 1994. A study of the microbiology status of fresh farm house cheeses with emphasis on selected pathogenic and spoilage microorganisms. *J. Applied Bacteriol.*, 77: 621-630.
- [26] Sobeih, A. M., AL-Hawary, I., Khalifa, E., & Ebied, N. (2020). Prevalence of Enterobacteriaceae in raw milk and some dairy products. *Kafrelsheikh Veterinary Medical Journal*, 18(2), 9-13.
- [27] Nyein; Mar-Mar, Khine TT and Thin KK (2002): Bacteriological aspects of milk and milk products in Yangon
- [28] during 2000, Myanmar. *Health. Sci. Res. J.*, 14 (1/3): 35-41
- [29] Branciaro R, Goga BTC, Rea S and Avellini P (2004): Evaluation of hygienic characteristics of Italian "fossa" cheese, food-safety assurance and veterinary-public-health volume 2: Safety assurance during Food Processing, 333
- [30] El-Mokadem, E. A., El-Leboudy, A. A., & Amer, A. A. (2020). Occurrence of Enterobacteriaceae in Dairy Farm Milk. *Alexandria Journal for Veterinary Sciences*, 64(2).
- [31] Coveney, H.M., G.F. Fitzgerald and C. Daly, 1994. A study of the microbiology status of fresh farm house cheeses with emphasis on selected pathogenic and spoilage microorganisms. *J. Applied Bacteriol.*, 77: 621-630.
- [32] Massa, S., Gardini, F., Sinigaglia, M., & Guerzoni, M. E. (1992). *Klebsiella pneumoniae* as a spoilage organism in mozzarella cheese. *Journal of dairy science*, 75(6), 1411-1414.

- [33] Araújo, V. S., Pagliares, V. A., Queiroz, M. L. P., & Freitas-Almeida, A. C. (2002). Occurrence of Staphylococcus and enteropathogens in soft cheese commercialized in the city of Rio de Janeiro, Brazil. *Journal of Applied Microbiology*, 92(6), 1172-1177.
- [34] Fleming, L. R., Bolzan, D. N., and Nascimento, J. S. (2010). Antimicrobial substances produced by coliform strains active against foodborne pathogens. *Foodborne Pathog. Dis.* 7, 243–247. doi: 10.1089/fpd.2009.0333
- [35] Bosco, K. J., Kaddu-Mulindwa, D. H., and Asiimwe, B. B. (2012). Antimicrobial drug resistance and plasmid profiles of Salmonella isolates from humans and foods of animal origin in Uganda. *Adv. Infect. Dis.* 2, 151–155. doi: 10.4236/aid.2012.24025
- [36] Murphy, C. P., Fajt, V. R., Scott, H. M., Foster, M. J., Wickwire, P., and McEwen, S. A. (2016). Scoping review to identify potential non-antimicrobial interventions to mitigate antimicrobial resistance in commensal enteric bacteria in North American cattle production systems. *Epidemiol. Infect.* 144, 1–18. doi: 10.1017/S0950268815000722
- [37] Rolain, J. (2013). Food and human gut as reservoirs of transferable antibiotic resistance encoding genes. *Front. Microbiol.* 4:173. doi: 10.3389/fmicb.2013.00173
- [38] Davin-Regli, A.; Lavigne, J.P.; Pagès, J.M. *Enterobacter* spp.: Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance. *Clin. Microbiol. Rev.* 2019, 32, e00002-19.
- [39] Annavajhala, M.K.; Gomez-Simmonds, A.; Uhlemann, A.C. Multidrug-Resistant *Enterobacter cloacae* Complex Emerging as a Global, Diversifying Threat. *Front. Microbiol.* 2019, 10, 44.
- [40] Al-Tawfiq, J.A.; Antony, A.; Abed, M.S. Antimicrobial resistance rates of *Enterobacter* spp.: A seven-year surveillance study. *Med.Princ. Pract.* 2009, 18, 100–104.
- [41] Wang, S.; Xiao, S.Z.; Gu, F.F.; Tang, J.; Guo, X.K.; Ni, Y.X.; Qu, J.M.; Han, L.Z. Antimicrobial susceptibility and molecular epidemiology of clinical *Enterobacter cloacae* bloodstream isolates in Shanghai, China. *PLoS ONE* 2017, 12, e0189713.
- [42] Jiménez-Guerra, G.; Borrego-Jiménez, J.; Gutiérrez-Soto, B.; Expósito-Ruiz, M.; Navarro-Marí, J.M.; Gutiérrez-Fernández, J. Susceptibility evolution to antibiotics of *Enterobacter cloacae*, *Morganella morganii*, *Klebsiella aerogenes* and *Citrobacter freundii* involved in urinary tract infections: An 11-year epidemiological surveillance study. *Enferm. Infecc. Microbiol. Clin. (Engl. Ed.)* 2020, 38, 166–169