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Detection of Klebsiella Pneumoniae in Milk and Milk Products of Karbala Province and Susceptibility of Antibiotics

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***Abstract*— The overall prevalence of *Klebsiella pneumoniae*, a critical zoonotic pathogen, within global dairy herds, with a potential risk of cross-species transmission between humans and cattle as a food-borne infection, remains to be clarified. This study aimed to determine the prevalence of *Klebsiella pneumoniae (K. pneumoniae)* in raw milk, cheese, butter, yoghurt, and cream collected from various local markets in Karbala, Iraq. A total of 375 random samples were examined, including products from City Center, Al-Hur, Al-Hindyia, Al-Hassainya, and Ain Al-Tamer. Samples were cultivated on MacConkey agar and CHROMagar Enterobacteria selective medium to isolate and identify *K. pneumoniae*. Confirmation was achieved through biochemical tests and the Vitek system. The results revealed a high contamination rate of *Klebsiella pneumoniae* in cheese (45.33%), followed closely by raw milk (42.67 %), and yoghurt exhibited the lowest contamination rate at (8 %). A test for antibiotic sensitivity was conducted to a number of isolates by using disc diffusion method against six antimicrobial agents (Penicillin G, Amikacin, amoxicillin , Ciprofloxacin , Tetracycline , and ampicillin/cloxacillin) , using Mueller Hinton agar (MHA) plates. *Klebsiella pneumoniae* isolates exhibited multiple resistance against 4 or more of different antibiotics. These findings highlight significant microbiological safety concerns regarding dairy products in the region, indicating an urgent need for improved hygiene practices in dairy processing and handling to safeguard public health.**

***Keywords* — *Klebsiella pneumoniae*, Milk, Food-borne infections, Antimicrobial resistance.**

INTRODUCTION

THE dairy industry is one of the almost all of advanced and profitable food industries. based on the Food and Agriculture Organization (FAO), more than 6 billion people consume milk globally (1).Statistics indicate that the majority of milk produced is sourced from cows., accounting for approximately 81%, while milk production from goats, sheep, and camels combined only exceeds 4% (2).

Milk occupies a prominent position among other foods, as it is considered the ideal source of fundamental nutrients for humans from infancy to senility (3). This is due to its excellent sensory properties and all the nutrients necessary for rapid growth, as well as its potential ability to prevent or reduce the risk of many illness resulting from malnutrition. However, because of its distinctive properties and composition (such as its richness in nutrients, high moisture content, and pH value), milk can function as not only as a unique growth medium for a diverse range of microbial flora like lactic acid bacteria (LAB) but it can also be a potential transmission source for some pathogens, such as *Propionibacterium*, *Staphylococcus*, *Streptococcus*, *Enterobacteriaceae* like *Klebsiella* , and others (4,5).

Klebsiella pneumoniae is a Gram-negative, facultative anaerobe, non-motile bacterium that typically produces a prominent polysaccharide capsule, contributing to its virulence. As a member of the *Enterobacteriaceae* family, it is frequently associated with opportunistic infections in humans and can be transmitted through the consumption of contaminated animal-derived food products such as beef, poultry, fish, and dairy. When cultivated on selective media such as MacConkey agar, *K. pneumoniae* exhibits a distinctive mucoid colony morphology due to its capsule, which plays a protective role against host immune defenses. Additionally, this bacterium is capable of fermenting lactose, a characteristic feature aiding in its laboratory identification (6,7).

In recent years, there has been a significant increase in the prevalence of multidrug-resistant (MDR) strains of *Klebsiella pneumoniae*, largely attributed to the overuse of traditional antimicrobial agents. These bacteria have evolved several mechanisms to resist various antibiotics, the most important of which are efflux pumps and the ability to form biofilms. Efflux pumps are protein structures that expel various toxic substances from the cell, while biofilms enable *K. pneumoniae* to evade from the host immune response and the effects of antibiotics (8).

Klebsiella pneumoniae is not commonly considered a foodborne germ, so the majority of foodborne strains studies focus on more common pathogens like *Escherichia coli* (*E. coli*), *Salmonella*, and *Shigella*. Consequently, there is still a lack of comprehensive data regarding the prevalence of *K. pneumoniae* in retail food and the traits of these strains, including their virulence properties and antibiotic resistance, even though such information is crucial for evaluating possible public health threats (9,10) .

Aims of the study

This study aims :

1. to determine the prevalence of *K. pneumoniae* in raw milk, cheese, butter , yoghurt and cream in different areas of Karbala.
2. to isolation and identification of *K.pneumoniae* .
3. to detection of the suitable antibiotics against *K.pneumoniae*

Materials and Methods

The study was conducted to detect *K. pneumoniae* in raw milk and dairy products in Karbala markets, during the period from October 2024 to April 2025. A total of 375 random samples of milk, cheese, butter, yogurt, and cream were collected from five areas (City center, Al-Hussynia, Al-Hur, Ain Altamor, Al-Hindyia), with 75 samples per area. The samples were transported in sterile vials inside iceboxes at 0–4°C directly to the microbiology laboratory for microbial analysis.

Isolation and bacterial identification were carried out using different culture media (MacConkey), CHROMagar Enterobacteria agar and Mueller Hinton agar (Himedia/India).
All samples were plated on MacConkey agar and incubated for 24 hours at 37°C , followed by culture of suspected samples on CHROMagar as an initial confirmation step. Biochemical tests were also performed, which included catalase, oxidase, indole, urease, citrate, and Triple sugar iron test. Pure colonies were then taken and streaked onto Mueller-Hinton agar (MHA) plates for an antibiotic susceptibility test. Pure cultures were preserved at 4°C for subsequent analysis. Subsequently, four suspicious isolates were selected for further analysis using the Vitek system.

K. pneumoniae isolates were then evaluated for sensitivity to six different antibiotics using the disc-diffusion method (11,12). Antibiotics included Penicillin G (10 µg), Ciprofloxacin (10 µg), Amikacin (10 µg), Tetracycline (10 µg) , amoxicillin (10 U), and ampicillin/cloxacillin (25/5 mcg).

Statistical analysis

Statistical analysis was done by using spss in level 0.05.

Result & discussion

All of 375 samples were cultured on MacConkey agar and the results of bacterial isolation showed colony development 24 h after culture for all types of samples. Growth features of *Klebsiella pneumoniae* on MacConkey and CHROMagar showed in figure(1 A). On MacConkey agar; the glistening pink, lactose fermenting mucoid dome shaped colonies while on CHROMagar, a metallic blue coloration, along with large and rounded colonies , were observed, were counted as Klebsiella pneumonia which consistents with the findings reported by (13,14), were mention the colony of bacteria were appered as lactose fermenting pink mucoid colony on MacConkey agar in figure (1 B). MacConkey agar contains lactose and neutral red dye and *K. pneumoniae* is capable of fermenting lactose, which lowers the pH and turns the dye pink. While the mucoid appearance results from the production of a dense mucous membrane (capsule), a characteristic feature of *K. pneumoniae* (15). While the CHROMagar is a culture medium containing chromogenic substrates that react with specific enzymes produced by bacteria. *Klebsiella pneumoniae* produces enzymes (such as β-glucosidase) that react with these substrates, producing a distinctive metallic blue color (16).

Figure 1. (A) Metallic blue rounded and large colonies of Klebsiella pneumonia on chrome agar
(B) Pink , mucoid colonies on MacConkey agar.

Biochemical identification of bacteria :

Biochemical tests were performed on all isolates, and the results are presented in table 1. The isolates showed a positive result for the catalase test, decomposing the hydrogen peroxide reagent into oxygen and water. The isolates were also tested for oxidase and the results were negative as since no color change occurred to crimson red, indicating that the isolates do not contain the enzyme cytochrome oxidase, which acts as a hydrogen acceptor. While the results of Urease test was positive as the isolates have urease enzyme, which breaks down urea into ammonia and carbon dioxide, raising the pH and turning the medium pink. Color changes in both surface and bottom of TSI slant agar to pinkish, respectively, that because *K. pneumoniae* ferments glucose and lactose/sucrose, causing the medium to turn yellow at the bottom and surface (A/A). It also produces gases that appear as bubbles or cracks, but it does not produce hydrogen sulfide (H_2S). The Citrate result was positive because the bacteria can use citrate as the sole carbon source, which leads to their growth and the color of the medium changes to blue. Lastly indole test was negative indicating that the bacteria lack the enzyme tryptophanase and therefore cannot convert tryptophan to indole (17,18,19,20).

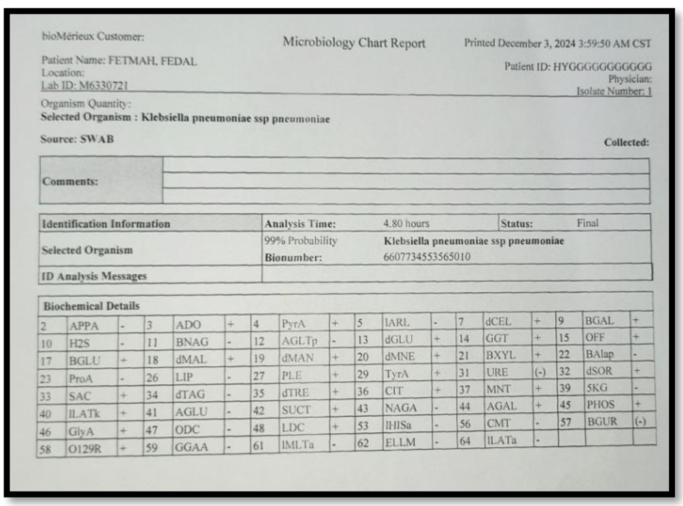
Table1. Identification of Klebsiella pneumonia by biochemical tests.

Test	Result
Oxidase	-ve
Indole	-ve
Catalase	+ve
Urease	+ve
Citrate	+ve
TSI	+ve

Vitek 2 System

To confirm the identity of bacterial isolates, the isolates of *K. pneumoniae* tested by Vitek 2 system, utilizing the GN ID card for Gram-negative bacteria. This system performs 64 tests within a period of 5 to 8 hours, a suitable time period that allows for accurate identification of isolates before any potential mutations occur.

The diagnostic results using the Vitek 2 System showed a matching rate for the isolates under study of 99% and 98% as shown in the figure (2).



bioMérieux Customer: Microbiology Chart Report Printed December 3, 2024 3:59:50 AM CST

Patient Name: FETMAH, FEDAL
Location:
Lab ID: M6330721

Patient ID: HYGGGGGGGGGGG
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: SWAB

Collected:

Comments:

Identification Information	**Analysis Time:** 4.80 hours	**Status:** Final
Selected Organism	99% Probability **Klebsiella pneumoniae ssp pneumoniae** **Bionumber:** 6607734553565010	
ID Analysis Messages		

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	-			

Figure 2 . Vitek 2 System results.

Prevalence of *Klebsiella pneumoniae*

The results revealed a high contamination rate of *K. pneumoniae* in cheese (45.33%), followed closely by raw milk (42.67%) , cream (25.33%) , butter (16%), and yoghurt exhibited the lowest contamination rate at (8%).

The percentage of contamination in raw milk was slightly similar to that obtained by a previous study conducted in Iran on healthy cows, where the percentage in raw milk was 40% (21).

Although contamination rates varied, a study conducted in Dohuk, Iraq, reported a particularly high level, with milk contamination reaching 57.5% (22), which is close to what was found in the current study.

The percentage of cheese contamination in the current study is close to the results of the study conducted in Egypt on Damietta cheese, which is similar in its manufacture to the local cheese in Iraq, except for the high salt concentration in it, which is perhaps what reduced the percentage of bacteria to 39%. (23).

Although there are no Iraqi previous studies directly documenting the presence of *Klebsiella pneumoniae* in local cow's cheese to compare results for the same type of cheese, there is a study in Baghdad (24), that showed results of soft cheese that 90% of samples were contaminated with coliforms. The presence of *Klebsiella pneumoniae* was not specifically mentioned, but it is classified within the coliform family, which opens the way for its presence.

The lowest contamination rate was in yogurt (8%), which is related to was found by (25), where 3 isolates from *K.pneumoniae* were isolated from 30 yogurt samples. This is likely due to the fact that the acidity of yogurt, due to the presence of lactic acid bacteria)LAB), may be an inhibitory factor for the growth of these bacteria. This was also indicated by (12).

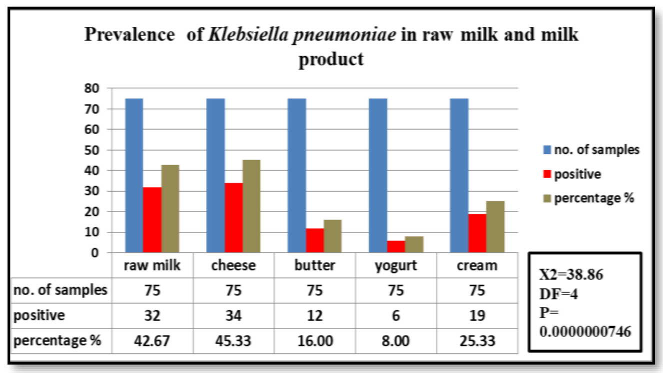


Figure 3 . Prevalence of *Klebsiella pneumoniae* in milk and milk product

Antibiotic susceptibility of *K. pneumoniae* isolates:

The results of the antibiotic susceptibility test for *K. pneumoniae* revealed that *K. pneumoniae* isolates exhibited varying degrees of sensitivity towards each of Ciprofloxacin and Amikacin, while all isolates were resistant to Amoxicillin. In addition, only one isolate was sensitive to Penicillin G, while other antibiotics, *K. pneumoniae* showed resistance against them to varying degrees, as shown in Table 2.

Table 2. The resistance and sensitivity percentage of *K. pneumonia* isolates.

Antibiotics	Concentration	Resistant (%)	Susceptible (%)
Penicillin G	(10 µg)	96.6	3.4
Amikacin	(10 µg)	40	60
Tetracycline	(10 µg)	56.6	43.4
Amoxicillin	(10 U)	100	0
Ciprofloxacin	(10 µg)	16.6	83.6
Ampicillin/cloxacillin	(25/5 mcg)	93.3	6.7

Ciprofloxacin showed the highest sensitivity rate (83.6%) among the antibiotics used, indicating that it remains a good treatment option against *K. pneumoniae* isolates. This finding is supported by a previous study by (12), were showed that fluoroquinolones such as ciprofloxacin remain effective on *K. pneumoniae* isolates in some areas in Libya.

Furthermore, the results revealed that (60%)of the isolates were sensitive to amikacin, indicating its partial efficacy. Amikacin is considered one of the most effective aminoglycosides against *K. pneumoniae,* a finding confirmed by (26,12), who found that amikacin remains effective against several Klebsiella isolates resistant to other antibiotics.

The results also showed (56.6%) resistance rate to tetracycline. Studies indicate that *K. pneumoniae* resistance to tetracycline is increasing, reducing the effectiveness of this antibiotic. For example, a study in Romania between 2019 and 2021 (27), showed an increase in *K. pneumoniae* resistance to antibiotics, including tetracycline.

On the other hand, very high resistance to β-lactam antibiotics, such as penicillin G (96.6%) and ampicillin/cloxacillin (93.3%), was observed, in addition to 100% resistance to amoxicillin. These results are expected given that *Klebsiella pneumoniae* possesses effective resistance mechanisms, such as production of the enzyme β-lactamase, which cleaves the β-lactam ring in the antibiotic structure, rendering it ineffective. These mechanisms have been documented in several previous studies, including (12, 28, 29,30) , which have been confirmed the innate resistance to unprotected penicillins in *Klebsiella pneumoniae*.

Figure 4 . diffusion test of *K. pneumoniae* isolates

CONCLUSION

The results of this study indicate a significant prevalence of *Klebsiella pneumoniae* in locally produced unpasteurized dairy products, reflecting a disruption in the production and distribution chains and posing a biohazard with health and epidemiological dimensions. The results also revealed a pattern of multidrug resistance, reinforcing concerns about the uncontrolled use of antibiotics in agricultural and veterinary settings.

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