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PREVALENCE OF ENTEROBACTER AEROGENES IN RAW MILK AND SOME MILK PRODUCTS

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ABSTRACT

Introduction: *Enterobacter aerogenes* is known as *Aerobacter aerogenes* and belongs to the family *Enterobacteriace*. It normally habitats the intestine of the animals so it is widely found in soil, sewage and water. It causes a wide variety of health problems for humans and animals. **Objective:** The following study was to detect *Enterobacter aerogenes* in milk, yoghurt and ice cream to assess their hygienic quality. **Methods:** The number of samples was 300 as follows: raw milk samples, 100 yoghurt samples and 100 ice cream samples. Samples were collected in Assiut Governorate and examined for the presence of *E. aerogenes.* **Results:** *E. aerogenes* were found in 13% of raw milk samples, and 5% of examined yoghurt samples, while detected in 6% of examined ice cream samples. **Conclusion:** *E. aerogenes* in milk is an index of direct fecal contamination of milk and milk products which is considered a public health hazard. Consumers and emphasizes must improve their hygienic standards to avoid *E. aerogenes*.

Keywords: Microbiology, Enterobacter aerogenes, Raw milk, Milk products.

INTRODUCTION

E. aerogenes known as Aerobacter aerogenes belong to the family Enterobacteriace. About its morphological characters, E. aerogenes is a Gram-negative, facultatively anaerobic, rod-shaped bacillus, and it is motile by peritrichous flagella (Anne Davin *et al.,* 2019). It normally habitats the intestines of large animals so it is widely spread in water, soil and sewage. It causes nosocomial infections including urinary tract infections, pneumonia, wound and burn infections, meningitis, eye infections, and lower respiratory tract infections. Outbreaks of E. aerogenes result in a very high mortality rate (Kulali *et al.,* 2019). E. aerogenes can also contaminate raw milk and milk products causing food-borne infections (Gao S *et al.,* 2014).

MATERIALS AND METHODS

This study was performed in the Microbiology and Immunology department, Faculty of Medicine, Assuit University from April 2021 to May 2023.

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1- Collection of samples:

Samples were collected through one year in the period between April 2021 to May 2022. The number of samples was 300 samples as follows: 100 raw milk samples, 100 yoghurt samples and 100 ice cream samples. Samples were collected in Assuit Governate and examined for the presence of *E. aerogenes*. Samples were collected from farmer's houses and supermarkets in Assuit Governate. The samples were preserved in clean and sterile containers and transferred to the laboratory within 1-2 hours to be examined.

2- Preparation of samples:

A) Milk samples:

Ten ml of milk samples were centrifuged at 5000 rpm for 10 minutes, and the supernatant was discarded. A loopful from the processed samples was incubated onto the MacConkey's agar for 24 hrs at 37˚C (S.E.Duncan *et al.,* 2012).

B) Yogurt samples:

The samples of yogurt were shaken vigorously to suspend the microbial content. A loopful from these samples was separately inoculated on MacConkey's agar and then incubated at 37˚C for 24 hrs (Salisu M.D *et al.,* 2016)

C) Ice cream Samples:

Ice cream samples were kept in a 45°C water bath, after thawing, 10 ml of ice cream was pipetted out and transferred into a glass bottle. One ml of previously prepared was pipetted out aseptically and transferred into a sterile test tube. This test tube contained 9 ml of distilled water to give a 1:10 dilution. Then the processed samples were inoculated on MacConkey's agar for 24 hrs at 37˚C (Abo El-Makarem *et al.,* 2017).

3- Isolation and identification of *E. aerogenes*:

a- Colony morphology:

1- On MacConkey's Agar:

The presumptive colonies of *E. aerogenes* appeared on MacConkey's agar as pink, round lactose fermenting colonies.

2- On Brilliance UTI Agar (Chromogenic Medium):

The pink colonies on MacConkey's agar were subcultured onto Brilliance UTI ager, Suspected isolates produce blue-colored colonies.

b- Microscopic appearance of Gram's-stained film:

The detection of Gram-negative, pink, rod-shaped bacilli was presumptive for *E. aerogenes* (Kevin Le *et al.*, 2013).

C. Biochemical reactions of isolates *E. aerogenes:*

1- Oxidase test: (Baron *et al.*, 1994).

Bacteriological differential discs for oxidase testing were used. It was performed by adding an isolated colony on an oxidase disc. The reaction was observed within seconds as a positive reaction detected by purple-blue color change while a negative reaction which is presumptive for *E. aerogenes* remained colorless.

2- Catalase activity test: (Baron *et al.*, 1994).

Pure growth was transferred with a sterile loop onto the surface of the glass slide then a drop of 3% H2O2 was placed onto the colony on the glass slide. The evolution of bubbles of gas indicates a positive result which is presumptive for *E. aerogenes.*

3- Citrate utilization test: (Winn *et al, 2005)*

Simon's Citrate agar tubes were tightly streaked with a pure culture of the organism. The tubes were inoculated for 24 hrs at 37˚C. blue coloration of the medium indicated a positive result which is presumptive for *E. aerogenes*.

4- Urease test (Benita Brink *et al., 2010)*

We used an inoculum from a 20-24 hrs culture to inoculate the broth. The tube was shaken and then incubated at 35˚C. Color change of the broth was observed at 24 hrs.

Urease production was indicated by a bright pink color throughout the broth while the negative result appeared yellow color which is presumptive for *E. aerogenes***.**

5- Motility test (Sagar Aryal *et al.*, 2022).
Bacterial motility was seen by a diffuse zone of bacterial growth which extended out from the line of inoculation. A needle touched a colony of 18-24 hrs then was grown on agar medium and stabbed it into the center of a semisolid agar. Incubate at 37˚C and examine after 24 hrs. A diffuse zone of growth is grown out from the line of inoculation causing cloudiness indicating positive motility which is presumptive for *E. aerogenes***.**

6- Ornithine decarboxylase (MacFaddin J.F *et al., 2000)*
Pure culture was inoculated for 18-24 hrs in an Ornithine decarboxylase broth tube and overlaid the tubes with 2 ml mineral oil. The inoculated tubes were inoculated at 37˚C for 24 hrs. Changing the color of the indicator in Ornithine decarboxylase broth from yellow to purple or violet is presumptive for *E. aerogenes*.

RESULTS

1- E. aerogenes was detected as follows:
a) Milk samples:
E. aerogenes was isolated from 13 milk samples. Table (1).

b) Yogurt samples:
The total number of positive samples for *E. aerogenes* in yogurt samples was 5 samples. Table (1).

c) Ice Cream samples:
The total number of positive samples for *E. aerogenes* in Ice cream samples was 6 samples. Table (1).

Table 1: *E. aerogenes* in the examined samples.

Types of samples	No. of examined samples	Positive samples for *E. aerogenes*	
		N.	%
Milk	100	13	13
Yogurt	100	5	5
Ice cream	100	6	6
Total isolates of *E. aerogenes*	300	24	8

2- Identification of *E. aerogenes*:
a- Culture media:

On MacConkey's agar:

E. aerogenes appeared as pink, round, lactose fermenting colonies as shown in Photo (1).

On Brilliance UTI ager:
E. aerogenes appeared as blue colored colonies as shown in Photo (2).

b- Microscopic appearance of Gram's-stained film: *E. aerogenes* was Gram-negative, pink, rod-shaped bacilli as shown in Photo (3).

Photo (1) MacConkey's agar inoculated with *E. aerogenes* showing pink, round, lactose fermenting colonies.

Photo (2): Brilliance UTI inoculated with *E. aerogenes* showing blue-colored colonies.

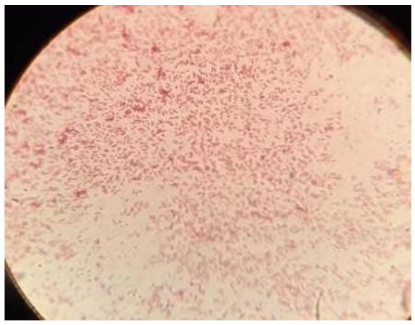

Photo (3): *E. aerogenes* Gram's stain showing Gram-negative rod-shaped bacilli.

c- Biochemical tests for isolated *E. aerogenes:*

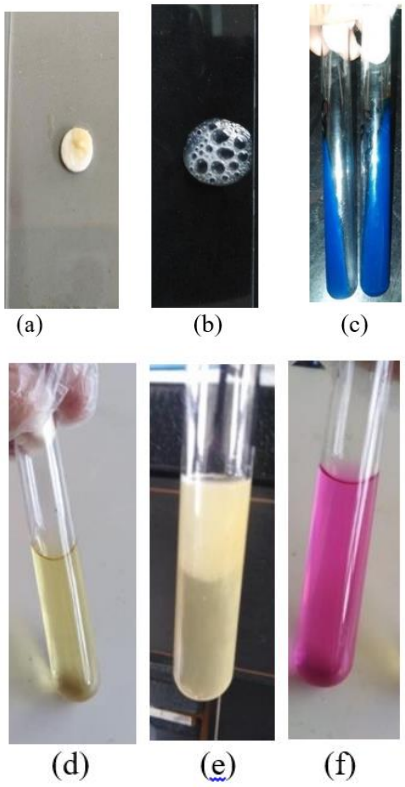

Photo (4) Biochemical tests used for identification of isolated *E. aerogenes:*

a. Oxidase test: *E. aerogenes* is oxidase negative indicated by no changing of disc color.
b. Catalase test: *E. aerogenes* is catalase positive indicated by the evolution of gas bubbles.
c. Citrate utilization: *E. aerogenes* is positive for citrate utilization indicated by the blue coloration of the medium.
d. Urease test: *E. aerogenes* is urease negative indicated by a yellow color.
e. Motility test: *E. aerogenes* is motile indicated by the appearance of the zone of growth grown out from the line of inoculation in the tubes causing cloudiness.
f. Ornithine decarboxylase test: *E. aerogenes* Positive for Ornithine decarboxylase test as the color appeared pink color.

DISCUSSION

E. aerogenes is a Gram-negative, facultative anaerobic, rod-shaped bacillus and motile by peritrichous flagella, it belongs to the family *Enterobacteriace*. *E. aerogenes* is a nosocomial bacterium that causes opportunistic infections. It is found in the human gastrointestinal tract and does not cause disease in healthy people but it infects immuno-compromised patients, old people, neonates and individuals that received antibiotic therapy. (Bilevicius *et al., 2001* Song *et al., 2010).*

E. aerogenes is a notorious hospital-acquired infection, some infections, result from *E. aerogenes* result from venous catheter insertions. Healthcare workers considered subjects that could transmit *E. aerogenes* infection. Bacteremia due to *E. aerogenes* was reported in cases associated with catheters and may cause sepsis and septic shock. (Bilevicius *et al.*, 2001). *E. aerogenes* causes respiratory tract infections and is implicated in urinary tract infections (Piagnerelli *et al.*, 2002). Also, associated

with meningitis (Désinor *et al.,* 2004), especially in people who had neurosurgery and medical devices in surgery. (Fooster *et al.,* 2005; Hamid *et al.,* 2007). The presence of *E. aerogenes* in milk indicates unfit for human consumption.

(Nyein *et al., 2002). E. aerogenes* are killed by pasteurization, there for it is recommended to pasteurized milk used for human consumption (Branciari *et al.,* 2004). A high count of *E. aerogenes* in ice cream and yogurt samples may attributed to a lack of attention to personal hygiene, so sanitation routines during production and handling are required. Ingredients must be of high quality especially when it added after heat treatment to ensure that it is free from pathogens. Attention should be given to the equipment that is in contact with yogurt and ice cream in order to be clean and dry (Omnia M. El-Tayeb *et al.,* 2023). The presence of *E. aerogenes* indicates post-pasteurization contamination, unsanitary manufacturing methods, and unhygienic pasteurization. *E. aerogenes was* reported to be mastitic in cows (Jain *et al.,* 1971). Healthy cows recovered within 14 days of infection but neutropenic cows had necrosis of the mammary gland (Wenz *et al.,* 2001). In the present study, the prevalence of *E. aerogenes* in raw milk was 13% which was similar to the results obtained by (Vivian Hoffmann *et al.,* 2022) and (Jaime Olivares-Pérez *et al.,* 2015) who reported 12% and 12.5% *E. aerogenes* in raw milk respectively. On the other hand, high results 17.5% were reported by (Eman A. El-Mokadem *et al.,* 2023), this may be due to a lack of routine sanitation of equipment particularly during handling of milk. In contrast, lower results of 6.54% were reported for *E. aerogenes* in raw milk by (Azza M.K. Sobeiha *et al.,* 2020), this may be due to good sanitary measures they used. Regarding yogurt and ice cream, the incidence of *E. aerogenes* was 5% and 6% respectively, similar results were reported by (Azza M.K. Sobeiha *et al.,* 2020) who reported 5.06% in yogurt and 7.27% in ice cream samples. In contrast, higher results 34% were reported for *E. aerogenes* in Ice cream by (T Masud *et al.,* 1989). On the other hand, lower results 2.6 were obtained by (S. Mathews *et al.,* 2023).

CONCLUSION

In the present study, the results concluded that the sanitary measures during the handling, production and distribution of the examined milk and milk products (yoghurt and ice cream) are neglected. Also, the presence of *E. aerogenes* is considered an index for fecal contamination of milk and milk products which is a public health hazard. So, Good hygienic measures, sanitation all operating procedures, standardized pasteurization and (HACCP) Hazard analysis and critical control points are effective methods to prevent food-borne infection caused by *E. aerogenes*.

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انتشار بكتيريا الانتيروباكتر ايروجينز في الحليب الخام وبعض منتجات الألبان

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مقدمة: عُرفت الانتيروباكتر ايروجينز باسم ايروباكتر ايروجينز وتنتمي إلى عائلة انتيروباكتيرياسي. عادة ما تكون موائل لأمعاء الحيوانات لذلك توجد على نطاق واسع في التربة والصرف الصحي والمياة, و تسبب مجموعة متنوعة من المشاكل الصحية للإنسان والحيوان.

الهدف من الدراسة التالية هي الكشف عن البكتيريا المعوية الهوائية في اللبن والزبادي والآيس كريم لتقييم جودتها الصحية.

الطريقة: بلغ عدد العينات 300 عينة وهي كالتالي: 100 عينة لبن خام ، 100 عينة زبادي و 100 عينة آيس كريم. تم جمع العينات من محافظة أسيوط وفحصها بحثاً عن وجود بكتيريا الانتيروباكتر ايروجينز
النتائج: تم العثور على الانتيروباكتر ايروجينز في 13٪ من عينات اللبن الخام، و 5٪ من عينات الزبادي التي تم فحصها، بينما تم الكشف عنها في 6٪ من عينات الآيس كريم التي تم فحصها.

الخلاصة : بكتيريا الانتيروباكتر ايروجينز في الحليب هي مؤشر للتلوث البرازي المباشر للحليب ومنتجات الألبان التي تعتبر خطرا على الصحة العامة. يجب على المستهلكين تحسين معاييرهم الصحية لتجنب العدوي ببكتيريا الانتيروباكتر ايروجينز.