# Bacteriological Investigation of *Aeromonas Hydrophila* in Dairy Cattle: A Preliminary Study

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**Abstract**

Many different kinds of bacteria flourish in milk due to its almost neutral pH, high moisture percentage, and abundance of nutrients. Milk provides a perfect environment for these bacteria. The pathogenic *A. hydrophila*, capable of causing gastroenteritis, can potentially be transmitted to people through milk. This study purposed to explore the prevalence of *Aeromonas hydrophila* in certain dairy farms, as well as its culture characteristics and biochemical identification. There were positive 22 milk samples of cattle origin out of 380 milk samples examined and the prevalence of *Aeromonas hydrophila* was 5.8%. The identification of *A. hydrophila* was assuerd consistent with phenotypic characteristics on specific culture medium and the diagnostic biochemical reactions. Both the possible risks to public health that could be posed by *Aeromonas* species found in milk and its derivatives, as well as the potential methods that could be used to combat these risks, have been considered.

**Keywords:** *Aeromonas hydrophila*, dairy cows, milk, public health.

## Introduction

Milk is usually assumed to be a stellar medium for the growth of microorganisms like *A. hydrophila*, due to the exuberance of moisture, pH near to neutral side (6.4-6.6), and high nutrient content. In fact, these factors made the production process of milk need high standards of hygiene and emergency in most nations where milk was the first food so, focus of food hygiene legislation was important (**Ahmed et al. 2014**)

Milk easily can transmit pathogenic *A. hydrophila*, which can cause gastroenteritis. Generally, some researches concentrated on the isolation of motile *Aeromonas* spp. from many sources; bulk tank raw milk, pasteurized milk, and street milk samples which are gathered from dairy manufacturing factories, dairy shops, and supermarkets (**Fernández Bravo, Ana. 2019**).

The control of such infection in dairy animals depends only on the rapid detection of the causative agent and rapid treatment of infected animals with the drug of choice **(ElBalat et al. 2014)**.

So, the resistance to bacterial pathogens is based mainly on the use of antibiotics **(Enany et al., 2013; Algammal et al., 2019; Algammal et al., 2021; Kareem et al., 2021; Algammal et al., 2022; Elbehiry et al., 2022; Algammal et al., 2023)**. Different parts of the world recorded the incidence of multi-drug resistant *A. hydrophila* to penicillin and ampicillin. On the other side, the organism was sensitive to aminoglycosides, chloramphenicol, quinolones, tetracycline, trimethoprim-sulfamethoxazole, as well as both second and third generation cephalosporins **(Aravena-Román 2012)**.

## Materials and methods

### 1. Sampling

The study was conducted on apparently healthy dairy cows (≈ 380) during different stages of lactation, using hand-milking twice a day, in private farms sited in El-sharkia province. Milk samples were aseptically collected from cows before milking. Firstly, 70% alcohol was applied on teat ends and then let to dry. The first streams of foremilk were discharged, and then collection of 25 ml milk from each aseptic mammary quarter was done into sterile bottles. Transportation of samples to the laboratory was immediately done in ice box.

### 2. Isolation of *A. hydrophila*

A loopful of each tested milk sample was directly streaked onto plates of nutrient agar, MacConkey agar, and *Aeromonas* base medium supplemented with ampicillin and aerobically incubated at 37° C for 18 to 24 hours while incubated at 28° C in case of nutrient agar **(Ahmed et al. 2018).**

### 3. Identification of A.hydrophila

The pure culture was prepared. Films from the pure colonies were stained by gram stain and examined microscopically. The suspected colonies were further examined biochemically through performing indole, catalase, oxidase, urease and sugar fermentation tests as well as motility was assured by stabbing the organism in a tube of motility medium and incubating at 37°C for 24°C hours.

### 4. Hemolytic activity

The hemolytic activity of *A. hydrophila* was assessed on blood agar that contains 5% sheep RBCs.

## Results

### 1. Prevalence and phenotypic traits of *A. hydrophila* among the examined milk samples

Based on cultural characteristics, the suspected typical colonies were translucent, convex and pale green. Good growth is also obtained on MacConkey agar and colonies are usually lactose-negative. Colonies were white to buff, circular, and convex on nutrient agar.

The results revealed 22 *A.hydrophila* isolates; gram-negative straight non-sporulated rods, and motile via single polar flagella. The phenotypic and biochemical reactions of obtained isolates as shown in Table 1.

**2.Hemolytic activity**

Fourteen isolates (14/22, 63.6%) displayed the picture of β-hemolysis on blood agar; small, smooth, convex beta-hemolytic colonies then become dark green after 3-5 days.

**Table 1:** *Phenotypic characters of the recovered A. hydrophila isolates*

| | |
|---|---|
| Macconky agar | Good growth with an inability to ferment lactose so, colonies appear pale in color. |
| Nutrient agar | White: buff colonies with optimum growth at 28° C. |
| *Aeromonas* base medium | Yellowish small colonies. |
| Gram staining | Negative |
| Motility | Motile |
| Oxidase | Positive |
| Catalase | Positive |
| Indole | Positive |
| MR test | Negative |
| Gelatin liquefaction | Positive |
| O-F test | Fermentative |
| VP test | Positive |
| Glucose fermentation | Positive |
| Urease | Negative |

**Discussion**

Food of animal origin plays a substantial role in the transmission of microorganisms from animals to humans; also animal feces could be considered a prime source of food contamination **(Akan et al., 1998)**.

In Turkey, aeromonads were obtained in 49.2% of 132 samples from bulk raw milk, 16% of 31 pasteurized milk samples and 40% of 25 raw milk samples that were obtained from the streets. *A. hydrophila* was the most frequently isolated species (90.2%) **(Yucel et al., 2005)**. Similarly, **Akan et al. (1996)** isolated motile *Aeromonas* spp. in 23 out of 80 bulk tank milk samples (28.7%) and mentioned that fifteen strains were *A. hydrophila* (65.22%). Furthermore, **Abdel-Raouf and Naima (2011)** and **Zeinhom and Abdel-Latef (2014)** reported higher prevalences of *A.hydrophila* (36 and 24%) respectively in apparently normal cow feces. Also, **Agarwal et al. (2000)** may proposed feceal contamination of raw milk as a risk factor. It seems that these previous investigations have linked the major isolation of *A. hydrophila* from milk samples with bulk tank raw milk and/ or pasteurized milk samples.

Our data demonstrated high rates of *A.hydrophila* that exhibited β-

haemolytic activity (63.6%) and this obtained result nearly similar to **Al-Fathawy and Al-Ammar (2013)**, **Joseph et al. (2013)**, **Taj-Aldeen et al. (2014)**, **Al-Oqaili et al. (2016)**, and **Simon et al. (2016)**. Contrarily, **Majeed (2011)**, **Manna et al. (2013)**, **Amsaveni et al. (2014)**, **Mansour et al. (2014)**, and **Sharma et al. (2015)** have mentioned lower results. Haemolytic activity, is considered an indicator of pathogenicity, and usually is linked to enterotoxigenic *Aeromonas* spp. **(Obi et al., 2007)**. Haemolytic activity provides a simpler and more cost-effective method for detecting Aeromonads pathogenicity than PCR assay, particularly in developing countries. In conclusion, this study revealed the presence of *A.hydrophila* in milk which indicates high-risk public health issues. In dairy farms, proper hygienic procedures are required to obtain healthy milk suitable for human consumption.

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## الملخص العربي

## التحري البكتريولوجي لبكتيريا ايروموناس هيدروفيلا في الأبقار الحلوب : دراسة أولية

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العديد من انواع البكتريا تنتواجد في الالبان و ذلك يرجع لنسبة الرطوبة العالية ، الرقم الهيدروجيني الذي يعد متعادل و وفرة المواد الغذائية. يوفر اللبن البيئة المثالية لهذه البكتريا و منها بكتريا الايروموناس هيدروفيلا المسببة للامراض و التي تسبب امراض الجهاز الهضمي فتنتقل للانسان عن طريق اللبنز تهدف هذه الدراسة لاكتشاف مدى ضراوة الايروموناس هيدروفيلا في مزارع محددة للماشية الحلابة والتعرف عليه من خلال دراسة الخصائص المميزة للميكروب عند زراعته و اجراء التفاعلات البيوكيميائية عليهم و تم التعرف علي 22 (5.8%) عترة للايروموناس هيدروفيلا من اصل 380 عينة لبن بقري قد تم فحصها و تم التاكد من ايجابية هذه العترات بعد اظهارها الخصائص المميزة للايروموناس ظاهريا و بيوكيميائيا بعد اجاء التفاعلات الاختبارات البيوكيميائية عليها. تم الاخذ في الاعتبار كلا من تأثيرالايروموناس علي الصحة العامة و الطرق الفعالة التي يمكنها موجهة هذه المخاطر.