

## INCIDENCE OF PLESIOMONAS SHIGELLOIDES IN RAW MILK (With 2 Tables)

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### ABSTRACT

*A total of 150 random samples of raw milk were collected from different localities in Assiut city. These samples were examined for the prevalence of P. shigelloides using 2 selective media; Plesiomonas agar (PL agar) and modified Rimler-Shotts medium. The results revealed that 4 (2.7 %) of the examined raw milk were contaminated with P. shigelloides using PL. agar. The incidence of P. shigelloides on modified Rimler-Shotts medium was 2 (1.3%) in the same samples. These findings could be attributed to the neglected sanitary control during the handling and distribution of raw milk. The public health significance of the organism and the recommended sanitary measures were also discussed.*

### INTRODUCTION

Over the past two decades, public health authorities in industrialized countries have encountered increasing numbers of food safety problems. In 1984, the Joint FAO/WHO) Expert Committee on Food Safety concluded that food borne illness are the most wide spread health problem world wide and an important cause of reduced economic productivity. In addition to problems recognizing food-borne disease public health officials are continually being challenged by new and emerging types of food - borne illness. Increased demand for ready-to-eat and minimally processed foods has resulted in the appearance of several new food borne pathogens. Such food including milk can rendered unsafe for human consumption in one of several ways, including

by contamination with disease producing bacteria.

Plesiomonas shigelloides is an important microorganism recognized recently as a potential human and animal pathogen. It was first named Paracolon C27 and related to the family Enterobacteraceae (**Ferguson and Henderson, 1947**), then the organism was included in the genus Aeromonas because the name of Plesiomonas (Plesio = neighbor, monas related to Aeromonas and belonged in the same family (**Hobs and Schubert 1962**). Recently, a new family was created, Plesiomonadaceae, with one species, P. shigelloides (**Ruymi et al, 1994**).

Plesiomonads are gram-negative, motile, non-spore forming bacilli, facultative anaer-

obic and oxidase positive (Miller and Koberger, 1995 and Varnam and Evans, 1991). Plesiomonas is globally distributed most of the reports on its isolation are from countries situated in the tropical or subtropical areas (Sanya, I et al., 1987 and Aldova, et al., 1999). This bacterium has also been called the "Asian" bacteria because of the high incidence of isolations in countries as Japan and Thailand.

*P. shigelloides* play an important role in gastro-enteritis and diarrhea cases (Tsukamoto, et al., 1978 and Food and Drug Administration, 1998). Symptoms associated with gastroenteritis caused by *P. shigelloides* are diarrhea which is the predominant symptoms occurring in (94%) of cases abdominal pain (74%) nausea (72 %) chills (49%) fever (37%) headache (34%) and vomiting (33%). The severity of symptoms varies and the duration is usually seven days or longer (Varnam and Evans, 1991). *P. shigelloides* can cause unusual clinical pictures since the organism invade the body. Other extra-intestinal infections caused by *P. shigelloides* are pyosalpinx cellulites migratory poly-arthritis, ocular infection and acute cholecystitis (Gupta, 1995; Butt, et al., 1977 and Roth et al., 2002).

Information on the incidence of *P. shigelloides* in raw milk in Egypt is lacking, therefore the present study was designed for the isolation and identification of *P. shigelloides* from raw milk.

## **MATERIALS AND METHODS**

### **I- Isolation of Plesiomonas shigelloides from raw milk:**

1- Collection of samples: A total of (150)

random samples of raw milk including farm milk, street vendors, supermarkets (50 samples each) were collected from different localities in Assiut city. The samples were collected in clean, dry and sterile containers. Collected samples were transferred in an ice box to the laboratory as soon as possible to be examined.

Each milk sample was mixed by inversion several times and tested for heat treatment using Storch's test (Lampert 1975).

2- Preparation of samples: According to the technique recommended by (A.P.H.A.1992)

3- Experimental procedures:

The technique adopted by (Freund, et al., 1988) was used.

a- Enrichment procedures: one milliliter of each homogenized sample was aseptically inoculated into a sterile tube containing 10 ml of tetrathionate broth and incubated at 35-37°C for 24 h.

b- Selective plating:

\* Incubated broth cultures were then streaked onto plates of both modified Rimler-Shotts medium and *Plesiomonas* agar (PL agar). Streaked plates were incubated at 35-37°C for 24-48 h.

\* Suspected colonies should be pink, opaque, of 1 mm in diameter, surrounded by an alkaline zone on PL agar and yellow colonies and turning medium greenish yellow due to production of Lysine and Ornithine Decarboxylase on modified Rimler-Shotts agar. Such colonies were picked up with a sterile platinum loop and streaked

onto Trypticase Soy Agar and incubated at 35°C for 24 h.

Von Gravenit & Bucher (1983) and Miller & Koburger (1986).

**II- Identification of the isolated *P. shigelloides*:**

Identification was carried out according to

**RESULTS AND DISCUSSION**

Results are shown in Tables 1 and 2.

**Table 1: Incidence of *P. shigelloides* in the examined raw milk samples collected from different sources.**

Source of samples	No. of examined samples	Positive samples on:			
		Pl. agar		Modified Rimler-Shotts	
		No.	%	No.	%
Street vendors	50	2	4	1	2
Dairy shops	50	2	4	1	2
Dairy farms	50	-	-	-	-
<b>Total</b>	<b>150</b>	<b>4</b>	<b>2.6</b>	<b>2</b>	<b>1.3</b>

**Table 2 : Incidence of *P. shigelloides* in the examined raw buffalo's and cow's milk samples.**

Source of samples	No. of examined samples	Positive samples on:			
		Pl. agar		Modified Rimler-Shotts	
		No.	%	No.	%
Buffalo's milk	75	1	1.33	--	--
Cow's milk	75	3	4	2	2.7

## DISCUSSION

Milk has long considered a valuable human food. Its nutrient composition makes it a suitable medium for bacterial growth; including pathogenic organisms to man.

Results recorded in Table 1 showed that *P. shigelloides* was isolated from 4% (2/50) of the examined raw street vendors milk samples using Plesiomonas agar (Pl agar), while by using modified Rimler-Shotts agar, it was isolated from 2% (1/50) of the examined samples, so it was detected in a higher percentage (4%) by using Pl agar.

Results achieved in Table 1 revealed that 4% (2/50) of the examined raw dairy shops milk samples were contaminated with *P. shigelloides* on PL agar. Also by using modified Rimler-Shotts medium, *P. shigelloides* could be isolated from 2% (1/50) of the same samples. In the present study, *P. shigelloides* could not be detected in the examined raw milk samples collected from dairy farms (Table 1). The absence of these microorganisms may reflect the good sanitation practices applied during milking process.

The presence of *P. shigelloides* in street vendors and dairy shops raw milk samples recorded in this study is expected as the produced milk is liable to contamination from different sources (dust, air, water, equipments, milkers and handlers), moreover, the prevailing of bad handling, poor sanitation of equipments and lack of cooling facilities during transportation. In dairy farms milk, no sample was found to be contaminated by *P. shigelloides* which indicated that dairy farm milk is fit for human consumption because it gave

a real indication for the good hygienic practice during production and handling.

In this study *P. shigelloides* was recovered only by 2.6% (4/150) from raw milk samples on PL agar and 1.3% (2/50) on modified Rimler-Shotts medium. Table 2 showed that the incidence of *P. shigelloides* in the examined raw milk samples was higher in cow's milk (4% on PL agar and 2.7% on Rimler-Shotts agar) than in buffalo's milk (1.33% on PL agar and non on Rimler-Shotts agar). This may be attributed to the bactericidal capacity of digestion products of milk triglycerides and membrane lipids which may protect against food-borne gastroenteritis (**Sprong et al., 2001**) on the basis of higher fat content of buffalo's milk (average 7.98%) than that of cow's milk (average 4%).

It is evident from the present work that the incidence of *P. shigelloides* in milk was low. Not only the milk quality but also the methods of isolation, seasonal, and geographic nature may have a major effect on the incidence rate.

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## الملخص العربى

### تواجد ميكروب بلزيموناس شيجيلويدز فى اللبن الخام بواسطة

أمال على عبدالحليم\* و لىلى مصطفى الملط\*\* و مارى رفعت حافظ\*

تبدو أهمية اللبن فى كونه يحتوى على العناصر الغذائية الأساسية التى يحتاجها جسم الإنسان فى جميع المراحل السنية وعلى الجانب الآخر فهو وسط جيد لنمو وتكاثر الميكروبات التى منها ماهو ممرض ومنها ما يؤدى إلى فساد الألبان ومنتجاتها إذا لم تراعى الظروف الصحية المناسبة أثناء الإنتاج أو التداول أو التوزيع، ولقد زاد الاهتمام بميكروب البلزيموناس شيجيلويدز كمسبب مرضى للإنسان ومايسببه من نزلات معوية، يعتبر هذا الميكروب من الميكروبات المسببة للتلوث الغذائى حيث يمكن تواجده فى مياه الشرب الملوثة بالبراز وكذلك المياه التى تستعمل فى مزارع ومصانع الألبان، ولذا كان من الضرورى الاهتمام بتحديد مدى تواجد هذا الميكروب فى الألبان.

تم جمع ١٥٠ عينة عشوائية من الألبان من (١) الباعة الجائلين ومن (٢) بيوت المربين ومن (٣) المزارع بواقع ٥٠ عينة من كل نوع للتعرف على مدى تلوثها بميكروب البلزيموناس شيجيلويدز، وقد أسفرت النتائج على تواجد هذا الميكروب بنسبة ٤ (٢٧٪) فى اللبن الخام وذلك باستخدام مستنبت البلزيموناس أجار (PL.Agar) وعند استخدام مستنبت رملر شوتز المتحور (modified Rimler-shotts) وجد أن ٢ (١٣٪) من العينات السابقة كانت ملوثة بميكروب البلزيموناس شيجيلويدز، وقد أعزى تلوث اللبن الخام إلى عدم إتباع القواعد الصحية السليمة أثناء إنتاج اللبن وتعبئته حتى يصل إلى المستهلك، هذا وقد تم مناقشة النتائج والشروط الصحية الواجب إتباعها لمنع تلوث الألبان حماية لصحة المستهلك.