

## دراسة عن الحالة الصحية للألبان في مدينة أسيوط (مدى انتشار الميكروبات المرضية في اللبن)

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قام الباحثون بفحص ٦٤ عينة لبن من الباعة الجائلين ومحال بيع الألبان بمدينة أسيوط.  
لمعرفة مدى انتشار الميكروبات المرضية باللبن ومدى خطورتها على الصحة العامة للمستهلكين .

وقد أشارت النتائج .

عزل ميكروبات مختلفة منها القولونية . . . . . وغيرها .

وكذلك الميكروب السعى الصيدي وميكروب العنقودي الذهبى .

كما ثبت وجود الميكروبات اللاهوائية بنسبة ١٤.٠٦٪

وقد أثبتت النتائج أن ٧١.٤٪ من الميكروبات المرضية المعزولة كانت من عينات اللبن  
المانوخذة من الباعة الجائلين . . . مما يؤكد خطورة الباعة الجائلين على الصحة العامة ومدى أهمية  
الفحص الدوري لهم والعناية بأدوات تعبئة اللبن وتوزيعه حرصا على سلامة المستهلكين .

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## STUDIES ON THE SANITARY CONDITION OF MARKET MILK IN ASSIUT PROVINCE, EGYPT

### Part II: The incidence of potentially pathogenic micro-organisms

(with 2 Tables)

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#### SUMMARY

64 milk samples marketed by street peddlers and dairy shops in Assiut City were examined bacteriologically. Regarding the enteric group of organisms (*E. coli*, *Alcaligenes faecalis*, *Alkaliscens dispar*, Arizona, *Klebsiella* and *Proteus* species were isolated in the following percentages : 21.8, 10.9, 4.6, 6.2, 7.8 and 7.8, respectively.

Other micro-organisms isolated were *Strept. pyogenes* (20.3%), *Strept. faecalis* (7.8%), *Staph. aureus* (17.1%) and *Pseudomonas fluorescens* (4.6%).

Moreover, Clostridial species (*Cl. welchii*) were detected in 9 milk samples (14.06%).

The results obtained from this study revealed that 71.4% of the isolates were from samples of milk distributed by street peddlers.

The public health significance of each isolate was discussed.

#### REVIEW OF LITERATURES

Milk-borne outbreaks have been recorded from time to time, at home and abroad, which are usually of an explosive and wide spread nature. Milk may be the vehicle of salmonella strains, staphylococcal toxigenic organisms, or any other pathogenic bacteria. The source of infection usually comes either through the diseased dairy animal, human contamination or through contamination of milk after leaving the udder. THOMASON (1956) revealed that 1% of farm milk samples contained enteropathogenic *E. coli* generally associated with gastro-enteritis. COLLINS *et al.* (1968) recorded an outbreak of salmonellosis which was traced to dry milk.

MILONNOJA (1968) isolated *Staph. aureus*, *Strept. faecalis*, *Proteus* species, *E. coli* and *Salmonellae* from raw market milk and milk products. In the same year, ZEHEREN and ZEHEREN alluded to a widespread outbreak of staphylococcal food poisoning.

In a previous contribution regarding the sanitary condition of market milk in Assiut City (Part I from this series), AHMED *et al.* (1974) have found that the total bacterial count ranged between 9 and 924 million bacteria per ml, while the coliform titre varied  $10^{-4}$  to  $10^{-5}$ . They concluded that the results achieved proved that such milk has been produced and handled under neglected hygienic conditions.

A trial therefore, was found to be necessary in order to provide information about the pathogenic agents that may find their way into raw milk marketed in Assiut City.

## MATERIAL AND METHODS

64 random samples of raw milk marketed in Assiut (29 samples from street peddlers and 35 from dairy shops) were collected in sterile stoppered bottles. The sample were taken (250 ml each) by means of a sterile dipper. Each sample was thoroughly mixed before being subjected to the following bacteriological examinations :

### 1. Detection of enteric pathogens :

About 2 ml of each milk sample were aseptically inoculated into sterile Selenite F broth and incubated for 16 hours at 37°C. Loopfuls from each inoculated enrichment medium were streaked on McConkey's and Modified Kauffmann's agar plates and incubated at 37°C for 24 hours. Different developing colonies of both lactose and non-lactose fermenting micro-organisms were picked up and subcultured for further identification according to EDWARDS and EWING (1962).

### 2. Isolation of *Staphylococci*, *Streptococci* and other pathogens :

A part of the milk sample was centrifuged in sterile centrifuge tubes for 30 minutes at 3000 r.p.m. From the sediment obtained, loopfuls were streaked on milk salt agar plates as well as blood agar plates and incubated for 24 hours at 37°C. Suspected colonies were further identified morphologically and biochemically according to CRUICKSHANK *et al.* (1969).

### 3. Stormy fermentation test :

A series of test tubes containing 10 ml of milk sample was heated to 80°C for 30 minutes and a layer of paraffine wax was poured on the surface. The tubes were incubated at 37°C for 2 days. A clot, very much disrupted by a large amount of gas produced indicates the presence of anaerobic micro-organisms.

## RESULTS AND DISCUSSION

Considering the pathogenic micro-organisms in raw market milk in Assiut City, the bacteriological examination of 64 samples revealed the isolation of the types shown in Table 1.

TABLE 1.—Percentage distribution of isolates

Type	No of isolates	% isolates	% samples
Strept. pyogenes . . . . .	13	18.6	20.3
Strept. faecalis . . . . .	5	7.1	7.8
Staph. aureus . . . . .	11	15.7	17.1
E. coli. . . . .	14	20.0	21.8
Arizona species . . . . .	4	5.7	6.2
Proteus vulgaris . . . . .	1	1.4	1.5
Proteus rettgeri . . . . .	3	4.3	4.6
Proteus morgani . . . . .	1	1.4	1.5
Klebsiella species . . . . .	5	7.1	7.8
Alkalescens dispar . . . . .	3	4.3	4.6
Alcaligenes faecalis . . . . .	7	10.0	10.9
Pseudomonas fluorescens . . . . .	3	4.3	4.6
Total . . . . .	70	—	—

*Strept. pyogenes* was isolated from 13 samples of milk (18.6%), of which 7 strains were isolated from street peddlers and 6 strains from dairy shops (Table II).

*Strept. pyogenes* was considered by TANNER and TANNER (1953) as a common cause of mastitis in dairy cattle, and several milk-borne outbreaks were traced to the diseased udder. Moreover, it is the causative agent of septic sore throat, scarlet fever, tonsillitis, and rheumatic fever, as well as a number of other pyogenic and septicaemic infections in man and animals (MERCHANT and PACKER, 1967). They stated that it may be a secondary invader in such diseases as diphtheria, tuberculosis, pleurisy and pneumonia.

TABLE 2.—Relation between the hygienic standards and frequency distribution of isolates

Type	No. of isolates	Street peddlers	%	Dairy shops	%
<i>Strept. pyogenes</i> . . . . .	13	7	53.8	6	46.2
<i>Strept. faecalis</i> . . . . .	5	5	100.0	—	—
<i>Staph. aureus</i> . . . . .	11	10	91.0	1	9.0
<i>E. coli</i> . . . . .	14	12	85.7	2	14.3
Arizona species . . . . .	4	3	75.0	1	25.0
<i>Proteus vulgaris</i> . . . . .	1	1	100.0	—	—
<i>Proteus rettgeri</i> . . . . .	3	1	33.3	2	66.7
<i>Proteus morgani</i> . . . . .	1	1	100.0	—	—
<i>Klebsiella</i> species . . . . .	5	4	80.0	1	20.0
<i>Alcaliscens dispar</i> . . . . .	3	2	66.6	1	33.4
<i>Alcaligenes faecalis</i> . . . . .	7	4	57.1	3	42.9
<i>Pseudomonas fluorescens</i> . . . . .	3	—	—	3	100.0
Total . . . . .	70	50	71.4	20	28.6

Staphylococci are still the organisms most commonly involved in milk and milk products - associated food poisoning (ANDERSON and STONE, 1955; HENDRICKS et al., 1959; MATTICK et al., 1959; FOLTZ, et al., 1960; MICKELSEN et al., 1961; KAPLAN et al., 1962; MICKELSEN, 1963; ZEHEREN and ZEHEREN, 1968 and US PUBLIC HEALTH SERVICE, 1970).

*Staph. aureus* could be isolated from 11 samples of raw milk (15.7%) of which 10 strains were isolated from milk marketed by street peddlers and one strain from samples collected from dairy shops. It may be found in milk as a result of contamination from the bovine udder or human sources. These organisms may grow especially in the absence of proper cooling, and hence the dosage of the pathogens delivered to the consumer, in addition to the potent enterotoxine production will constitute a significant health problem.

Arizona organisms were isolated in a percentage of 5.7 as shown in Table 1. Three strains could be isolated from milk distributed by street peddlers and one strain from samples of dairy shops (Table 2). These organisms are responsible for a number of outbreaks in poultry, particularly turkeys and hens. Moreover, they were implicated by EDWARDS *et al.* (1959) in some outbreaks of food poisoning. They produce similar clinical syndroms, and can be regarded as very similar from epidemiological point of view as well as the prophylactic measures as *Salmonellae* (SMITH and CONANT, 1960 and EDWARDS and EWING, 1962).

Evidence that faecal contamination of milk as well as the presence of excessive dust and dirt during milking was provided in the present investigation by the isolation of *Strept. faecalis* (7.1%), *Alcaligenes faecalis* (10.0%), *E. coli* (20.0 %), *Alkaliscens dispar* (4.2%), *Proteus* species (7.1 %), *Pseudomonas fluorescens* (4.2%) and *Klebsiella* species (7.1%) as shown in Table 1.

Enterococci have been implicated as aetiological agents of food-borne illness. *Strept. faecalis* was previously isolated from raw and pasteurized milk by WHITE AND SHERMAN (1944). The symptoms include nausea, colic and in some cases vomiting. COOPER AND RAMADAN (1955) indicated that *strept. faecalis* is a dominant species in human beings, which may contaminate milk through hands of milkers. In this study, *Strept. faecalis* was isolated from 5 samples (7.8%), all of them were isolated from milk samples collected from street peddlers, the presence of which is a definite proof that faecal contamination of milk had occurred.

*E. coli* was isolated from 14 samples (20.0%), a percentage which is considered higher than that previously recorded by THOMASON (1956). This group of organisms may be rapidly spread by hands or fomites contaminated with faeces containing large numbers of *E. coli*. As shown from Table 1, 12 strains were isolated from milk samples marketed by street peddlers and 2 strains from milk distributed by dairy shops.

*E. coli* isolated from a wide variety of cases of calf dysentery. It is also associated with pyelonephritis, naval infection, joint infection, cystitis, mastitis and metritis in cattle. Moreover, it has been isolated from suppurative exudates from wound infections and abscesses of various animals (MERCHANT and PACKER, 1967). In man, this organism produces fatal intestinal infection in infants, peritonitis, gall bladder infections, inflammation of the pancreas, infections involving uro-genital tract and pneumonia. It also found in the majority of the abscesses and fistulae involving the perineal region of man (MERCHANT and PACKER, 1967).

*Alcaligenes faecalis* is a normal inhabitant of the intestinal tract of man and animals however, it is occasionally associated with enteritis, and have been isolated from the liver, kidneys, biliary ducts, blood and lymph nodes of animals (SMITH and CONANT, 1960). Four strains of *Alcaligenes faecalis* were isolated from milk samples collected from street peddlers and 3 strains from dairy shops.

*Pseudomonas* species were encountered in cases of urinary tract infections, severe enteritis and diarrhoea (SMITH AND CONANT, 1960, and MACKIE and McCARTNEY, 1962). It was isolated from 3 milk samples dispatched from dairy shops.

HORVATH *et al.* (1964) reviewed a food-borne outbreak due to *Klebsiella* organisms. Four strains were isolated in this investigation from milk sample collected from street peddlers and one strain from milk distributed by dairy shops (Table II).

*Proteus* species have been encountered in some cases of summer diarrhoea of infants. It was also isolated from urinary tract infection. In Egypt, MOSTAFA *et al.* (1948) reported that 30 of 125 food-borne outbreaks were attributed to *Proteus* species. The incrimination of *Proteus* has usually been based on the presence of large numbers of the organisms in milk (SMITH and CONANT, 1960).

As shown from Table II, *Proteus vulgaris* and *Proteus morganii* were isolated from samples collected from street peddlers only, while *Proteus rettgeri* was isolated from both dairy shops and street peddlers .

The presence of *Clostridium welchii* was detected in this study in 9 samples of raw milk (14.06%). *Cl. welchii* is usually found in the alimentary tract of nearly all warm-blooded animals, and therefore, its presence in milk indicates faecal contamination. Rezk (1962) reported cases of fatal gangarinous mastitis in cattle due to *Cl. perfringens*, which is excreted in milk. Moreover, this organism causes gas gangarone in man, food poisoning, lamb dysentery and various other diseases, often intestinal (HAGAN and BRUNER 1961).

The results obtained revealed that 71.4 % of the isolates were from samples of milk marketed by street peddlers.

It is clear therefore, that the potential for disease outbreaks associated with milk and dairy products still exists, and public health workers and the dairy industry must be conizant of that potential. Obviously the animal is not the only source of contamination of milk with organisms implicated in milk-borne diseases. Contamination of milk generally arises through faulty cleaning and sterilization of utensils, improper preparation of the animal, dust contamination or the use of polluted water supply.

Man can be a source of contamination and subsequent infection to any point in the processing untill the product is actually consumed.

It is concluded that milk improperly handled provides a ready medium for the transmission of certain types of diseases.

The pathogens so carried may result in disease outbreaks of epidemic properties. A well organized sanitary control programe is essential to guard against such possibilities. Moreover, in order to establish an effective milk control porgrame, standards must be established, effective enforcement must be provided and education must be extended to producers, distributers and consumers.

Last in the series of these investigations, an effort will be made focussed on the isolation of *Mycobacteria* from raw market milk in Assiut City.



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