



## Some antibiotic resistant bacteria of public health hazard isolated from raw milk sold in some Assiut City markets

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### ABSTRACT

A total of 50 raw milk samples were collected in this study from some Assiut City markets, Egypt, and examined for isolation of some human hazard pathogens. The percentages of the isolated pathogens were 46, 76, 78, 4 and 24% for *Staph. aureus*, *Enterococcus faecalis*, *E. coli*, *Citrobacter freundii* and *Yersinia enterocolitica*, respectively. In vitro Antibiogram was carried out on all isolates against (8) different antimicrobial agents; moreover, all of these isolates showed multi-drug resistance against two or more of the tested antibiotics. The public health hazards of the isolated pathogens were also discussed.

### INTRODUCTION

Milk is one of the few food stuffs consumed in its natural state. It is the only article in the diet, with the exception of honey, whose sole function in nature is to serve as a food. It has a special importance as a component of the diets of both young children and old people. Milk is a compulsory part of daily diet for the expectant mothers as well as growing children (Javaid *et al.*, 2009). Milk is synthesized in specialized cells of the mammary gland and is virtually sterile when secreted into the alveoli of the udder (Tolle, 1980). In addition of being a nutritious food for human beings also serves as a good medium for the growth of many microorganisms. Therefore, the quality of milk is of great importance than the quality of most other foods. Contamination of milk with microorganisms comes from indigenous source from the udder due to clinical and subclinical mastitis or exogenous sources during milking, air, milking equipment, feed, soil, feces, grass as well as handling and purchasing of milk. Some diseases associated with the consumption of milk include *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter*,

*Yersinia*, pathogenic *Escherichia coli* and *Clostridium botulinum* (Hahn, 1996).

Antibiotics are usually used to treat cattle diseases and their indiscriminate use has led to the development of multi-antibiotic resistant strains of bacteria thereby rendering antibiotic treatment ineffective (Johnston *et al.*, 1983).

Antibiotic-resistant strains of bacteria cause humans to become more virulently ill for longer periods of time than their antibiotic-susceptible counterparts. Treatment failure occurs as a consequence and there is the need for expensive and/or toxic alternative drugs which in most cases are more expensive (WHO, 2007).

The evolution of antibiotic resistance strains in microbial communities is enhanced by horizontal transfer of resistance genes over species and genus borders by conjugative plasmids, transposons, the possession of integrand and insertion elements, as well as lytic and temperate bacteriophage. Several mechanisms of resistance against a particular antibiotic have evolved including enzymatic inactivation of the antibiotic (e.g. by  $\beta$ -lactamases, amino glycoside acetyl-, nucleotidyl and phosphoryltransferases), restricted import of antibiotics (e.g. penicillin

binding proteins), active export of antibiotics (e.g. by membrane inserted ATP-dependent efflux systems) or target modification (e.g. methylation of 23S rRNA, mutation of amino acid sequence of topoisomerase) For certain pathogenic and potentially pathogenic bacteria like staphylococci and enterococci, the evolution of highly resistant clones has inaugurated a crisis in antibiotic resistance (Huys et al., 2004).

Due to milk act as a good vehicle for microorganisms and sometimes these microorganisms may be antibiotic resistant, therefore, the aim of this study was to determine the prevalence of some microorganisms of public health hazard importance as *Staphylococcus aureus*, *Enterococci spp*, *Listeria monocytogenes*, *E. coli*, and *Yersinia enterocolitica* isolated from raw milk sold in some Assiut City markets and testing them for antimicrobial sensitivity test.

## MATERIALS AND METHODS

A total of 50 raw milk samples were collected randomly from some Assiut City markets, Egypt, during summer 2013. Each sample was collected in sterile plastic bag and transported in an ice box as rapid as possible to the laboratory.

The apparently normal raw milk samples were mixed thoroughly and tested for heat treatment by Storch test according to A.P.H.A. (1985) before being subjected to examination for isolation of:-

- Isolation and identification of *Staphylococcus aureus* (Quinn et al., 2004).
- Isolation and identification of *Enterococci* (Quinn et al., 2004)
- Isolation and identification of *Listeria monocytogenes* (Finegold and Martin, 1982).
- Isolation and identification of *E. coli* (Koneman et al., 1992).
- Isolation and identification of *Yersinia enterocolitica* (Schiemann, 1979).

Testing of all the isolates to antimicrobial susceptibility test was carried out by agar disk diffusion methods according to (NCCLS, 2001). By using Amoxicillin (25 µg), Oxacillin (1 µg), Gentamicin (10 µg), Ceftriaxone (30 µg), Erythromycin (15 µg), Tetracycline (30 µg), trimethoprim-sulphamethoxazole (SXT) (25 µg) and Ciprofloxacin (5 µg). (Bioanalyse-Turkey).

## RESULTS

**Table 1.** Percentage of some bacterial pathogens isolated from the raw milk samples sold in some Assiut City Markets (No. 50).

Isolated bacterial pathogens	No./50	%
<i>Staphylococcus aureus</i>	23	46
<i>Enterococcus faecalis</i>	38	76
<i>Listeria monocytogenes</i>	0	0
<i>E. coli</i>	39	78
<i>Citrobacter freundii</i>	2	4
<i>Yersinia enterocolitica</i>	12	24

- Isolation and identification of *Staphylococcus aureus* (Quinn et al., 2004).

**Table 2.** Percentage of antimicrobial resistant bacterial pathogens isolated from raw milk samples sold in some Assiut City markets according the type of antibiotic used.

Type of antibiotic used	Isolated bacterial pathogens																			
	<i>Staph. aureus</i> No. 23				<i>Enterococcus faecalis</i> No. 38				<i>E. coli</i> No. 39				<i>Citrobacter freundii</i> No. 2				<i>Yersinia enterocolitica</i> No. 12			
	Resist		Susceptible		Resist		Susceptible		Resist		Susceptible		Resist		Susceptible		Resist		Susceptible	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Amoxicillin	16	69.6	7	30.4	18	47.4	20	52.6	30	76.9	9	23.1	2	100	0	0	12	100	0	0
Oxacillin	0	0	23	100	38	100	0	0	39	100	0	0	2	100	0	0	12	100	0	0
Gentamicin	4	17.4	19	82.6	17	44.7	21	55.3	0	0	39	100	0	0	2	100	0	0	12	100
Ceftriaxone	8	34.8	15	65.2	30	78.9	8	21.1	0	0	39	100	0	0	2	100	0	0	12	100
Erythromycin	0	0	23	100	6	15.8	32	84.2	35	89.7	4	10.3	2	100	0	0	5	41.7	7	58.3
Tetracycline	2	8.7	21	91.3	11	28.9	27	71.1	10	25.6	29	74.4	0	0	2	100	5	41.7	7	58.3
SXT	0	0	23	100	17	44.7	21	55.3	4	10.3	35	89.7	0	0	2	100	4	33.3	8	66.7
Ciprofloxacin	0	0	23	100	0	0	38	100	0	0	39	100	0	0	2	100	0	0	12	100

\*SXT = Trimethoprim-sulphamethoxazole

**Table 3.** Percentage of multi-antimicrobial resistant bacterial pathogens isolated from raw milk samples sold in some Assiut City Markets.

Multi-antimicrobial resistant bacterial pathogens isolated from raw milk									
<i>Staph. aureus</i> No./23		<i>Enterococcus faecalis</i> No./38		<i>E. coli</i> No./39		<i>Citrobacter freundii</i> No./2		<i>Yersinia enterocolitica</i> No./12	
No.	%	No.	%	No.	%	No.	%	No.	%
8	34.8	33	86.8	37	94.9	2	100	12	100

## DISCUSSION

*Bacteriological* examination of 50 raw milk samples sold in some Assiut City Markets (Egypt) revealed that, *Staph. aureus* was isolated in percentage of 46% (Table 1). Nearly similar results 46.7 and 45% were reported by **Mohamed and El-Zubeir (2007)** and **Vyletĕlova et al. (2011)**, respectively. However, lower results were estimated by **Yagoub et al. (2005)**, **Singh et al. (2011)**, **Khudor et al. (2012)**, **Meshref (2013)**, **Thaker et al. (2013)**, and **Vahedi et al. (2013)** who reported incidences of 30, 21.4, 28.5, 23.7, 6, and 22% respectively. While, higher results 80, 75, 68, 52 and 56.66% were found by **Al-Tarazi et al. (2003)**, **Ekici et al. (2004)**, **Oliveira et al. (2011)**, **Pourhassan and Taravat (2011)** and **Gwida and El-Gohary (2013)**, respectively.

*Presence of Staph. aureus* in raw milk may be due to infection of the animals by subclinical mastitis where *Staph. aureus* was the most isolated bacteria, while, the milk is apparently normal when secreted from the udder or due to human sources from milkers and milk handlers during milking of animal and handling of milk. Also, inadequate refrigeration and long storage of milk before use are some factors which enhance the presence of *Staph. aureus* (**Asperger, 1994**). In addition, negligence of hygienic condition such as improper cleaning of bulk tank, dirty udder, milking equipments, milk handling technique and improper storage will increase the proportion of Gram-positive and Gram negative bacteria in the bulk tank milk (**Bonfoh et al., 2003**).

*In vitro* testing of all isolated *Staph. aureus* strains for antibiotic sensitivity by using disk diffusion methods revealed that, all of *Staph. aureus* isolates were resistant to amoxicillin, gentamicin, ceftriaxone and tetracycline in different percentages ( 69.6, 17.4, 34.8 and 8.7% ), respectively; while, 30.4, 82.6, 65.2 and 91.3% were sensitive to the used antibiotics, respectively; moreover, all of them were sensitive to Oxacillin, erythromycin, SXT and ciprofloxacin in percentage of 100% (Table 2). From table 2 it is clear that, no resistance to oxacillin,

erythromycin, SXT and ciprofloxacin was reported for all *Staph. aureus* isolates. This observation is coincided with **Daka et al. (2012)** who reported no resistance of tested *Staph. aureus* strains to ciprofloxacin. Furthermore, this results agreed with **Farzana et al. (2004)** and **Dharmilk and Gomashe. (2011)** as the authors reported that erythromycin was the most effective antibiotic against *Staph. aureus*. Moreover, it is clear that most of tested strains were resist to amoxicillin (69.6%) followed by ceftriaxone (34.8%) (Table 2).

From the tested strains 34.8% had multi-antibiotic resistance (resist simultaneously for two or more antibiotics) (Table 3) and the resistance is mostly to amoxicillin, gentamicin and ceftriaxone simultaneously. Organisms that are resistant to multiple anti-infective drugs are not unusual (**Levy, 1998**). The results of resistance are very serious in terms of increased mortality, with a doubling of mortality being observed in some resistant infections as well as a need for an increase in the length of treatment with the more expensive anti-infective drugs or drug combinations (**WHO, 2007**). In addition, these strains may represent a big problems during treatment of human being infected by these strains due to consumption of milk or milk products contaminated by such microorganisms. Furthermore, Staphylococcal contamination of milk and milk products is associated with enterotoxicity in humans (**Chao et al., 2007**).

*The indiscriminate* use of antibiotics agents for prophylactic as well as other therapeutic purpose could be the reasons for increased antimicrobial resistance of *Staph. aureus*. A relatively high number of *Staph. aureus* strains in this study are resistant to the antibiotics commonly used in the therapeutic protocols of many human and animal infections which indicate the need for continuous surveillance of antibiotic sensitivity pattern of *Staph. aureus* with a view to selecting appropriate therapy. Also, the indiscriminate use of antimicrobials can lead to the selection of resistant bacteria in food animals that could subsequently be transmitted to humans, a serious public health problem (**McEwen and Fedorka-Cray, 2002**).

With regard to *Enterococcus faecalis*, out of the examined 50 raw milk samples, 38 samples (76%) gave positive result for isolation *Enterococcus faecalis* (Table 1). Lower result (51.5%) was reported by **Kročko et al. (2011)**. Concerning antibiogram of *Enterococcus faecalis*, out of 38 tested strains, 47.4, 44.7, 78.9, 15.8, 28.9, and 44.7% were resist to amoxicillin, gentamicin, ceftriaxone, erythromycin, tetracycline and SXT, respectively; while, 52.6, 55.3, 21.1, 84.2, 71.1, and 55.3% were sensitive to the used antibiotics, respectively (Table 2). From the obtained result it is obvious that, all the tested *Enterococcus faecalis* strains were completely resist to oxacillin while all of them were sensitive to ciprofloxacin (Table 2). This result coincided with (**Al-Marjani, 2013 and Yameen et al., 2013**) as the authors stated that, all the tested *Enterococcus faecalis* strains were 100% resist to oxacillin. Concerning gentamicin, nearly similar result (36.2%) was reported by **Gajan et al. (2013)**, while for erythromycin, **Bulajić and Mjačević (2004)** and **Olawale et al. (2010)** reported 31.46 and 35% resistance of *Enterococcus faecalis*, respectively. For tetracycline, a relatively higher results of resistance 47 and 83% were reported by **Olawale et al. (2010)** and **Gajan et al. (2013)**, respectively.

From Table (2) it is worth to state that, all the tested *Enterococcus faecalis* strains in this study were 100% sensitive to ciprofloxacin and this may be due to low usage of this type of antibiotic in animal treatment by most veterinarian in Assiut Governorate, therefore, the microorganisms stile sensitive to it. While, **Olawale et al. (2010)** and **Oli et al. (2012)** reported 20 and 14.10% resistance for Ciprofloxacin, respectively. From the tested *Enterococcus faecalis*, 33 strains (86.8%) had multi-antibiotic resistance (Table 3) and the resistance is mostly to amoxicillin, oxacillin, gentamicin, ceftriaxone and SXT. Nearly similar result (50%) was reported by **Oli et al. (2012)**.

The presence of *Enterococcus faecalis* in milk is an indication of faecal contamination from milked animals and this may occur through the hands of milk collectors or other human sources. High

contamination levels of Enterococci are considered to cause the deterioration of organoleptic properties in some cheese (**Thompson and Marth, 1986 and Lopez-Diaz et al., 1995**). Moreover, Enterococci produce esterases, which can play an important role in flavour formation (**Tsakalidou et al., 1993**). In addition, the presence of *Enterococcus faecalis* in food is linked with a wide variety of human infections such as endocarditis, urinary and genital tract infections, meningitis and septicemia (**Mannu et al., 2003**).

From Table (1) it is clear that, *Listeria monocytogenes* strains could not be isolated in this study. This result coincided with **Vahedi et al. (2013)** as the authors examined 100 milk raw milk samples and failed to isolate *Listeria monocytogenes*. In contrary, **Mugampoza et al. (2011)** isolated *Listeria monocytogenes* from bulked raw milk in percentage of 13%. The failure of *Listeria monocytogenes* isolation in this study may be due to the small number of examined milk samples or due other factors which need further investigation.

Concerning *E. coli*, out of the examined 50 raw milk samples 39 (78%) of samples gave positive result to presence of *E. coli* (Table 1). Nearly similar results 76, 73 and 75% were estimated by **Hassan and Elmalt (2008)**, **Ahmad et al. (2011)** and **Pourhassan and Taravat (2011)**, respectively. While, lower results 57, 12.5, 63, 32.14, 38, 32.5, 36.66, 52.6 and 42% were recorded by **Soomro et al. (2002)**, **Thabet (2003)**, **Ali and Abdelgadir (2011)**, **Singh et al. (2011)**, **Thaker et al. (2012)**, **Bali et al. (2013)**, **Gwida and El-Ghohary (2013)**, **Meshref (2013)** and **Vahedi et al. (2013)**, respectively. In contrary, higher result 94.4% was reported by **Skočková et al. (2012)**.

Isolation of *E. coli* from milk in this study is a good indicator of faecal pollution (**Benkerroum et al., 2004**), bad hygienic measures during production and handling of milk or milk from animals having subclinical mastitis where in this case the milk appear normally. *E. coli* is a known causative agent of diarrhea and other food-borne related illnesses through the ingestion of contaminated foodstuffs. In addition, presence of *E. coli* in milk and milk products indicates the presence of enteropathogenic

microorganisms, which constitute a public health hazard. Furthermore, enteropathogenic *E. coli* can cause severe diarrhea and vomiting in infants and young children (Anon., 1975).

Concerning antibiotic sensitivity of *E. coli*, out of 39 tested strains 76.9, 89.7, 25.6, and 10.3% of *E. coli* strains were resist to amoxicillin, erythromycin, tetracycline and SXT respectively, while, 23.1, 10.3, 74.4, and 89.7 % of tested strains were sensitive to the tested antibiotics, respectively; moreover, all of them were completely sensitive to gentamicin, ceftriaxon and ciprofloxacin but completely resist to oxacillin (Table 2). Relatively similar results were estimated by Yagoub *et al.* (2005) as the authors detected that, 40 and 50% of tested *E. coli* were resist to amoxicillin and erythromycin, respectively. From Table 2 one can easily concluded that, gentamicin, ceftriaxone and ciprofloxacin were superior in effect to *E. coli* than the other antibiotics used in this study as they gave 100% sensitivity. Nearly similar result was reported by Virpari *et al.* (2013) where the authors detected that, 96.25 of tested *E. coli* strains were sensitive to gentamicin and only 11.25% were resist to ciprofloxacin. In contrary, Mahami *et al.* (2011) reported that, 100 and 87.5% of the tested *E. coli* strains were resist to gentamicin and ceftriaxone, respectively. Concerning tetracycline, the obtained result relatively agreed with Skočková *et al.* (2012) as they reported that, 54.4% of tested *E. coli* strains were resist to tetracycline. The discrepancy of antibiotic sensitivity test from one study to other may be due to the difference of the localities in where the tested strains were investigated.

From Table (2) it is clear that, 100% of tested *E. coli* strains were resist to oxacillin. This is due to oxacillin is narrow spectrum antibiotic that affect only on Gram positive bacteria (*Staph. aureus*). It is logic to not perform this antibiotic for testing *E. coli* (Gram negative), but the authors preferred to fix all the parameters that performed in this study. Out of the 39 tested *E. coli* strains 37 (94.9%) were multi-antibiotic resistant strains (Table 3) and the resistance is mostly to amoxicillin, oxacillin, erythromycin and tetracycline simultaneously. This result is coincided with Yagoub *et al.* (2005), Nováková *et al.* ((2010) and Mahami *et al.*

(2011), as the authors detected that, *E. coli* had multi-antibiotic resistant to various antibiotics. As mentioned earlier, multi-antibiotic resistant microorganisms represent a big hazard for human being.

Out of 50 raw milk samples examined, *Citrobacter freundii* isolated in percentage of 4% (Table 1). Performing antibiotic sensitivity test on *Citrobacter freundii*, all of the isolates were completely resist to amoxicillin, oxacillin and erythromycin while, sensitive to gentamicin, ceftriaxone, tetracycline, SXT and ciprofloxacin (Table 2). Also, all of the tested strains had multi-antibiotic resistant (Table 3) and resist to amoxicillin, oxacillin and erythromycin simultaneously.

Concerning *Yersinia enterocolitica*, 12 samples (24%) were positive for *Yersinia enterocolitica* (Table 1). Nearly similar result 20% was estimated by Hussien *et al.*, (2013). While, lower result 10.6% was reported by Subha *et al.*, (2009). On contrast, El-Leboudy (1989); El-Kholy (1990); Abdel-Hady (1993) and Basha *et al.*, (2008) failed to detect *Yersinia* organisms in Egyptian raw milk samples. On the other hand, higher result 81.4% was reported by Vidon and Delmas (1981).

With regard to *Yersinia enterocolitica* antibiotic sensitivity, 41.7, 41.7 and 33.3% of the tested isolates were resist to erythromycin, tetracycline and SXT, respectively; moreover, all of the tested isolates were completely sensitive to gentamicin, ceftriaxone and ciprofloxacin but completely resist to amoxicillin and oxacillin (Table 2). It is clear that, 100% of the tested isolates were resist to amoxicillin which is coincide with Akhila *et al.* (2013) as the authors reported that *Yersinia enterocolitica* was 100% resist to amoxicillin. Also, oxacillin showed 100% resistance (Table 2) while, Subha *et al.* (2009) reported 50% resistance to oxacillin. On the other hand, gentamicin, ceftriaxone and ciprofloxacin gave 100% effect on *Yersinia enterocolitica* and this result agreed with Pugazhenthii *et al.* (2013) as the authors estimated that, ciprofloxacin was the most effective against *Yersinia enterocolitica*. With regard to erythromycin and tetracycline they gave 41.7% resistance for each (Table 2). Nearly similar result was observed by Akhila *et al.* (2013) as the authors reported 55 and 64%

resistance of *Yersinia enterocolitica* for the two antibiotic, respectively.

It is worth to indicate that, all the tested *Yersinia enterocolitica* isolates in this study had 100% multi-antibiotic resistance and the resistance is to amoxicillin, oxacillin, erythromycin, tetracycline and SXT simultaneously (Table 3). This result coincided with **Akhila et al. (2013)** as they reported that, most of *Yersinia enterocolitica* isolates were multi-antibiotic resistance. Pathogenic strains of *Yersinia enterocolitica* are capable of causing illness in human with a wide range of symptoms. In children and adolescents, symptoms of gastroenteritis, mesenteric lymphadenitis and pseudoappendicitis are predominate, where in adults, symptoms of acute abdominal disorders and arthritis (**Larsen, 1980; Roberts et al., 1995 and Marth and Steele, 2001**). Also, psychotropic bacteria, example *Yersinia spp.*, are associated with Crohn's disease (**Hugot et al., 2003**).

## CONCLUSION

The obtained results in his study indicated that, microbial quality and safety of raw milk sold in some Assiut City markets were unsafe. The presence of faecal indicator organisms (*E. coli* and *Enterococcus faecalis*) not only indicate the poor hygiene but also itself may be pathogenic. The pathogenic bacteria such as *Staph. aureus*, *E. coli*, *Citrobacter freundii* and *Yersinia enterocolitica* may pass to the milk; this suggests that raw milk should be considered as a vehicle for the transmission of potentially pathogenic bacteria. Also, all of these pathogens were multi-antibiotic resistant which impact a hazard on human health.

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## الملخص العربي

### بعض البكتريا ذات الخطورة علي الصحة العامة والمقاومة للمضادات الحيوية المعزولة من اللبن الخام المباع في بعض محلات مدينة أسيوط

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تم في هذه الدراسة تجميع عدد ٥٠ عينة من اللبن الخام المباع في بعض محلات مدينة أسيوط - مصر. وقد تم فحص هذه العينات لعزل بعض البكتريا ذات الخطورة علي الصحة العامة وكانت نسب العزل هي ٤٦، ٧٦، ٧٨، ٤ و ٢٤% لكل من المكور العنقودي الذهبي، المكور السبحي ألبرازي، الميكروب القولوني، ستروباكتز فروندياي واليرسينيا انتيروكوليتيكا علي الترتب. وبفحص جميع العترات المعزولة لاختبار مقاومتها باستخدام ٨ أنواع من المضادات فقد أظهرت جميع العترات مقاومتها لمضادين أو أكثر من المضادات الحيوية. وقد تم مناقشة الأهمية الصحية لهذه الميكروبات.