



Stenotrophomonas species in Milk and some Dairy Products

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ABSTRACT

Stenotrophomonas maltophilia is a multidrug-resistant nosocomial pathogen that is difficult to identify by using current methods. This study aimed to detect *S. maltophilia* in raw milk and some dairy products. A total of 90 raw milk samples including dairy farms, dairy shops and street vendors (30 samples each) were examined. Also, 60 cheese samples (30 Damietta cheese and 30 Kareish cheese), 30 cream and 30 cooking butter samples were examined. Results showed that 25 and 14 *Stenotrophomonas* isolates were recovered from milk and some milk products samples and identified as *S. maltophilia* by biochemical tests and PCR assay, respectively.

Keywords:

S. maltophilia
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Introduction

Stenotrophomonas maltophilia, a global emerging Gram-negative bacteria mostly associated with human infections in the respiratory tract (Barchitta *et al.*, 2009; De Vrankrijker *et al.*, 2010; Brooke, 2012). *S. maltophilia* was initially described as *Bacterium bookeri*, which had been isolated from pleural fluid in 1943 and was subsequently classified as a member of the genus *Pseudomonas* as *Pseudomonas maltophilia* (Hugh and Ryschenkow, 1961) and later changed to *Xanthomonas maltophilia* (Swings *et al.*, 1983) finally coming to rest in *S. maltophilia* (Palleroni and Bradbury, 1993).

Cells of *S. maltophilia* are straight or slightly curved non sporulating Gram negative bacilli that are 0.5 to 1.5 µm long. They occur singly or in pairs and do not accumulate poly-β-hydroxybutyrate as intracellular granules. They are motile by means of several polar flagella. Their colonies are smooth, glistening with entire margins and are white to pale yellow. Moreover, *S. maltophilia* is an obligate aerobe and growth does not

occur at temperatures lower than 5°C or higher than 40°C, the optimal growth temperature is 35°C (Gilardi, 1971).

The Greek word *Stenotrophomonas* comes from *Stenos*, Greek: narrow; *trophos*, Greek: one who feeds; *monas*, Greek: a unit, monad; i.e., a unit feeding on few substrates; and *malt*, Old English: malt; *philos*, Greek: friend; i.e., a friend of malt. The type strain ATCC 13637 isolation in 1958 from an oropharyngeal swab from a patient with oral carcinoma was described (Hugh and Leifson, 1963).

S. maltophilia is not a highly virulent pathogen but it has emerged as a serious nosocomial pathogen associated with crude mortality rates ranging from 14 to 69% in patients with bacteremia (Victor *et al.*, 1994). Infections associated with *S. maltophilia* include (most commonly) respiratory tract infections as pneumonia and acute exacerbations of chronic obstructive pulmonary disease, bacteremia, biliary sepsis, infections of the bones and joints, urinary tract, soft tissues, endophthalmitis, eye infections (keratitis, scleritis, and dacryocystitis), endocarditis and meningitis (Sefcick *et al.*, 1999). In animals, *S. maltophilia* has caused respiratory infections with chronic coughing in horses, canines, and felines (Winther *et al.*, 2010). Other species of *Stenotrophomonas* as *S. africana*, *S. nitritireducens*, *S. acidaminiphila* and *S. rhizophila* were also

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identified as a significant nosocomial pathogen (Ryan et al., 2009).

S. maltophilia is an environmental multiple-drug-resistant organism and it has been isolated from aqueous-associated sources both inside and outside the hospital or clinical setting. *S. maltophilia* occurs ubiquitously and may be isolated from materials used in clinical laboratories and medical practice, hemodialysis water and dialysate samples, contaminated chlorhexidine-cetrimide topical antiseptic, cannulae, prosthetic devices, dental unit waterlines, and nebulizers (Hoefel et al., 2005), foods (Qureshi et al., 2005), water, soil, plants, animals, raw and microfiltered milk (Rasolofa et al., 2010). In addition, *S. maltophilia* exhibits resistance to a broad array of antibiotics, including TMP-SMX (trimethoprim-sulfamethoxazole)-lactam antibiotics, macrolides, cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, chloramphenicol, tetracyclines, and polymyxins.

Owing to, *Stenotrophomonas* species became a common cause of opportunistic infections in humans particularly *S. maltophilia*, this work was planned to determine the incidence of *Stenotrophomonas* species in raw milk and some dairy products.

Materials and methods

A total of 240 random samples of raw milk and some milk products were collected from different localities including dairy farms, dairy shops and street vendors in Assiut Governorate, Egypt, then transferred to the laboratory as soon as possible.

Enrichment procedure

One ml of the homogenized milk samples/1g of prepared sample of milk products was aseptically inoculated into sterile cotton plugged test tube, containing 10 ml of nutrient broth and incubated at 37oC for 24-48 h (Bollet et al., 1995).

Selective plating and identification of isolates

A loopfull from the incubated broth was streaked on plates

of Steno medium agar as described by Goncalves-Vidigal et al. (2011). Streaked plates were incubated at 37oC for 24-48 h followed by identification of isolates by catalase test, oxidase test, lactose and maltose fermentation, hydrolysis of arginine and oxidation fermentation test (Hugh and Leifson, 1953; Speck, 1976; Collins and Lynes, 1989; Land et al., 1991; Baron et al., 1994).

Detection of *S. maltophilia* by using PCR

The 23S rRNA gene was chosen due to the higher variability in this region among species of the *Stenotrophomonas* genus compared to the 16S rRNA gene (Gallo et al., 2013).

Results and Discussion

Presence of *Stenotrophomonas* is rarely documented in milk and milk products, so this study was planned to investigate its presence in milk and milk products. We aimed to detect *Stenotrophomonas* species in milk of different places in Assiut city to have overall prospective on its spread.

The use of steno medium agar has proved to be the most effective selective agar for *Stenotrophomonas* as it actually suppresses the growth of other bacteria.

From the data summarized in Table 1, it's obvious that the highest incidence of *Stenotrophomonas* spp. in Kareish cheese samples (90%) due to the primitive way during its production, processing and handling. Additionally, the incidence of *Stenotrophomonas* spp. were recovered descendingly as 83.33% for each Damietta cheese and ice-cream, 82.22% for raw milk, 70% for cooking butter and the lowest incidence was detected in cream samples (56.67%).

It is evident from the results in Table 2 that 25 and 14 *Stenotrophomonas* isolates were recovered from milk and some milk products samples were identified as *S. maltophilia* by biochemical tests and PCR assay, respectively.

These findings could be attributed to adulteration by addition of water to milk to increase its amount for consumers where *Stenotrophomonas* spp. had been isolated from a wide range of sources including water and soil (Ryan et al., 2009). Moreover, a marked seasonal variation, where the incidence

Table 1. Incidence of *Stenotrophomonas* spp. in the examined samples of milk and milk products.

Examined samples	No. of examined samples	Positive samples	
		No.	%
Raw milk	90	74	82.22
Damietta cheese	30	25	83.33
Kareish cheese	30	27	90.00
Ice-cream	30	25	83.33
Cream	30	17	56.67
Cooking butter	30	21	70.00

Table 2. Incidence of *S. maltophilia* in the examined milk and milk products samples according to the biochemical tests and PCR assay.

Examined samples	No. of examined samples	Biochemical tests		PCR assay	
		No.	%	No.	%
Dairy farms	30	1	3.33	1	3.33
Dairy shops	30	2	6.66	1	3.33
Street vendors	30	4	13.33	2	6.67
Damietta cheese	30	7	23.33	4	13.33
Kareish cheese	30	4	13.33	3	10.00
Ice-cream	30	4	13.33	2	6.67
Cream	30	0	0	0	0
Cooking butter	30	3	10.00	1	3.33
Total	240	25	10.42	14	5.83

peak of *S. maltophilia* infection occurring in the wet season (Heath and Currie, 1995).

The presence of *Stenotrophomonas* spp. in Damietta cheese may be due to the use of raw milk in its production or its contamination after pasteurization and processing. Furthermore, Damietta cheese may be kept for a short period in pickled solution in the groceries.

Conclusion

The results of this investigation clarified that the incidence of *Stenotrophomonas* species in milk, white cheeses (Damietta and Kareish) and ice cream samples was higher than the other samples and that may be due to the environmental condition surrounding milk and cheese production. Therefore, raw milk may be a potential source of infection. The presence of *Stenotrophomonas* in milk is of great concern because of its capability to grow at low temperature and because *S. maltophilia* has emerged as a serious opportunistic pathogen affecting primarily the hospitalized and debilitated host with considerable morbidity and mortality in immunosuppressed patients.

To safeguard consumers from infection we should make health examination of persons who handle milk to prevent transmission of *Stenotrophomonas* spp. by food handlers into the food chain and observation of aqueous-associated environment and regular cleaning and disinfection of surfaces of milking machines, bedding and milk room environment.

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