Original article

Bacteriological Quality of Infant Milk Formula in Tripoli City, Libya

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Abstract

The World Health Organization (WHO) has estimated that a huge number of cases related to food-borne diseases occurs in every part of the world annually. Infant milk formula is known to be a reservoir of many organisms and contributes to neonatal infections. Hence, we decided to characterize and examine the infant formula's quality. Fifty samples of infant milk formula were collected from various locations in Tripoli city, Libya to be tested for its bacteriological quality. The maximum of total aerobic count (TAC) was 4.9 log cfu ml^{-1}, with a mean value 4 log cfu ml^{-1}. 52% of samples contained coliform organisms. The maximum coliform count (MPN/100 ml) was 5.7 log; , with a mean value of 4.4 log. Enteropathogenic *Escherichia coli* (O_{124}:K_{72}: B_{17} and O_{111}:K_{58}:B_4) were isolated from 2 samples. *Enterobacter aerogenes, Ent. Cloacae, Ent. Liquifaciens, Klebsiella aerogenes, K. ozaenae, K. edwardsii* and *K. rhino-scleromatis* were isolated at varying percentages ranging from 4% to 24%. *Staphylococcus aureus* was isolated from 3 samples, while *Proteus spp.* was detected in 56% of examined samples. The total *Enterococci* count/ml of infant milk ranged from 4.3 to 4.6 log cfu ml^{-1} with a mean value of 3.7 log. Therefore, it is concluded that the presence of such microorganisms in infant formula should not be neglected and considered a serious risk with the possibility of the organism to multiply during the preparation and holding time before the consumption of the product. The public health importance of existing microorganisms as well as suggestive control measures was discussed.

Keywords: Infant formula, Microorganisms, Coliform

Introduction

Over the past century, infant formula based on cow's milk has shown steady increase and improvement. Although the Infant milk powders are generally considered as product of good microbiological quality with no risk of spoilage, infant formula milk has been implicated as a source of several organisms in neonatal infections (Farber and Forsythe 2008). In addition, several factors may play a role and contribute to change its physical and chemical properties which reduce shelf-life and thus its commercial value. The shelf life of infant milk, storage stability, flavor quality and high nutritional value of infant milk formula are impaired when it is stored at high temperature over a long period. The manufacture of infant milk formula based on milk powder mixing with various ingredients such as milk powder, vegetables powder, fruits, cereals and other nutritional additives (Rajput *et al.* 2009). Serious health hazards may be found if good manufacture and hygienic practices are not sufficient. Many gastrointestinal disturbances have been attributed to consumption of such contaminated infant milk especially in summer time (Rajput *et al.* 2009).

Although food technology developments and the extreme focus on food safety have led to increase the microbiological standards of food, low risk foods cannot be always ensured. For example, the microorganisms struggle or even unable to grow in infant milk due to its low moisture content and do not play any direct role in their spoilage. However, their occurrence in infant milk powder is of great significance and serves as an index of hygienic standards maintained during production, processing and handling (Lehner and Stephan 2004). The infant milk provides a highly nutritious substrate that can support the wide variety of bacteria as well as yeast and molds for their growth and reproduction (Philips and Griffiths 1990).

There have been many reports explaining the fact of the contamination role of organisms throughout the preparation time of infant milk powder. For instance, economic consequences could be significantly high when the thermophiles exceed specification limits and may lead to down grading of the products (Rueckert *et al.* 2005b; Rueckert *et al.* 2005a).

The microbiological quality of infant milk has not yet been tackled especially concerning the incidence of organisms implicated in cases of gastrointestinal disturbances. The presence of pathogenic bacteria in infant formula milk is of particular concern because of its use as breast milk substitute. The former reason including the lack of researches on this matter led this study to examine and investigate the effect of post process contamination or/and poor handling of infant formula milk particularly in developing countries with warm climate.

Materials and Methods

Fifty random samples of infant milk powder were randomly collected from different groceries, pharmacies and hospitals in Tripoli city, Libya and examined

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bacteriologically for total colony count (cfu/ml), coliform count (MPN/100ml) and *Enterococci* count, as well as for incidence of *Staphylococci* and *Enterococci*.

Preparation of samples, decimal dilutions, culturing and enumeration techniques of bacteria were performed according to the methods described by the American Public Health Association (APHA) (Morton 2001; Swanson *et al.* 2001). Briefly, 25 gm of the sample was transferred to a sterile Stomacher® bag (Stomacher 400, Seaward medicals, UK) under aseptic conditions. The sample was then diluted to a 10^{-1} dilution with 225 Peptone Water (M0216, Park scientific limited, Northampton) and stomached for 2 min by using a Seward's Stomacher® 400 Circulator (Stomacher 400, Seaward medicals, UK.). Then serial dilutions were made using sterile 0.1% peptone water. Determination of the total aerobic count (TAC) was performed using plate count agar (Difco Laboratories, Detroit, MI) which were inoculated with serial dilutions and incubated at 37 °C for 48 h. Countable plates are those containing from 30 to 300 colonies. Most Probable Numbers (MPN) technique was also used in order to determine the actual coliform count by using 3 fermentation tubes containing Lauryl Sulfate Tryptose Broth (LST), supplemented with inverted Durham tubes (Swanson *et al.* 2001). In addition, the identification of isolated E. coli strains was observed by the serological technique (Denka Seiken, Tokyo, Japan).

Enumeration of total *Staphylococci* and *S. aureus* counts were performed using Baird-Parker agar (Park scientific limited, Northampton). The inoculums were surface plated using sterile bent glass streaking rod. Plates were inverted and incubated at 37 °C for 48 h. Suspected *S. aureus* colonies are circular, smooth, convex, moist, 2-3 mm in diameter on un-crowded plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone; colonies have buttery to gummy consistency when touched with inoculating needle. *S. aureus* isolates were identified by microscopical examination and biochemical test (catalase test, coagulase test and thermostable nuclease production test).

Whereas, the enumeration of *Enterococci* was performed using Enterococci Selective Differential media agar (ESD) (Efthymiou *et al.* 1974). The inoculum was surface plated using sterile bent glass rod.

Statistical Analysis

Analysis of variance (ANOVA) was performed by one way ANOVA with *P* value of <0.05 using Microsoft excel 2010 software.

Results and Discussion

Microbiological monitoring of the infant formula is an important aspect of the quality assurance policy, which makes it possible to take corrective measures when an unsatisfactory result is related to infant hospital (Tudela *et al.* 2008).

Table 1 and Figure 1 revealed that maximum colony count of TAC in examined samples was 4.9 log cfu/ml^{-1} , the minimum was 4.6 log, with a mean value of 4 log ±3.5 log cfu/ml^{-1}, the highest frequency distribution (68%) lies within the range 2-4 log cfu/ml^{-1} (Table 2). These results indicate the serious risk of neonatal infections due to such babies' food contamination.

Table 1. Statistical analytical results of different types of microorganisms in examined samples

Type of bacterial count	No. of sample	Positive samples		Min.	Max.	Mean	±SE
		No.	%				
Aerobic TAC log cfu ml^{-1}	50	50	100	4.6	4.9	4	3.4
Coliform MPN/100ml	50	26	52	35	5.6	4.4	4.1
Enterococci TEC log cfu ml^{-1}	50	30	60	32	4.6	3.7	3
DEC log cfu ml^{-1}							
E. faecalis	50	20	40	31	4.3	3.6	2.8
E. faecium	50	12	24	100	3.8	3.1	2.5
E. intermediate	50	20	40	220	4.2	3.6	2.8

cfu= Colony Forming Unit

TAC/ml =Total Aerobic Count cfu/ml

MPN/100ml = Most Probable Number /100ml.

TEC/ml = Total Enterococci Count cfu/ml.

DEC/ml = Differential Enterococci Count cfu/ml

SE = Standard error

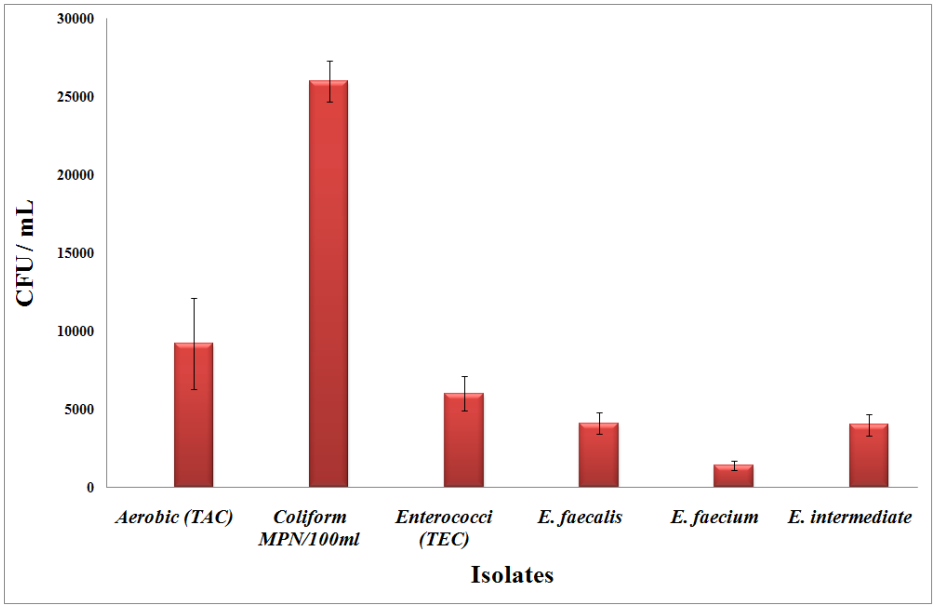

Figure 1. Mean counts of contaminating bacteria of the examined infant milk formula (cfu/ml or MPN/100ml). Error bar represents stander error (SE)

Figure 2 showed that out of 50 samples examined, 26 samples (52%) contained coliforms with a mean count (MPN/100ml) of 4.4 log ± 4.1 log where the highest frequency distribution (69.23%) lies within the range between 2 log ± 4 log (Table 2). The results obtained from this study have shown higher coliform counts in infant milk compared to previous study which was conducted by (Leznik *et al.* 1973).

Table 2. Frequency distribution of examined samples based on their microbial content

Rang	Frequency											
	Aerobic TAC cfu/ml		Coliform MPN/100ml		*E. faecalis*		*E. faecium*		*E. intermediate*		*Enterococci* TEC cfu/ml	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
$10 - 10^2$	9	18	4	15.39	1	5	0	0	0	0	1	3.33
$10^2 - 10^3$	16	32	12	46.15	4	20	9	75	4	20	10	33.33
$10^3 - 10^4$	18	36	6	23.08	12	60	3	25	14	70	12	40
$10^4 - 10^5$	7	14	2	7.69	3	15	0	0	2	10	7	23.34
10^5 - 10^6	0	0	2	7.69	0	0	0	0	0	0	0	0
Total	50	100	26	100	20	100	12	100	20	100	30	100

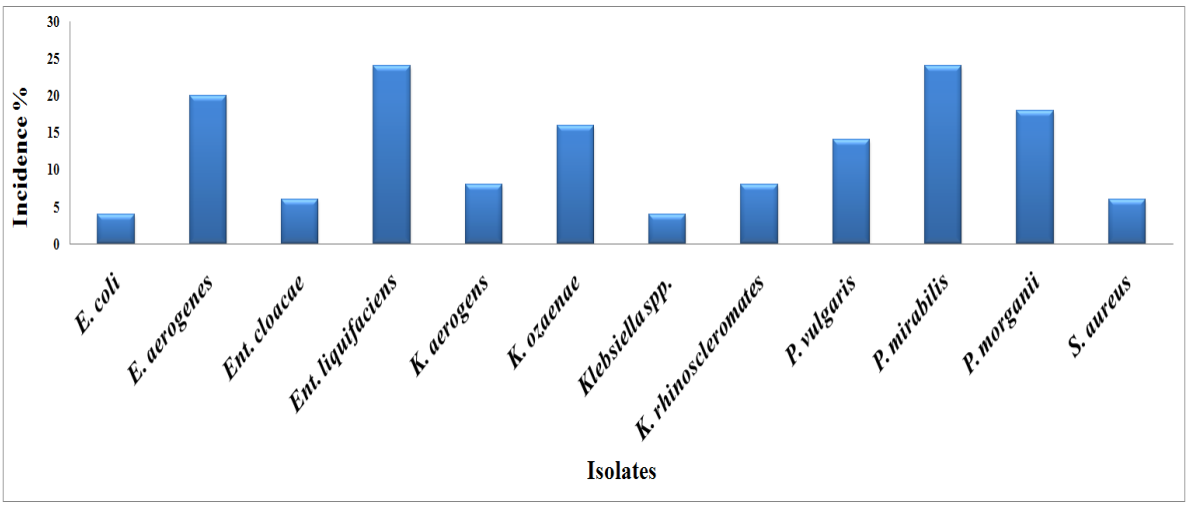

Figure 2. Incidence of isolated organisms in examined samples

Results presented in Figure 2 showed that 4% of total examined samples contained *E. coli*. Different percentages (4% - 24%) of strains belong to O_{124}:K_{72}:B_{17} and O_{111}:K_{58}:B_4. *Enterobacter aerogenes, Ent. Cloacae, Ent. Liquifaciens, Klebsiella aerogenes, K. ozaenae, K. edwardsii V. ed.* and *K. rhinoscleromatis* were isolated. The finding of coliforms persist in any foods article is indicative of sanitary neglected measures during its production, processing and handling; besides they may at times constitute a public health hazard which is a big concern (Mardaneh and Dallal 2013).

The results in this study supported the findings of Tudela *et al*. (2008) in a hospital infant formula room, Of the 156 formulas analyzed, a higher percentage of the premature formulas infants were positive for Bacillus species (54%) while only 19% of the other formulas. The authors suggested that Bacterial contamination could be due to poor handling by operators during reconstitution of the powdered formulas.

Proteus vulgaris, *P. mirabilis*, and *P. morganii* were isolated from 14%, 24% and 18% of examined samples respectively (Fig. 2). Results given in Table 1 and Fig. 1 reveal that the total enterococci count/ml. of infant milk ranged from 4.3 log to 4.6 log, with a mean value of 3.7 log ±3 log. The highest frequency distribution (73.33%) lies within the range2 log-4 log Table 2. The mean counts of *E. faecalis*, *E. faecium* and *E. intermediate* was 3.6 log ±2.8 log , 3.1 log ± 2.5 log and 3.6 log ± 2.8 log per ml/ respectively (Table1 and Fig. 1).

These results suggested that *Enterococci* being a normal inhabitant in the intestinal tract of man and animal, thus their presence in any food article are indicative of fecal contamination. Moreover some species can grow at wide range of temperature and can with stand heat treatment; hence, they may be implicated in cases of food poisoning (Silliker 1980).

Staphylococcus aureus was isolated only from 3 out of the 50 tested samples (6%). Theses isolated strains proved to be strong coagulase positive, meanwhile only two strains of them produced DNase proving to be enterotoxigenic. It should be taken into account that the presence of these strains in infant milk gives a huge opportunity to grow easily and multiply rapidly producing thermostable enterotoxin in the product inducing symptoms of poisoning. The incidence of *Staph.aureus* and its enterotoxin in infants milk has already been reported long time ago by (Hoppner *et al.* 1972). However, the problem remains in many countries especially developing ones including Libya.

Basically, there is absolutely no hesitation in raising the alarm about babies' food being at risk or lead to serious consequences. For example, the most common cultured microorganisms from the neonatal intensive care unit (NICU) were coagulase negative *Staphylococcus* and *Klebsiella pneumoniae*, which were responsible for septicemias at NICU. There were strong relationships between environmental culture results and the agents responsible for the outbreak of septicemia at the NICU. The formula heater at the pediatrics clinic also revealed the same microorganisms with the blood cultures of 3 patients in the same clinic (Buyukyavuz *et al.* 2006).

The results achieves allow to conclude that infant milk was proved to contains different types of organisms at various rates, which can be attributed to low quality ingredients and/or unsatisfactory methods of preparation and handling of such foods. Most of isolated organisms proved to be of public health importance. It is worth mentioning that most infants suffering from gastrointestinal disturbances were fed on infant milk food highly contaminated with coliforms.

It would seem the biggest challenge facing the modern dairy industry is the pressure to improve the product handling alongside the excellent processing environment which leads to produce sterile infant milk formula. Recently there has been considerable concern related to the presence of pathogenic bacteria, in particular *Enterobacter sakazakii*, in powdered infant formula milk. The bacteria in these products at point of sale, with reference to current microbiological testing and the need for good hygienic practice in their subsequent preparation before feeding should be considered. The ingestion of raised numbers of *E. sakazakii* resulting from temperature abuse after reconstitution is highlighted as well as the uncertain routes of *E. sakazakii* product contamination (Forsythe 2005). Up to now, there is lack of information about the mechanism of *E. sakazakii* including fully understanding of its characterization. Therefore, we strongly believe a further study is needed in order to ensure the quality and safety of infant milk.

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