World Applied Sciences Journal 30 (1): 93-98, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.30.01.81255

Detection of Baby Milk Powder Contamination by Microorganisms

[1,2]M.M.M. Ahmed, [2]E.E. Hafez, [2]Mona M. Hassan, [3]Hagar A. Abdelrrassoul and [3]Y.M. Mabrouk

[1]Department of Biological Sciences, Faculty of Science, P.O. Box 80203,
King AbdulazizUniversity, Jeddah, 21589, Saudi Arabia
[2]Nucleic Acids Research Department, Genetic Engineering and Biotechnology Research Institute
(GEBRI), City for Scientific Research and Technology Applications. Alexandria, Egypt
[3]Genetics Department, Faculty of Agriculture, Alexandria University, Egypt

Abstract: Food poisoning is a common, yet distressing and sometimes life-threatening problem for millions of people all over the world. More than 250 different diseases can cause food poisoning. Gram's staining test on these colonies to detect the bacterial type. This step showed that all bacterial samples were gram positive bacteria and confirmed that high degree of similarity between the aerobic and faculatitative aerobic samples in the shape .Six different antibiotics were used to study the resistance of the isolated bacteria against these antibiotics. Different concentrations were used in the culture media starting with 0.005 mg/ml. The MIC was according to the antibiotic activity against the examinated bacteria. The lowest minimum inhibition concentration (MIC) of tetracycline (0.005 mg/ml).then , ampicillin and kanamycin (0.025 mg/ml), then neomycin and spectinomycin (0.08 mg/ml) the highest MIC was in case of chloromphenicol (0.125 mg/ml). This result was confirmed that different degree of antibiotic resistance affecting on the bacterial growth, bacteria have the highest resistance to chloromphenicol and moderate resistance to ampicillin , kanamycin, neomycin and spectinomycin while have the lowest resistance to tetracycline.

Key words: Baby · Milk · Contamination · Microorganisms · Stain · Antibiotics

INTRODUCTION

Food poisoning is a common, yet distressing and sometimes life-threatening problem for millions of people all over the world. More than 250 different diseases can cause food poisoning. The most common diseases are infections caused by bacteria, such as *Campylobacter, Salmonella, Shigella, E. coli, Listeria* and *Cl.Botulinum* toxin. Foodborne illness results from eating food contaminated with bacteria (or their toxins) or other pathogens such as parasites or viruses. The illnesses range from upset stomach to more serious symptoms, including diarrhea, fever, vomiting, abdominal cramps and dehydration.

The conventional microbiological methods to detect these bacteria are time consuming, usually include multiple subcultures and biotype or serotype-identification steps. Rapid and easy detection of pathogenic organisms will facilitate precautionary measures to maintain healthy food [1].

The occurrence of antibiotic-resistant bacteria in food animals is a major public health threat. Antibiotics are used to treat diseases of cattle and as preservatives in milk as well. The indiscriminate use of antibiotics in milk has led to the development of multiple antibiotic resistant populations of bacteria thereby rendering antibiotic treatment ineffective [2, 3] reported high incidence of multiple antibiotic resistant (MAR) pathogenic bacteria in unpasteurized and pasteurized packaged milk.

Milk is an excellent bacteriological medium for a large number of microorganisms. At the time it is drawn from the udder of a healthy animal, milk contains organisms that have entered the teat canal through the teat opening. They are mechanically flushed out during milking. The number present at the time of milking has been reported to range between several hundred and several thousand per milliliter. The source of contamination includes the air in the environment, the milking equipment and the personnel. A variety of diseases are potentially transmissible through milk.

Corresponding Author: Ahmed, M. M. M., Department of Biological Sciences, Faculty of Science, P.O. Box 80203, King Abdulaziz University, Jeddah, 21589, Saudi Arabia.

Important factor influencing the microflora of milk powder is the heat treatment given the milk before the drying process and the method milk drying . Where comparatively severe exposure to heat occurs (for example, in roller-drying)the resulting powder shows a more restricted flora than does one in which the temperatures involved are less extreme (for example, spray-dried powder). In spray-drying the amount of heating to which contaminating bacteria may be exposed is insufficient for the drying process to decontaminate the milk even in respect of relatively heat-sensitive pathogens such as *Salmonella*. Therefore, milk for spry-drying should be pasteurized first and care should be taken that there is no possibility of recontamination between pasteurizer and drier. Further factors influencing the microflora of the powder are the extent of contamination from the milk-plant and the extent to which microbial multiplication can occur before the drying process. When powdered milk is reconstituted, any surviving microorganisms are capable of growth and milk should therefore not be kept for longer than fresh milk once reconstituted [4].

Bacteria of the genus *Enterococcus*are an important group of lactic acid bacteria (LAB), which have a predominant habitat in the gastrointestinal tract of humans and animals. They also persist in the extraenteral environment and can colonize diverse niches due to their high heat tolerance and ability to survive under adverse environmental conditions. Thus, *Enterococci* occur in extended range of foods, especially those of animal origin, such as fermented sausages and cheeses. It is well known, that *Enterococci*are an important part of the bacterial population of several cheeses,such as Manchego [5-10].

Ruminococcus is Gram-positive bacteria. Work has been started on the *Ruminococcus* genome structure. In 1995,*Ruminococcus*are anaerobic bacteria. They obtain nutrients by breaking down cellulose that comes through the digestive system of the host organism.*Ruminococcus* inhabits the rumen of cattle, sheep and goats. [11] Simmering *et al.* [11],proposed a new species; *Ruminococcusluti*, found not in the rumen of cattle, but in human feces.

The aim of present study was application of microbial technology to detect baby milk food infection with microorganisms.

MATERIALS AND METHODS

Materials

Powder Milk Samples: In the present study twelve powder milk samples were purchased from pharmacies belong to twelve different companies. Ten samples were selected according to their growth of microorganisms on solid media, for further tests, the samples and their respective codes were represented in Table (1).

Table 1.The Number of powder baby milk samples and codes.

Sample No.	Sample code
1	N
2	BS1
3	LN
4	SG
5	NA
6	BS2
7	PG
8	A2
9	A1
10	P

Table 2: Kinds of antibiotic

Antibiotic. No.	Antibiotic name	Company	Country
1	Neomycin	Fulka	Switzerland
2	Tetracycline	Sigma	USA
3	Kanamycin	Fulka	Switzerland
4	Chloromphenicol	Sigma	USA
5	Ampicillin	Sigma	USA
6	Spectinomycin	Sigma	USA

Table 3: Antibiotics name, stock solution and reference

Antibiotics	Stock solution(g/ml)	Reference
Ampicillin	50 (H_2O)	Meniates,first part -page
Chloamphenicol	34 (EtOH,MetOH)	A.6 table A.1
Kanamicin	50 (H_2O)	
Neomycin	10 (H_2O)	
Tetracycline	5-12.5 (EtOH,70%EtOH)	
Spectinomycin	10 (H_2O)	

Preparation of solid L.B media: Ten g NaCl, 5g yeast extract, 10gm peptone and 16 gm agar were added to ddH2O,and completed up to 1000 ml, pH was adjusted at 7.2. The mixture was heated till complete dissolving and then autoclaved, after that the media were poured in Petri dishes and were leaved to cool down.

Preparation of L.B liquid media: Teng NaCl, 5gm yeast extract, 10gm peptone was added to ddH2O up to 1000 ml and pH was adjusted at 7.2. They were heated till complete dissolving and then autoclaved, after that the media were poured in test tubes and later were leaved to cooldown.

Microbiology Tests: Gram staining as procedure routine work

Antibiotic Sensitivity Test: Six antibiotics were used in this study. Ampicillin, chloramphenicol, kanamicin, neomycin, tetracycline and spectinomycin. They were chosen randomly were used,Table (3).

Table 4: Minimum inhibition concentration:

Antibiotics	MIC(mg/ml)
Neomycin	0.08
Tetracycline	0.005
Kanamycin	0.025
Chloromphenicol	0.102
Ampicillin	0.025
Spectinomycin	0.08

Table 5: Type of bacterial growth

		Type of growth	
Bacterial code	Growth	Aerobic	Anerobic
N	+	Na	Nan
BS1	+	BS1a	BS1an
LN	+	LNa	--
SG	+	SGa	SGan
NA	-	--	--
BS2	+	BS2a	BS2an
PG	+	PGa	PGan
A2	-	--	--
A1	-	--	--
P	+	Pa	--

Different concentrations from antibiotics were prepared to get MIC(Minimum inhibition concentration). In this experiment (1ml) of solid media:(1µl) antibiotic, 1:2, 1:3, 1:4 and 1:8 were used Table (4).

Fig. 1: Morphological shape of the bacterial isolates using a plate of LB medium.

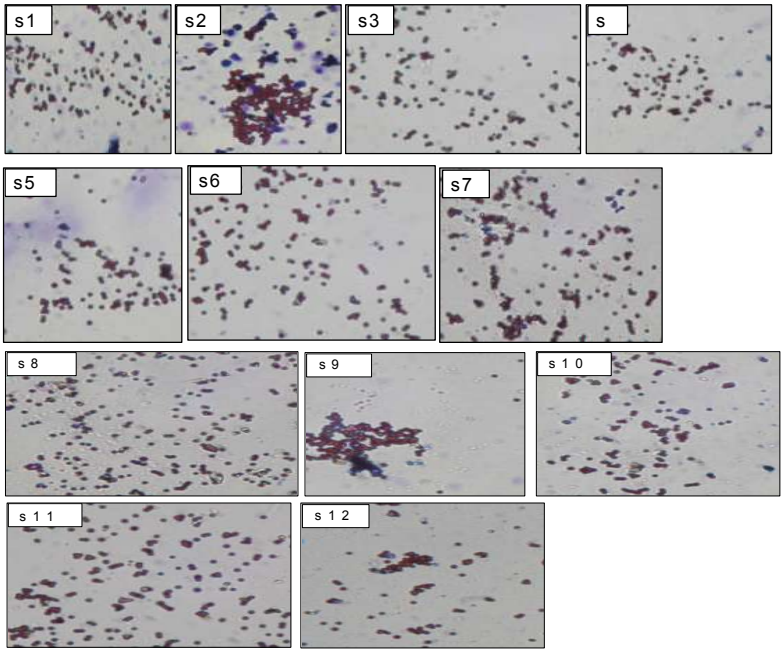
RESULTS

Biological Strains on the Bacterial Isolates

Isolation and Cultures: After collecting samples of milk powder, they were dissolved in water and were streaked onL.B agar and were incubated at 37°Cfor 24 h and then examined for bacterial growth. A colony was inoculated in L.B liquid media then the growth was incubated and shake overnight at 37°C to be used in molecular tests

Morphological Shape: The samples were classified into two groups, the first one contains aerobic bacteria (N a, BS1 a, LN a, SG a, BS2 a, PG a and P a) the second has facultativeanaerobic bacteria (N an, BS1 an, BG an, SG an, BS2 an and PG an).All the selected isolates have the same morphological shape after growing on LB medium as shown in Figure (1)

Gram's Stain: The samples were stained with Gram stain, the two groups resulted in the same morphological shape, all of them were Gram positive. This was shown at Figure (2)



a

b

Fig. 2: a, b. Groups 1&2 show similarity using gram stain as in group a and b.

Table 6: The resistant rate of bacterial isolates against antibiotic

	Resistant Rate			
		Degrees of resistance		
Antibiotics	The ratio between media and antibiotic	R+++	R++	R+
Neomycin	1/1	1a,2a,3a,6a,7a,10a,6b,7b	1b,2b,4b	4a
	1/2	7a,10a,2a,3a,	6a,4a,1a,1b	2b,7b,6b,4b
	1/3	7a,10a,2a,3a,1a	6a,4b,6b,7b,2b,1b,4a	---
	1/4	7a,6a,4a,1a,3a,2a	10a,1b,7b,6b,4b,2b	---
	1/8	---	---	---
Tetracyclin	1/0.5	1a,3a,7a,10a,1b,2b,4b,7b	4a,6a,2a,6b	---
	1/1	---	---	---
Kamycinna	1/0.25	3a,4a,6a,7a,10a,1b,2b,7b	1a,2a,6b,4b	---
	1/0.5	---	---	---
Choloramphnicol	1/1	---	---	4a
	1/2	---	4a	---
	1/3	---	---	---
Ampicillin	1/0.25	1a,2a,3a,7a,6a,10a,1b,2b,7b,7b	2a,4a,6b	---
	1/0.5	---	---	---
Spectenomycin	1/1	1a,2b,1b,4b	2a,7a,10a,3a,6a,4a	---
	1/2	1a	4b,2b,7b,6b,1b,4a,6a,3a,	10a,7a,2a
	1/3	1a,4b	2a,3a,4a,6a,1b,2b,6b,7b	10a,7a
	1/4	1a,4b,2a,3a,6b,2b,7b,1b	4a,6a,10a,7a	---
	1/8	---	---	---

Table 7: The MIC values of the six antibiotics

Antibiotics	MIC(mg/ml)
Neomycin	0.08
Tetracycline	0.005
Kanamycin	0.025
Chloromphenicol	0.102
Ampicillin	0.025
Spectinomycin	0.08

The Antibiotic Resistance: The antibiotics were used to resist the present bacteria in these samples. The antibiotics were (Neomycin, Tetracycline, Kanamycin, Chloromphenicol, Ampicillin and Spectinomycin) they prepared with different concentrations to specify the resistance in bacteria in samples and to determine MIC (Minimum Inhibition Concentration).

Different concentrations of antibiotics from (1ml media / 0.25 µl antibiotic) to (1ml media / 8 µl antibiotic) were used to determine MIC the results were shown in Table (6).

Six different antibiotics were used to study the resistance of the isolated bacteria against these antibiotics. Different concentrations were used in the culture media starting with 0.005 mg/ml. The MIC was according to the antibiotic activity against the examined bacteria. The lowest minimum inhibition concentration (MIC) of tetracycline (0.005 mg/ml).then , ampicillin and kanamycin (0.025 mg/ml). then neomycin and spectinomycin (0.08 mg/ml) the highest MIC was in case of chloromphenicol (0.125 mg/ml). This result was confirmed that different degree of antibiotic resistance affecting on the bacterial growth, bacteria have the highest resistance to chloromphenicol and moderate resistance to ampicillin , kanamycin, neomycin and spectinomycin while have the lowest resistance to tetracycline.

DISCUSSION

The aim of the present work was to identify and detect the food pathogenic bacteria among different types of milk powdersamples using different microbiological techniques, which proved to be efficient tools for milk pathogenic bacteria identification. Also to estimate genetic diversity between these bacteria.

Microbiological Techniques

Gram Stain Test: Gram staining test results showed that all the selected isolates have the same morphological shape (gram positive bacteria) using LB media. This result confirmed that high degree of similarity between the aerobic and faculatitative aerobic samples were found.

Antibiotic Resistance Test: In the current study, six different antibiotics were used to study the resistance of the isolated bacteria against these antibiotics. Different concentrations were used in the culture media

starting with 0.005 mg/ml. The MIC was according to the antibiotic activity against the exanimate bacteria. The lowest minimum inhibition concentration (MIC) of tetracycline (0.005 mg/ml). Ampicillin and kanamycin (0.025 mg/ml) then neomycin and spectinomycin (0.08 mg/ml) the highest MIC was in case of chloromphenicol (0.125 mg/ml). This result was confirmed that different degree of antibiotic resistance affecting on the bacterial growth, bacteria have the highest resistance to chloromphenicol and moderate resistance to ampicillin , kanamycin, neomycin and spectinomycin while have the lowest resistance to tetracycline.These finding are in agreement with the results of [12-14] who said that multiple drug resistant bacteria have been reported all over the world and they can transfer the resistances to a sensitive bacterium by conjugation. Such type of resistance is known as R plasmid mediated antibiotic resistance and is quite dangerous from view point of chemotherapy. R plasmids have also been detected in isolates from milk and they are quite dangerous from public health point of view [15, 16]. Other studies showed that the resistance to ampicillin, tetracycline, oxytetracycline, erythromycin and streptomycin is very high, one of the reasons is the frequent use of these antibiotics in treating the infections of cows, given to increase the yield of milk and to prevent contamination by adding them in bulk quantities of raw milk [17, 18].

As pointed out differences in the extent of bacterial resistance to various antibiotics may reflect the history of antibiotic application and may allow bacterial drug resistance to be used as an indicator of antibiotic application. The indiscriminate use of antibiotics is reported to be one of the major factors that have resulted in the emergence of antibiotic resistant strains of bacteria.

The number of antibiotics/ drugs against which resistance was observed ranged from two to sevenm.Grewal and Tiwari [19] found four different resistance patterns in *E.coli* isolated from foodstuffs. Singh *et al*. [20] found six different resistance patterns in *E.coli*isolated from orange juice and four different types of resistance patterns in *E.coli* isolated from sugarcane juice.

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