

Evaluation of Some Antibiotics Against Pathogenic Bacteria Isolated from Infant Foods in North Africa

M. Shadlia-Matug¹, K.E. Aidoo*¹, A.A. Candlish¹ and A.M. Elgerbi²

¹School of Biological and Biomedical Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow G4 0BA, UK

²Department of Food Technology, Sebha University, Faculty of Engineering and Technology, Brack Ashati, P.O. Box 68, Libya

Abstract: Eighty four samples of commercial infant foods in Libya were examined for microbiological quality. *Bacillus cereus*, *B. stearothermophilus*, *B. licheniformis*, *Staphylococcus xylosum*, *S.lentus*, *Enterobacter sakazakii*, *E. aerogenes* were isolated from the samples. Over 64.3 % of the samples contained high counts of *Bacillus* spp ($\geq 2 \log_{10}$ CFU/g), 42.9% *Staphylococcus* spp ($\geq 2 \log_{10}$ CFU/g) and 26.3% *Enterobacteriaceae* ($\geq 2 \log_{10}$ CFU/g). The moulds isolated were mainly of the genera, *Aspergillus* and *Penicillium*. In relation to antibiotic resistance *Bacillus* spp showed the highest level of resistance to bacitracin (63.6%), ampicillin (54.5%), cephalosporin (36.4%), penicillin (18.1%) and nalidixine acid (18.2%). Corresponding values for *Staphylococcus* spp were bacitracin 60%, erythromycin 30%, penicillin 30%, cephalosporin 10%, nalidixine acid 10% and ampicillin 10%, respectively. *Enterobacteriaceae* strains were resistant to bacitracin (100%), erythromycin (62.5%), ampicillin (37.5%), cephalosporin (25%) and nalidixine acid (12.5 %). *Bacillus* spp, *Staphylococcus* spp and *Enterobacteriaceae* were susceptible to chloramphenicol, kanamycin, gentamicin and streptomycin.

Keywords: Infant food, *Bacillus* spp., enterobacteriaceae, *Staphylococcus* spp., antimicrobial resistance.

INTRODUCTION

Contamination of infant food by microorganisms and natural toxins such as enterotoxins and mycotoxins has been the most common problem resulting in several outbreaks of diseases [1-4]. In most studies reviewed, contamination of infant food formula by pathogenic microorganisms at some points during production resulted in several outbreaks of diseases worldwide. The use of infant formula in developing countries has caused higher rates of diarrhoeal morbidity and mortality, possibly because contaminated water is often used to prepare infant formula and because the high nutrient contents of infant formula provide a good growth medium for bacterial pathogens [5].

Lehner *et al.* [6] reported that when reconstituted formula was stored for about 10 h the bacterial count increased from $< 10^1$ to 10^7 CFU/100 ml. Schmitt *et al.* [7] reported two cases of food poisoning that resulted from the consumption of powdered milk products. Muytjens *et al.* [8] and Forsythe [9] reported the isolation of the *Enterobacteriaceae* from powdered infant formula. The *Enterobacteriaceae* isolated included *Enterobacter agglomerans*, *E. cloacae*, *E. sakazakii*, *Citrobacter koseri* and *Klebsiella oxytoca*.

In New Zealand, there were four identified cases of *E. sakazakii* infection in premature babies - one in 1986, two in 1991 and one in 2004 have been linked to consumption of infant formula [10, 11]. *Enterobacter sakazakii* causes major

infections especially among neonates [2, 12]. Recently the Food Standards Agency, UK reported on the presence of *E. sakazakii* in a Ugandan baby food product [13].

Improperly stored cereal-based product may also become contaminated with fungi and under conditions of high humidity, poor ventilation and warm temperature growth of certain fungi may result in the production of toxic substances (mycotoxins) which are known to be carcinogenic. Studies on occurrence of microorganisms and potential microbial metabolites in baby food and feed formula have received little attention particularly in developing countries.

The aim of the present study was to evaluate the microbiological contamination of commercial infant food available in North Africa and to determine the susceptibility of the isolated bacteria against some antibiotics.

MATERIALS AND METHODS

Samples and Media

Eighty four samples of baby food consumed by Libyan infants were collected from several local sources including retailers, factories and stores. Samples which consisted of imported and locally produced products were stored dry at room temperature ($22 \pm 2^\circ\text{C}$) and examined to determine their level of contamination. The samples contained rice flour, wheat flour, mixed grain cereal contained, wheat, rice, barley, and oat flour, skimmed milk powder or whole milk powder and in various combination. The samples were examined for bacteria species such as *Bacillus* spp, *Staphylococcus* spp, *Enterobacteriaceae* and fungi. Standard methods were used for isolation, enumeration and identification of bacteria and fungi [14, 15].

*Address correspondence to this author at the School of Biological and Biomedical Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow G4 0BA, UK; E-mail: K.Aidoo@gcal.ac.uk

Table 1. Total Bacterial and Fungal Counts and Occurrence of *Bacillus* spp., Enterobacteriaceae and *Staphylococcus* spp. in Infant Foods

Product Number	Number of Product Examined	Main Ingredients	Range log ₁₀ CFU/g of Total Count	Range log ₁₀ CFU/g of Fungi	Number and Percentage of Samples Positive for		
					<i>Bacillus</i> spp.	Enterobacteriaceae	<i>Staphylococcus</i> spp.
P1	8	Rice flour, maltodextrin.	<1.0 – 3.67	1.0 - 3.67	3 (37.5 %)	ND	4 (50 %)
P2	3	Rice flour, malt, maltodextrin.	<1.0 – 3.43	<1.0 – 2.63	1 (33.3%)	ND	ND
P3	1	Wheat flour, sugar, vegetable.	3.81	3.0	1 (100 %)	ND	ND
P4	5	Cereals with milk.	<1.0 – 5.28	<1.0 - 3.3	3 (100 %)	ND	2 (40%)
P5	4	Rice flour.	<1.0 – 3.91	<1.0 – 2.52	3 (75%)	2 (50%)	2 (50%)
P6	7	Wheat flour, banana, skimmed milk powder, whole milk powder, malt extract, milk fat.	<1.0 - 3.54	<1.0 – 3.4	3 (42.9%)	2 (28.6%)	3 (42.9 %)
P7	3	Wheat flour, vegetables (carrot, tomato, peas, spinach), skimmed milk powder, malt extract, milk fat.	<1.0 – 6.43	2.36 – 3.43	2 (67 %)	1 (33.3%)	3(100%)
P8	7	Wheat flour, corn starch.	<1.0 – 3.39	<1.0 – 2.82	4 (57.1 %)	ND	ND
P9	9	Milk, cereal, orange and honey, skimmed milk, wheat flour.	<1.0 – 4.06	<1.0 – 3.32	6 (66.7 %)	5 (55.6%)	6 (66.7%)
P10	3	Wheat flour, skimmed milk powder, banana, malt extract, milk fat.	2.69 – 5.81	2.69 – 3.39	3 (100 %)	ND	2 (66.6 %)
P11	3	Skimmed milk, wheat flour, fruit concentrates (orange, banana, lemon).	<1.0 – 3.82	<1.0 – 2.83	2 (66.6 %)	1 (33.3%)	1 (33.3%)
P12	1	Rice flour.	3.57	2.91	1(100 %)	ND	1 (100 %)
P13	9	Wheat flour, nuts.	1.89 – 5.58	<1.0 – 3.57	5 (55.6%)	4 (80%)	2 (22.2 %)
P14	2	Rice flour, nuts.	2.75 – 2.79	2.43 – 2.80	2 (100 %)	1 (50%)	1 (50 %)
P15	5	Rice flour.	2.44 – 4.41	0.0 – 3.64	3 (60%)	1 (20 %)	1 (20%)
P16	14	Ground nuts and mixed grains.	2.91 – 5.34	0.0 – 3.43	8 (57.1%)	6 (42.9 %)	6 (42.9 %)
Total	84				61.9 %	27.4 %	40.5 %

ND = not detected.

Samples were reconstituted in maximum recovery diluents (MRD). Total aerobic bacterial counts were determined using plate count agar. *Bacillus cereus* was isolated and enumerated using *Bacillus cereus* selective agar (PEMBA) and *Bacillus cereus* agar base. Coliforms and Enterobacteriaceae were isolated and enumerated using Violet red bile glucose (VRBG) agar. *Enterobacter sakazakii* was isolated by using Chromocult® *Enterobacter sakazakii* agar. *Staphylococcus* spp was isolated and enumerated using Baird-Parker agar. Selective enrichment broth (RV) and XLD were used for isolation of *Salmonella*. spp. Moulds and yeast were isolated and enumerated using malt extract agar (MEA) and potato dextrose agar (PDA). All media and diluents were purchased from Oxoid (Basingstoke, UK).

Antimicrobial Compounds Tested on Isolates

The following antimicrobial agents: penicillin, cephalosporin, bacitracin, polymyxin B, streptomycin, tetracycline and gentamicin were purchased from Sigma, (Dorset, UK) and their minimum inhibitory concentrations (MIC) evaluated against some of the bacteria isolated.

Studies on antimicrobial resistance of the isolates were carried out with the following antibiotic test discs purchased from Oxoid (Basingstoke, UK): penicillin G10 units, cephalosporin 30µg, bacitracin 10units, streptomycin 10µg, tetracycline 30µg, gentamicin 10µg, erythromycin 15µg, ampicillin 10µg, chloramphenicol 30µg, kanamycin 10µg and nalidixic acid 30µg.

Cultivation, Enumeration and Identification of Microorganisms

Sterile maximum recovery diluent (225 ml) was added to 25 g of the sample, and then mixed in a stomacher for 60s. The bacteria and fungi present were isolated and enumerated by plating out in a serial dilution for each sample in triplicate onto PCA, PEMBA, Baird-Parker agar, XLD agar and VRBG agar for bacteria and, MEA and PDA for fungi. The plates were incubated at 37°C for 48h and 25°C for 5 days respectively. The results were reported as the percentage of the samples positive for each organism.

Colonies obtained on the culture media were also examined for the following properties: Gram staining, catalase reactions, haemolytic reaction and motility. In addition to these tests each isolate was confirmed using the API 20 E, API staph and API 50 CH tests (Biomerieux, Basingstoke, UK). Fungal isolates were identified according to the method described by Samson *et al.* [15] size, colour and morphology of colonies on media were recorded after incubation at 25° C for 5 days.

Inhibition Assays

Determination of MIC and susceptibility of the isolated bacteria to various antibiotics was performed following National Committee for Clinical Laboratory Standard recommendations (NCCLS, 1993) [16]. Thirty one bacterial strains were used; twenty eight were isolated from the samples and three were type cultures used for control purposes (*Staphylo-*

coccus aureus, NCTC6571 and *E. coli*, NCTC9001 obtained from the National Collection of Typed Cultures and *E. sakazakii* NCIMB 8272 purchased from the National Collection of Industrial and Marine Bacteria Ltd, Aberdeen, Scotland).

Bacterial isolates were cultivated in nutrient broth at 35°C for 2 – 5 h until an absorbance of 0.2 was obtained at wavelength, 450 nm, and equivalent to cell density of 10^8 CFU/ml as described by Carmen *et al.* [17] with some modifications. Swabs were dipped into standardised bacterial suspension and then streaked in three directions over the surface of plate agar and allowed to dry for 5 min before the discs (13 mm) and antibiotics were applied. An aliquot of 0.1 ml antibiotic was placed onto each disc. The inhibition zones (mm) were recorded after incubation at 35 °C for 24 h.

Data Analyses

Tests were carried out in triplicates. Colonies were counted and expressed as \log_{10} CFU/g. Mean and SD was calculated using Microsoft Office Excel 2003 software (Microsoft Corporation, Redmont, Washington, USA). Susceptibility test results were considered when diameter for strains was within the ranges accepted by the National Committee for Clinical Laboratory Standard.

RESULTS AND DISCUSSION

The general microbiological quality of the infant food and feed samples is given in Table 1. The total counts varied over the range ≤ 1.0 to $6.4 \log_{10}$ CFU/g with the mean total count of $3.4 \log_{10}$ CFU/g. Of the eighty four samples examined, 60% were considered microbiologically satisfactory because the total aerobic mesophilic count were $\leq 4.0 \log_{10}$ CFU/g [18]. Nearly 10% (8) of the samples, were deemed unsatisfactory for infant consumption because they contained a total viable count of $\geq 5.0 \log_{10}$ CFU/g powder. The total mould count in most samples was equal to or less than $3.7 \log_{10}$ CFU/g. Average counts of *Bacillus* spp, *Staphylococcus* spp and *Enterobacteriaceae* were 4.4, 4.5 and $3.8 \log_{10}$ CFU/g respectively.

Occurrence of *Bacillus* spp. Enterobacteriaceae and *Staphylococcus* spp. in the samples was 61.9%, 27.4% and 40.5% respectively. Of the 10 (60%) strains of *Bacillus* spp. were identified as *Bacillus cereus*. More than 64.3% of the samples contained high count of *Bacillus* spp. ($\geq 2 \log_{10}$ CFU/g). Becker *et al.* [19] reported that when samples of infant food distributed in 17 countries were examined for *B. cereus*, 54% of them were contaminated with *B. cereus* with levels of $\leq 6 \times 10^2$ viable cells/g, much lower than results found in this study. Dried milk products, such as milk powder, infant milk formula and infant cereal products, contaminated with *B. cereus* should be considered as potential vehicle for foodborne *B. cereus* disease [19, 20]. These products often contain high level of carbohydrates (starch, sucrose or lactose) and minerals which can promote proliferation and enterotoxin production when they are reconstituted and held at ambient temperature for extended periods, potentially even at refrigeration temperature [21-23]. *Bacillus* spp isolated were identified as *B. cereus*, *B. licheniformis*, *Geobacillus stearothermophilus*, *B. subtilis*, *Brevi. Laterosporus* and some unidentified *Bacillus* spp. isolated were similar to that reported by Rowan *et al.* [21].

In this study, it was found that more than 26.3% of the samples contained *Enterobacteriaceae* ($\geq 2 \log_{10}$ CFU/g).

Salmonella was not present in any of the samples tested. Other studies showed occurrence of Enterobacteriaceae in different types of infant food products although *Salmonella* spp were not detected in any of the infant samples examined [8, 10, 24]. Iversen and Forsythe [9] also reported the absence of *Salmonella* spp in powdered infant milk formula. Other pathogens such as *Listeria monocytogenes* and *Salmonella* spp have been shown to be able to tolerate the spray drying, a process used in the production of a number of infant food formulae [25]. Enterobacteriaceae isolates from the products examined were identified as *K. pneumoniae*, *E. aerogenes*, *E. sakazakii*, *K. oxytoca*, *E. coli*, *Aeromonas hydrophila*, *E. cancerogenus*. Two of the samples which showed the presence of *E. sakazakii* were both manufactured in North Africa. Occurrence of *E. sakazakii* in formula and infant feed has been reported [8, 26].

Over 42 % of the samples contained *Staphylococcus* spp ($\geq 2 \log_{10}$ CFU/g). This is in agreement with the study by Benda and Vyletelova [27]. They reported that *S. aureus* counts from milk samples collected from a baby food factory were 2 to $3 \log_{10}$ CFU/g. Also Silvia, *et al.* [28] reported isolating *S. aureus* from 11 samples of milk with counts greater than 10^5 CFU/g. Sugimoto, *et al.* [29] investigated raw and powdered milk samples collected from various milk processing factories for microbial quality and found that viable counts in raw milk ranged from 10^3 to 10^6 CFU/ml and < 10 to 10^3 CFU/g in powdered samples. *Staphylococcus aureus* and *B. cereus* accounted for 79 and 26.3% of the isolates respectively. They isolated 143 strains of *S. aureus* and 13 (9.1%), 5 (3.5%) and 2 (1.4%) of the strains produced enterotoxins B, A and C respectively. Staphylococci are among the most significant pathogens that cause wide spectrum of diseases in both humans and animals. *Staphylococcus aureus* is capable of producing enterotoxins which are resistant to most cooking temperature [30]. The toxin is only produced in sufficient quantities when the bacterial numbers reach 10^5 to 10^8 cells/g or ml of contaminated foods (Leterre, *et al.* [31]. The 27 isolated strains of *Staphylococcus* spp. were identified as *Derma nishinomiyen*, *S. xylosus*, *Kocuria varians*, *S. lentus*, *Kytococ sedenarius*, and *Kocuria rosea*.

Sixty isolates comprising *Bacillus* spp., *Staphylococcus* spp. and *Enterobacteriaceae* were identified by API system (Biomérieux Limited, Basingstoke, UK). All identified isolates were tested for their resistance to antibiotics (Tables 2-4).

Based on MIC break point analysis, a high percentage of bacterial resistance was observed among Gram positive bacteria with some of the antimicrobials tested. For instance 80% of *Bacillus* spp. strains were resistant to cephalosporin (MIC $\geq 2.048 \mu\text{g/ml}$), 60% of strains were resistant to penicillin (MIC ranged 0.128 - $0.256 \mu\text{g/ml}$), while 40% of strains were resistant to bacitracin (MIC ranged 0.128 - $1.024 \mu\text{g/ml}$). Antibiotic susceptibility data with *Bacillus* spp. confirm previous reports that Gram positive bacteria are generally resistant to penicillin and cephalosporin [32-34]. The majority of the Gram positive strains were found to be resistant to penicillin, bacitracin and cephalosporin, probably in part by means of β -lactamase production [34]. For other antimicrobial tested, the tetracycline MIC was $0.128 \mu\text{g/ml}$ and gentamicin MIC was $0.0002 \mu\text{g/ml}$ for *Bacillus* spp.

Table 2. Minimum Inhibitory Concentration (MIC) of Some Antibiotics for *Bacillus* spp. Isolated from Infant Foods

Species	MIC (µg/ml) ^b / Diameter zone (mm ± SD) ^a				
	Gentamicin	Tetracycline	Cephalosporin	Bacitracin	Penicillin
<i>Brevi. laterosporus</i>	0.002 ^a / (22± 0.0) ^b	0.016 ^a / (21 ± 0.5) ^b	-	0.032 ^b / (13± 0.3) ^b	0.064 ^a (18 ± 0.13) ^b
<i>Bacillus</i> .spp	0.001 ^a / (23 ± 0.17) ^b	-	2.048 ^a / (20 ± 0.44) ^b	0.512 ^a / (20± 0.0) ^b	0.00025 ^a (28 ± 0.1) ^b
<i>B. cereus</i> (1)	0.002 ^a / (17 ± 0.0) ^b	0.016 ^a / (15± 0.1) ^b	-	0.016 ^a / (1.5 ± 0.22) ^b	0.256 ^a / (15 ± 0. 1) ^b
<i>B. cereus</i> (2)	0.002 ^a / (20 ± 0.1) ^b	0.016 ^a / (16 ± 0.0) ^b	2.048 ^a / (12 ± 0.1) ^b	0.008 ^a / (14 ± 0.0) ^b	0.128 ^a / (19 ± 0.0) ^b
<i>B. cereus</i> (3)	0.002 ^a / (19 ± 0.2) ^b	0.128 ^a / (20 ± 0.1) ^b	-	1.024 ^a / (16 ± 0.0) ^b	0.256 ^a (18 ± 0.0) ^b
<i>Geobacillus stearothermophilus</i>	0.001 ^a / (15± 0.0) ^b	0.008 ^a / (16 ± 0.1) ^b	2.048 ^a / (16± 0.35) ^b	0.128 ^a / (15 ± 0.33) ^b	0.004 ^a (20± 0. 18) ^b
<i>B. cereus</i> (4)	0.005 ^a / (15 ± 0.5) ^b	0.032 ^a / (16 ± 0.0) ^b	-	0.032 ^a / (16 ± 0.0) ^b	0.256 ^a / (16 ± 0. 1) ^b
<i>B. cereus</i> (5)	0.0002 ^a / (17 ± 0.0) ^b	0.016 ^a / (17 ± 0.3) ^b	2.048 ^a / (15 ± 0.0) ^b	0.032 ^a / (15± 0.1) ^b	0.128 ^a (18 ± 0. 1) ^b
<i>B. licheniformis</i>	0.002 ^a / (19 ± 0.4) ^b	0.016 ^a / (16 ± 0.0) ^b	-	0.004 ^a / (15 ± 0.0) ^b	0.256 ^a (19 ± 0. 2) ^b
<i>B. subtilis</i>	0.0005 ^a / (18 ± 0.32) ^b	0.032 ^a / (20 ± 0.5) ^b	0.256 ^a / (26 ± 0.0) ^b	0.00025 ^a / (14 ± 0. 5) ^b	0.0002 ^a (32± 0.0) ^b
<i>B. cereus</i> (6)	0.001 ^a / (16± 0.12) ^b	0.004 ^a / (19± 0.2) ^b	2.048 ^a / (18± 0.0) ^b	0.032 ^a / (15 ± 0.4) ^b	0.016 ^a / (15± 0. 3) ^b

a=MIC (µg/ ml), b=Diameter Zone (mm ± SD), - = no effect.

Table 3. Minimum Inhibitory Concentration (MIC) of some Antibiotics for *Staphylococcus* spp. and *Micrococcus* spp. Isolated from Infant Foods

Species	MIC (µg/ml) ^a / Diameter zone (mm) ^b				
	Gentamicin	Tetracycline	Cephalosporin	Bacitracin	Penicillin
<i>S. aureus</i> (NCTC 6571)	0.002 ^a / (19 ± 0.4) ^b	0.016 ^a / (25 ± 0.0) ^b	0.512 ^a / (18 ± 1.02) ^b	0.064 ^a / (15 ± 0.0) ^b	0.004 ^a / (40 ± 0.76) ^b
<i>S. xyloso</i>	0.0005 ^a / (16 ± 0.0) ^b	0.016 ^a / (21 ± 0.3) ^b	0.512 ^a / (19 ± 0.0) ^b	0.256 ^a / (18 ± 0.54) ^b	0.064 ^a / (17 ± 0.57) ^b
<i>S. lentus</i>	0.001 ^a / (15 ± 0.1) ^b	0.008 ^a / (24 ± 0.8) ^b	0.512 ^a / (22 ± 0.2) ^b	0.008 ^a / (21± 0.42) ^b	0.0002 ^a 5 / (23 ± 1.02) ^b
<i>Kytococ. Sedenarius</i>	0.0005 ^a / (18 ± 0.5) ^b	0.002 ^a / (13 ± 0.0) ^b	0.512 ^a / (34 ± 0.7) ^b	0.0005 ^a (13 / ± 0.61) ^b	0.00025 ^a / (30 ± 0.9) ^b
<i>Derma.nishinomiyaen</i>	0.0005 ^a / (16 ± 0.36) ^b	0.032 ^a / (21 ± 0.3) ^b	0.512 ^a / (26 ± 0.58) ^b	0.128 ^a / (25 ± 0.0) ^b	0.128 ^a / (20 ± 0.82) ^b
<i>Microoccus</i> . spp (1)	0.00025 ^a / (23 ± 0.5) ^b	0.008 ^a / (17 ± 0.0) ^b	0.512 ^a / (38 ± 0.24) ^b	0.512 ^a / (20 ± 0.5) ^b	0.004 ^a / (23 ± 0.01) ^b
<i>Microoccus</i> . spp (2)	0.001 ^a / (18 ± 0.6) ^b	0.032 ^a / ± (18 ± 0.59) ^b	0.512 ^a / (36 ± 0.53) ^b	0.0002 ^a 5 / (25 ± 0.54) ^b	0.0002 ^a / (33 ± 0.31) ^b
<i>Koc.varians / rosea</i> (1)	0.002 ^a / (13 ± 0.67) ^b	0.032 ^a / (25 ± 0.53) ^b	-	0.032 ^a / (21 ± 0.0) ^b	0.512 ^a / (23 ± 0.35) ^b
<i>Koc.varians / rosea</i> (2)	0.004 ^a / (20 ± 0.2) ^b	0.016 ^a / (19 ± 0.4) ^b	0.512 ^a / (32 ± 0.42) ^b	-	-
<i>S. capitis</i>	0.002 ^a / (21 ± 0.32) ^b	0.008 ^a / (15 ± 0.0) ^b	-	-	2.048 ^a / (15 ± 0.55) ^b

a = MIC (µg/ ml) b = Diameter Zone (mm ± SD) - = no effect.

Table 4. Minimum Inhibitory Concentration (MIC) of some Antibiotics for Enterobacteriaceae Isolated from Foods

Species	MIC (µg/ml) ^a / Diameter zone (mm) ^b				
	Gentamicin	Tetracycline	Cephalosporin	Streptomycin	Penicillin
<i>E. coli</i> (NCTC 9001)	0.002 ^a / (20 ± 0.0) ^b	0.032 ^a / (23 ± 0.2) ^b	0.512 ^a / (22 ± 0.19) ^b	0.004 ^a / (18± 0. 1) ^b	0.128 ^a / (14 ± 0.0) ^b
<i>K. pneumoniae</i> (1)	0.002 ^a / (19 ± 0.0) ^b	0.032 ^a / (16 ± 0.11) ^b	0.256 ^a / (17 ± 0.0) ^b	0.008 ^a / (17 ± 0.01) ^b	0.256 ^a / 15 ± 0.2) ^b
<i>K. pneumoniae</i> (2)	0.002 ^a / (17 ± 0.24) ^b	0.064 ^a / (17 ± 0.0) ^b	0.512 ^a / (21 ± 0.2) ^b	0.004 ^a / (14 ± 0.0) ^b	0.256 ^a / (13 ± 0.1) ^b
<i>E. aerogenes</i>	0.002 ^a / (17 ± 0.33) ^b	0.128 ^a / (19 ± 0.4) ^b	-	0.008 ^a / (12 ± 0. 4) ^b	0.256 ^a / (18 ± 0.03) ^b
<i>E. sakazakii</i>	0.002 ^a / (18 ± 0.0) ^b	0.128 ^a / (18 ± 0.13) ^b	-	0.008 ^a / (16 ± 0. 5) ^b	-
<i>Aero. hydrophila</i>	0.002 ^a / (20 ± 0.15) ^b	0.032 ^a / (19 ± 0.2) ^b	0.128 ^a / (20 ± 0.36) ^b	0.008 ^a / (20 ± 0.14) ^b	0.064 ^a / (18 ± 0.18) ^b
<i>E. cancerogenus</i>	0.0005 ^a / (16 ± 0.5) ^b	0.004 ^a / (19 ± 0.0) ^b	0.128 ^a / (17 ± 0.0) ^b	0.004 ^a / (15 ± 0.22) ^b	0.0128 ^a / (19 ± 0.0) ^b
<i>K. oxytoca</i>	0.004 ^a / (23 ± 0.23) ^b	0.004 ^a / (23 ± 0.3) ^b	0.032 ^a / (21 ± 0.20) ^b	0.256 ^a / (23 ± 0.0) ^b	0.128 ^a / (19 ± 0.5) ^b

a = MIC (µg/ ml), b = Diameter Zone (mm ± SD), - = no effect.

Table 5. The Susceptibility of *Bacillus*. spp Isolated from Various Infant Foods to some Antibiotics

Antimicrobial Agent	<i>Brevi. laterosporus</i>	<i>Bacillus</i> spp.	<i>B. cereus</i> (1)	<i>B. cereus</i> (2)	<i>B. cereus</i> (3)	<i>Geobacillus stearothermophilus</i>	<i>B. cereus</i> (4)	<i>B. cereus</i> (5)	<i>B. licheniformis</i>	<i>B. subtilis</i>	<i>B. cereus</i> (6)
Penicillin G 10 units	9 (I)	29 (S)	8 (I)	10 (I)	0 (R)	10 (I)	9 (I)	8 (I)	7 (R)	10 (I)	12 (I)
Streptomycin (10 µg)	22 (S)	26 (S)	21(S)	25 (S)	20 (S)	24 (S)	25 (S)	22 (S)	20 (S)	26 (S)	24 (S)
Erythromycin (15 µg)	30 (S)	34 (S)	29 (S)	32 (S)	27 (S)	30 (S)	31 (S)	30 (S)	28 (S)	38 (S)	21(S)
Ampicillin (10 µg)	10 (R)	30 (S)	10 (R)	12 (I)	0 (R)	14 (I)	10 (R)	9 R)	10 (R)	30 (S)	12 (I)
Cephalothin (30 µg)	16 (I)	49 (S)	14 (R)	14 (R)	18 (S)	17 (I)	15 (I)	14 (R)	14 (R)	46 (S)	17 (I)
Chloramphenicol (30 µg)	25 (S)	37 (S)	25 (S)	24 (S)	8 (R)	34 (S)	35 (S)	24 (S)	21(I)	35 (S)	27 (S)
Kanamycin (10 µg)	25 (S)	30 (S)	19 (S)	25 (S)	20 (S)	26 (S)	25 (S)	19 (S)	19 (S)	30 (S)	27 (S)
Nalidixic acid (30 µg)	12 (R)	23 (S)	20 (S)	22 (S)	18 (I)	14 (I)	21(S)	19 (S)	17 (I)	21(S)	11(R)
Bacitracin (10 units)	11(R)	10 (R)	18 (S)	21 (S)	0 (R)	12 (R)	13 (R)	19 (S)	22 (S)	13(R)	9 (R)
Tetracycline (30 µg)	27 (S)	21(S)	18 (I)	20 (S)	13 (R)	27 (S)	25 (S)	22 (S)	15 (I)	20 (S)	30 (S)
Gentamicin (10 µg)	23 (S)	36 (S)	22 (S)	21 (S)	20 (S)	25 (S)	22 (S)	22 (S)	22 (S)	29 (S)	24 (S)

R = Resistance, I = Intermediate, S= susceptible.

Table 6. The Susceptibility of *Staphylococcus* spp. and *Micrococcus* spp. Isolated from Various Infant Foods to some Antibiotics

Antimicrobial Agent	Zone Diameter (mm)									
	<i>S. aureus</i> (NCTC 6571)	<i>S. xylosus</i>	<i>S. lentus</i>	<i>Kytococ sedenarius</i>	<i>Derma nishinomiyaen</i>	<i>Micrococcus</i> spp.	<i>Koc varians / rosea</i>	<i>Koc varians / rosea</i>	<i>Micrococcus</i> spp.	<i>S. capitis</i>
Penicillin G 10 units	40 (S)	15 (I)	25 (S)	32 (S)	45 (S)	30 (S)	0 (R)	0 (R)	29 (S)	0 (R)
Streptomycin (10 µg)	18 (S)	23 (S)	16 (S)	16 (S)	29 (S)	15 (S)	18 (S)	19 (S)	13 (I)	18 (S)
Erythromycin (15 µg)	30 (S)	37 (S)	35 (S)	31(S)	45 (S)	35 (S)	10 (R)	13 (R)	28 (S)	0 (R)
Ampicillin (10 µg)	40 (S)	25 (S)	24 (S)	32 (S)	49 (S)	32 (S)	13 (I)	14 (I)	25 (S)	11(R)
Cephalothin (30 µg)	40 (S)	28 (S)	26 (S)	30 (S)	55 (S)	57 (S)	18 (S)	29(S)	25 (S)	6 (R)
Chloramphenicol (30 µg)	30 (S)	19 (S)	36 (S)	52 (S)	42 (S)	3.8 (S)	28 (S)	18(S)	30 (S)	29 (S)
Kanamycin (10 µg)	24 (S)	30 (S)	29 (S)	32 (S)	38 (S)	30 (S)	25 (S)	20(S)	15 (S)	21(S)
Nalidixic acid (30 µg)	30 (S)	17 (I)	19 (S)	25 (S)	30 (S)	24 (S)	21(S)	22(S)	0 (R)	20 (S)
Bacitracin (10 units)	24 (S)	13 (R)	19 (S)	13 (R)	24 (S)	12 (R)	0 (R)	0 (R)	21(S)	0 (R)
Tetracycline (30 µg)	30 (S)	30 (S)	30 (S)	24 (S)	25 (S)	24 (S)	19 (S)	20(S)	24 (S)	20 (S)
Gentamicin (10 µg)	22 (S)	30 (S)	26 (S)	30 (S)	35 (S)	31 (S)	22 (S)	22(S)	19 (S)	20 (S)

R = Resistance, I = Intermediate, S= susceptible.

Table 7. The Susceptibility of Enterobacteriaceae Isolated from Various Infant Foods to some Antibiotics

Antimicrobial Agent	Zone Diameter (mm)									
	<i>K. pneumoniae</i> (1)	<i>E. aerogenes</i>	<i>K. pneumoniae</i> (2)	<i>E. sakazakii</i> (1)	<i>K. Oxytoca</i>	<i>E. sakazakii</i> (2)	<i>Aero hydrophila</i>	<i>E. cancerogenus</i>	<i>E. coli</i> (NCTC 9001)	<i>E. sakazakii</i> (NCIMB 8272)
Penicillin G (10 units)	0 (R)	0 (R)	8 (I)	0 (R)	10 (I)	5 (R)	12 (I)	12 (I)	8 (I)	6 (R)
Streptomycin (10 µg)	19 (S)	17 (S)	13 (I)	20 (S)	21(S)	21(S)	24 (S)	19 (S)	20 (S)	22 (S)
Erythromycin (15 µg)	10 (R)	13 (R)	10 (R)	0 (R)	20 (I)	17 (I)	20 (I)	10 (R)	20 (I)	15 (I)
Ampicillin (10 µg)	8 (R)	10 (R)	18 (S)	5 (R)	18 (S)	25 (S)	20 (S)	22 (S)	18 (S)	25 (S)
Cephalothin (30 µg)	25 (S)	0 (R)	16 (I)	0 (R)	30 (S)	21(S)	17 (I)	20 (S)	23 (S)	20 (S)
Chloramphenicol (30 µg)	29 (S)	26 (S)	25 (S)	25 (S)	29 (S)	33 (S)	25 (S)	29 (S)	30 (S)	35 (S)
Kanamycin (10 µg)	23 (S)	21(S)	24 (S)	22 (S)	21(S)	23 (S)	24 (S)	22 (S)	25 (S)	22 (S)
Nalidixic acid (30 µg)	23 (S)	21(S)	13 (R)	23 (S)	27(S)	20 (S)	23 (S)	20 (S)	27 (S)	30 (S)
Bacitracin (10 units)	6 (R)	0 (R)	6 (R)	6 (R)	7 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Tetracycline (30 µg)	22 (S)	19 (S)	21(S)	23 (S)	23 (S)	34 (S)	26 (S)	22 (S)	25 (S)	38 (S)
Gentamicin (10 µg)	22 (S)	20 (S)	23 (S)	24 (S)	25 (S)	29 (S)	25 (S)	23 (S)	25 (S)	30 (S)

R = Resistance, I = Intermediate, S= susceptible.

Antibiotic resistance profiles of the isolates are shown in Tables 5 to 7. The percentage resistance of Enterobacteriaceae was 100% with bacitracin, 50% erythromycin and penicillin, 30% ampicillin, 20% cephalosporin and 10% nalidixic acid. Cameiro *et al.* [35] reported that 33% of Enterobacteriaceae isolated from 18 infant formula samples were resistance to ampicillin, amoxicillin/ clavulanic acid, cefoxitin and cephalotin. The percentage resistance of *Bacillus* spp was 54.5% ampicillin, 63.6% bacitracin, 36.4% cephalosporin, while 18.2% was resistant to penicillin and nalidixic acid (Table 5). The percentage resistance by *Staphylococcus* spp. was 60% bacitracin and 30% penicillin and erythromycin (Table 6). The typed strain was susceptible to all antibiotics tested. Antibiotic resistance among the Enterobacteriaceae family varied (Table 7). Enterobacteriaceae, *Staphylococcus* spp. and *Bacillus* spp. strains were highly susceptible (100%) to chloramphenicol, kanamycin, gentamicin and streptomycin. These results indicate that there is a considerable variation in susceptibility depending on the species. In most cases there were some antibiotics such as tetracycline, gentamicin, kanamycin and nalidixic acid which were highly active against *Bacillus* spp., *Staphylococcus* spp. and also active against Enterobacteriaceae.

CONCLUSION

Bacterial contamination is of the major concern since they tend to proliferate once a feed is reconstituted with wa-

ter under warm environmental conditions. Also most isolated strains were resistance to some antibiotic agents such as cephalosporin, bacitracin and penicillin. Many of the locally produced infant foods investigated in this study are still produced by small to medium scale manufacturers whose premises, food processing and quality of ingredients may lack a thorough food safety protocol and quality assurance systems in the preparation and/or manufacture of these infant foods. Application of Hazard Analysis Critical Control Point (HACCP) system in the manufacturing processes would improve the quality of such products. An important aspect of the study, yet to be undertaken, is the mycological profile and presence of mycotoxins in the infant food especially when stored under simulated environmental conditions.

ACKNOWLEDGMENTS

SM gratefully acknowledges financial support from the Libyan Embassy, London for research studentship.

REFERENCES

- [1] Michanie A, Frank L, Avarez P, Olivo A. Critical control points for food prepared in households in which babies had *Salmonella*. Int J Food Microbiol 1987; 5: 337-54.
- [2] Lai K. *Enterobacter sakazakii* infection among neonates, children, and adults. Medicine (Baltimore) 2001; 80: 113-22.
- [3] Schocken-Iturrino RP, Carneiro MC, Kato E, Sorbara JO, Rossi OD, Gerbasi LE. Study of presence of the spore of *Clostridium botulinum* in honey in Brazil. FEMS Immun Med Microbiol 1999; 24: 379-82.

- [4] Razzazi-Fazeli E, Noviandi C, Porasuphatana A, Bohm J. A survey of aflatoxins B1 and total aflatoxins contamination in baby food, peanut and products sold at retail in Indonesia analyzed by ELISA and HPLC. *Mycotoxins Res* 2004; 20: 51-8.
- [5] Fone M, Larry R, Michael P, *et al.* Survival and growth of *Shigella flexneri*, *Salmonella enterica serovar Enteritidis*, and *Vibrio cholerae* O1 in reconstituted infant formula. *Am J Trop Med* 2002; 66: 782-6.
- [6] Lehner A, Tasara T, Stehan R. 16S rRNA gene based analysis of *Enterobacter Sakazakii* from different sources and development of a PCR as identification. *BMC Microbiol* 2004; 4: 43.
- [7] Schmitt N, Bowmer EJ, Willoughby BA. Food poisoning outbreak attributed to *Bacillus cereus*. *Can J Public Health* 1976; 67: 418-22.
- [8] Muytjens HL, Roelofs-Willemsse H, Jasper GH. Quality of powder substitutes for breast milk with regard to members of the family *Enterobacteriaceae*. *J Clin Microbiol* 1988; 26: 743-46.
- [9] Iversen C, Forsythe S. Isolation of *Enterobacter sakazakii* and other *Enterobacteriaceae* from powdered infant formula milk and related products. *Food Microbiol* 2004; 21: 771-6.
- [10] International Baby Food Action Network (IBFAN). Urges for better information on infection risks related to baby food. The world Health Assembly held its 58th meeting from 16-25 May 1998 in Geneva. Babies cannot wait 2005 Available at: <http://www.gifa.org>
- [11] Vasavada PC. Testing *Enterobacter sakazakii*: The quest for Testing 2005. Available at: <File://E:\Food safety Magazine.htm>
- [12] Nazaowec M, Farber J. *Enterobacter sakazakii*: a review. *Int J Food Microbiol* 1998; 34: 103-13.
- [13] Health Protection Scotland (HPS. HPS weekly reports) Warning on Ugandan baby food. <http://www.documents.hps.scot.nhs> 2007.
- [14] Roberts D, Greenwood M, Hooper W. Confirmatory biochemical tests. *Practical food microbiology: methods for the examination of food for micro-organisms of public health significance*. 3rd ed: Blackwell Publishing 2004.
- [15] Samson R, Hoekstra E, Frisvad J, Filtenborg O. *Introduction to Food Borne Fungi*. 6th ed. Centraalbureau voor Schimmelcultures: Utrecht Netherlands 2002.
- [16] NCCLS Document M2.A5. Performance standards for Antimicrobial Disk Susceptibility Tests-Fifth ed. Approved Standard 1993; vol. 13: p. 24.
- [17] Carmen D, Rosa M, Borobio M. Application of a spectrophotometric method for determination of post-antibiotic effect and comparison with viable counts in agar. *J Antimicrob Chemother* 2001; 47: 391-8.
- [18] Institute of Food Science and Technology (IFST). *Development and use of Microbiological criteria for foods*: (ISBN 0905367162) London 1991.
- [19] Becker H, Schaller G, Wiess W, Terplan G. *Bacillus cereus* in infant foods and dried milk products. *Int J Food Microbiol* 1994; 23: 1-5.
- [20] Kramer JM, Gilbert JL. *Bacillus cereus* and others *Bacillus* species. *Food-borne Bacterial Pathogens*. Marcel Dekker: New York 1989; pp. 21-70.
- [21] Rowan N, Anderson J, Anderton A. The bacteriological quality of Hospital-prepared infant feeds. *J Hosp Infect* 1997; 36: 259-67.
- [22] Jaquette CB, Beuchat LR. Survival and growth of psychrotrophic *Bacillus cereus* in dry and reconstituted infant rice cereal. *J Food Prot* 1998; 61: 1629-35.
- [23] Juan ER, Jose MB, Manuel RG, Mari OR. Prevalence of *Bacillus cereus* in dried milk products used by Chilean School Feeding Program. *Food Microbiol* 2007; 24: 1-6.
- [24] Chantal M, Martina WR, Leon G, Gorris M, Olivier G, Mike S. Occurrence of *Enterobacter sakazakii* in food production environments and households. *Lancet* 2004; 363. Available at: <http://www.thelancet.com>
- [25] In-tveld PH, Soentoro PSS, Delfgou-Van Asch EHM, Notermans S. Influence of reconstitution on isolation and enumeration of *Listeria monocytogenes* from milk powder used for reference samples. *J Food Protect* 1991; 54: 124-6.
- [26] Van Acker J, De Smet F, Muyldermans G, Anne Naessens A, Lauwers S. Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. *J Clin Microbiol* 2001; 39: 293-7.
- [27] Benda P, Vyletelova M. *Staphylococcus aureus* in bulk milk samples. *Vet Med (Praha)* 1995; 40: 221-6.
- [28] Silvia M, Frank LB, Persia A, Auria BO. Critical control points for foods prepared in households in which babies had Salmonellosis. *Int J Food Microbiol* 1987; 5: 337-54.
- [29] Sugimoto Y, Suzuki A, Ito A, *et al.* Characterization of microflora in raw milk and powdered milk. *J Antibact Antifung Agents* 2004; 32: 243-50.
- [30] Stephen JF. *Enterobacter sakazakii* and other bacteria in powdered infant milk formula. *Matern Child Nutr* 2005; 1: 44-50.
- [31] Letertre C, Perelle F, Fach P. Identification of a new putative enterotoxin SEU encoded by the *egc* cluster of *Staphylococcus aureus*. *J Appl Microb* 2003; 95: 38-43.
- [32] Sabath LD, Abraham EP. Cephalosporinase and penicillinase activity of *Bacillus cereus*. *Antimicrob Agents Chemother* 1996; 39: 2-7.
- [33] Dieter A. Global antibiotic resistance in *Streptococcus pneumoniae*. *J Antimicrob Chemother* 2002; 50: 1-5.
- [34] Cloete T. Resistance and mechanisms of bacteria to antimicrobial compound. *Hyg Disinfect* 2003; 51: 277-82.
- [35] Cameiro LA, Silva AP, Merquior VL, Oueiroz M. Antimicrobial resistance in Gram-negative bacilli isolated from infant formulas. *FEMS Microbiol Lett* 2003; 228: 175-9.

Received: October 14, 2008

Revised: October 20, 2008

Accepted: November 14, 2008

© Shadlia-Matug *et al.*; Licensee Bentham Open.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.