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

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Abstract: Eighty four samples of commercial infant foods in Libya were examined for microbiological quality. *Bacillus cereus, B. stearothermophilus, B. licheniformis, Staphylococcus xylosus, S.lentus, Enterobacter sakazakii, E. aerogenes* were isolated from the samples. Over 64.3 % of the samples contained high counts of *Bacillus* spp (≥2 $\log_{10}$ CFU/g), 42.9% *Staphylococcus* spp (≥2 $\log_{10}$ CFU/g) and 26.3% *Enterobacteriaceae* (≥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 bacittracin (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%, nalidixic 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 ± 2°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].

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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		
					Bacillus spp.	Enterobacteriaceae	*Staphylococcus* 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 concen-trates(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, polymxin 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 naldixic 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 37ºC for 48h and 25ºC 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 ≤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 ≥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. (≥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 ≤ 6 x 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* (≥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 (≥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 (Letertre, *et al.* [31]. The 27 isolated strains of *Staphylococcus* spp. were identified as *Derma nishinomiyaen, 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 (Biomerieux 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 ≥ 2.048 µg/ml), 60% of strains were resistant to penicillin (MIC ranged 0.128-0.256 µg/ml), while 40% of strains were resistant to bacitracin (MIC ranged 0.128-1.024 µg/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 ß- lactamase production [34]. For other antimicrobial tested, the tetracycline MIC was 0.128 µg/ml and gentamicin MIC was 0.0002 µg/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
Brevi. laterosporus	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]
*Bacillus.*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]
B. cereus (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]
B. cereus (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]
B. cereus (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]
Geobacillus stearothermophilus	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]
B. cereus (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]
B. cereus (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]
B. licheniformis	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]
B. subtilis	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]
B. cereus (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
S. aureus (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]
S. xylosus	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]
S. lentus	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]
Kytococ. Sedenarius	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]
Derma.nishinomiyaen	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]
Micrococus. 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]
Micrococus. 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 5 [a] / (25 ± 0.54) [b]	0.0002 [a] / (33 ± 0.31) [b]
Koc.varians / rosea (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]
Koc.varians / rosea (2)	0.004 [a] / (20 ± 0.2) [b]	0.016 [a] / (19 ± 0.4) [b]	0.512 [a] / (32 ± 0.42) [b]	-	-
S. capitis	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
E. coli (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]
K. pneumoniae (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]
K. pneumoniae (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]
E. aerogenes	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]
E. sakazakii	0.002 [a] / (18 ± 0.0) [b]	0.128 [a] / (18 ± 0.13) [b]	-	0.008 [a] / (16 ± 0. 5) [b]	-
Aero. hydrophila	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]
E. cancerogenus	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]
K. oxytoca	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	*Brevi. laterosporus*	*Bacillus* spp.	*B. cereus* (1)	*B. cereus* (2)	*B. cereus* (3)	*Geobacillus stearothermophilus*	*B. cereus* (4)	*B. cereus* (5)	*B. licheniformis*	*B. subtilis*	*B. cereus* (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)									
	S. aureus (NCTC 6571)	*S. xylosus*	*S. lentus*	*Kytococ sedenarius*	*Derma nishinomiyaen*	*Micrococcus* spp.	*Koc varians / rosea*	*Koc varians / rosea*	*Micrococcus* spp.	*S. capitis*
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)									
	K. pneumo-niae (1)	*E. aerogenes*	*K. pneumo-niae* (2)	*E. sakazakii* (1)	*K. Oxy-toca*	*E. sakazakii* (2)	*Aero hydrophila*	*E. cancero-genus*	*E. coli* (NCTC 9001)	*E. sakazakii* (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% nalidixine 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 nalidixine acid which were highly active against *Bacillus* spp., *Staphylococcus s*pp. and also active against Enterobacteriaceae.

CONCLUSION

Bacterial contamination is of the major concern since they tend to proliferate once a feed in reconstituted with water 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.

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