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MICROBIOLOGICAL ANALYSIS AND SAFETY EVALUATION OF VARIOUS CANNED FOODS IN ADDIS ABABA

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ABSTRACT: The microbiological quality and safety of canned foods available to consumers on the market in Addis Ababa were evaluated and the fate of contaminating pathogens in the canned foods, when stored at ambient and refrigerator temperatures, was also assessed. A total of 100 samples of canned foods comprising 20 each of fish, meat, vegetables, milk and soups were collected from Addis Ababa super markets and grocer's shops. The aerobic mesophilic bacteria were dominated by *Bacillus* spp., *Micrococcus* spp. and other Gram positive rods. Staphylococci were encountered in some fish and meat canned food products. *Salmonella* and *Shigella* spp. were not detected in any of the 100 samples. A total of 250 aerobic spore-forming bacilli were isolated from canned foods. *B. cereus*, *B. firmus*, and *B. licheniformis* were the most frequent isolates. Antibiotic susceptibility studies showed that over 88% of Bacillus isolates were sensitive to chloramphenicol, erythromycin, gentamycin and kanamycin. Most isolates exhibited resistance to amoxicillin and ampicillin. Multiple antibiotic resistance (resistance to two or more drugs) was seen in 139 (53%) isolates. The most widespread pattern was Amo, Amp, Cep, Met, PenG, Sxt, Tet. In a challenge study, both *Shigella flexineri* and *Salmonella thyphimurium* grew and multiplied luxuriously in canned meat, fish, vegetable and soup at refrigeration and ambient temperatures.

Key words/phrases: Antimicrobial susceptibility, *Bacillus* spp., Canned foods, Microflora.

INTRODUCTION

Shelf stable canned foods are packed in hermetically sealed containers and are commercially sterile. The application of heat alone or in combination with other treatments to canned foods would result in the elimination of microorganisms capable of growing in the food under normal conditions of distribution and storage.

Currently, there are various types of canned foods produced worldwide. These include canned meat and vegetable salads, powdered milk, canned

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baby foods, mayonnaise and salad dressing products, pickles, jams, jellies and related products, canned soups, canned meat and poultry products, canned sea food products, canned dry pack products, canned juices, fruit drinks and water, canned fruits and canned vegetables.

Despite thermal processing, however, canned foods are subject to microbial spoilage. Spoilage of canned foods is usually caused by growth of microorganisms following leakage or under-processing. A viable mixed bacterial flora is indicative of leakage, which may usually be confirmed by can examination (Warren *et al*., 1998). Under-processing may be caused by undercooking, inaccurate or improperly functioning thermometer, excessive contaminations of the product for which normally adequate processes are insufficient, and product resulting in a more viscous or tighter packing in the container. Under-processed and leaking cans are of major concern and both pose potential health hazards (Warren *et al*., 1998).

Canned foods have also been involved in enteric infections and food poisoning incidents, including cases of typhoid fever, botulism, salmonellosis and staphylococcal poisoning (Foster, 1997). Problems had occurred relating to spoilage of consignments of canned foods from a variety of countries (Foster, 1997).

The main objective of this study was, therefore, to evaluate the microbiological quality and safety of canned foods available to consumers in grocer shops and supermarkets in Addis Ababa. The study also assessed the fate of contaminating pathogens in the canned foods at different storage temperatures and evaluated the distribution of drug resistance among the dominant bacterial groups isolated from the canned foods.

MATERIALS AND METHODS

A total of 100 samples of canned foods comprising 20 each of fish, meat, vegetable, Infant Formula powder milk and soup products were collected from Addis Ababa supermarkets and grocer shops. Date of production and expiration, country of production and ingredients of each sample were recorded. The samples were kept at ambient temperature until further analysis.

Before opening, cans were visually examined for presence of double seam and side seam defects, flaws and physical damage and pertinent observations were recorded. The non-coded end of the metal can was cleaned with alcohol-socked towel. This was flamed using a laboratory burner (Robert *et al*., 1996).

The contents were mixed by shaking cans prior to opening. A sterilized opening device was used to cut the desired size entry hole. Under aseptic conditions, 25 g of samples were removed from the centre and placed into a sterile stomacher bag, 225 ml of sterile bacteriological peptone water (BPW) were added to it and homogenized for 2 minutes using a stomacher (Model 400, Seward). The homogenized samples were serially diluted.

Microbiological enumeration

Appropriate dilution (0.1 ml) of homogenized samples were surface plated in duplicates on pre-dried surfaces of the following media (Oxoid) for microbial enumeration: Plate Count (PC) agar for aerobic mesophilic bacteria (AMB), Violet Red Bile (VRB) agar for coliforms, and Mannitol Salt agar for staphylococci. The plates were incubated under aerobic conditions for 1 to 2 days at 30-32°C. Chloramphenicol Bromophenol Blue (CBB) agar was similarly plated and incubated at 25-28^{0}C for 3 to 4 days for yeasts count (CBB consisted of: yeast extract, 6.0g; glucose, 20.0g; chloramphenicol, 0.1g; bromophenol blue, 0.01g; agar, 15g; distilled water, 1000 ml; pH, 6.0-6.4).

After colony counting, 10 to 15 colonies were randomly picked from countable PC agar plates for further characterization. After repeated purification on Nutrient agar (Oxoid), isolates were microscopically characterized by cell shape, cell grouping, motility, and presence or absence of endospores. Gram reaction of isolates was tested by the KOH test (Gregersen, 1978). Production of the enzyme oxidase was tested according to Kovacs (1956) and formation of catalase was determined by flooding young colonies with 3% solution of H_2O_2. Oxidative or fermentative utilization of glucose by each isolate was assessed by the O/F test (Hugh and Leifson, 1953). The testing medium consisted of (g/l): Peptone, 2g; yeast extract, 1g; NaCl, 5g; K_2HPO_4, 0.2g; glucose, 10g; Bromophenol blue, 0.08g; agar, 2.5g; distilled water, 1000 ml, pH, 7.1. The pH was determined from the original sample using pH meter.

Isolation and characterization of *Salmonella* and *Shigella* spp.

Samples (25 g) were removed from the centre and placed into 225 ml of sterile Buffered Peptone Water (BPW) and incubated at 32^{0}C for 18-24 hours for primary enrichment. After pre-enrichment in BPW, 1 ml of culture was transformed into a tube containing 10 ml of Selenite broth, and 0.1 ml in to a tube containing 10 ml of Rappaport-Vassiliadis (RV) broth for secondary enrichment. Selenite broth was incubated at 37^{0}C and RV broth at

43^{o}C for 48 hours in water bath.

Salmonella-Shigella (SS) Agar and Xylose Lysine Deoxycholate (XLD) medium (Oxoid) were used for plating purposes. A loopful of culture from selective enrichment broth was streaked separately on to each of the solid media and incubated at 37^{o}C for 18-24 hours.

Pure culture of *Staphylococcus* isolates from counting plates were sub-cultured into 5 ml of Nutrient Broth and incubated at 32^{0}C for 24 hours. An overnight broth culture (0.5 ml) was added to a tube containing 0.5 ml of sheep plasma. The tube was rotated gently and incubated in water-bath at 37^{o}C for 2-4 minutes.

Identification and characterization of *Bacillus* spp.

Isolates which were identified as *Bacillus* spp. were purified and tentatively identified using the following biochemical tests (Berkley *et al*, 1984). Growth of culture at 50^{o}C and 65^{o}C was noted after 3 days and 5 days, respectively. Growth in Nutrient broth tubes containing 7% NaCl was observed for 7 to 14 days after incubation at 32^{o}C. Hydrolysis of starch was detected on Nutrient Agar containing 20% soluble starch. The Voges Proskauer test was made in MR-VP broth. Utilization of citrate was detected on Simmon's Citrate Agar slant.

Antimicrobial susceptibility testing for *Bacillus* spp.

Antimicrobial susceptibility testing was done for 256 isolates. From Nutrient Agar plates, two to three pure colonies were inoculated into Nutrient Broth and incubated at 32^{o}C for 18-24 hrs. The growth was standardized to optical density of 0.5 McFarland Standard to bring the cell density to about 10^{7}-10^{8}cfu/ml (Jorgenson *et al*., 1999). The cultures were spread evenly over the entire surface of the Muller Hinton Agar plate by swabbing. The plates were allowed to dry before applying antimicrobial discs.

The following antibiotic discs (all from Oxoid) were used in this study: Erythromycin (Ery) (15µg), Kanamycin (Kan) (30µg), Sulfamethoxazole-Trimethoprim (Sxt) (25µg), Amoxycilin (Amo) (2µg), Ampicillin (Amp) (10µg) Gentamycin (Gen) (10µg), Streptomycin (Str) (10µg), Methicilin (Met) (5µg), Chloramphenicol (Chl) (30µg), Tetracyclin (Tet) (30µg), Vancomycin (Van), (30µg), Penicillin G (Pen G) (l0iu), Polymyxin B (Pol B) (30 iu) and Cephalothin (Cep) (30µg).

Plates were incubated at 32^{o}C for 18-20 hours. Diameter of zones of

inhibition was measured in mm and interpreted as susceptible (S), or resistant (R) (Jorgenson *et al*., 1999). All intermediate results were considered sensitive for the purpose of interpretation.

Determination of Growth Potential of pathogens in canned foods

This study was made to asses the potential hazards related to possible kitchen contaminations after cans are opened and left-overs are kept for later consumption. The growth potential of *Salmonella typhimurium* and *Shigella flexneri* was assessed in canned tuna, farm style vegetable, asparagus, creamy chicken soup, and Borena corned beef. Two cans of each food item were opened aseptically and separately innoculated with overnight culture of the test strains to give an initial inoculum level of 10^2-10^3 cfu/gm. The inoculated foods were mixed thoroughly in their respective cans aseptically and incubated at ambient (20-25^{o}C) and refrigeration (4^{o}C) temperature for 48 hours and 5 days, respectively. To determine the initial inoculum level, freshly inoculated foods (10g each) were homogenized separately into 90 ml of BPW and 0.1 ml of appropriate dilutions was spread plated on McConkey Agar (Oxoid). Portions of inoculated food items (10g each) were further sampled aseptically at six-hour intervals for 48 hours for ambient temperature and at one-day intervals for five days for refrigeration temperature.

RESULTS

Various types of canned foods are imported to Ethiopia in different brand forms. Limited types of canned foods are also produced locally (Table 1). Supermarkets and grocer's shops are the major outlets where the canned products were made available to the consumer. Some canned foods are sold only in supermarkets while others such as Sardines, Tuna, and powder milk were available in both supermarkets and grocer's shops. Venders keep all canned foods at room temperature. Each canned food was labeled with date of production and expiration, ingredients, and appropriate condition of storage. In some canned foods date of production was not given. All canned foods had no seams on cans by visual examination.

The aerobic mesophilic count of the canned products ranged between 2.8 and 5.6 log cfu/g (Table 2). Higher counts (5.6 log cfu/g) were noted in farm style vegetable samples. No significant variation in counts was observed between all farm style vegetable samples (CV<10%). But counts within samples were significantly variable in most other canned foods (CV>10%).

The mean pH of most food items ranged between 5.1 and 5.9. Beef and

vegetable soup was slightly more acidic (pH, 4.7) whereas both milk products had pH values ≥6.2. Values within samples did not show significant variation in most cases (CV<10%).

Table 1. Type and description of canned foods.

	Type	Produced in	Ingredients	Remarks
Fish	Sardine	Unknown	Sunflower oil, fish, salt	Date of expiration and production was labeled
	Tuna	Unknown		
Meat	Borena corned Beef	Ethiopia	Corned beef, salt, sodium nitrite, spices	Date of expiration given Date of production not given
	Beef-in-Jelly	Ethiopia	Beef, jelly, salt, spice	Date of production not given Date of expiration given
Vegetable	Aspargus	South Africa China	Peeled Asparagus, water, salt	Date of production not given on SA products
	Farm style vegetable	USA	water, tomatoes, carrots, potatoes, processed peas, butter, cornstarch, barely, salt, cane sugar, vermicelli, onions and spices	Cooking instructions given
	Mixed vegetables	South Africa	water, carrot, potatoes, celery, sweat peas, green beans, corn, lima beans, salt, onion, and flavoring calcium chloride	Recommended to be consumed without further cooking.
Soup	Creamy chicken soup	Australia, England and Netherlands	chicken stock, chicken thickener (modified maize starch), skim milk, wheat, flour, cream, salt, flavors, sugar, spices, Natural colour (B- carotene, Riboflavin) and water. No preservatives and artificial flavors	Cooking instruction given, boiling not recommended
	Beef and vegetable soup	USA	water, tomatoes, carrots, beef, beans, barley, cornstarch, butter, peas, salt, onions, flavoring agents, cane sugar and spices	Unused soup was suggested to be refrigerated properly
Powder milk	Infant formula I	Netherlands, Dubai	Cow milk and various other nutritional additives	
	Infant formula II		Cow milk and various other nutritional additives	For infants not being breast-fed,

Table 2. Mean pH and counts (log cfu/g) of aerobic mesophilic bacteria of canned food samples.

Food type and number	Sample type	pH	Microbial count	
		Mean ± SD	Mean ± SD	%CV
Fish (20)	Tuna	5.7 ± 0.11	4.14 ± 0.54	13.0%
	Sardine	5.8 ± 0.13	4.22 ± 0.64	15.0%
Meat (20)	Borena corned beef	5.9 ± 0.04	3.68 ± 0.38	10.0%
	Beef in jelly	5.7 ± 0.11	3.86± 0.53	14.0%
Vegetables (20)	Mixed Vegetable	5.2 ± 0.29	4.91 ± 1.32	27.0%
	Asparagus	5.1± 0.39	3.47 ± 1.05	30.0%
	Farm style Vegetable	5.6 ± 0.01	5.56 ± 0.03	0.60%
Soup (20)	Creamy chicken Soup	5.3 ± 0.29	4.24 ± 0.55	13.0%
	Beef and Vegetable soup	4.7 ± 0.02	3.75± 0.39	10.0%
Powder milk (20)	Infant formula I	6.4 ± 0.01	3.25 ± 0.56	17.0%
	Infant formula II	6.2 ± 0.02	2.79 ± 0.23	8%

A total of 679 bacterial isolates were obtained from 93 canned foods and were characterized to various genera and bacterial groups (Table 3). The aerobic microflora was dominated by *Micrococcus* spp. (40.3%), *Bacillus* spp. (37.7%) and other Gram positive Rods (18.3%). *Micrococcus* and *Bacillus* species were isolated from all samples at varying frequencies (Table 3). *Micrococcus* had the highest frequency of isolation from vegetables, soups and powder milk, whereas *Bacillus* species were most frequently isolated from fish and beef products

Table 3. Frequency distribution (%) of dominant bacteria in various canned foods.

Sample	No of isolates	No (%) of isolates				
		Bacillus spp.	*Micrococcus* spp.	*Streptococcus* spp.	*Staphylococcus* spp.	OGPR[1]
Tuna	65	28(43.1)	34(52.3)	2(3.1)	-	1(1.5)
Sardine	68	42(61.8)	9(13.2)	-	-	17(25.0)
Borena corned beef	65	35(53.8)	16(24.6)	-	4(6.2)	10(15.4)
Beef in jelly	61	27(44.3)	16(26.2)	-	2(3.3)	16(26.2)
Mixed Vegetable	48	7(14.6)	30(62.5)	-	6(12.5)	5(10.4)
Asparagus	73	32(43.8)	33(45.2)	-	-	8(10.9)
Farm style Vegetable	17	6(35.3)	8(47.1)	-	2(11.7)	1(5.9)
Creamy chicken Soup	80	28(35.0)	36(45.0)	2(2.5)	-	14(17.5)
Beef and Vegetable soup	49	14(28.6)	22(44.9)	-	4(8.1)	9(18.4)
Infant formula powder milk	73	17(23.3)	35(47.9)	1 (1.4)	-	20(27.4)
Regular powder milk	80	20(25.0)	35(43.7)	2(2.5)	-	23(28.7)
Total	679	256(37.7)	274(40.3)	7(1.0)	18(2.6)	124(18.3)

A total of 256 *Bacillus* species, isolated from the various types of canned foods, were tentatively identified to the species level using a simplified identification scheme based on Berkely *et al*., (1984). A total of 17 different species were identified. *B. cereus*, *B. firmus, and B. licheniformis* were the most common isolates from among the dominant *Bacillus* spp. (Table 4). *B. cereus* was more dominant in beef samples, *B. brevis* in soup samples and *B. firmus* in fish samples.

Salmonella and *Shigella* were not isolated from all samples of canned foods. None of the *Staphylococcus* isolates produced the enzyme coagulase.

Table 4. Distribution of *Bacillus* isolates in canned foods.

	Canned food items					
Isolates	Vegetables	Beef	Soup	Fish	Milk	Total
B. cereus	8	12	8	10	5	43
B. firmus	0	6	5	23	1	35
B. licheniformis	5	11	2	10	2	30
B. brevis	7	1	10	1	5	24
B. macerans	1	9	8	3	2	23
B. larvae	6	2	2	7	4	21
B. subtilis	5	7	1	4	2	19
B. pumilus	6	2	3	2	4	17
B. circulans	3	9	0	0	3	15
Other species	4	3	3	10	9	29

A total of 228 *Bacillus* species were tested for their antimicrobial resistance. Over 60% of the isolates were resistant to Amo and Amp, 55% to PenG and about 40% to Cep, Met, Sxt and Tet. More than 50% *B. cereus* were resistant to Amo, Amp, Cep, Met, PenG, Sxt and Tet. Only <4% of all isolates developed resistance to Van, Str, Kan, Gen, Ery. About 50% of *B. firmus* were resistant to Amo, Amp, Cep, Met, PenG and SXT. All isolates were susceptible to Ery and Van (Table 5). Nearly half of the 30 isolates of *B. licheniformis* were resistant to Amo, Amp, Cep, Met, PenG and Tet (Table 5). However, almost all were sensitive to Chl, Ery, Gen, Kan, PolB, Str and Van. Most of the other species were sensitive to Ery, Gen, Kan and Van. Resistance to Amo, Amp, PenG and Tet was common among these species.

Table 5. Antibiotic resistance of *Bacillus* isolates from various canned foods.

Bacterial Species	No. of strains	Amo	Amp	Cep	Chl	Ery	Gen	Kan	Met	PenG	PolB	Str	SXT	Tet	Van
B. brevis	24	14	19	3	1	-	-	-	5	7	3	-	4	6	-
B. cereus	43	37	33	24	1	-	7	-	27	38	12	1	28	22	1
B. circulans	15	7	8	6	1	-	1	-	6	9	2	1	3	5	-
B .firmus	35	31	34	15	1	-	-	-	14	16	7	1	19	11	-
B. larvae	21	8	9	8	1	1	-	2	6	9	2	2	4	7	2
B. licheniformis	30	16	15	15	2	1	-	2	12	15	2	1	9	12	1
B. macerans	23	15	16	8	3	1	-	-	15	16	5	-	15	15	1
B. pumilus	18	6	9	5	-	-	-	-	5	8	2	-	-	7	-
B. subtilis	19	10	9	9	1	-	1	-	6	10	6	1	5	6	-
TOTAL	228	144	152	93	11	3	9	4	96	126	41	7	87	91	5
(%)		63.1	66.7	40.8					42.1	55.3	18		38.1	39.9	

A total of 26 different MDR patterns were seen among the *Bacillus* species encountered in this study (Table 6). Between two and nine different patterns were observed for the different species. The most widespread pattern was Amo,Amp,Cep,Met,PenG,Sxt,Tet seen in 16 isolates belonging to seven different species followed by Amo,Amp,Cep,Met,PenG,Sxt seen in nine isolates belonging to six species. Other widespread patterns included Amo,Amp,Cep,Met,PenG,PolB,Sxt (six isolates and four species) and Amo,Amp,Cep,Met,PenG,PolB,Sxt (seven isolates and four species). Among the species which were frequently isolated, *B. cereus* showed seven different MDR patterns, *B. firmus* showed nine, *B. macerans* five and *B. licheniformis* four MDR patterns.

Table 6. Multi-drug resistance pattern of *Bacillus* isolates from various canned foods.

Resistance against	Resistance pattern	Species (number) showing the pattern
9 drugs	Amo,Amp,Cep,Gen,Met,PenG,PolB,Sxt,Tet	*B. cereus* (2)
	Amo,Amp,Cep,Kan,Met,PenG,PolB,Sxt,Tet	*B. licheniformis* (1)
	Amo,Amp,Cep,Met,PenG,PolB,Sxt,Tet,Van	*B.macerans* (1)
	Amo,Amp,Cep,Met,PenG,PolB,Str,Sxt,Tet	*B.sphericus* (2)
8 drugs	Amo,Amp,Cep,Met,PenG,PolB,Sxt,Tet	*B. alvae I*(1), *B. circulans* (3), *B. larvae (*1), *B. macerans* (1)
	Amo,Amp,Cep,Gen,Men,PenGSxt,Tet	*B.cereus* (2)
	Amo,Amp,Cep,Met,PenG,Str,Sxt,Tet	*B.larvae* (1)
7 drugs	Amo,Amp,Cep,Met,PenG,PolB,Sxt	*B.cereus* (2) *B.firmus* (2), *B.polymixa* (1), *B.subtilis* (2)
	Amo,Amp,Cep,Gen,Met,PenG,Sxt	*B.circulans* (1)
	Amo,Amp,Cep,Met,PenG,Sxt,Tet	*B.cereus* (6), *B.circulans* (1), *B.firmus* (2), *B.lentimorbis* (1), *B.licheniformis* (3), *B.macerans* (1), *B. subtilis* (2)
	Amo,Amp,Cep,Chl,Met,PenG,Tet	*B. circulans* (2)
	Amo,Amp,Cep,Chl,PenG,Sxt,Tet	*B. firmus* (1)
	Amo,Amp,Cep,Met,PenG,Sxt,Str	*B. firmus* (1)
	Amo,Amp,Cep,PenG,PolB,Sxt,Tet	*B. firmus* (1)
	Amo,Chl,Kan,Met,PenG,Str,Van	*B. larvae* (1)
	Amo,Amp,Cep,Ert,PenG,Sxt,Van	*B. larvae* (1)
	Amo,Amp,Cep,Met,PenG,PolB,Tet	*B. sphericus* (1)
6 drugs	Amo,Amp,PenG,PolB,Sxt,Tet	*B. brevis* (2)
	Amo,Amp,Cep,PenG,Sxt,Tet	*B. brevis* (1) *B firmus* (2)
	Amo,Amp,Cep,Met,PenG,Sxt	*B. cereus* (1), *B. firmus* (2) *B. lentimorbis* (1), *B.lichenifirmis* (2), *B. macerans* (2), *B. polymixa* (1)
	Amo,Amp,Cep,Met,PenG,Tet	*B. circulans* (1)
	Amo,Amp,Cep,PenG,PolB,Tet	*B. larvae* (1)
	Amo,Amp,Met,PenG,Sxt,Tet	*B. macerans* (6)
	Amo,Amp,Cep,Met,Sxt,Tet	*B. larvae* (1)
5 drugs	Amo,Amp,Cep,PenG,Sxt	*B. cereus* (2), *B. firmus* (3)
	Amo,Amp,PenG,PolB,Tet	*B. subtilis* (2)

Where, Ery, Erythromycin; Kan, Kanamycin; Sxt, Sulfamethoxazole-Trimethoprim ; Amo, Amoxycilin; Amp, Ampicillin; Gen, Gentamycin; Str, Streptomycin; Met, Methicilin; Chl, Chloramphenicol; Tet, Tetracyclin; Van, Vancomycin; Pen G, Penicillin G; Pol B, Polymyxin B; and Cep, Cephalothin.

At ambient temperature storage, the growth of *Shigella flexneri* increased by 1 log unit in all canned food products within 6 hours of inoculation. Its growth markedly increased between 6 and 12 h in Tuna, Asparagus, and Creamy Chicken Soup. The fastest growth rate of the test strain was seen in all canned food products between 12 and 24 hours. Maximum counts of 9 log cfu/g were reached at 24 h in Borena Corned Beef and Asparagus. In the other canned foods, the test strain reached highest counts at 36 h. (Fig 1). At refrigeration temperature storage, *Shigella flexneri* showed growth starting from day 1 in tuna, farm style vegetable and asparagus (Fig 2). On the other hand, growth was not detected until day 3 in refrigerated Creamy chicken soup and Borena corned beef. Growth after 3 days was steady in all cases and highest numbers were reached at day 6 ($\geq 10^7$ cfu/g).

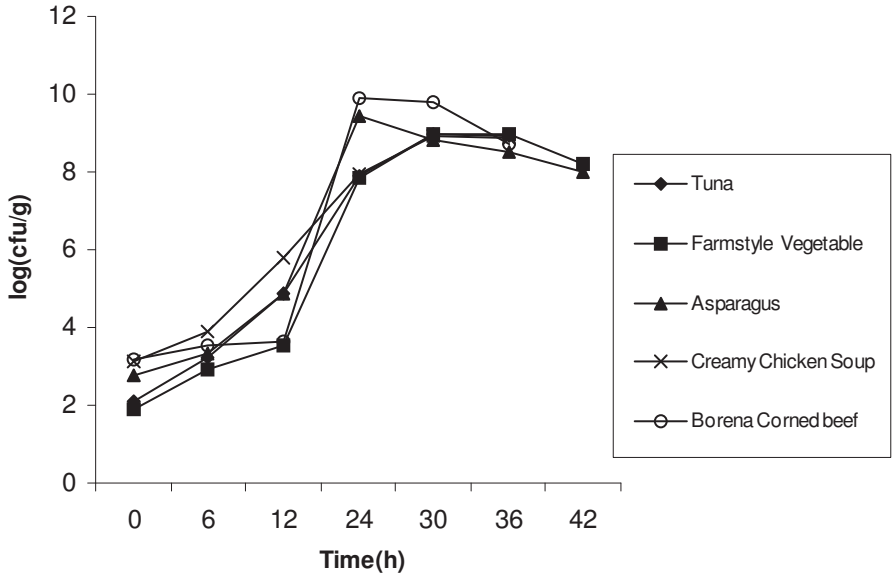

Fig 1. Growth pattern of *Shigella flexineri* at ambient temperature in five canned food products.

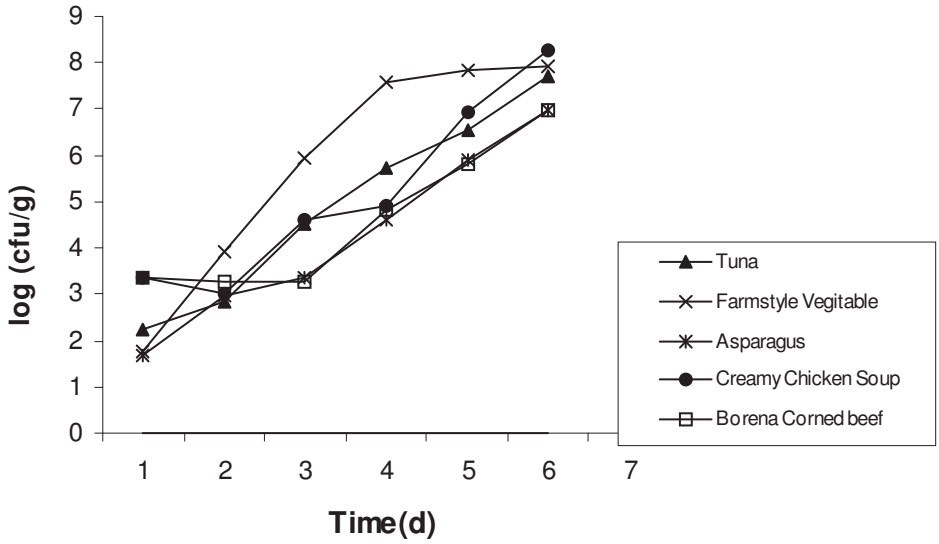

Fig 2. Growth pattern of *Shigella flexineri* at refrigeration temperature in five canned food products.

Under room temperature storage, *Salmonella thyphimurium* increased by about l log unit in the first 6 hours and then showed a steady growth up to 12 hours in all canned foods. Its growth rate in farm style vegetable and creamy chicken soup was relatively higher than in the other canned food products. In most of the canned food products, the highest counts ($>10^8$ cfu/g) were reached at 24 hours. In creamy chicken soup and farm style vegetable, however, the highest count was reached at 30 hours. The *Salmonella* test strain still had counts as high as log 8 cfu/g after 36 hours of storage (Fig. 3). At refrigeration temperature, *Salmonalla thyphimurium* grew steadily in Farm Style Vegetable from day 1. In the other products, growth was markedly low until day 2. Growth was steady in all cases thereafter with final count reaching $>10^7$ cfu/g (Fig. 4).

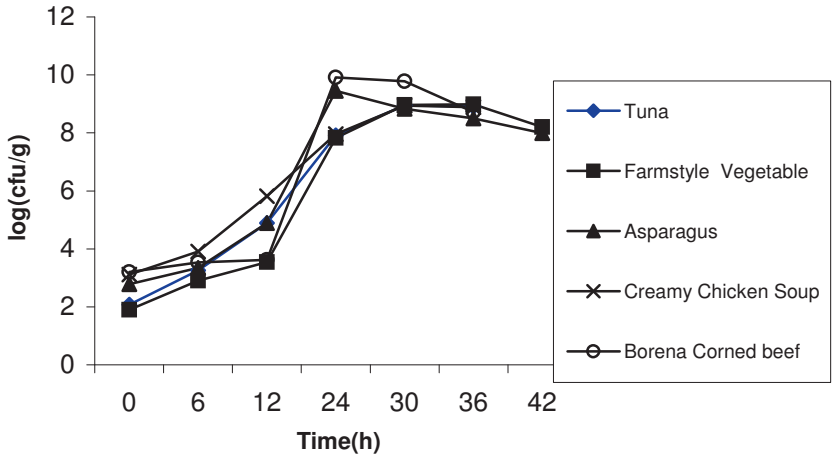

Fig 3. Growth pattern of *Salmonella thyphimurium* at ambient temperature in five canned food products.

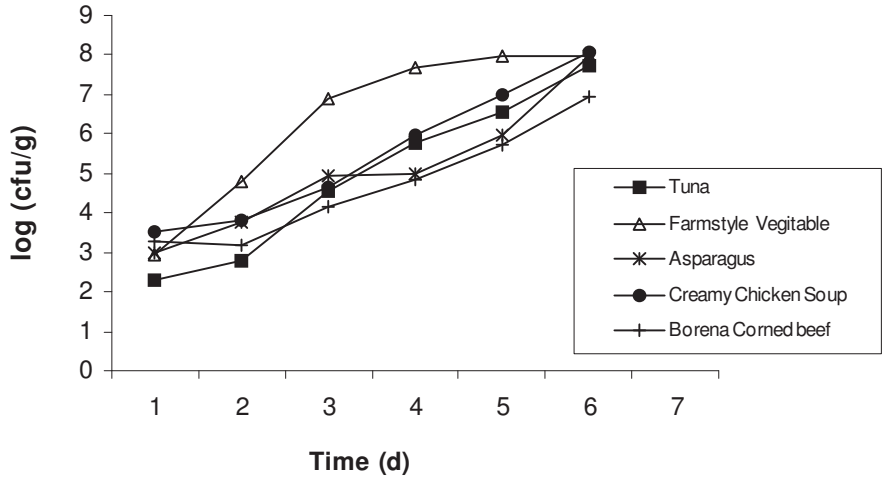

Fig 4. Growth pattern of *Salmonella thyphimurium* spp. at refrigeration temperature in five canned food products.

DISCUSSION

Canning aims to have a product, which is free from pathogens and other microorganisms, which might spoil the food in the container. Canned foods are considered to be commercially sterile. However, as seen in this study, canned foods can be subjected to microbial contamination due to various reasons.

Spoilage of canned foods is usually caused by growth of microorganisms following leakage or under processing. External examination of our samples showed no physical damage of the cans. Thus under-processing may be a possible explanation for the high microbial count, particularly of the non-sporing type. All canned foods in this study were not spoiled, however they were not sterile. Spoilage is not the only cause of abnormal cans. Some microorganisms that grow in canned foods do not produce gas and therefore cause no abnormal appearance of the can. Nevertheless, they cause spoilage of the product (Warren *et al*., 1998).

The canned foods were maintained at ambient temperature at point of display. During warmer seasons in Addis Ababa, spoilage may be aggravated. High summer temperature and low altitude increase the degree of spoilage (Warren *et al*., 1998).

Almost all canned foods contained heat resistant *Micrococcus* spp., *Bacillus* spp. and, some of them had *Staphylococcus* spp., coliforms and yeasts. The presence of coliforms particularly indicated that the canned foods were not properly heat-treated as coliforms could be eliminated at temperatures around 80^{o}C.

The pH values of all samples were >4.6, and, thus, could be considered as low acid canned foods. The pH values permit the growth of most pathogen. In addition, the products are nutritious and can support the growth of a variety of microorganisms. For these products, heat treatment after canning would be the only control factor with respect to microbial safety or spoilage. Factors such as pH and water activity of the heating medium greatly affect the survival of heated cells (Mafart, 2000). Moreover, if initial contamination is excessive, normal adequate processes are insufficient to eliminate contaminants. This could cause the consequent lengthening of the heat penetration time.

The aerobic mesophilic counts of canned fish products (Tuna and Sardine) were high for heat treated products. Similar investigations carried out elsewhere showed that tins of sterilized canned fish were non sterile and

cultures of seeded materials under specified conditions made it possible to establish the presence of residual microflora in the tins of fish (Todorov, 1977). Fish is largely harvested from the marine origin and could be subjected to environmental contamination, including pathogens from the harvested site and on-board-ship handling practice (USFDA, 2001). High protein content, and low acidic nature of the fish products (pH >5.6) could allow these contaminants to grow and produce toxins.

Bacillus spp. were the dominant flora of Sardine products while Tuna products were dominated by heat-resistant *Micrococcus* spp. followed by *Bacillus* spp. *Bacillus* spp. are common in marine environments (Boeye and Aerts, 1976; Ivanova *et al.,* 1999). Presence of *Micrococcus* spp. might be due to contamination during processing or under-processing.

Borena Corned Beef and Beef-in-Jelly were locally produced canned meat products. Borena Corned Beef was a cured product, while Beef-in-Jelly was not. Both products were not commercially sterile. The mean pH value of the beef products was within the pH standards (5.5-6.0) as stated in Warren *et al*. (1998). Addition of sodium chloride and sodium nitrite in canned meat inhibits the out-growth of bacterial spores. Lowering pH and increasing sodium chloride concentration enhance inhibitory action of sodium nitrate (Krumm *et al.,* 1998). Although Borena Corned Beef contained these additives the product was not sterile. This might be due to improper curing. When spoilage occurs, it is usually the result of improper curing rather than inadequate heating (Krumm *et al.,* 1998).

Another study on canned meat showed that some canned meat products contained microbial load with a level of about 10^7/g, mainly dominated by Enterobacteriaceae and *Staphylococcus* spp. (Brock and Bijker, 1976). In the same study the contents of another can contained *Bacillus* spp. at a level about 10^5/g. Inadequate sterilization and errors in processing were suggested as possible causes (Brock and Bijker, 1976). *Bacillus* spp. were the dominant isolates in both canned meat products. The dominant strains were *B. licheniformis*, *B. cereus B. macerans* and *B. circulans*. Inadequate heat treatment, contamination during processing and addition of non-sterilized spices could be suggested as the possible explanation for the presence of these bacteria. Some of the strains of *Bacillus* spp. were frequently isolated from some Ethiopian sauce spices and included *B. circulans, B. licheniformis,* and *B. cereus.* Some of the isolates from the canned products were reported to show proteolytic activities (Feleke Moges and Mogessie Ashenafi, 2000).

Canned infant powder milk formulas normally contain many kinds of dry food additives to improve the nutritional values. They are added after heat-treatment (Richter and Vedamuthu, 2001). Mild heat treatment, addition of non-sterilized additives and absence of good manufacturing practice could be possible reasons for the microbial load of the canned milk products in the present study. The flora of *Bacillus* spp. of the canned milk products was dominated by *B. sphaericus*, *B, cereus* and *B. brevis*. Except *B. polymixa* the other strains were reported to be isolated from human infections (Richter and Vedamuthu, 2001). If leftover milk was consumed, spores could germinate and infect the consumer.

The mean pH values of Asparagus, Mixed Vegetables and Farm Style Vegetables were within the standards for canned vegetables as given in Warren *et al*. (1998). Considering the fact that generally canned vegetables in this study had heavy microbial contamination, special attention should be given to Mixed Vegetables because contents are consumed without further cooking according to the labelled instruction.

The canned soups considered in this study were condensed products and highly viscous. The viscous nature may not allow further proliferation of microorganisms due to lack of available moisture. The presence of microorganisms in the soup products might be due to under-processing and physical effects of their viscosity. Abdul *et al.* (1999), using high viscous liquid as a model liquid food, showed that heat treatment was not evenly distributed and this may explain the microbial quality of the products.

Although most isolates of *Bacillus* spp. were reported as human infectious agents (Kenneth, 2005), *Bacillus cereus* is the most prominent strain that causes food poisoning. Another study on spoiled canned foods reported the presence of *B. subtilis*, *B. megaterium* and *B. brevis* (Kotzekidou, 1996). In semi-canned meat, 50% of the bacilli were determined as *B. licheniformis*, 26% *B. subtilis*, 20% *B. pumilus* and 4% *B. cereus* (Petrova, 1975). These studies indicated that *Bacillus* species diversity was not uniform in canned foods.

B. cereus and *B. thuringiensis* produce a broad spectrum β-lactamase and are, thus, resistant to penicillin, and cephalosporins. Recently, much emphasis has been placed on bacterial gene transfer and acquisition of antibiotic resistance in the environment. Studies have investigated that resistance genes present in *Bacillus* spp. in manure could transfer to indigenous soil bacteria (Agerso *et al*., 2002). This study showed that various patterns of multiple drug resistance were detected in the various

Bacillus spp. As *Bacillus* is widely present in nature, it could contaminate various types of foods. Such foods, thus, would be major sources for transferable multiple resistance genes to pathogens in the gut environment.

Some of the canned foods were challenged with food borne pathogens to evaluate the safety of the foods when and where storing left-overs for a later consumption might be necessary. The findings showed that left-over canned foods, if contaminated, could support the growth of pathogens at room temperature or refrigeration storage. Thus, left-over canned foods must be kept and handled carefully to avoided contamination.

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