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ORIGINAL ARTICLE

Molecular identification of isolated fungi, microbial and heavy metal contamination of canned meat products sold in Riyadh, Saudi Arabia

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Abstract Several studies have shown that canned meat products may be contaminated with fungal elements, bacteria and even heavy metals which may occur during the transportation, storage and handling processes. We conducted this study to determine the fungal, microbial and heavy metal contents of canned meats in Saudi Arabia. Of the 13 canned meat samples studied, *Aspergillus* and *Penicillium* were found in more than 70% of the total samples. Sequences of *Penicillium* species isolated from meat samples generated a phylogenetic tree which shows that the studied isolates were clustered in four groups. No bacterial contamination was noted in all of the samples. Nine of the 13 samples had iron concentrations above the permissible limit. All samples had zinc and copper levels below the maximum permissible limit. Four samples had cadmium levels above the maximum permissible level. All samples had levels of lead above the maximum permissible levels. These results indicate that fungal elements and higher levels of heavy metals such as lead and cadmium can be found in canned meat products. This may pose as a real danger to consumers, since canned meat products are readily accessible and convenient in Saudi Arabia.

1. Introduction

The market for canned foods in Saudi Arabia has increased over the years, with the canned fish/seafood category leading the canned food market in Saudi Arabia (Market Indicator Report, 2011). Meats, when exposed to biological and chemical contamination, can serve as an excellent culture media for growth of microorganisms, since they are a good source of amino acids and nitrogens (BD Diagnostics Manual, 2009). When these microorganisms multiply in food, they produce toxins that are hazardous and even lethal to humans (Billy and Wachsmuth, 1997). Canning of meat may exacerbate contamination by microorganisms when processing practices are poor, especially in states of low acidity and incubation at temperatures above 37 °C. Contamination may also occur during transportation, storage and handling processes. Several bacterial species known to contaminate canned meats include *Escherichia coli*, *Clostridium*, *Staphylococcus aureus*, *Listeria* and *Bacillus* species (Blake et al., 1977; Cragg and

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Andrews, 1973; Mitrica and Granum, 1979). For one, *Clostridium botulinum* can grow at pH above 4.6 and is able to form very heat resistant spores and a lethal toxin (Brooks, 2012).

Several studies have shown that despite strict regulations on processing and canning of food products, a large number of cases of poisoning are reported primarily due to the consumption of canned foods (Czerwiński et al., 2012). In March 1995, a large outbreak of food poisoning among 188 inmates in an institution occurred in Singapore. The incident strongly implicated imported canned pork, as the most probable cause of food poisoning (Ng et al., 1997). It has been shown that mutagens can be produced during heat-processing of canned foods (Krone and Iwaoka, 1984). Furthermore, canned cured meats are more prone to spoilage and potentially hazardous if they are temperature abused after a period of refrigerated storage (Tompkin et al., 1978).

Besides microbial contamination of canned meats, there is also a widespread concern on heavy metal contamination of these canned meat products. Several studies have shown that canned products may have high levels of lead (Pb), the source of which originates from the solder used in the canning process (Mol, 2011). Other heavy metals particularly mercury (Storelli et al., 2010) and cadmium (Cd) have been broadly studied (Shiber, 2011; Storelli et al., 2010; Gutiérrez et al., 2007). Levels of heavy metals in canned fish have been widely reported (Ashraf et al., 2006; Tahvonen and Kumpulainen, 1996). It has also been shown that storage of canned meat gradually increased the concentration of iron (Fe), copper (Cu), lead (Pb) and zinc (Zn) with time in some canned meats (Arvanitoyannis, 1990).

This study was conducted to quantitatively and qualitatively evaluate the microbial and heavy metal contents of canned meats in Riyadh, Saudi Arabia.

2. Materials and methods

Thirteen (13) samples of canned foods comprising three of each of different brands of canned meat within the expiry date as indicated on the cans were randomly (without specific order) collected from supermarkets and shopping malls within Riyadh City, Saudi Arabia between October and November 2012. Samples were taken to the laboratory for analysis. The information on the container/labels was recorded to include NAFDAC (National Agency for Food and Drug Administration and Control) number, manufacture and expiry dates, batch number, manufacturer's address, preservative(s) and compositions. Cans were examined for evidence of bloating, leakage and physical damage.

Details of these samples are listed in the following table.

Sample number	Type of processed meat samples	Producing company and country
1	Corned beef	Queen's Way, Brazil
2	Corned beef	Americana, Packed in Brazil under authority from Food Industries, Dubai, UAE
3	Chicken luncheon meat	Robert (Hot spiced), Damkjaer, Denmark
4	Chicken luncheon meat	Union, Siniora Food Industries, Jordan
5	Pure Beef Luncheon meat	Freshly. Orient Provision & Trading Co., LTD, Brazil
6	Corned beef	Target, Brazil
7	Chicken luncheon meat	California Garden, Packed in Holland under authority from Food Industries
8	Mortadele de Poulet	Danborg, Robert, Damkjaer, Denmark
9	Corned beef	Bordon, Brazil
10	Corned beef	California Garden, Pampeano Alimentos Hulha Negra/RS, Brazil
11	Vienna canned sausages – chicken brand	Picnic, USA, San Diego, California, Food Industries
12	Beef Luncheon	Siniora Food Industries, Jordan
13	Corned beef	Target, Brazil

Prior to analysis, the surface of the container was cleaned with 70% ethanol and tincture of iodine. Containers were opened near the flame of the Bunsen burner to avoid contamination. The pH of the samples was recorded using a pH meter.

3. Bacteriological analysis

Ten gram portions of the foods was blended in a sterile warring blender and inoculated into triplicate tubes of 90 ml Brain Heart Infusion (BHI) broth (Oxoid) and Cooked Meat medium (Oxoid) and into MacConkey and EMB agar plates that were incubated aerobically at 37 °C and 45 °C for Coliforms. At the end of incubation time, colonies were counted using colony counter (Stuart Scientific, UK). Results were expressed as cfu/g. Characteristic colonies on plates were Gram stained, purified by repeated subculturing and stored on agar slants or agar stab if anaerobic, until further characterization. Identification of isolates was done by Gram staining, indole test, urease test, catalase test, methyl red test, citrate utilization test, Vogues-Proskauer test, gelatin liquefaction, starch hydrolysis, sugar fermentation tests, motility and cultural characteristics on culture media.

Confirmatory identification was based on the following methods; Plain agar for total bacterial count (TBC), Violet Red Bile agar for total and fecal coliform count (FCC), Baird Parker agar for *Staphylococcus* count, *Bacillus cereus* agar for *Bacillus cereus* count, Brilliant green agar for Salmonella count and Rose Bengal agar for Total mold count.

4. Mycological analysis

The dilution plate method described by Pitt and Hocking in 2009 was employed for this purpose (Easa, 2010). Proper dilution rates ranged from 1/2 to 1/10 (weight/volume). Cultures in 5 replicates per sample were prepared and incubated at 28 °C for 7–10 days after which the growing colonies were counted, identified and isolated in pure cultures.

5. Isolation media

Two types of media were chosen for the mycological analysis of the processed meat samples as used by Taniwaki et al. (2009), King et al. (1979), Pitt and Hocking (2009) and Samson et al. (2004). These media were:

5.1. Dichloran Rosebengal Chloramphenicol agar (DRBC)

The medium contains (g/l): peptone 5 g, glucose 10 g, KH_2PO_4 1 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, dichloran (2,6-dichloro-4-nitroaniline) solution (0.2% (w/v) in ethanol) 1 ml, Rose Bengal 0.025 g, chloramphenicol 0.1 g, agar 15 g, and distilled water 1000 ml. Final pH should be 5.6. Ingredients are mixed, heated to dissolve agar and sterilized by autoclaving at 121 °C for 15 min. The medium is allowed to cool to 45 ± 1 °C in a water bath prior to pour plating. DRBC agar is especially useful for analyzing sample containing "spreader" molds (e.g. *Mucor*, *Rhizopus*, etc.), since the added dichloran and rose bengal effectively slow down the growth of fast-growing fungi, thus readily allowing detection of other yeast and mold propagules, which have lower growth rates.

5.2. Dichloran 18% Glycerol agar (DG18)

It comprises (g/l): peptone 5 g, glucose 10 g, KH_2PO_4 1 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, dichloran (0.2% in ethanol) 1 ml, glycerol 220 g, chloramphenicol 0.1 g, agar 15 g, and distilled water 1000 ml. These media were autoclaved at 120 °C for 20 min. Mix the above items and steam to dissolve agar, then bring the volume to 1000 ml with distilled water. Add 220 g glycerol and sterilize by autoclaving at 121 °C for 15 min. The final pH should be 5.6 and the final a_w, 0.955. This medium is used as a general purpose mold enumeration medium and is preferred when the a_w of the analyzed food is 0.95 or lower. The low water activity of this medium reduces interference by bacteria and fast-growing fungi.

5.3. Identification of fungal cultures

Macro- and microscopic morphological features were used to identify the different fungal isolates contaminating the processed meat samples. The following references were consulted to check correct identification: de Hoog et al. (2002), Samson et al. (2004) and Pitt and Hocking (2009).

6. Evaluation of toxic heavy metal contamination

All 13 samples were analyzed for toxic heavy metal contamination. Microwave-assisted acid digestion was used for all of the samples, and the elemental contents and their infusions were determined by FAAS and ICP-AES. Microwave digestion procedure was applied under optimized conditions for dissolution of herbal plants.

6.1. Acid digestion, FAAS and ICP-AES

In 400 ml cylinder, 300 ml of concentrated HCl and 100 ml of concentrated HNO_3 were added. The mixture was transferred to a 1000 ml volumetric flask and the volume was made up with diH_20. The mixture was inverted to mix and allowed to stand.

Samples were dried at 70 °C for 24 h after which they were grinded in a Spex mill. Crucibles and caps were prepared by washing in 10% HNO_3 and muffled at 75 °C for 2 h. Ground samples of 0.5 g were placed in each crucible using a Mettler (four-place) balance and placed in muffle furnace to bring down to ashing temperature (45 °C) slowly for 90 min and ashed for 4 h. Crucibles were allowed to cool. Linear range of concentrations was determined for the wavelength to be used and an appropriate sample dilution scheme was devised. Diluted extract was measured for metal content by an atomic absorption spectrophotometry.

In the analyses of heavy metal contamination, the permissible concentrations were set as follows; maximum of 0.15 mg/kg for nickel (Ni), maximum of 0.2 mg/kg for lead (Pb), maximum of 0.5 mg/kg for cadmium (Cd), maximum of 10 mg/kg for copper (Cu), maximum of 50 mg/kg for zinc (Zn) and maximum 20 mg/kg for iron (Fe) (Salama and Radwan, 2005; Mariam et al., 2004; CAC, 2003; Demirezen and Uruc, 2006).

7. Genotypic identification of fungi isolates

Nucleotide sequencing of rRNA genes of some *Penicillium* species was done with the help of SolGent Company, Daejeon, South Korea. Primers used for gene amplification have the following composition: ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′), and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). Then the amplification was carried out in a thermal cycler under the following conditions: one round of denaturation at 95 °C for 15 s followed by 30 cycles of denaturation at 95 °C for 20 s, annealing at 50 °C for 40 s and extension at 72 °C for 1 min, with a final extension step at 72 °C for 5 min. The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. Purified PCR products were reconfirmed (using size marker) by electrophoreses on 1% agarose gel. Then these bands were eluted and sequenced with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture. Each sample was sequenced in the sense and antisense directions using ITS1 and ITS4 primers (White et al., 1990). Sequences were further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

8. Results

The mycological analysis of the 13 samples of canned meat revealed the isolation of 13 fungal species belonging to five genera (Tables 1,2,3). The total fungal population on DRBC was relatively higher than that on DG18 (1912 versus 1288 colonies/g fresh meat). *Aspergillus* and *Penicillium* were the most common genera on both medium types (Tables 1–3).

On DRBC medium, *Penicillium* was the major fungus contaminating all samples recording the highest total count among all fungi, accounting to 77.8% of total population (Table 3). *Penicillium* was represented by four species of which *Penicillium chrysogenum* was the most prevalent (69.23% of samples matching 63.6% of total fungal count). The remaining *Penicillium* species (*Penicillium citrinum*, *Penicillium gris-*

Table 1 Counts (colonies/g fresh meat) of fungal genera and species isolated from canned meat samples on Dichloran Rosebengal Chloramphenicol agar (DRBC) at 28 °C.

Genera and species	1	2	3	4	5	6	7	8	9	10	11	12	13	TC	F
Aspergillus (total)	0	0	108	6	22	14	10	0	170	26	2	8	32	398	10
A. flavus	0	0	0	6	0	0	0	0	2	14	2	6	32	62	6
A. flavus var. *columnaris*	0	0	108	0	0	0	0	0	0	0	0	0	0	108	1
A. niger	0	0	0	0	0	0	0	0	168	12	0	2	0	182	3
A. ochraceus	0	0	0	0	22	14	10	0	0	0	0	0	0	46	3
Cladosporium sphaerospermum	2	22	0	0	0	0	0	0	0	0	0	0	0	24	2
Penicillium (total)	72	88	84	0	150	10	136	112	600	180	2	20	34	1488	12
P. aurantiogriseum	0	0	0	0	18	0	0	0	0	0	0	0	0	18	1
P. chrysogenum	72	88	84	0	132	10	0	0	600	180	0	16	34	1216	9
P. citrinum	0	0	0	0	0	0	136	112	0	0	0	0	0	248	2
P. griseofulvum	0	0	0	0	0	0	0	0	0	0	2	4	0	6	2
Scopulariopsis brevicaulis	2	0	0	0	0	0	0	0	0	0	0	0	0	2	1
Total count	76	110	192	6	172	24	146	112	770	206	4	28	66	1912	–

TC, total count; F, frequency out of 13 samples.

Table 2 Counts (colonies/g fresh meat) of fungal genera and species isolated from canned meat samples on Dichloran 18% Glycerol (DG18) agar medium at 28 °C.

Genera and species	1	2	3	4	5	6	7	8	9	10	11	12	13	TC	F
Aspergillus (total)	0	4	82	0	14	152	4	0	0	2	0	2	0	260	7
A. flavus	0	0	10	0	0	0	0	0	0	0	0	0	0	10	1
A. flavus var. columnaris	0	0	72	0	0	0	0	0	0	0	0	0	0	72	1
A. niger	0	4	0	0	0	0	0	0	0	2	0	2	0	8	3
A. ochraceus	0	0	0	0	14	152	4	0	0	0	0	0	0	170	3
Cladosporium (total)	0	2	0	0	0	36	4	0	12	20	0	0	0	74	5
C. sphaerospermum	0	2	0	0	0	0	0	0	0	0	0	0	0	2	1
C. variabile	0	0	0	0	0	36	4	0	12	20	0	0	0	72	4
Eurotium amstelodami	0	0	0	0	0	0	4	0	2	2	0	0	6	14	4
Penicillium (total)	412	18	0	4	82	68	110	6	90	30	118	2	0	940	11
P. chrysogenum	8	18	0	0	82	68	0	0	90	30	0	2	0	298	7
P. citrinum	0	0	0	0	0	0	110	6	0	0	0	0	0	116	2
P. griseofulvum	404	0	0	0	0	0	0	0	0	0	118	0	0	522	2
P. oxalicum	0	0	0	4	0	0	0	0	0	0	0	0	0	4	1
Yeasts	0	0	2	0	0	0	0	0	0	0	0	0	0	2	1
Total count	412	24	84	4	96	256	122	6	104	54	118	4	6	1290	–

TC, total count; F, frequency out of 13 samples.

eofulvum and *Penicillium aurantiogriseum*) occurred in low incidences in meat samples. *Aspergillus* appeared in 10 out of 13 samples (77.0%) matching 20.81% of total fungal count. Among the four species of *Aspergillus*, *Aspergillus flavus* was the commonest (46.15% of samples) followed by *Aspergillus niger* and *Aspergillus ochraceus* (23.08% of samples for each). The remaining fungal genera and species were less frequently encountered from canned meat and were represented by *Cladosporium sphaerospermum* and *Scopulariopsis brevicaulis*.

Utilization of G18 medium revealed the isolation of 11 fungal species belonging to four genera of which *Cladosporium variabile*, *Eurotium amstelodami* (30.77% of samples for each), *Penicillium oxalicum* and unidentified yeast species (7.69% for each) were only encountered on this medium and were not isolated on DRBC. Other fungi such as *P. aurantiogriseum* and *S. brevicaulis* which occurred in low incidence on DRBC were not isolated on DG18. The incidence of the genera *Aspergillus* and *Penicillium* was slightly lower on DG18 (53.85% and 84.62% of samples respectively) than on DRBC (84.63% and 100% respectively).

Application of DRBC medium showed that the most contaminated sample was No. 9 which was a corned beef imported from Brazil. It yielded 770 colonies per gram fresh weight (Table 1) with the majority identified as *P. chrysogenum* and *A. niger* (600 and 168 colonies, respectively). On the other hand, sample No.1 (corned beef from Brazil) yielded the highest number of colonies when DG18 medium was used (412 colonies/g of sample). Sample No 6 (corned beef from Brazil was also rich in fungi (256 colonies/g) with *P. chrysogenum* and *A. ochraceus* being the most common species.

The lowest number of fungal colonies (4/g on DG18 or 6/g on DRBC) was detected in sample No. 4 which was a chicken luncheon meat manufactured by Siniora Food Industries, Jordan. A markedly low number of colonies (4–28 colonies/g) also were obtained from sample No. 11 (chicken sausage from USA) and No. 12 (beef luncheon from Jordan) as indicated in Tables 1 and 2.

Nucleotide sequencing of rRNA genes of some of the *Penicillium* species was done. Fig. 1 shows the dendrogram generated based on the similarity values of three strains as well

Table 3 Total counts (colonies/g in all samples) of fungal genera and species isolated from canned meat samples on DRBC and DG18 media.

Genera and Species	DRBC				DG18			
	TC	%TC	F & OR	%F	TC	%TC	F & OR	%F
Aspergillus (total)	398	20.81	10 H	77.00	260	20.16	7 H	53.85
A. flavus Link	62	3.24	6 M	46.15	10	0.78	1 L	7.69
A. flavus var. *columnaris* (Raper and Fennell, 1965)	108	5.64	1 L	7.69	72	5.58	1 L	7.69
A. niger Van Tieghem	182	9.51	3 M	23.08	8	0.62	3 M	23.08
A. ochraceus Wilhelm	46	2.41	3 M	23.08	170	13.19	3 M	23.08
Cladosporium (total)	24	1.26	2 L	15.38	74	5.74	5 M	38,46
C. sphaerospermum Penzig	24	1.26	2 L	15.38	2	0.16	1 L	7.69
C. variabile (Cooke) de Vries	0	0	0	0	72	5.58	4 M	30.77
Eurotium amstelodami Mangin	0	0	0	0	14	1.09	4 M	30.77
Penicillium (total)	1488	77.81	12 H	92.31	940	72.87	11 H	84.62
P. aurantiogriseum Dierckx	18	0.94	1 L	7.69	0	0	0	0
P. chrysogenum Thom	1216	63.60	9 H	69.23	298	23.10	7 H	53.85
P. citrinum Thom	248	12.97	2 L	15.38	116	8.99	2 L	15.38
P. griseofulvum Dierckx	6	0.31	2 L	15.38	522	40.47	2 L	15.38
P. oxalicum Currie and Thom	0	0	0	0	4	0.31	1 L	7.69
Scopulariopsis brevicaulis (Saccardo) Bainier	2	0.10	1 L	7.69	0	0	0	0
Yeasts	0	0	0	0	2	0.16	1 L	7.69
Total count	1912	–	–	–	1288	–	–	–

TC, total count; %TC, percentage total count; F, Frequency out of 13 samples; %F, percentage Frequency; OR, occurrence remark; H, high occurrence = 7–13 cases; M, moderate occurrence = 3–6 cases; L, low occurrence = 1, 2 cases.

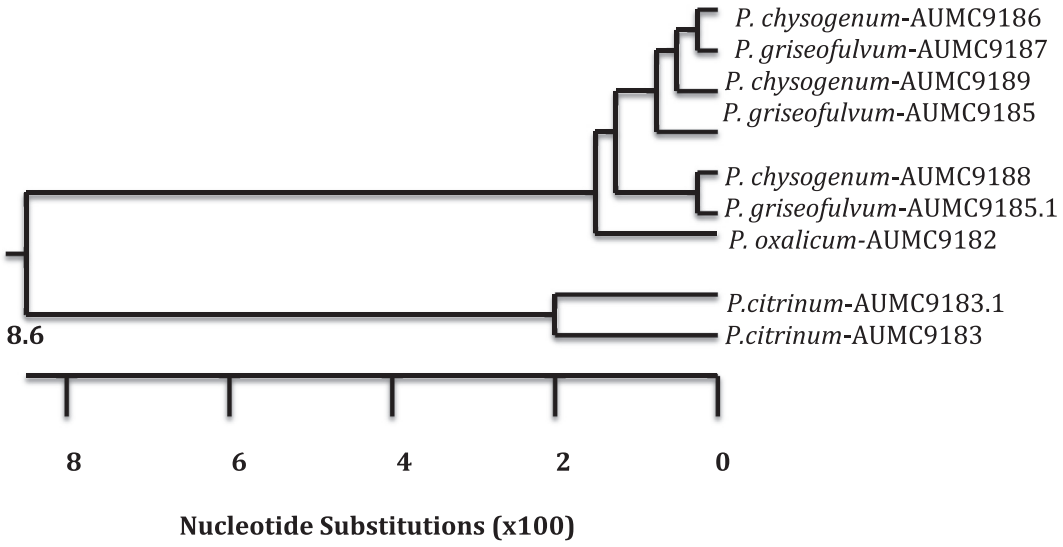

Figure 1 Phylogenetic tree of *Penicillium* species isolated from meat samples (given AUMC numbers).

as reference strains obtained from the GenBank. Table 6 presents the fungi isolated from meat compared with closely related fungi obtained from the GenBank. The generated phylogenetic dendrogram (Fig. 1) shows that the studied isolates were clustered in four groups.

Table 4 presents the colony counts in colonies per gram of samples of bacterial genera and species. There were no detection of bacterial colonies in most of the samples except the presence of yeast in sample No. 3 (42×10^3 colonies/g), sample No. 5 (15×10^3 colonies/g), and *Bacillus* spp. in sample No. 3 (1×10 colonies/g), sample No. 5 (2×10 colonies/g), sample No. 8, 11 and 13 (all had 1×10 colonies/g).

It is noteworthy that all samples were negative for pork DNA. All samples were positive of DNA of a ruminant meat. Samples 2, 3, 9, 13 were positive for DNA of fish while the rest of the samples were negative for such DNA.

Table 5 presents the concentrations of iron (Fe), zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb) and nickel (Ni) found in the 13 brands of canned meat products. The concentration of Fe in different brands ranged from 11.8 to 39.1 mg/kg. The highest concentration of Fe was in sample No. 13. Nine of the 13 samples had Fe concentrations above the permissible limit. The concentration of Zn ranged from 3.6 to 28.0 mg/kg with the highest level shown in sample No. 1. All

Table 4 Total counts (colonies/g in all samples) of bacteria genera and species isolated from canned meat samples.

Sample No.	TPC (cfu/g)	TCC (cfu/g)	FCC (cfu/g)	TFC (cfu/g)	Yeast	Staph.	*Bacillus*	Salmonella	PCR			
									Listeria monocytogenes	*Campylobacter* spp.	*Yersinia enterocolitica*	*Staphylococcus aureus*
1	2×10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	42×10^3	ND	1×10	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	15×10^3	ND	2×10	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	ND	ND	ND	ND	ND	ND	1×10	ND	ND	ND	ND	ND
9	14×10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	ND	ND	ND	ND	ND	ND	1×10	ND	ND	ND	ND	ND
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
13	3×10	ND	ND	ND	ND	ND	1×10	ND	ND	ND	ND	ND

TPC, total plate count; TCC, total coliform count; FCC, fecal coliform count; TFC, total fungal count; cfu, colony forming units.

Table 5 Levels of heavy metals in 13 samples of canned meat products in Riyadh, Saudi Arabia.

Sample	Fe	Zn	Cu	Cd	Pb	Ni
1	36.8[a]	28.0	0.59	0.61[a]	0.73[a]	Not detected
2	11.8	8.3	0.43	0.16	1.12[a]	Not detected
3	15.3	3.6	Not detected	Not detected	0.94[a]	Not detected
4	24.2[a]	5.3	0.25	0.45	Not detected	Not detected
5	29.1[a]	8.3	Not detected	0.62[a]	0.49[a]	Not detected
6	34.3[a]	23.5	0.38	0.48	0.42[a]	Not detected
7	25.4[a]	5.2	0.01	0.61[a]	0.59[a]	Not detected
8	19.9	7.1	0.07	0.27	0.27[a]	Not detected
9	30.6[a]	22.7	0.28	0.53[a]	0.78[a]	Not detected
10	32.5[a]	22.4	0.21	0.18	0.59[a]	Not detected
11	16.9	7.9	Not detected	0.14	0.93[a]	0.28[a]
12	24.4[a]	6.9	0.08	0.36	0.41[a]	0.77[a]
13	39.1[a]	20.5	0.09	0.33	0.36[a]	0.21[a]

[a] High concentration.

samples had Zn levels below the maximum permissible limit. The concentration of Cu ranged from 0.01 to 0.59 mg/kg. The highest concentration of Cu was seen in sample No. 1 at 0.59 mg/kg. All samples had Cu concentrations below the maximum permissible level. Three samples (No. 3, 5 and 11) showed undetectable levels of Cu. The concentration of Cd ranged from 0.16 to 0.62 mg/kg. The highest level of Cd concentration was seen in sample No. 5 (0.62 mg/kg). Four samples (No. 1, 5, 7, 9) had Cd levels above the maximum permissible level. Sample No. 3 had undetected level of Cd. The concentration of Pb ranged from 0.27 to 1.1 mg/kg, the highest concentration found in sample No. 2 (1.1 mg/kg). Sample No. 4 had undetectable level of Pb. All samples had Pb levels above the maximum permissible level. All samples had undetectable levels of Ni except samples No. 11, 12 and 13, which had Ni levels above the maximum permissible level.

9. Discussion

This study showed that the fungal load per sample might be correlated with the type of medium on which the samples were cultured. The two isolation media (DRBC and DG18) showed to be suitable for culturing food borne fungi contaminating meat samples. As shown in Table 2, the high counts were probably mainly due to an outbreak in the counts of *P. griseofulvum*. All canned samples of meat had no growth of fungi observed visually by the naked eye. All samples were apparently in good condition when sampled. The presence of fungal units (spores, hyphae or budding cells) can be expected as contamination from the environment during meat processing and canning. If sterilization is not enough such fungi may remain viable and cultivable when conditions become favorable for the growth of fungi.

Table 6 shows that there was a high level of similarity between fungi isolated from meat samples and their related equivalents obtained from the GenBank. All fungal DNA bands identified and displayed similarities to GenBank data were of *Penicillin* spp. Three were *P. chrysogenum*, two were *P. citrinum*, three were *P. griseofulvum* while *P. oxalicum* were found in one isolate each. Contamination of meat by these species has been previously reported in the literature (Dorn-In et al., 2013).

Table 6 Fungi isolated from meat compared with closely related fungi in the GenBank.

Fungi isolated from meat samples				Related fungi recorded in the GenBank		Similarity (%)
AUMC No.	Identification	Number of nucleotides with ITS1 primer	Number of nucleotides with ITS4 primer	Accession No.	Fungal species	
9189	*Penicillium chrysogenum*	544	556	AY213669.1	*Penicillium chrysogenum*	99
9186	*Penicillium chrysogenum*	545	557	AY213669.1	*Penicillium chrysogenum*	99
9188	*Penicillium chrysogenum*	547	557	AY213669.1	*Penicillium chrysogenum*	99
9183.1	*Penicillium citrinum*	514	538	KF758800.1	*Penicillium citrinum*	99
9183	*Penicillium citrinum*	517	511	KF758800.1	*Penicillium citrinum*	99
9185.1	*Penicillium griseofulvum*	547	556	HQ012499.1	*Penicillium griseofulvum*	99
9185	*Penicillium griseofulvum*	547	557	HQ012499.1	*Penicillium griseofulvum*	99
9187	*Penicillium griseofulvum*	543	555	HQ012499.1	*Penicillium griseofulvum*	99
9182	*Penicillium oxalicum*	551	558	DQ681323.1	*Penicillium oxalicum*	98

The sources of such contaminants are uncertain, as it is difficult to predict whether the contamination happened at the origin of production, during processing or by adding some ingredients that are used to enhance flavor in meat products such as yeast or other ruminant meat or fish (Bockelmann et al., 2008; Pollmer, 2011).

Commercially canned foods are supposed to be considered safe because they are processed under carefully controlled conditions. Canned meat may contain toxins if not properly processed. Table 1 shows the mean of total viable bacterial count to be 1912 cfu/g and Table 2 has a mean of 1290 cfu/g. These may be considered higher than expected and it may be due to processing practices, which might have exacerbated contamination through poor hygienic practices. The presence of fungal elements and bacteria (Tables 1–3) in some samples reveals the presence of unsanitary condition in the processing plants and their numbers were considered to be a more practical indicator of the hygienic efficiency and microbiological status of processing the canned products.

Our study showed that in some of the samples, the levels of heavy metals were above the maximum permissible levels (Table 5). The high levels of lead in most of the samples are particularly worrying. Soldering is a source of lead contamination in the canning process. Lead is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults. The toxic effects of lead, like those of mercury, have been principally established in studies on people exposed to lead in the course of their work. Short-term exposure to high levels of lead can cause brain damage, paralysis (lead palsy), anemia and gastrointestinal symptoms. Longer-term exposure can cause damage to the kidneys, reproductive and immune systems in addition to effects on the nervous system. The most critical effect of low-level lead exposure is on intellectual development in young children and, like mercury, lead crosses the placental barrier and accumulates in the fetus. Infants and young children are more vulnerable than adults to the toxic effects of lead, and they also absorb lead more readily. Even short term, low-level exposures of young children to lead are considered to have an effect on neurobehavioral development. Consumption of food containing lead is the major source of exposure for the general population (Mahalakshmi et al., 2012).

To some extent, four of our samples had Cadmium levels above the maximum permissible limit. Cadmium may accumulate in the human body and may induce kidney dysfunction, skeletal damage and reproductive deficiencies. The principal toxic effect of cadmium is its toxicity to the kidney, although it has also been associated with lung damage (including induction of lung tumors) and skeletal changes in occupationally exposed populations. Cadmium is relatively poorly absorbed into the body, but once absorbed it is slowly excreted, like other metals, and accumulates in the kidney causing renal damage. The kidneys of consumed animals are a major source of cadmium in the diet although lower levels are found in many other foods (Mahalakshmi et al., 2012).

10. Conclusion

The results indicate that fungal elements and higher levels of heavy metals such as lead and cadmium were found in canned meat products in Saudi Arabia. This may pose a real danger to consumers, since canned meat products are readily accessible and very convenient. There is clearly a need to improve quality control in the processing of these canned meat products and also imposition of stricter guidelines from the Saudi authorities on the importation and sale of canned meat products contaminated with fungal elements, bacteria and heavy metals.

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