

## Microbiological profile of retail sliced dry sausages in Ethiopia

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### Abstract

Sausage production in Ethiopia has only a recent history. Previously, dry sausages were produced mainly from pork and hence there was low consumer demand due to religious reasons in relation to pork eating. Currently, dry sausages (commonly known as *mortadella*) made from beef, veal and chicken are also made available to consumers and there is a growing consumer demand towards these products. These dry sausage products are usually maintained under refrigeration and sold as vacuum-packed cylindrical rolls in casings or as slices. They can be consumed either heated or cold. However, there is no any report regarding microbiological quality and safety of these products. In this study, the microbiological profile of retail sliced dry sausages (pork, veal and chicken) collected from different retail shops in Addis Ababa, Ethiopia was determined. Mean pH values of retail dry sausages ranged from 6.09 to 6.33 and moisture content values ranged from 35 to 41%. Mean microbial count values (log cfu/g) ranged from 4.87 to 5.18 (aerobic mesophilic bacteria, AMB), 2.02 to 2.50 (*Enterobacteriaceae*), 1.73 to 2.24 (coliforms), 2.46 to 3.04 (enterococci), 3.09 to 3.76 staphylococci, 5.31 to 5.68 (lactic acid bacteria) and 3.28 to 3.87 (for yeasts). The aerobic mesophilic bacterial flora of retail sliced dry sausages was dominated by Gram-positive organisms. *Salmonella* was isolated from two sausage samples. Spoilage of sliced dry sausages, after the vacuum package was opened, was detected within 3 to 4 days during aerobic storage at ambient temperature (22°C on average) and within 12 to 20 days at refrigeration storage (4°C). The storage conditions were intended to reflect what normally would happen in routine food handling in home kitchen environments and food service establishments. Generally, the majority of retail sliced dry sausages showed the presence of high microbial load, which indicated contamination during or after processing of the products.

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### Introduction

Heat processed large diameter (90-120 mm) sausages produced by Ethiopian sausage processors are commonly known as *mortadella*. Originally, *mortadella* was a traditional cured, cooked Italian sausage (Abdullah, 2004) but the version produced in Ethiopia is an emulsion type sausage. *Mortadella* is an increasingly popular meat product in many countries because of its pleasant taste and texture, high nutritional value and ease of incorporation into sandwiches (Al-Shubi, 1999).

Traditional sausage (wakalim) production for household consumption is practiced among the Harari people in Ethiopia. Some microbiological studies have also been made on this product (Bacha *et al.*, 2007, 2010, 2011). Small scale commercial sausage production started in Ethiopia recently. Initially, pork was used to produce dry sausages. But this resulted in low consumer demand as pork eating was a religious taboo among the orthodox Christian and Muslim communities. Currently, dry sausages made from beef, veal and chicken are also made available to consumers, and there is a growing

consumer demand towards these products. In general, the common ingredients, which are added in the production of these products are spices, preservatives (nitrates, sodium chloride and polyphosphates), and other supplements such as oil, sugar, milk solids, wheat flour, blood and vine (Savic, 1985). However, the amount of each ingredient in these sausages is unknown as processors do not usually label their products with required information.

Dry sausage products are usually maintained under refrigeration and sold as vacuum-packed cylindrical rolls in casings or as slices. They can be consumed either heated or cold. Despite the increasing popularity of sausages, there is no report concerning the microbiological status of these retail meat products. Hence the objective of this study was to determine the microbiological quality and safety of these products as they are made available to consumers.

### Materials and Methods

#### Samples

A total of 90 sliced dry sausage samples

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comprising 30 each of pork, veal and chicken were collected from various retail shops in Addis Ababa, Ethiopia. Pork dry sausage products were produced by three different processors and samples were collected in a manner to represent processors. Sausage samples were purchased from retail shops immediately after arrival from producers. Purchased samples were handled aseptically and immediately transported to the laboratory with an ice box for microbiological analysis. Microbiological analysis was conducted within 1 to 3 hours of sample purchase.

#### *Microbiological analysis*

A 25 g sample of each dry sausage type was placed aseptically in a sterile stomacher bag containing 225 ml of sterile 0.1% bacteriological peptone (Oxoid) water and homogenized for 1-3 minutes using a Stomacher lab blender (model 400, Seward JAC, London). Serial ten-fold dilutions were also prepared for surface plating.

Aerobic mesophilic bacteria (AMB) were counted using Plate Count Agar (Oxoid) plates incubated at 30°C for 72 hrs. For *Enterobacteriaceae*, Violet Red Bile Glucose Agar (Oxoid) was used and plates were incubated at 30°C for 24 hrs. All purple colonies were counted as members of *Enterobacteriaceae*. Coliforms were counted on Violet Red Bile Agar (Oxoid) and plates were incubated at 30°C for 24 hrs. Red to pink colonies surrounded by precipitated bile were counted as coliforms. Enterococci were enumerated on Bile Aesculin Agar (Oxoid). The plates were incubated at 30°C for 24 hrs and colonies surrounded by blackened zone were counted. For staphylococci, Mannitol Salt Agar (Oxoid) was surface plated and incubated at 30°C for 36 hrs. Lactic acid bacteria were counted on MRS Agar (Oxoid) plates incubated in anaerobic jar using gas generating kit at 30°C for 48 hrs. Counts of yeasts were determined on Chloramphenicol Bromophenol Blue Agar plates incubated at 25-28°C for 3 to 5 days. Chloramphenicol-Bromophenol Blue Agar consisted of (g/l distilled water) yeast extract (Oxoid) 5.0, glucose 20, chloramphenicol 0.1, Bromophenol-blue 0.01, agar 15, pH 6.0-6.4. Smooth (non-hairy) colonies without extension at periphery (margin) were counted. Microbial counts were transformed to log<sub>10</sub> cfu/g.

About 5 to 7 colonies were picked randomly from countable PC plates and inoculated into tubes containing 5 ml Nutrient Broth No 2 (Oxoid). These were incubated at 30-32°C over-night. Cultures were purified by repeated plating and were characterized to the genus level using the following tests: cell morphology, KOH test (Gregerson, 1978), oxidation

fermentation (O/F) test (Hugh and Leifson, 1953), catalase test and cytochrome oxidase test (Kovacs, 1956).

#### *Isolation of Salmonella spp.*

Sausage samples (25 g) were homogenized in 225 ml buffered peptone water using a Stomacher lab blender and incubated at 37°C for 24 hrs for primary enrichment. For secondary enrichment, 1 ml of primary enrichment culture was transferred into separate tubes each containing 10 ml of Selenite broth (Oxoid), 10 ml of Tetrathionate broth (Oxoid), 10 ml of Mannitol Selenite broth (Oxoid) or 10 ml of Muller-Kauffmann Tetrathionate broth (Oxoid). A volume of 0.1 ml of culture was also transferred into a separate tube containing 10 ml of Rappaport Vassiliadis (RV) broth (Merck). Selenite and Mannitol Selenite broths were incubated at 37°C for 24 hrs and Tetrathionate, RV and Muller-Kauffmann Tetrathionate broths were incubated at 43°C for 48 hrs in water bath. Culture from each enrichment broth was separately streaked on plates of MacConkey Agar, Salmonella-Shigella (SS) Agar and Xylose Lysine Desoxycholate (XLD) medium (all from Oxoid). Characteristic colonies from each selective medium were picked, purified and tested biochemically on Triple Sugar Iron Agar (Oxoid), Lysine Iron Agar (LIA) (Oxoid), Urea Agar (Oxoid), Simmons Citrate Agar (Oxoid) and SIM Medium (Oxoid). The ability of *Salmonella* to ferment mannitol, glucose or sucrose was assessed using a fermentation broth containing the corresponding sugars. Fermentation tubes contained inverted Durham tubes to detect gas production. Presumptive *Salmonella* isolates were further confirmed by using API 20E (BioMerieux, France) identification system as described by the manufacturer.

#### *Determination of keeping quality of sausages during aerobic storage at ambient and cold temperatures*

To determine the keeping quality of sausages after their vacuum package was opened, each sausage type was separately stored at ambient and refrigeration temperatures. The storage conditions were intended to reflect what normally would happen in routine food handling in home kitchen environments and food service establishments. Samples were collected from retail shops immediately after their arrival from processors. Each dry sausage type was then separately stored at ambient (22°C on average) and cold temperatures (4°C). Aerobic mesophilic bacteria (AMB), *Enterobacteriaceae*, Lactic acid bacteria and yeasts were monitored during the storage time. Counts were made at 8 hrs intervals for sausages stored at ambient temperature and at 48 hrs intervals

for those stored at refrigeration temperature.

### Measurement of pH and moisture content

The pH of each dry sausage sample was determined by blending 10 g dry sausage in a stomacher with 100 ml distilled water. The pH value of the homogenate was measured using a digital pH-meter. The moisture content of the sliced dry sausages was determined by allowing the samples to dry to constant weight at 35°C. Other physicochemical parameters such as protein and fat content of the sausages and the level of preservatives added were not analyzed in this study.

### Statistical analysis

To see if there was significant variation in counts within samples in each type of dry sausage, coefficient of variation (CV) was calculated. Difference in microbial counts among pork dry sausage samples produced by three processors was analyzed by analysis of variance (ANOVA) and means were separated by Tukey HSD test. Significance was determined at the 5% level.

## Results

### Microbiological analysis

The mean aerobic mesophilic bacterial count of retail sliced dry sausages was around 5 log cfu/g (Table 1). *Enterobacteriaceae* and coliforms had mean counts of < 3 log cfu/g. However, variation in counts within samples was significantly high (CV > 10%). The sliced dry sausages harboured high counts of lactic acid bacteria (LAB) with mean counts as high as 5 log cfu/g. The majority of pork and more than 60% of veal and chicken sliced dry sausage samples had yeast counts with mean values as high as 3 log cfu/g (Table 1).

Varying frequency of distribution of the dominant microbial groups was observed in all retail sliced dry sausages (Table 2). Many sausage samples had mean counts of > 5 log cfu/g for the various microbial groups (Table 2). About 47% of sliced dry sausages had aerobic mesophilic bacterial counts > 5 log cfu/g. Enterococci were also encountered at counts > 5 log cfu/g in 11% of the different dry sausage products. Fifteen percent of our sausage samples had staphylococci counts > 5 log cfu/g. About 60% of all sausage types had also LAB counts > 5 log cfu/g. Furthermore, yeast counts > 5 log cfu/g was found in 16% of our sausage samples.

There was no significant difference in the counts of aerobic mesophiles, staphylococci, LAB and yeasts among the products from the three processors (P > 0.05). However, significant differences were detected in the counts of *Enterobacteriaceae*, coliforms and

Table 1. Microbial counts (log cfu/g) of retail sliced dry sausages in Addis Ababa

Bacterial groups	Pork dry sausage (n=30)				Veal dry sausage (n=30)				Chicken dry sausage (n=30)			
	Min	Max	Mean	%CV	Min	Max	Mean	%CV	Min	Max	Mean	%CV
AMB*	3.27	7.64	4.93	21.09	3.27	7.79	5.18	28.96	3.27	6.95	4.87	26.28
<i>Enterobacteriaceae</i>	<2.00	6.37	2.50	54.40	<2.00	4.49	2.07	58.45	<2.00	4.44	2.02	59.04
Coliforms	<2.00	6.30	2.24	62.50	<2.00	4.25	1.73	65.89	<2.00	4.31	1.87	60.42
Enterococci	<2.00	5.00	2.56	53.51	<2.00	5.66	3.04	57.89	<2.00	6.04	2.46	59.35
Staphylococci	<2.00	6.83	3.09	59.22	<2.00	6.31	3.67	42.50	<2.00	6.13	3.76	38.29
Lactic acid bacteria	3.17	9.79	5.68	29.04	<2.00	8.76	5.31	30.32	3.62	7.51	5.34	22.47
Yeasts	<2.00	5.62	3.87	25.84	<2.00	6.51	3.30	58.18	<2.00	7.54	3.28	67.38

\*AMB, Aerobic Mesophilic bacteria

n in parenthesis indicates the number of samples

CV, Coefficient of variation

Table 2. Frequency distribution (%) of counts of microbial groups in all sausage samples

Microbial groups	Count (log cfu/g)			
	<2.00	2.00-4.99	5.00-7.99	>8.00
Aerobic mesophilic bacteria (AMB)	0	53	47	0
<i>Enterobacteriaceae</i>	45	53	2	0
Coliforms	57	42	1	0
Enterococci	37	42	11	0
Staphylococci	23	62	15	0
Lactic acid bacteria	2	40	54	4
Yeasts	24	60	16	0

Table 3. Microbial counts of retail sliced pork dry sausages from three processors (n=30)

Microbial groups	Log cfu/g (Mean±S.D.)		
	Processor 1	Processor 2	Processor 3
Aerobic mesophiles	5.43±1.21a	4.82±0.83a	4.54±0.91a
<i>Enterobacteriaceae</i>	3.44±1.46a	1.89±1.01b	2.17±1.14ab
Coliforms	3.33±1.45a	1.56±0.92b	1.82±1.14b
Enterococci	3.53±1.22a	2.37±1.26b	1.78±1.09ab
Staphylococci	3.72±2.18a	2.89±1.69a	2.66±1.58a
Lactic acid bacteria	6.19±1.89a	5.98±1.59a	4.86±1.23a
Yeast	4.37±0.59a	3.91±0.78a	3.34±1.28a

Means in rows followed by the same letters

are not significantly different (P > 0.05).

S.D. = Standard Deviation.

enterococci (P < 0.05) (Table 3). Relatively high counts of these microbial groups were obtained in samples from processor 1.

### Frequency distribution of dominant bacteria in retail sliced dry sausages

A total of 459 bacterial isolates were cultured from retail sliced dry sausages. The aerobic mesophilic bacterial flora of almost all (99.5%) samples was dominated by Gram positive bacteria (Table 4). *Bacillus* spp. (34%) were dominant followed by *Micrococcus* (26%) and *Staphylococcus* spp. (17%).

### Isolation of *Salmonella* spp.

*Salmonella* spp. was isolated from two of the 90 dry sausage samples (2.2%) investigated in this study (data not given).

### pH and moisture content

The mean pH values of dry sausage samples in this study were above 6.00 (Table 5). Pork sausages had a mean moisture content of 41% and those of veal and chicken sausages were 35% and 38%, respectively. However, moisture content variation within samples was high for pork and chicken sausages (CV > 10%) (Table 5).

### Keeping quality of sausages during aerobic storage at ambient and cold temperatures

Sliced dry pork and dry veal sausage samples spoiled within 80 hrs during storage at ambient temperature (Figures 1 and 2). In both sausage types, LAB dominated the spoilage flora at counts over



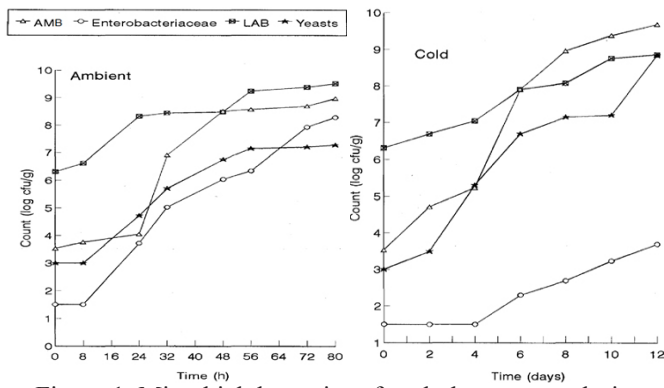


Figure 1. Microbial dynamics of pork dry sausage during ambient and cold storage

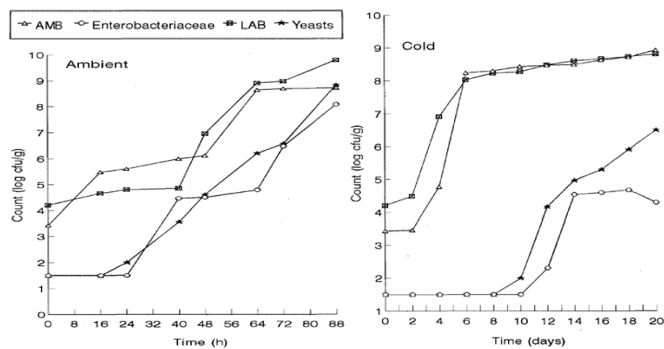


Figure 2. Microbial dynamics of veal dry sausage during ambient and cold storage

9 log cfu/g, although initial LAB counts of fresh sausages were over 6 log cfu/g. Aerobic mesophilic bacteria were also part of the dominant spoilage flora. *Enterobacteriaceae* increased by 6 log units and yeasts by 4 log units until 72 hrs (Figures 1 and 2).

At refrigeration temperature, pork dry sausage spoiled after 12 days. Aerobic mesophilic bacteria dominated the spoilage flora at counts of 10 log cfu/g, followed by LAB and yeasts at counts of 9 log cfu/g. Yeasts showed an increase by 6 log units until day 12. The count of *Enterobacteriaceae* remained below 4 log cfu/g during cold storage.

Spoilage was detected in veal dry sausages after 20 days of cold storage. LAB and aerobic mesophilic bacteria reached counts of 8 log cfu/g at day 6, and remained at this level throughout the storage period. Yeasts did not show any marked increase until day 10, but increased by over 5 log units thereafter. A similar pattern was observed for *Enterobacteriaceae*, but final counts did not go beyond 4 log cfu/g until the end of storage (Figure 1 and 2). Spoilage in these products was expressed as green discoloration and development of off-odour (especially buttery odour in the case of veal dry sausage).

## Discussion

### Microbiological analysis

The recommended reference value for the aerobic

Table 4. Frequency distribution (%) of dominant bacteria in retail sliced dry sausages

Sausage type	(n)	% of isolates						
		<i>Bacillus spp</i>	Micrococci	Staphylococci	Streptococci	Lactobacilli	<i>Enterobacteriaceae</i>	Other Gram +ve rods
Pork dry sausage	181	28	25	20	12	9	2	4
Veal dry sausage	137	38	26	16	12	7	-	0.7
Chicken dry sausage	141	38	26	14	10	10	-	2
Total/mean	459	34	26	17	12	9	0.4	2

n = number of isolates

Table 5. The pH and moisture content (%) values of retail sliced dry sausages

Sample type (n)	pH				Moisture content (%)			
	Min	Max	Mean	%CV	Min	Max	Mean	%CV
Pork dry sausage (30)	5.72	6.59	6.18	4.25	31	63	41	15.8
Veal dry sausage (30)	5.21	6.52	6.09	5.26	32	40	35	7.22
Chicken dry sausage (30)	5.74	6.63	6.33	3.16	32	47	38	11.3

n = number of isolates

mesophilic bacterial count of ready-to-eat foods was indicated in several studies to be < 5 log cfu/g (Solberg *et al.*, 1990; Shapton *et al.*, 1991; Jay *et al.*, 2005). In this study, the mean aerobic mesophilic bacterial counts of retail sliced dry sausages were around 5 log cfu/g. Thus, about 53% of pork, 48% of veal and 40% of chicken dry sausage samples (Table 2) exceeded the typical aerobic mesophilic bacterial count value set for ready-to-eat food products.

Although variations in coliform counts within the samples were significant, the counts were still higher than what was reported in another study (Apaydin *et al.*, 2003) for bologna type sausages in which all samples had coliform counts under detectable levels (< 2 log cfu/g). In fact, heat treatment should eliminate coliforms and all other members of *Enterobacteriaceae* during processing. Therefore, the presence of these microbial groups in the dry sausages was indicative of lack of appropriate heat treatment or contamination thereafter.

Enterococci are not generally desirable in processed meat products, because they cause spoilage (Giraffa, 2002). Enterococci were encountered in all sausages, and counts were > 5 log cfu/g in 11% of the different dry sausage products. It is reported that enterococci can be found as spoilage contaminants in processed meats, either by surviving heat processing due to their thermoduric nature (Franz *et al.*, 1999) or by cross-contamination at the final stages of processing, such as slicing and packaging (Hugas *et al.*, 2003). On the other hand, enterococci were not detected in any of the samples of bologna type sausages sold in Turkey (Apaydin *et al.*, 2003).

The presence of staphylococci at counts > 5 log cfu/g is a point of concern (Jay *et al.*, 2005). At this level toxin producing staphylococci may render the sausages unsafe for consumption. The majority of the sliced dry sausages harboured lactic acid bacteria with count higher than that reported for bologna type sausage samples sold in Turkey in which 80% of the samples had counts < 2 log cfu/g (Apaydin *et al.*, 2003). Lactic acid bacteria (LAB) are reported

to dominate the spoilage flora of emulsion sausages (Korkeala *et al.*, 1989). Although there was not any observable spoilage on the purchase day of these products, the presence of such high counts might limit their shelf life.

The high count of yeasts in the majority of pork, veal and chicken samples contribute to the spoilage of the products. Yeasts are spoilage organisms in cooked meat products (Jay *et al.*, 2005). In a study elsewhere, 67% of bologna type sausage samples had yeast and mould counts  $< 2 \log \text{ cfu/g}$  (Apaydin *et al.*, 2003). Dry sausage products are usually sold sliced. Slicing might pose a microbiological risk because of the potential for recontamination via the slicing blade and board. It is reported that cooked meat slicing machines, if inappropriately cleaned, could be sources of contamination and cross-contamination (Little and De Louvois, 1998; Elson *et al.*, 2004).

Counts of aerobic mesophiles, staphylococci, LAB and yeasts were consistently high among the products from the three processors, although significant differences were detected in the counts of *Enterobacteriaceae*, coliforms and enterococci. The relatively high counts of these microbial groups in samples from processor 1 might indicate inadequate heat-processing or poor hygienic practices during handling or storage of products.

#### *Frequency distribution of dominant bacteria in retail sliced dry sausages*

The dominance of *Bacillus* spp. among the microbial flora might be attributed to their virtue of having resistant endospores that confer tolerance to adverse conditions and various other stresses (Kramer and Gilbert, 1989). Additives such as spices were suggested as possible sources of aerobic spore forming bacteria to these types of products (IOM, 1985).

#### *Isolation of Salmonella spp.*

The rate of isolation of *Salmonella* spp. in our study was much higher than that reported in another study (Little and De Louvois, 1998) in which *Salmonella* was isolated in 0.1% of cooked sliced meats purchased from butchers' premises in the United Kingdom. None of cold ready-to-eat sliced meats taken from catering establishments in the United Kingdom were positive for *Salmonella* (Gillespie *et al.*, 2000). The presence of salmonellae in ready-to-eat meat or poultry products is a potential health hazard (Tompkin, 1983). To inactivate bacterial pathogens, it is recommended that a temperature of 70°C should be achieved in all parts of the sausage for a minimum of 2 min (Gaze *et al.*, 1989). Thus,

detection of *Salmonella* spp. in the sausage samples could indicate insufficient heat processing or post cooking contamination due to improper handling. Contamination may also occur in retail shops via slicing blades.

#### *Keeping quality of sausages during aerobic storage at ambient and cold temperatures*

Although LAB dominated the spoilage flora of our sausage samples at ambient temperatures, the rate of proliferation of aerobic mesophilic bacteria was notably higher during spoilage at ambient temperatures. The active proliferation of aerobic mesophilic bacteria was indicative of abundant formation of metabolites responsible for spoilage. However, the contribution of metabolic products from yeasts and LAB should not be undermined. Aerobic mesophilic bacteria were also important in refrigeration spoilage of our samples. Although they were counted after incubation at 30°C, their role in spoilage could be as psychrophilic. Lactic acid bacteria and yeasts with psychrophilic role could also have contributed to spoilage of the sausage products. The final counts of *Enterobacteriaceae* indicated that these microbial groups were not important in refrigeration spoilage (Gram *et al.*, 1999)

Spoilage was manifested through off-odor production and greenish discoloration. Green discoloration can be caused by different members of LAB and enterococci (Grant *et al.*, 1988; Borch *et al.*, 1988). According to a study (Jay *et al.*, 2005), in spite of the discoloration, the green product is not known to be harmful when eaten. In pork sausage, appearance of surface growth of yeasts towards the later stages of cold storage resulted in product no more desirable for consumption. Usually dry sausage slices are consumed immediately after purchase. They are not kept at ambient temperature for three or more days. Thus, microbial spoilage may not be a serious problem.

#### *pH and moisture content*

The majority of dry sausage samples in this study had pH values above 6.00 despite the high number of lactic acid bacteria. This could be due to the marked presence of the other microbial groups with proteolytic activities that could counteract the acid produced by LAB. Acceptable pH values for dry sausages range between 4.7 and 5.4 (Jay *et al.*, 2005). Some sausages had also a high moisture content which is beyond what dry sausages are expected to have (30-40%) These might make the products susceptible for growth of spoilage microorganisms and pathogen, as it could allow the multiplication of

bacteria, yeasts and molds (Jay *et al.*, 2005).

## Conclusion and Recommendations

Generally, the majority of retail sliced dry sausages analyzed in this study showed the presence of high microbial load, which indicated contamination during or after processing of the products. High variability in the counts of all microbial groups within the sample of each dry sausage type might also indicate the lack of constant processing parameters and quality control during manufacturing and/or post-production handling of these products. This study also pointed out a possible microbial safety problem related to the consumption of locally produced dry sausages, because few contained target pathogens such as *Salmonella*. Therefore, appropriate control methods should be implemented in the sausage production system to get safe products with good keeping quality. Prevention of cross contamination and proper cold storage practices are also essential in retail shops found in Addis Ababa.

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