Original article

Microbial load and incidence of *Salmonella* spp. in 'kitfo', a traditional Ethiopian spiced, minced meat dish

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Abstract: Raw and medium-cooked 'kitfo' samples were collected from ten food establishments in Addis Ababa and analyzed for their microbial load and incidence of *Salmonella* spp. in 1996 and 1997. All raw 'kitfo' samples from all food establishments had high aerobic mesophilic counts ranging from $2x10^7$ to $2x10^8$ cfu/g. Coliforms, staphylococci, lactic acid bacteria, yeasts and molds and aerobic spores had counts of $>10^4$, 10^6, 10^5, 10^4 and 10^3 cfu/g, respectively**.** Variations in aerobic mesophilic counts in the various food establishments were not significant (C.V.<10%). The microbial load of medium-cooked ('leb-leb') 'kitfo' was also high ($>10^6$cfu/g). Well-cooked 'kitfo' yielded no vegetative microorganisms. Salmonellae were isolated from 21 of 50 raw 'kitfo' samples. All medium-cooked or well-cooked samples were free from salmonellae. *Salmonella* test strains used in this study grew to the level of 10^7 cfu/g within 12 h. This study indicated the need to train food handlers in basic principles of hygiene. Keeping minced meat for several hours at ambient temperatures must also be avoided. The best alternative to avoid infection by *Salmonella* from 'kitfo' is to consume well-cooked 'kitfo'. [*Ethiop. J. Health Dev.* 1998;12(2):135-140]

Introduction

Meat is the most perishable of all important foods. It contains abundant nutrients for microbial growth. The surface of meats is contaminated with a variety of microorganisms (1). Where meat is cut into pieces, more microorganisms are added to the surfaces of the exposed tissue. Raw meats, particularly minced meats, have very high total counts of microorganisms and salmonellae are likely to be present in large numbers.

Animals, eggs, meats and meals of animal origin are found to be origins of various strains of *Salmonella* (2). Over 10% of raw sausage meat was found to contain salmonellae (3) and 27% of muscle of veal calves contained salmonellae (4). According to O'Toole (5) incidence of *Salmonella* in minced meat was 1.8% from samples in the U.S.A., 2% from samples in England, 5.3% from German samples and 20% from Canadian samples.

In Ethiopia, where meat is consumed either raw or cooked, salmonellae were isolated from the different organs of animals, such as spleen (6) and fresh raw beef (7). One traditional way of preparing meat dish is in the form of 'kitfo', where a chunk of lean meat is minced and mixed with 'kibe', a traditional butter containing specific types of spices. The traditional way of mincing meat for 'kitfo' preparation provides new surface for microbial contamination. Considering the relative unhygienic status of food handlers and kitchen utensils used for 'kitfo' preparation in various food establishments in Addis Ababa, it is very likely that 'kitfo' would have a high microbial load and would harbour *Salmonella*, which is closely associated with raw

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meat. In addition, the preference of a considerable number of Ethiopians for raw 'kitfo' dishes makes raw 'kitfo' a real health hazard.

The purpose of this study was, therefore, to evaluate the microbial load and microflora of 'kitfo' as made available to the consumer in some food establishments in Addis Ababa. The effect of partial

or complete cooking on the microflora and the growth potential of *Salmonella* test strain in 'kitfo' was also assessed.

Methods

Sample collection: Fifty samples of raw 'kitfo' were collected from 10 different non-star hotels, bars and restaurants in Addis Ababa as made available to the consumer. Five samples were collected aseptically from each site using sterile plastic bags. Sites of collection were Hotel A (Lideta), Hotel B (Sidist Kilo), Hotels C,D,E, and F (Arat Kilo), Hotel G (Kazanchis), Restaurant A (Giorgis), Bars A and B (Merkato). Medium-cooked ('leb-leb') and well done ('tibs') 'kitfo' samples were also collected from the same sites. The samples were collected between November 1, 1996 and February 15, 1997. Samples were immediately taken to the laboratory for bacteriological examination.

Swab samples were also aseptically collected from an area of 100 cm^2 of solid meat purchased from a butcher's shop using sterile cotton swabs and introduced into buffered peptone water (10 ml).

Enumeration of microorganisms: Twenty five grams of 'kitfo' and 225 ml of 0.1% sterile Buffered Peptone Water (BPW) were homogenized in a stomacher lab blender for 1-3 min. (8). Appropriate dilutions (0.1 ml) were plated on the following media (all from Oxoid) for microbial count:

Aerobic mesophilic bacteria were counted on Plate Count (PC) agar after incubation at 32°C for 24-48 h. Violet Red Bile agar was used to count coliforms. After 24 h incubation at 32°C, purplish red colonies surrounded by red zone of precipitated bile were counted as coliforms. Staphylococci were counted on Mannitol Salt agar after incubation at 32°C for 36 h. Lactic acid bacteria were counted on de-Mann, Rogossa and Sharp (MRS) agar plates after incubation in an anaerobic jar at 32°C for 48 h. Yeasts and molds were counted on chloramphenicol-bromophenol blue agar plates (yeast extract, 5.0g; glucose, 20.0g; chloramphenicol, 0.1g; bromophenol blue, 0.01g; agar, 15g; distilled water, 1000 ml; pH, 6.0-6.4). Colonies were counted after incubation at 28-30°C for five days. For spore counting, appropriate dilutions were heat-treated at 80°C for 10 min in a water bath, and plated on PC agar. Colonies were counted after incubation at 32°C for 48 h.

Isolation of Salmonella spp.: BPW, after homogenization, was incubated at 32°C for 16-20 h for primary enrichment. For secondary enrichment, one ml of culture was inoculated in a tube containing 10 ml of Selenite broth and into another tube containing an equal volume of Tetrathionate broth. In addition 0.1 ml of BPW was also inoculated in a tube containing 10 ml RappaportVassiliadis broth. Selenite broth was incubated at 37°C and Tetrathionate broth and RappaportVassiliadis broth at 43°C for 24 h in a water bath (9).

A loopful of culture from the enrichment broths was inoculated onto Salmonella-Shigella (SS) agar, Brilliant Green (BG) agar, and Xylose Lysine Deoxycholate (XLD) agar for the isolation of *Salmonella* and incubated at 37°C for 18-24 h.. Based on their characteristic appearnace, *Salmonella*looking colonies were picked from the respective selective media and, after repeated purification, were inoculated into the following biochemical tubes for identification: Triple Sugar Iron (TSI) agar, Lysine Iron (LI) agar, Urea agar, Simmon's Citrate agar, Sulphur-Indol-Motility (SIM) medium, Glucose broth (1%), Sucrose broth (1%) and Mannitol broth (1%). Incubation was at 37°C for 1824 h.

Isolates which met the minimum biochemcial profile of *Salmonella* were subjected to serological identification using polyvalent O and H antisera (Difco)

Swab samples were similarly processed for the counting of aerobic mesophilic bacteria, coliforms, staphylococci, lactic acid bacteria, yeasts and molds, spores and *Salmonella* spp. *Determination of the growth potential of Salmonella test strains in 'kitfo'*: 'Kitfo' was

Table 1: **Counts (log cfu/g) of aerobic mesophilic bacteria (AMB), coliforms, and staphylococci in raw 'kitfo' samples**

Sample source	AMB*			Coliforms			Staphylococci		
	X	S	%CV	X	S	%CV	X	S	%CV
Hotel A	7.36	0.99	13.45	4.51	0.87	19.29	6.81	0.80	11.74
Hotel B	8.31	0.84	10.10	6.77	0.76	11.22	7.21	1.10	15.25

Hotel C	7.83	0.64	8.17	6.18	0.96	15.53	6.99	0.94	13.44
Hotel D	7.32	0.34	4.64	6.39	0.66	10.32	6.42	0.67	10.43
Hotel E	7.42	0.03	0.40	61.4	0.36	5.89	6.90	0.50	7.24
Hotel F	8.10	0.96	11.85	5.72	0.59	10.31	6.83	0.48	7.02
Hotel G	7.81	0.54	6.91	5.57	0.65	11.66	6.22	0.76	12.21
Res A	7.30	0.70	9.58	5.33	0.69	12.94	6.18	0.41	6.63
Bar A	8.33	0.79	9.48	4.66	0.45	9.65	7.29	1.00	13.71
Bar B	7.87	0.42	5.33	5.89	0.64	10.86	6.93	0.76	10.96

*AMB, aerobic mesophilic bacteria; X, mean; S, standard deviation; CV, coefficient of variation

prepared in the laboratory and pasteurized to kill any *Salmonella*, which might be present in the meat. Then 200g of 'kitfo' was inoculated with overnight cultures of *Salmonella* test strains to give an inoculum level of 10^2 - 10^3 cfu/g. The inoculated 'kitfo' was mixed thoroughly and incubated at ambient temperatures for 24 h. To determine the initial inoculum level, freshly inoculated 'kitfo' (20g) was homogenized in 180 ml diluent and 0.1 ml of appropriate dilutions were plated on MacConkey agar. Samples were further processed for counting at 4 h intervals until 24 h. The test strains used in this study were those isolated from raw 'kitfo'. Counts were mean values of three observations.

Statistical analysis: Coefficient of variation (C.V.) was calculated by dividing the standard deviation by the mean to see the level of variation in counts of the microbial groups within the same sampling site. Values greater than 10% were considered significant variations.

Results

All raw 'kitfo' samples from all food establishments had high aerobic mesophilic counts ranging from $2x10^7$ to $2x10^8$ cfu/g. Coliforms, staphylococci, lactic acid bacteria, yeasts and molds and aerobic spores each had counts $>10^4$, 10^6, 10^5, 10^4 and 10^3 cfu/g, respectively, (Tables 1 and 2). In most of the food establishments considered in this study, variations in aerobic mesophilic count were not significant (coefficient of variation, CV < 10%) within an establishment. However, significant variations (CV>10%) were observed in counts of coliforms, staphylococci, lactic acid bacteria and aerobic spores within an establishment in most or all establishments. No significant variation was observed in mean aerobic mesophilic counts, counts of staphylococci, and lactic acid bacteria among the ten establishments (CV<10%). However, mean counts of coliforms, yeasts and molds, and aerobic spores varied significantly among the establishments (CV>12%).

The microbial load of medium-cooked ('leb-leb') 'kitfo' was not markedly low either. Counts of aerobic mesophilic bacteria and staphylococci were > $1.0x10^6$ cfu/g, coliform and aerobic spore counts were > $1.0x10^5$ cfu/g, and the other groups had counts of > $1.0x10^4$ cfu/g (Table 3). Variations in the counts of aerobic mesophilic bacteria, staphylococci, and lactic acid bacteria among the ten establishments were not significant (CV < 10%).

Well-cooked 'tibs kitfo' collected from the various establishments yielded no vegetative microorganisms (data not shown).

Salmonellae were isolated from 21 of the 50 raw 'kitfo' samples collected from the various establishments. All samples from Hotel B and Bar A, collected at different days, yielded *Salmonella* spp.. Three of the five samples collected from Hotel C and Bar B were positive for *Salmonella* and two of the five samples from Hotels A and F contained

Table 2: **Counts (log cfu/g) of lactic acid bacteria (LAB), yeasts and molds and aerobic spores in raw 'kitfo' samples**

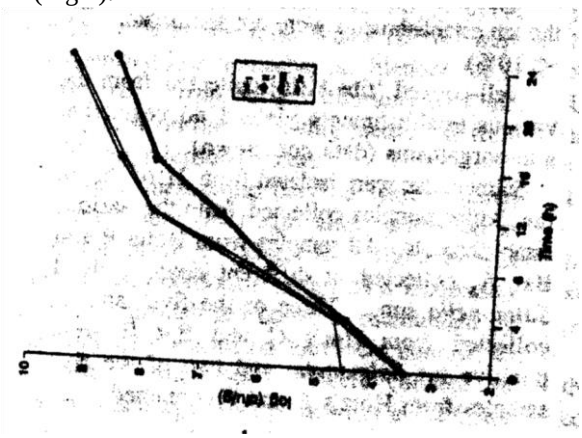
Sample source	LAB*			Yeasts and molds			Aerobic spores		
	X	S	%CV	X	S	%CV	X	S	%CV
Hotel A	7.36	0.99	13.45	4.51	0.87	19.29	6.81	0.80	11.74
Hotel B	8.31	0.84	10.10	6.77	0.76	11.22	7.21	1.10	15.25
Hotel C	7.83	0.64	8.17	6.18	0.96	15.53	6.99	0.94	13.44

Hotel D	7.32	0.34	4.64	6.39	0.66	10.32	6.42	0.67	10.43
Hotel E	7.42	0.03	0.40	61.4	0.36	5.89	6.90	0.50	7.24
Hotel F	8.10	0.96	11.85	5.72	0.59	10.31	6.83	0.48	7.02
Hotel G	7.81	0.54	6.91	5.57	0.65	11.66	6.22	0.76	12.21
Res A	7.30	0.70	9.58	5.33	0.69	12.94	6.18	0.41	6.63
Bar A	8.33	0.79	9.48	4.66	0.45	9.65	7.29	1.00	13.71
Bar B	7.87	0.42	5.33	5.89	0.64	10.86	6.93	0.76	10.96

Salmonella. Only one sample from Hotel G was positive for *Salmonella*. In samples from Hotels D, E and Restaurant A, no *Salmonella* spp. was isolated at any time. All medium-cooked or wellcooked 'kitfo' samples were free from salmonellae.

Analysis of swab samples of raw meat surface revealed that the average count of aerobic mesophilic bacteria and staphylococci was around 10^5 and 10^3 cfu/cm^2, respectively. Coliforms, lactic acid bacteria, molds and yeasts, and aerobic spores had $<10^3$ cfu/cm^2 (Data not shown).

The *Salmonella* test strains used in this study grew luxuriously in 'kitfo'. They grew to the level of 10^7 cfu/g within 12 h. A sharp increase in growth was noted between 4 h and 12 h. Growth has not declined even at 24 h (Fig 1).



Discussion. Figure 1: **Growth dynamics of four *Salmonella* test strains i, ii, iii, and iv in 'Kitfo'.**

The mean aerobic mesophilic count of raw 'kitfo' from food establishments in Addis Ababa was much lower than that reported for raw beef samples from butchers' shops in Awassa (7). The high load of microorganisms indicated that the level of contamination was very high. This could be the result of unhygienic handling and processing using unclean knives, cutting boards, and storage bins added to the poor hygienic status of food handlers. The high number of staphylococci, which is usually related to human skin and clothing, is indicative of this situation. At this level, presence of *Staphylococcus aureus* may lead to food intoxication.

The markedly high count of microorganisms in medium-cooked or 'leb-leb' 'kitfo' showed that this type of heat treatment was not a satisfactory one as far as the microbiological status of the food item is concerned. The presence of a significant number of coliforms indicated that the heat treatment was not sufficient to make a marked difference in microbial load. Preparation of wellcooked 'kitfo' appeared to be sufficient to guarantee a wholesome dish.

The frequency of isolation of *Salmonella* from raw 'kitfo' in this study was 42% (21/50). This was much higher than the 1.8%, 2.0%, 5.3% and 20% of minced meat reported from U.S.A., England, Germany, and Canada, respectively (5). It was also higher than the 9% incidence in raw beef reported from Awassa (7) and the 5.1% and 7% from organs of cattle in Addis Ababa and Dire Dawa

Table 3: **Counts of various microbial groups from medium-cooked ('leb-leb') 'kitfo' samples collected from different food establishments**

Sample source	(log cfu/g)					
	AMB	Coliforms	Staphylo cocci	LAB	Yeasts and molds	Aerobic spores
Hotel A	6.07	3.65	5.76	3.97	3.91	4.82
Hotel B	7.79	5.68	6.62	4.38	3.57	5.59
Hotel C	6.53	5.31	5.94	4.66	3.98	5.63
Hotel D	6.92	5.66	5.77	4.88	3.72	3.81
Hotel E	6.68	5.33	5.95	4.60	4.79	5.30
Hotel F	7.90	4.56	5.69	3.85	3.63	4.76
Hotel G	6.95	4.77	5.53	4.30	4.90	3.47
Res A	6.76	4.55	5.74	3.67	3.51	4.35
Bar A	7.88	3.92	6.96	4.80	3.97	5.00
Bar B	6.79	4.81	6.88	4.83	3.81	5.08

abattoirs, respectively (6). The higher prevalence rate in this study could be attributed to the mincing process in 'kitfo' preparation which guaranteed contamination of new surface areas. On the other hand, the frequency of isolation of *Salmonella* obtained in this study was lower than the 55% reported by Chau et al. (10) from pig carcass in Hong Kong and the 45% reported by Reilly et al. (11) from chicken carcass. The prevalence of *Salmonella* obtained in our study was beyond the range of 0.2% to 21% reported by D'Aoust (12) from beef carcass, and within the range of 0.4% to 66.3% from pig carcass and 5.0% to 79% from poultry. Pigs and poultry, however, are more exposed to *Salmonella* through their feeding habits than are cattle.

The constant isolation of *Salmonella* from some food establishments in this study indicated that these establishments could have constant sources of contamination with *Salmonella*. Food handlers, who may be carriers of *Salmonella*, are definite sources of contamination if they are not following basic hygienic principles during food handling. Other possible sources of contamination could be cutting boards, which could harbour *Salmonella*, as they are not cleaned thoroughly and are usually moist. Salmonellae may establish a niche in a crack in these woody cutting boards, proliferate, and continue to contaminate whatever comes in contact with the cutting board. Observation of the kitchen environments in the food establishments showed that the hygienic condition of the kitchens and the food handlers in most cases was very unsatisfactory. Although *Salmonella* was not isolated from medium-cooked or 'leb-leb kitfo' in our study, the presence of coliforms indicated that *Salmonella* could also survive the heat treatment if it were there.

The load of microorganisms in swab samples was markedly less than that of raw 'kitfo'. Solid meat chunks have less exposed surface area than minced meat. Accordingly the level of contamination from external sources is lower. Internal meat tissue is normally considered to be sterile.

Our *Salmonella* test strains grew to a level of 10^7 cfu/g within 12 h. This was, however, slightly lower than that in fermenting 'ergo', a traditional sour milk (13), and that in legume-based traditional sauces (14). In 'kitfo' *Salmonella* spp. could reach infective doses within 4 to 8 hours, where less than 100,000 cells are required to initiate successful infection.

This study indicated the need to train food handlers in basic principles of hygiene. The hygienic status of food handlers and the hygienic handling of kitchen utensils could improve the microbial status of food prepared in food establishments. Food esablishments should also follow strict sanitary control programmes for identifying and excluding *Salmonella* carriers in food preparation. Keeping minced meat for several hours at room temperature must also be avoided. The best alternative consumers seem to have to avoid infection by *Salmonella* is to consume wellcooked 'kitfo'.

Acknowledgements

The financial assistance for MT by SAREC is acknowledged.

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