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SALMONELLA, SHIGELLA AND GROWTH POTENTIAL OF OTHER FOOD-BORNE PATHOGENS IN ETHIOPIAN STREET VENDED FOODS
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ABSTRACT

Objective: **To evaluate the bacteriological safety of food items sold by street vendors with regard to *Salmonella* and *Shigella* and to assess the growth potential of some foodborne pathogens in some street foods.**
Design: **Collection of street-vended foods and laboratory based microbiological analysis.**
***Setting*: Microbiology Laboratory, Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia.**
Results: **Most of the street food samples had aerobic mesophilic counts $>10^7$ cfu/g. Nine "kitfo" and one "egg sandwich" samples yielded *Salmonella*. *Shigella* was isolated from three "macaroni" samples. The *Salmonella* isolates were sensitive to all ten drugs tested but the *Shigella* isolates had multiple resistance against five drugs. In a challenge study, *Salmonella typhimurium, Shigella flexneri* and *Staphylococcus aureus* grew in street-vended food samples to hazardous levels within eight to twelve hours.**
Conclusion: **Street foods are heavily contaminated with micro-organisms and are potential sources of food borne infections. Health hazards from street foods may be significantly minimised by consumption within four hours of preparation.**

INTRODUCTION

Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors, especially on streets and other public places. Types of vending sites encompass stalls, a variety of push-carts, roadside stands, hawkers with head-loads and other arrangements depending upon the ingenuity of the individual, resources available, type of food sold and the availability of other facilities(1).

Street vendors are common in both developing and industrialised countries with a considerable expansion in the former. Evidently, in large cities of developing countries, various food items of animal and plant origin are commonly vended at areas with busy economic activities and heavy movement of people(2-6). These areas include transportation centres, large construction sites, schools, factories, hospitals, churches, checkpoints and other similar business centres. Both males and females are involved actively in the activities of street vending(1,4,5).

In spite of numerous advantages offered by street foods, there are also several health hazards associated with this sector of the economy. Multiple lines of evidence reveal that foods exposed for sale on roadsides may become contaminated by either spoilage or pathogenic micro-organisms(2,3,7). This constitutes a serious health hazard, particularly in economically disadvantaged countries where food surveillance centres are rudimentary or not there at all. Evidently, street vended foods have shown epidemiological links with illness(8-10) and laboratory results have shown alarmingly high counts and presence of food-borne pathogens in street-vended foods(5,11,12).

Studies made by FAO(5) in Africa, Asia and Latin America pointed out that the important aspect of street foods is their safety. The studies suggested that the problems associated with safety of street foods are real and need to be addressed to protect consumers. In Ethiopia, various foods were reported to carry pathogens or allow the growth of pathogens in them(13-16). There is, however, limited information on the microbial load and safety of street foods, in spite of the widespread use of such food items in the country(3,17,18). As bacterial food-borne diseases pose considerable problems in countries like Ethiopia, there is a need to generate data on the safety of street foods in big cities such as Addis Ababa. Such information is useful to appreciate the safety problems related to street foods so that regulatory agencies may take appropriate steps to improve safety and sanitation with respect to this economic sector. Therefore, the present study was initiated to evaluate the bacteriological safety of food items sold by street vendors in Addis Ababa with regard to *Salmonella* and *Shigella* and assess the growth potential of *Salmonella tyhpimurium, Shigella flexneri* and *Staphylococcus aureus* in some of these foods.

MATERIALS AND METHODS

Sample collection: A total of 150 samples of street foods comprising 30 each of "sambussa", "macaroni", "lentil sandwich", "kitfo" (raw minced meat) and "egg sandwich", were collected from different sale outlets in Addis Ababa, Ethiopia between August, 1998 and April, 1999. Samples were collected in sterile aluminum plates using aseptic techniques. Samples of whole meals were purchased during late breakfast, lunch or supper. All samples were immediately brought to the laboratory and microbiological analysis was conducted within one to three hours of collection.

Enumeration: Twenty grams of whole meals were separately weighed and mixed to 180 ml Buffered Peptone Water (BPW) (Oxoid) and homogenised for one to three minutes using a stomacher lab blender (model 400, Seward JAC, London). From appropriate dilutions, 0.1 ml aliquots were spread plated in duplicates on Nutrient Agar (Oxoid) plates and incubated at 30-32°C for 24-48 hours for aerobic mesophilic count. In cases of "sambussa", "lentil sandwich" and "egg sandwich", the bread part was discarded and only the internal contents were microbiologically analysed. "Kitfo" and "macaroni" samples were directly processed for analysis.

Isolation and characterisation of Salmonella and Shigella spp: For primary enrichment of *Salmonella* and *Shigella,* the homogenate in BPW was incubated at 37°C for 18-24 hours for the metabolic recovery and proliferation of injured cells in the food samples and to raise the population of target organisms to a detectable level.

Selenite Broth (SB), Tetrathionate Broth (TB), both from Oxoid, and Rappaport Vassiliadis (RVS) broth (Merck) were employed for secondary enrichment purpose. After pre-enrichment in BPW, one millilitre of culture was each transferred into a tube containing 10 ml of SB and into another tube containing an equal volume of TB. However, 0.1 ml of BPW culture was inoculated into a tube containing 10 ml of RVS broth. SB was incubated at 37°C and TB and RVS broth at 43°C for 48 hours in water bath.

MacConkey agar No. 3, *Salmonella-Shigella (SS)* agar and xylose lysine desoxycholate (XLD) medium (all from Oxoid) were used for plating purpose. A loopful of culture from selective enrichment broth was streaked separately on to each of the solid media and incubated at 37°C for 18-24 h. Un-inoculated culture plates were incubated to check for sterility of the solid media.

Colonies typical of *Salmonella* or *Shigella* were picked from each plate and characterised biochemically following standard methods(19). Colonies that met the minimum biochemical profile of *Salmonella* or *Shigella* were confirmed by serogrouping with slide agglutination using polyvalent antisera (Murex Diagnostic Ltd, England).

Antimicrobial susceptibility test was done on Mueller Hinton Agar (Oxoid) plates following the standardised disk diffusion technique(20), with Oxoid drug disks: ampicillin, 10 µg; cephalothin, 30 µg; chloramphenicol, 30 µg; kanamycin, 10 µg; polymyxin B, 300 units; streptomycin, 30 µg; sulphadiazine, 1 mg; tetracycline, 30 µg; and trimethoprim sulphamethoxazole, 25 µg. The reference strain *Escherichia coli* (ATCC 25922), sensitive to all drugs was routinely tested. Interpretation of readings as sensitive, intermediate and resistant was made according to a chart(21). Intermediate readings were few and therefore considered as sensitive for the purpose of assessing the data.

Determination of growth potential of test strains in street foods: The growth dynamics of *Salmonella typhimurium, Shigella flexneri* and *Staphylococcus aureus* was assessed in "egg sandwich", "lentil sandwich" and "macaroni". Two hundred grams of each food item was steamed at 100°C for one minute to kill any vegetative cell, including *Salmonella, Shigella* and *Staphylococcus* species, which might be present in the items. Steamed food (10 g each) was examined for aerobic mesophilic bacteria and aerobic bacterial spores. Then 100 g of each street food item was challenged separately with overnight culture of the test strains to give an inoculum level of 10^2 - 10^3 cfu/g. The challenged foods were left at ambient temperature for 24 hours. To investigate the initial inoculum level, freshly inoculated foods (10 g each) were homogenised separately in 90 ml of BPW and 0.1 ml of appropriate dilutions were spread plated on SS Agar to count *Salmonella* and *Shigella* and Mannitol Salt Agar (Oxoid) to count *Staphylococcus aureus.* Portions of food samples were further sampled aseptically at 4 hour intervals from 0 - 24 hours.

RESULTS

All food items, except "sambussa" samples, had counts $>10^7$ cfu/g. *Salmonella* was isolated from nine samples of "kitfo" and one sample of "egg sandwich" collected from street vendors. *Shigella flexneri* was encountered in three "macaroni" samples (Table 1). Eight of the ten *Salmonella* isolates were sensitive to all ten drugs tested. The remaining two were resistant only to sulphadiazine. The three *Shigella* isolates showed a similar multiple resistance pattern against ampicillin, trimethoprim-sulphamethoxazole, chloramphenicol, streptomycin and tetracycline.

Table 1

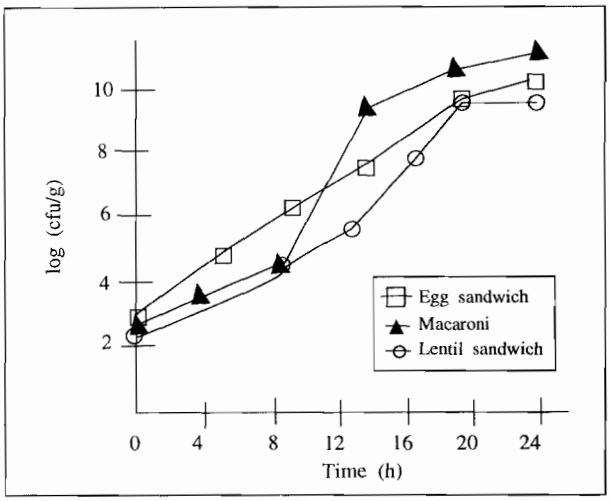
Counts of aerobic mesophilic bacteria and incidence of Salmonella and Shigella isolates in foods collected from street vendors in Addis Ababa.

Food type	No.	Aerobic mesophilic count (log cfu/g)			Positive for	
		$\bar{X}$	SD	% CV	*Salmonella*	*Shigella*
Sambussa	30	5.21	1.1	21.11	–	–
Macaroni	30	7.93	0.80	10.09	–	3
Lentil sandwich	30	7.20	0.63	8.79	–	–
Kitfo	30	7.71	0.94	12.19	9	–
Egg sandwich	30	8.38	1.31	15.58	1	–

Steamed foods had no vegetative cells and counts of aerobic spores were $<10^2$ cfu/g. In challenge studies, *Salmonella typhimurium* reached counts $>10^8$ cfu/g within 24 hours in all the food items tested. Counts increased by about 1 log unit in the first 4 hours and showed a steady growth thereafter. Its growth rate in "lentil sandwich" was relatively lower than that in the other food items (Figure 1).

Figure 1

Growth potential of salmonella typhimurium in some street-vended foods



Growth of *Shigella flexneri* was markedly fast in the first 4 hours in "macaroni" compared to "egg sandwich" and "lentil sandwich". Final counts in the food items varied slightly and higher counts were noted in "macaroni" followed by "lentil sandwich". Growth rate in "lentil sandwich" was relatively steadier. Initial inoculum level of the test strain was much lower than that of the other test strains (Figure 2).

Figure 2

Growth potential of shigella flexneri in some street-vended foods

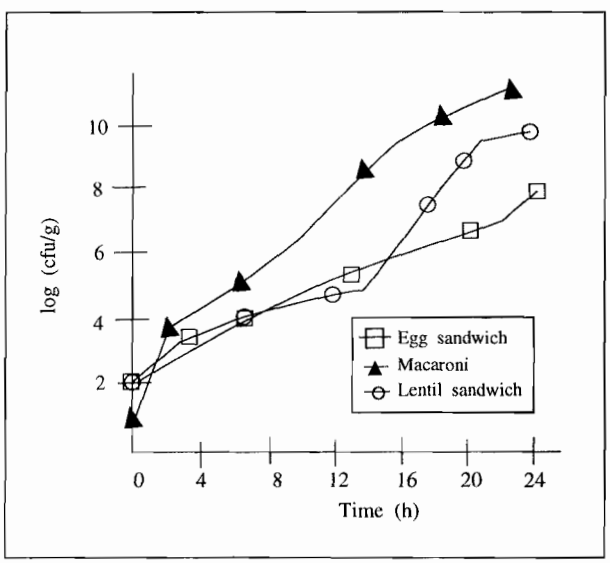
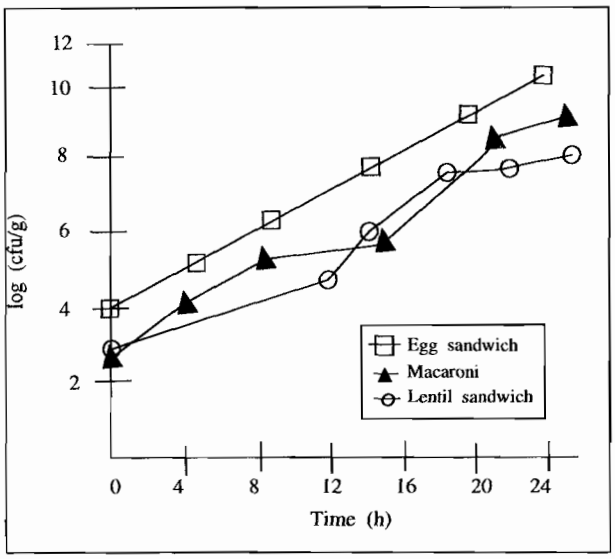


Figure 3

Growth potential of staphylococcus aureus in some street-vended foods



Staphylococcus aureus initially had relatively higher counts and increased by 2 log units within eight hours in all the three food items. Growth rate in the first eight hours was markedly high. Counts as high as 10^5 cfu/g were attained after eight hours, reaching final counts of $>10^9$ cfu/g at 24 h (Figure 3).

DISCUSSION

Despite the fact that most of the street vended food items considered in this study were cooked foods, the high aerobic mesophilic count was indicative of post cooking contamination, although a small number of surviving spores and heat resistant species might also be part of the flora. *Shigella* was isolated only from "macaroni" samples with incidence rates lower than what was reported from a similar study in Ethiopia(3). Other workers, however, reported that cereal based dishes collected from street vendors did not yield *Shigella* spp(8). *Shigella* in "macaroni" would most likely be killed during cooking process, but post-cooking contamination from food handlers is possible because reservoir host, other than human beings, is not reported for *Shigella spp.* Chronic carriers who are food handlers are important reservoir of pathogens, and play a role in the epidemiology of food-borne diseases(22). Despite the poor hygienic status of the vending environment, "macaroni" did not yield *Salmonella spp.* In another study, however, *Salmonella* was isolated from ready-to-eat spaghetti in an open market in Awassa, Ethiopia(3).

Samples of "kitfo" did not yield *Shigella.* Similar observations were made in other studies(2,8,9). However, the possibilities of cross-contaminations should not be undermined. *Salmonella* was, however, isolated from samples of "kitfo". Similarly, Bryan *et al*(2, 23), reported the isolation of this pathogen from ground raw meat. Other

workers also isolated *Salmonella* from minced meat in Addis Ababa(15,24). As a pathogen of zoonotic importance, this organisms could be a contaminant of the raw meat from the animal, or could be acquired from hands of the vendors or during the mincing process during "kitfo" preparation, which normally results in the contamination of sterile internal tissues. Cutting boards, knives, unclean multi-purpose cloth (used to rub food serving trays and spoons after use) and storage pans could also serve as possible sources of cross contamination. Isolation of *Salmonella* from chopping board has been reported elsewhere(23).

Despite the fact that egg is known to be a good source of *Salmonella,* only one egg sandwich was positive for *Salmonella* in this study. In another study, *Salmonella* was encountered in samples of egg shell and street-vended cooked chicken(23). Similarly lentil sandwich and sambussa samples were all free from *Salmonella* and *Shigella.* The external bread portion of these sandwiches could be protective from external contamination.

Challenge studies showed that *Salmonella typhimurium* multiplied more than 100-fold in the food items within 12 hours. This rate was, however, lower than that obtained in legume-based traditional sauces(25) or "kitfo"(15). In all the three food items, *S. typhimurium* could attain the infective dose level within 4 to 8 hours, where fewer than 100 viable cells are needed to be ingested to produce the disease syndrome.

Shigella flexneri also showed a steady growth in the tested food items. There has not been any report on growth dynamics of *Shigella* in traditional foods in Ethiopia. Hence, comparison of our results with previous studies was not possible. This test strain could, however, grow to a level of infective doses within 4 to 8 hours. At this cell number *Shigella* could initiate a successful infection.

The growth of *Staphylococcus aureus* was enhanced considerably in the three food types and reached a count greater than 10^6 cfu/g within 12 hours. Its growth was remarkably fast and attained higher counts than reported for a traditional fermenting gruel as observed in another study(26). At a level exceeding 5.6×10^5 cfu/g, *Staph. aureus* was reported to secret staphylococcal enterotoxin type A in food samples of potatoes obtained from street vendors in Pakistan(27). It has also been reported that *Staph. aureus* counts ranging from 10^5 to $>10^9$ cfu/g were recovered from foods implicated in food poisoning outbreaks(28). Considering the high level of carrier state of *S. aureus* among the human population, contamination of the food items with *S. aureus* is far from being a remote possibility. Thus street foods are likely to be causes of food poisoning if held for more than four to eight hours at ambient temperatures.

Our ten *Salmonella* isolates were all sensitive to all antimicrobials and all the three *Shigella* isolates showed multiple resistance to five antimicrobials. Unfortunately the number of our isolates was too small to make any reasonable comparisons with other studies. Other workers from Ethiopia reported that a good number of *Salmonella* isolated from minced meat and chicken exhibited resistance to one or more antimicrobials(24)

Street foods are sources of nutrition for many low-income groups at affordable prices in large urban areas. Nevertheless, there are also several health hazards associated with them. Street foods, in general, act as main vehicle for transmission of severe and fatal diseases. Moreover, *Salmonella* and *Shigella* species have been recovered considerably from various dishes obtained from street vendors(1-3). This study also indicated that street foods in Addis Ababa are heavily contaminated with aerobic mesophilic bacteria and there were also incidences, although low, where pathogens were isolated from some of them. In addition, the study has shown the potential of some food-borne pathogens to grow in them during holding at ambient temperature. Health hazards from street-vended foods may be minimised by avoiding long-holding at ambient temperatures.

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