MICROBIOLOGICAL QUALITY OF "KHEBAB" CONSUMED IN THE ACCRA METROPOLIS

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SUMMARY

The study was carried out in 2003 to evaluate the microbial load in "khebab", meat products from pork, and beef, which are vended in most of the streets and some public drinking places, either with alcoholic or non-alcoholic drinks.

Osu (Alata), Nima-Kotobabi and Central Accra (Adabraka – very close to the main lorry station), all in the Accra Metropolis, were selected for the investigation. The main reason for the selection of these sites was based on the population density as well as patronage for the khebab. Our main interest for this investigation was to assess the microbial load in khebab as far as enteric pathogen and other pathogenic micro-organisms reported earlier in the raw meat are concerned. Thirty samples of khebab were bought from these sampling points. Results obtained from samples at Osu recorded mean total plate count (TPC) of 5.02, Accra Central samples had TPC of 4.08 and those from Nima had TPC of 4.80 $\log_{10}$ colony-forming units (cfu) per gram of khebab. Samples from Accra Central recorded the highest mean coliform count (5.12) whist samples bought from Osu and Nima recorded 4.41 and 3.70 $\log_{10}$ cfu/g respectively. Accra Central samples again recorded the highest faecal coliforms (4.4 $\log_{10}$ cfu/g) as compared to 3.98 and 3.80 log10 cfu/g for samples bought from Osu and Nima respectively. *Salmonella ssp* were not isolated from the samples bought at the three sampling sites. Khebab samples from sites were contaminated with *E. coli*, other gram-negative bacteria and *Staphylococcus* species, whose virulence factor(s) are yet to be determined. The faecal coliforms enumerated could originate from either humans or the animals slaughtered for the khebab.

Staphylococcus species could originate from the vendors. Vendors have to be educated on hygienic practices which could help reduce risks of food-borne infection. Skin disinfection can be done by a thorough wash. Vendors could also be educated to stop selling their products to customers once they have bouts of diarrhoea, vomiting and "fever". Washing of their hands with soap and water before serving their customers could also help reduce the risk of food-borne infection from eating their products.

Keywords: Khebab, *Escherichia coli, Salmonella*, food safety, meat product.

INTRODUCTION

Food-borne infections still remain one of the conditions of public health importance worldwide. Though data from different countries seem to report increases in the incidence of food-borne diseases, these data may not always represent the actual fact on the ground[1]. Food production, processing and distribution in the world, differ from country to country. These practices depend on local consumer preference and the influence of other country's practices on the local consumer's lifestyle[2].

Illness, caused by the consumption of contaminated food, could be the result of the presence of pathogenic organisms (food-borne infection) or, it could be the result of the presence of toxic chemicals. The main symptoms of food-borne infection/intoxication include nausea, vomiting, colic and diarrhoea. There are different types of organisms which are known to cause food-borne infections. The most common bacterial agents are the *Camplyobacters, Salmonellae*, toxigenic/verocytotoxic *E. coli, Shigellae*, toxigenic *Staphylococcus* and *Clostridiae* worldwide[3,4,5]. Human cases of botulism have been found to originate from a considerable variety of preserved foods, for example, ham, sausage, canned meats, vegetable products etc.

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Modes of spread of alimentary tract infections, caused by pathogenic microbes, are through the oro-faecal route. Deterioration of meat begins soon after the slaughtering of the animal. This deterioration is the consequence of microbial, chemical and physical processes (Pobee 2001 unpublished). Freshly slaughtered animals may harbour few bacteria. But, the surface of the meat is, in most cases, exposed to contamination during slaughter, evisceration and other operations after slaughter[6]. Sanitation, proper refrigeration and proper handling of the meat are of paramount concern, if contamination is to be minimized and microbial activity is to be curtailed. This study was initiated to determine the microbial load (mainly *Salmonella, Shigellae, E. coli,* and *Staphylococcus*) in khebab samples bought from Osu, Nima and Accra Central.

MATERIALS AND METHODS

Prior to starting the study, visits were paid to the selected sites in May 2003 to explain to the vendors what was going to be done with their khebab samples. These vendors were recruited into the study after the owners of the vending places had given their approval and the vendors were assured of confidentiality. Samples of about 100g of khebab which were being sold to consumers were bought and with sterile knife an forceps, sliced into separate sterile containers. The samples were then transported to the laboratory in the Bacteriology Unit of the Noguchi Memorial Institute for Medical Research on ice packs within two hours after collection and kept at 4^0C until they were taken through bacteriological examination. Sample collection took place between 11:00 in the morning to 12:30 post meridian. All of the samples were examined within 24 hours after arriving at to the laboratory. Ten grams of the samples were weighed aseptically into sterile plastic stomacher bags and macerated. A 1:10 dilution was prepared by adding 90ml of phosphate-buffered saline (PBS, Oxoid Dulbecco ABR, UNIPATH, Basingstoke, England, pH 7.0).

The 1:10 suspension was thoroughly mixed and further ten-fold serial dilutions were carried out by aseptically transferring 1ml of this into 9ml of the diluent, and serially diluted in the same buffer solution, starting from 1:10 though 1:100,000. Half a milliliter volume of the dilutions were inoculated onto various agar plates and spread using sterile glass spreader. Total aerobic mesophillic bacteria counts were carried out using plate count agar (Oxoid CM 453). MacConkey agar (Oxoid CM 7) was used for coliforms and faecal coliform counts. Agar plates for enumeration of total mesophillic bacteria and coliforms were incubated at 37^0C whereas plates for the faecal coliforms were incubated at 44^0C and incubated aerobically between 16 and 24 hours.

Fifty millilitre portions of 1:10 suspension were centrifuged at 10,000 rpm for 30 minutes in a refrigerated centrifuge (4^0C). After decanting the supernatant, a loop-full of the pellet was streaked onto sheep blood agar, MacConkey and *Salmonella/Shigella* (SS) agar (Oxoid CM 533) and incubated at 37^0C for 18-24 hours under aerobic condition. Portions of the pellet were also inoculated into selenite – lactose broth (Oxoid CM 395) and incubated for 18 to 24 hour. Two loop-fulls were streaked onto the media indicated above and incubated for the same time. Gram method was used for preliminary identification of the isolates. The Gram method was complemented by standard biochemical test[7]. The same procedure described above was used on other serially diluted suspensions of the macerated khebab. Plates which had between 30-300 colonies were selected for the determination of colony forming unit per gram (cfu/g). Bacterial isolates were suspended in PBS and fixed by adding 2 or 3 drops of 40% formaldehyde per 10ml and mixed thoroughly by vortexing. Bacteria counts were carried out suing colony counting chamber (Gallenkamp, UK). The number of cfu/g in the khebab was calculated by multiplying the number of bacteria by the dilution factor.

RESULTS

Results obtained from samples bought at Osu recorded mean total plates count (TPC) of 5.02, Accra Central samples had TPC of 4.08 and those from Nima had TPC of 4.80 $\log_{10}$ cfu/g of khebab. Samples from Accra Central recorded the highest mean coliform count (5.12) whilst samples bought from Osu and Nima recorded 4.41 and 3.70 $\log_{10}$ cfu/g respectively. Accra Central samples again recorded the highest faecal coliforms (4.4 $\log_{10}$ cfu/g) as compared to 3.98 and 3.80 $\log_{10}$ cfu/g for samples bought from Osu and Nima respectively. *Salmonella* spp were not isolated from the samples bought at the three sampling sites. Khebab samples from the sites were contaminated with *E. coli*, other gram-negative bacteria and *Staphylococcus* species. Figure 1 shows the mean microbial colony count of the khebab samples bought from the three selected sampling sites. Results on the extent of contamination of khebab bought from the vending sites in the Accra Metropolis are presented in Table 1.

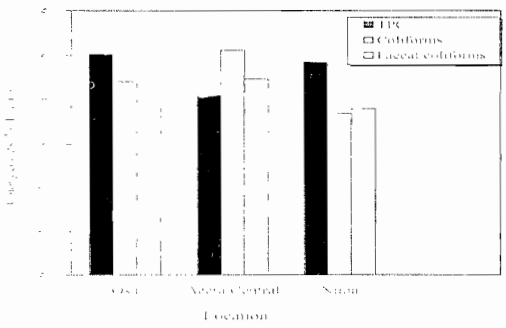

Figure 1 Mean microbial colony count of khebab samples from selected parts of Accra metropolis.

Table 1 Microbial load of khebab samples from three selling centres in the Accra metropolis

Location	Sample no.	Total count (log_{10} cfu)	Coliforms (log_{10} cfu/g)	Faecal coliform (log_{10} cfu/g)
Osu	001	4.20	0.00	4.28
	002	5.65	5.41	5.28
	003	6.25	4.36	4.84
	004	5.55	4.32	4.41
	005	5.85	4.00	4.30
	006	3.36	5.38	6.46
	007	4.89	4.72	0.00
	008	4.77	5.30	0.00
	009	4.97	5.34	5.04
	010	4.74	5.36	5.15
Accra Central	011	5.84	4.97	4.46
	012	5.38	4.46	4.28
	013	4.61	3.74	5.15
	014	4.23	5.11	4.30
	015	5.61	6.32	5.41
	016	5.28	5.40	5.41
	017	5.22	5.60	0.00
	018	4.93	5.38	5.23
	019	3.90	5.11	5.26
	020	5.65	5.11	5.26
Nima	021	4.91	3.86	3.65
	022	5.87	0.00	3.80
	023	5.95	0.00	0.00
	024	3.95	0.00	4.36
	025	4.39	5.30	4.89
	026	4.48	5.38	5.04
	027	5.23	7.00	4.23
	028	3.54	4.71	3.98
	029	4.74	3.69	3.72
	030	4.97	7.01	3.95

DISCUSSION

Khebab samples bought from Osu had the highest total plate count (TPC) which ranged between 3.36–6.25 log_{10}cfu/g. TPC for Central Accra and Nima had ranges of 3.90–5.84 and 3.54–5.95 log_{10}cfu/g respectively. TPC only refers to the number of mesophilic bacteria growing on the plates. The mean, as reported in this paper, have been based on these ranges as well as the number of samples from each sampling site. These findings could be attributed to the extent to which the samples were exposed to the environment and the handling procedures employed by the individual khebab vendor. Nima, a community known for its overcrowding in the Accra Metropolis and regarded to have unhygienic surroundings rather recorded the lowest mean coliform count of 3.70 and mean faecal coliform count of 3.8log_{10}cfu/g as compared to mean coliform and faecal counts at Osu (4.41 and 3.98) and Accra Central (5.12 and 4.46) respectively for the same test parameters in all of the samples which were examined. One possible explanation for these observations and results could be that khebab produced by the vendors at Nima were bought and consumed readily, on the same day as it was prepared, so that left-over khebabs were not kept for re-heating and sale on the following day. We have not analysed the components that these vendors used for the seasoning of the meat prior to processing. They may also have some effect on the bacterial load. The roasting temperatures, as well as the thickness of the slices of meat are the prerogative of each vendor. Thickly sliced meat could not have more heat penetrating the khebab. Thinly sliced meal will allow sufficient heat penetration which will reduce the microbial load. Samples 007 and 008 from Osu were free of faecal coliforms (Table 1). It was sample 017 from Accra Central which was not contaminated with faecal coliforms. With regard to samples bought from Nima, sample 023 was free from both coliforms and faecal coliforms, but has *Staphylococcus* spp. Normal commensal bacterial flora of the skin which are made up of a few species of resident organisms, mainly coagulase-negative *Staphylococcus* which grow in the glands and hair follicles cannot be removed entirely. The presence of *Staphylococcus* on the khebab samples bought from some of the selected sampling sites could come from the resident organisms from the vendors. Ghana Standard Board recommendation for pre-cooked meat is 1.0×10^4 i.e. 4.0 log_{10}cfu/g for total viable count which also pointed to the fact that there should no faecal coliforms or *Salmonella* species contaminating such meat. An earlier study carried out in Accra (the old Accra slaughterhouse, the Accra abattoir and a typical traditional slaughterhouse) on raw beef and raw chevron samples revealed contamination of beef with 15 bacteria species whilst the chevron was contaminated with

14 different bacteria species[8]. It should be emphasized here that neither *Shigellae* nor *Salmonellae* strains were isolated from khebab samples from any of the sampling sites. There is paucity in the literature with regard to the West African Region as well as Ghana on this type of investigation and the methodology used. Most people in Ghana do "overcook" their meat, especially in soups and in stews. The problem could only be with the khebab which has not been heated sufficiently in order to destroy most of the microbes. If the TPC is less than 5.0 $\log_{10}$cfu/g and the coliform counts are less than 3.0 $\log_{10}$cfu/g, the khebab could be classified as having low risk as far as transmission of pathogenic bacteria to the consumer is concerned. Anything higher than these counts puts the consumer at risk. Results obtained from this preliminary investigation seem to point to the fact that it could be risky eating khebab from some of these sampling sites. It should also be emphasized here that since the incubation of some of the media were carried out at 37°C, the coliforms could be atypical coliforms which may not be pathogenic. After all, we all have these atypical coliforms in our gut. Nevertheless, vendors have to be educated on various hygienic practices. It may not be economical for these vendors to be using 70% ethanol daily for skin disinfection. Skin disinfection can easily be done by thorough wash. It is also possible to educate the vendors who might have bouts of diarrhoea, vomiting and 'fever' to stop their sales to the public until there is improvement in their condition.

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