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Bacteriological aspects of some food available in Yangon area

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Bacterial pathogen contamination of 81 food samples from Pazundaung, Mingalar Taungnyunt and Pabedan Townships was carried out from June 2002 to May 2004. These samples were 26, 21, 19 and 15 of noodles, bread, biscuits and cookies respectively. The total bacterial count, total coliform and faecal coliform count were tested in all those food samples using conventional methods. Plasmid isolation was done on *Escherichia coli* isolates. In this study, the Colony Forming Units (CFU) of bacteria counted were $>7.5 \times 10^5$ organisms/gram in 27 samples (33.33%); 1.5×10^4 to 7.5×10^5 CFU/gram in 26 samples (32.09 %); and <2 CFU/gram in 28 samples (34.56%). Coliforms (100%) and faecal coliforms (61.53%) were identified from noodles. From bread, coliforms (71.42 %) and faecal coliforms (28.57%) were identified. From biscuits, coliforms (71.42 %) and faecal coliforms (21.05 %) were identified. Also, coliforms (46.66 %) and faecal coliforms (20.0%) were identified in cookies. From all foods tested, coliforms were isolated in 72.83% and faecal coliforms in 35.80%. *Staphylococcus* species was isolated from 46.15 % of noodles and 37.50 % of bread, however, none from biscuits and cookies. *Bacillus* species was isolated from 92%, 76.19 %, 21.05%, and 33.33% of noodles, bread, biscuits and cookies respectively. *Escherichia coli* was isolated from 100%, 71.42%, 21.05% and 53.33% of noodles, bread, biscuits and cookies respectively. Nine isolates of *E. coli* were serogrouped as O55K59 (5 isolates) and one each of O8K25, O26K50, O114K90 and O142K+. They were isolated from 6, 2 and 1 samples of noodles, bread and cookies respectively. However, *Salmonella*, *Shigella* and *Vibrios* (O1 and O139) were not isolated in this study. The plasmid size of *E. coli* isolates in this study was 23 kilo base pairs.

INTRODUCTION

Suspect foods are those that are implicated by an attack-rate table or other epi-demiological data or that have a history of being mishandled or mistreated. Potentially hazardous foods are those that readily support rapid and progressive growth of pathogens because of their properties (pH, nutrients, water activity) or that have a history of being vehicles in outbreaks of food-borne disease. The problems of food hygiene and sanitation have been reported in the various studies and it was recognized that there existed a high potential for serious health problems related to the preparation and handling of street foods. Studies from some countries (Indonesia, Nigeria, Pune, Bombay, Columbia) confirmed both microbial and chemical contamination of food being sold by street venders. There have been no recent epidemiological studies to suggest that street foods contribute to a significant number of food poisoning. In spite of this, there have been several documented outbreaks attributed to the consumption of Loh Shee Yum (rice noodles) bought from different hawkers. In 1981, a cholera epidemic in Pune City, India was attributed to contaminated sugar cane juice with ice. In this case, ice was found to be contaminated with *Vibrio cholerae.* In Singapore, there were 25 notified cases of food poisoning related to food center during 1987. The smallest infective dose of *Vibrio cholerae* is

10^2 to 10^3 organisms. Many reports were recorded with regard to microbiological profile of food served by street vendors in other countries [1-5].

In our country 277,871, 112,352, 4,128 and 4,477 cases of diarrhea, dysentery, typhoid and food poisoning respectively were reported in 1996 . In 1999, it was reduced to 24118, 11681, and 3562 cases of diarrhoea, dysentery and food poisoning cases respectively. In Yangon Division, 1517, 26 and 7 cases of diarrhea, dysentery and food poisoning respectively, were reported in 1999 [6]. Thus, this study was carried out to know the level of contamination of food sold around Yangon area.

General objective

To identify the contamination of bacterial pathogens from wheat product foods

Specific objectives

1. To identify the bacterial count of those food
2. To define the coliforms and faecal coliforms from food
3 .To diagnose the specific bacterial pathogens of food.

Study period

June 2002 to May 2004

Preparation and processing of food samples for isolation of pathogens

They were weighed as required for identification of pathogens aseptically soaked in their respective buffers, media and processed as according to the standard procedures [7].

Total bacterial count

The plate count expresses the number of all colony-forming bacteria. It is of limited value by itself, but as a supplementary test, it provides information about the amount and type of organic matter in food which may be useful in indicating the efficiency of the process of food. Specific bacterial count was done on their selective media. The plates were incubated at 37º C and 25º C for bacteria and fungi respectively [8].

Presumptive test fo r coliforms and faecal coliforms

The presence of total coliforms was isolated using multiple tube method of MacConkey broth purple (double strength), incubated in waterbath at 37º C. For faecal coliforms, the usual method as described above was performed while the incubation temperature was 44.5º C [7].

Isolation of Staphylococcus species, Escherichia coli, Salmonella species and Shigella species and Vibrio species

Twenty grams of food samples were weighed aseptically and mixed well with 180 ml of phosphate buffer. For primary culture, Mannitol salt agar, MacConkey agar, Salmonella-Shigella agar and Thiosulphate Citrate-Bile sucrose (TCBS) agar plates were used and Selenite F enrichment and alkaline peptone water were used for secondary culture. The numbers of colonies identified for specific organisms multiply by the dilution factors express the value as number of cells per gram of food [8].

Isolation of Bacillus species from food

Ten grams of homogenized food were mixed thoroughly with 90 ml Mannitol egg yolk polymixin B broth. Approximately 0.02 ml of culture suspension was streaked onto Mannitol egg yolk polymixin B agar and incubated at 25-30º C for 24-48 hours. Growth of eosin pink lecithinase positive colonies after biochemical tests and Gram staining revealed *Bacillus* species..

Isolation of fungi

Twenty grams of food sample after blending aseptically were soaked and mixed with 180 ml of phosphate buffer. After 10 fold dilutions for 5-tubes, 0.02 ml each of the suspension was placed onto Sabouraud Dextrose agar. The suspension was allowed

to dry at room temperature and kept up to five days. Growth of moulds and yeasts was examined every day. The colonies were stained and examined under microscope and germ tube test was performed.

Plasmid analysis

Rapid extraction of plasmid DNA was carried out from all enteropathogenic *Escherichia coli* isolates by the modified Birnboim procedure [9].

RESULTS

Total Plate count

The total bacteria count ranges from <2 to 10^6 (uncountable) per gram of food (Table 1).

Table 1. Total bacterial count in different kinds of food

Bacterial count CFU/gram	Noodles n=26	Bread n=21	Biscuits n=19	Cookies n=15	Total
<2	1 (3.84)	3 (14.28))	14 (73.68)	10 (66.66)	28 (34.56)
1.5x 10^4 to 7.5 x 10^5	8 (30.76)	8 (38.09)	5 (26.31)	5 (33.33)	26 (32.09)
7.5 x 10^5 to uncountable	17 (65.38)	10 (47.61)	0	0	27 (33.33)

Figures in parenthesis denote percentages

Determination of presumptive coliforms and faecal coliform counts

Out of 81 food samples, coliforms and faecal coliforms were identified from 72.83 % and 35.80% respectively . Coliforms were identified from noodles (100%), bread (71.42%) biscuit (57.89%) and cookies (46.66%). Faecal coliforms were isolated from noodles (61.53%) bread (28.57%), biscuits (21.05%) and cookies (20%) (Figure1).

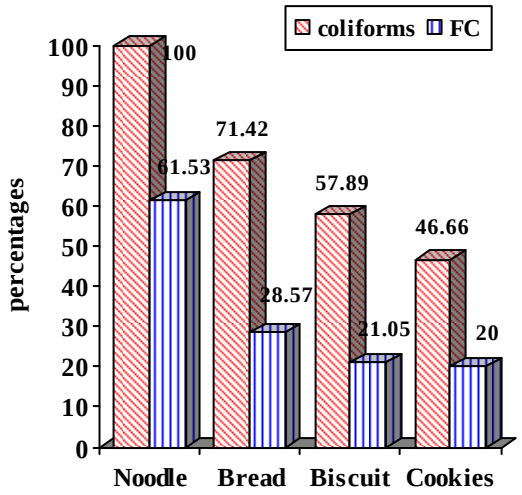

Fig 1. Coliforms and faecal coliforms from noodles, bread, biscuits and cookies from different areas of Yangon

Distribution of Staphylococcus species, Escherichia coli, Salmonella species, Shigella species and Vibrio species

Staphylococcus species was isolated from noodles (46.15 %) and from bread (37.5%). *Bacillus* species was isolated from noodles (92%), bread (76.198%), biscuits (21.05%) and cookies (33.33%). Similarly, *E. coli* was isolated from noodles (100 %), bread (71.42%), biscuits (21.05 %) and cookies (53.33%). Serogroups of *E. coli* isolated from noodles were O55K59 (from 3 isolates) and O8K25, O26K50 and O114K90 (one isolate each). From bread samples isolates, *E. coli* serogroups were O55K59 and O142K+. *Escherichia coli* O55K59 was identified from cookies. None of the *E. coli* from biscuits could be serotyped (Table 2). *Salmonella* spp, *Shigella* spp, *Vibrio* cholerae O1 or O139 were not isolated from all the food samples.

DISCUSSION

Recent studies indicated that cholera outbreak occurred in Mpumalanga Province, Mozambique, South Africa and in Liberia in 2003. They have shown that cholera outbreak occurred especially during the rainy season due to population movement

and the lack of safe water. Cholera and vibrio - associated diarrhoea responsible for diarrhoeal diseases are *V. cholerae* O-Group 1, non-O Group I, *V. cholerae* (non-epidemic strains), *V. parahaemolyticus, V. alginolyticus,* and 'Group F vibrios". It had been shown that cholera is food-borne transmission via street vendors, and claimed that transmission was carried through shell fish and water [3].

Table 2. Distribution of *Staphylococcus* species, *Bacillus* species, *Escherichia coli* serogroups in noodles, bread, biscuits and cookies

Types of food	*Staphylo-coccus* species	*Bacillus* species	*Escherichia coli*	serogroups
Noodles n=26	12 (46.15)	24 (92.00)	26 (100.0)	O8K25 O26K50 O55K59 (3 Nos) O114K90
Bread n=21	6 (37.50)	16 (76.19)	15 (71.42)	O55K59 O142K+
Biscuits n=19	0	4 (21.05)	4 (21.05)	nil
Cookies n=15	0	5 (33.33)	8 (53.33)	O55K59
Total n=81	18 (22.22)	49 (60.49)	53 (65.43)	9 (11.11)

Figures in parenthesis denote percentages

In this study, *Vibrio* species was isolated, however, they were not serotyped with O1 and O139 antisera. Thus, it is indicated they were termed as non-O1, non O139 strains and it may be of other species. The food to be consumed should not contain any kind of vibrios. Thus proper handling, use of clean water and utensils should be encouraged. Stool cases of children attending Yangon Children's hospital during 2003 with diarrhoea were also isolated with *Vibrio cholerae* and it is assumed that food is one of the risk factors in transmission of this strain [10].

The epidemiology of *E. coli* that causes diarrhoea suggest that most infections are acquired through food and water. The high inoculum of *Escherichia coli* (10^8) is required to induce diarrhoea in healthy volunteers to support the observations. Animals (cattles and pigs) are frequently infected with *Escherichia coli* that causes diarrhoea in man. Animals and foods of animal origin have been incriminated as enterotoxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC) infection [11].

Although acute diarrhoeal disease occurs at all ages, incidence is greatest among children less than five years of age and fatality is high, strikingly so in less developed countries. Besides, the so-called classic dyspepsia coli such as O26, O55, O86, O111 O127 and, many other types have been found. In this study, the serogroups O8, O26, O55, O114, O142 were identified.

In this study, *E. coli* is the most common organisms isolated with serogroup discrepancy. These serogroups encountered were within the well-known classical sero-groups accepted world wide as pathogenic organisms. Thus, every food must be prepared safely not to be contaminated by pathogens especially in children's food.

The presence of *S. aureus* indicates contamination from skin, mouth or nose of food handlers. The presence of *S. aureus* in food handlers which produced enterotoxin was shown [12]. They obtained 35 out of 102 samples (34%) had *S. aureus* and 19 out of 35 strains (54%) produced enterotoxins.

In this study, also *S. aureus* and other species were isolated representing that raw noodles are not safe for eating. Recent studies also emphasis on *B. cereus* food poisoning. In Accra, Ghana, *B. cereus* was isolated from 5.5 % of street foods [4]. In this study the isolation rate of *Bacillus* species is quite high (60.49 %). Nowadays, food safety is becoming an important public health issue. Thus, more works need to be carried out in this area [13,14].

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