

Isolation of anaerobic Gram-positive bacilli and detection of *Clostridium perfringens* alpha toxin from some foods

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Isolation of anaerobic Gram-positive bacilli and detection of *Clostridium perfringens* alpha toxin were done on 120 samples of different food collected from shops available around Yangon area during 2003. After culturing in Trypticase Glucose Yeast (TGY) media, *Clostridium perfringens* alpha toxin was detected by using ELISA kit obtained from Bio-X Diagnostics, Belgium. Anaerobic Gram-positive bacilli was isolated from 31 samples (25.83%). They were isolated from 16.67%, 25%, 29.17%, 20.83% and 37.5% of noodles, fermented vegetables, fermented fish and prawns, salted fish paste (ngapi) and pickled tea respectively. The toxins were detected from 20 samples of food: 4.17% (1/24 samples of noodles), 45.80% (11/24 samples of fermented vegetables such as salted/fermented beans) and 4.17% (1/24 samples of fermented fish & prawns), 29.19% (7/24 from ngapi) respectively. Thus, this study indicates that toxin could be elaborated in protein rich preserved foods (fish / prawn paste).

INTRODUCTION

Food and beverages sold in streets which are affordable sources of nourishment for students, low income workers and others contain substantial amount of valuable nutrients. However, accessibility, availability and quality need to be maintained for the control of foodborne transmission via street vendors. A number of cases of foodborne bacterial infection and intoxication had been transmitted through street foods. Cholera, typhoid, staphylococcal food poisoning, hepatitis and other diseases can be transmitted through such foods [1-5].

Food poisoning is the general description, although two recognized types of illnesses are caused by two distinct metabolites. The diarrhoeal type of illness is caused by a large molecular weight protein, while the vomiting (emetic) type of illness is believed to be caused by a low molecular weight, heat stable peptide. Enterotoxemia due to *Clostridium perfringens* is intestinal and involves types A, B, C or D. Type A has

been implicated in rare outbreaks of gastritis and haemolytic disease of ruminants (enterotoxigenic jaundice, the yellows, yellow lamb disease) and the haemorrhagic enteritis in cattle, horses, dogs, and infant alpacas. *Clostridium perfringens* type A causes necrotic enteritis in poultry and a mild form of food poisoning in humans. Demonstration of alpha toxin in the contents of the small intestine is the only way to definitely diagnose enterotoxemia. By using ELISA method, it is possible to detect alpha toxin in biological fluid or in culture filtrates. Thus, this study was undertaken to isolate Gram-positive bacilli and detect *Clostridium perfringens* alpha toxin from some food available around Yangon area.

MATERIALS AND METHODS

Food samples

One hundred and twenty four samples of different kinds of food including noodles (wheat and rice) fermented fish and prawns, fermented vegetables, fish paste from Pabedan, Mayangone, Kyauktada and

Botahtaung were randomly collected and used for isolation of anaerobic Gram-positive species.

Study period

January to September, 2003.

Study design

Random descriptive study

Bacteriological cultural methods

For culture and isolation of anaerobic Gram positive bacilli, Trypticase Glucose Yeast extract (TGY) with L-cysteine broth and agar was used. The plates were incubated at 37°C for 5 days in an anaerobic jar supplemented with Gaspak (BBL). Biochemical tests, Gram, acid fast and spore staining were carried out whenever necessary.

Detection of Clostridium perfringens alpha toxin from culture supernatant

The 96-well microtitration plates sensitized by specific antibodies for the alpha-toxin were used for the test. The culture supernatants were added and incubated for 60 minutes at room temperature. The plates were washed and incubated for 60 minutes with the conjugate which was a peroxidase labeled anti-alpha-toxin specific polyclonal antibody. After the second incubation, the plates were washed again and the enzyme substrate (hydrogen peroxide) and the chromogen (tetramethyl benzidine, TMB) were added. If the alpha-toxin is present in the tested samples, the conjugate remains bound to the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of alpha-toxin in the sample. Enzymatic reaction was stopped by acidification and resulting optical density was measured at 450nm using the microplate reader. The signals recorded for the negative control wells were subtracted from the corresponding positive microwells (Bio-X Diagnostics, Belgium). The optical density

at wave length 450nm with the cut-off value in controls was calculated after subtracting the optical density of corresponding negative control results. The limit of positivity for the antigen is 0.150. Any sample that yields a difference in optical density that is greater than or equal to 0.150 is considered positive. Conversely, any sample that yields a difference in the optical density that is less than 0.150 is considered negative.

RESULTS

Based on the morphology and cultural appearances, they were roughly identified as *Clostridium* species when they fall into the following categories. After culturing anaerobically on Trypticase Glucose Yeast agar, small, compact, shiny and translucent colonies which are similar to the features of *Clostridium perfringens* were picked and stained. They were Gram-positive rods in young cultures and not acid fast, catalase negative and produced spores that were heat resistant. The motility of anaerobes was easy to detect. The shape and position of spores when present, calls for subjective judgement and it may well be making a distinction to describe one spore as subterminal and another as central. In Cowan and Steel, they avoided this issue by indicating only those strains that produce terminal spores, and assumed that all others produced central or subterminal spores. Isolation of Gram-positive bacilli rate is high (16.67% to 37.5% (Table 1).

Detection of Clostridium perfringens alpha toxin from culture supernatants of different kinds of food

Alpha toxin from culture supernatants of different kinds of food is shown in Table 2. Out of 24 samples of each category of food tested, 1, 11, 1 and 7 samples of noodles, fermented vegetables, fermented fish and prawn; and ngapi (shrimp) produced *Clostridium perfringens* toxin (alpha toxin) respectively.

Table 1. Distribution of anaerobic Gram-positive bacilli in foods

Types of food	Pabedan	Mayangone	Kyauktada	Botahtaung	Total
Noodles, n=24	0	1	1	2	4(16.67)
Fermented vegetables, n=24	1	-	4	1	6(25.00)
Fermented fish & prawns, n=24	3	1	2	1	7(29.17)
Ngapi (shrimp), n=24	3	-	-	2	5(20.83)
Pickled tea, n=24	1	2	2	4	9(37.5)
Total, n=120	8	4	9	10	31(25.83)

Figures in parenthesis denote percentages

Table 2. Detection of *Clostridium perfringens* alpha toxin in foods

Types of food	Pabedan	Mayangone	Kyauktada	Botahtaung	Total
Noodles, n=24	0	1	0	0	1(4.17)
Fermented vegetables, n=24	3	4	2	2	11(45.80)
Fermented fish & prawns, n=24	0	1	0	0	1(4.17)
Ngapi (shrimp), n=24	1	2	1	3	7(29.19)
Pickled tea, n=24	0	0	0	0	0
Total, n=120	4	8	3	5	20(16.67)

Figures in parenthesis denote percentages

DISCUSSION

In Myanmar, diarrhea, dysentery, food poisoning, typhoid and paratyphoid fever are at the top of the list of Disease Under Surveillance (Notifiable Diseases) and they are all food borne diseases. The World Health Organization's data have shown that each year some 1500 million episodes of diarrhoea occur in children under the age of five, resulting in 3million deaths [6-8]. Most of the aetiological agents are associated with seasonal changes, environmental and personal hygiene. The ingestion of food or water is considered to be the principle mode of transmission of enteric pathogens. Suspect foods are those that are implicated by an attack-rate table or other epidemiological data or that have a history of being mishandled or mistreated. A food borne disease outbreak (FBDO) is defined as an incident in which two or more persons

experience a similar illness resulting from the ingestion of a common food. These include a wide range of diseases including diarrhoeal and parasitic diseases. They represent one of the most widespread and overwhelming public health problems in the world especially in developing countries.

Samples of food, chosen randomly from unopened original packages collected for evaluations as described in safe food handling [9], in this study might help overcome the problems associated with educating food handlers. In this study, Neurotoxicogenic *Clostridium butyricum* was isolated from the food implicated in an outbreak in China from salted and fermented paste made of soy beans and wax gourds [10]. In food borne botulism, toxin types caused by *Clostridium botulinum* were found locally in soil, suggesting that the organism may exist in soil in the area where the food was prepared.

In this study, isolation of Gram-positive bacilli rate is high (16.67% to 37.5%). Detection of alpha toxin is also recorded (4.17 to 45.80%). Among them, the fermented vegetables, fish and prawn are usually eaten raw in this locality.

The results indicated that food tested in this study, heavily contaminated with bacilli and with alpha toxin highlights the importance of food safety in this locality. Thus, preventive measures should be carried out through health education to consumers as well as to the sellers.

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