

SEROLOGICAL DETECTION OF ENTEROTOXIN FROM FOOD POISONING STRAINS OF *STAPHYLOCOCCUS AUREUS* ISOLATED IN MALAYSIA

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SYNOPSIS

Cultures of *Staphylococcus aureus* from eight food poisoning incidents in Malaysia were examined for their ability to produce enterotoxins. Five of the eight strains were found to be enterotoxigenic, the enterotoxins detected being A and E (three strains), A and C (one strain), and C (one strain). Penicillinase production was observed in four of the five enterotoxigenic strains; the penicillin-sensitive strain was also found to be coagulase-negative. The bacteriological and epidemiological investigations for confirming staphylococcal food poisoning are presented. The preventive measures to be taken in reducing such outbreaks are emphasized.

INTRODUCTION

Staphylococcal food poisoning is an acute gastroenteritis due to the ingestion of enterotoxin produced in food by certain strains of *Staphylococcus aureus*. The most common symptoms, which include nausea, vomiting, abdominal pain and diarrhoea, appear 2-6 hours after eating the contaminated food. In more severe cases, prostration and dehydration occur. The illness is relatively mild, normally lasting only a few hours to a day, with a high morbidity but a low mortality rate. All the five serologically distinct enterotoxins, A, B, C, D and E, have been implicated in food poisoning outbreaks.

In the U.S.A., enterotoxigenic *S. aureus* is the commonest cause of food poisoning (1). In Britain, however, staphylococcal food poisoning accounts for about 2% of all reported food poisoning incidents and about 5% of the reported cases (2).

Although food poisoning is a notifiable disease in Malaysia, the true incidence of staphylococcal food poisoning in this country is not known. The majority of the outbreaks are not investigated by the laboratories but are reported based on clinical and epidemiological findings. Moreover, many of the cases are never seen by a physician and hence go unrecognised.

As we were interested to find out the types of enterotoxin responsible for staphylococcal food poisoning in this country, a request was made to all the hospital laboratories to send us *S. aureus* strains isolated during the outbreaks. This paper reports the results obtained from studying the strains associated with eight food poisoning incidents during a two-year period, 1982-83.

MATERIALS AND METHODS

Cultures

S. aureus strains from food poisoning incidents 2, 4, 6 and 8 were received from the hospital bacteriologists and strains from incidents 1, 3, 5 and 7 were isolated in our laboratory (Table 1).

Biochemical and Antibiotic Sensitivity Tests

All the strains were tested for coagulase activity using human plasma, deoxyribonuclease activity on DNase agar (Difco), phosphatase activity on phenolphthalein phosphate agar (Oxoid), and mannitol fermentation on mannitol salt agar (Difco).

Six out of the eight strains were tested for their sensitivities to erythromycin (15 µg), lincomycin (2 µg), methicillin (10 µg), penicillin G (2 units) and tetracycline (10 µg) by a comparative disc-diffusion method (6). Strains producing penicillinase were recognised either by the absence of an inhibition zone around the penicillin disc or by the presence of characteristic heaped-up, clearly defined zone edges. They were then confirmed by the iodometric test (7) for β-lactamase production.

RESULTS

The results of enterotoxin production and antibiotic sensitivity tests on the *S. aureus* strains implicated in eight food poisoning incidents are given in Table 1. Enterotoxin A with enterotoxins E and C were found in three and one incidents, respectively. In another incident, enterotoxin C was produced by the strain studied. No enterotoxins were detected in three incidents.

All the strains tested were DNase-positive,

TABLE I
ENTEROTOXIN PRODUCTION AND ANTIBIOTIC SENSITIVITIES FROM EIGHT INCIDENTS OF STAPHYLOCOCCAL FOOD POISONING

| Incident | Place | Source | Enterotoxin produced | Antibiotic sensitivity pattern* |
|----------|--------------------------|----------------------|----------------------|---------------------------------|
| 1 | Kajang, Selangor | Nasi lemak | Not found | ND |
| 2 | Kota Baru, Kelantan | Fish curry | A and E | P |
| 3 | Klang, Selangor | Brinjal curry | Not found | ND |
| 4 | Seremban, Negri Sembilan | Chocolate cake | A and E | E |
| 5 | Klang, Selangor | Fried noodle | C | S |
| 6 | Grik, Perak | Fish curry | Not found | L |
| 7 | Kuala Lumpur | Vomit | A and C | P |
| 8 | Grik, Perak | Food (not specified) | A and E | P |

*ND = Not done.

P = Resistant to penicillin but sensitive to the other four antibiotics tested.

L = Resistant to penicillin and lincomycin but sensitive to erythromycin, methicillin and tetracycline.

E = Resistant to penicillin, erythromycin and tetracycline but sensitive to lincomycin and methicillin.

S = Sensitive to all antibiotics tested.

Enterotoxin Production

The cellophane-over-agar method of Hallander (3), as applied by Jarvis and Lawrence (4), was adopted to grow the strains. The supernatants obtained were tested for enterotoxins A — E.

Enterotoxin Detection

Supernatants were examined by the optimal sensitivity plate method of Robbins *et al.* (5). Plates were flooded with 0.1 M H₃PO₄ to increase the visibility of the precipitin lines before reading.

phosphatase-positive, and fermented mannitol. One of the strains was found to be coagulase-negative.

In the antibiotic sensitivity tests, five of the six strains studied showed resistance to penicillin. The coagulase-negative strain was also found to be sensitive to all the antibiotics tested.

DISCUSSION

In this study, it was found that enterotoxins were produced by *S. aureus* strains from five of the eight food poisoning incidents. Enterotoxin A with enterotoxins C or E were detected in four out of the five en-

terotoxigenic strains investigated. The predominant types causing staphylococcal food poisoning in the UK (2), USA (8), and Canada (9) have been reported as enterotoxin A or both enterotoxins A and D. The common cause of outbreaks in India is enterotoxin C (10) and in New Zealand, it is enterotoxin D (11). Enterotoxin B is implicated infrequently in staphylococcal food poisoning (2, 12, 13). In our study, enterotoxin B was not detected from any of the food poisoning strains examined.

The cultures tested by us did not produce enterotoxin A alone but together with enterotoxins C or E. This is consistent with our earlier studies with strains isolated from foods not associated with food poisoning (14) and human strains isolated from clinical specimens (15).

Practically all the food poisoning outbreaks are due to coagulase-positive staphylococci. However, enterotoxin-producing, coagulase-negative staphylococci have been implicated in outbreaks (16, 17), although only very rarely. In one of the incidents investigated by us, the enterotoxin C-producing staphylococcal strain was found to be coagulase-negative.

Penicillinase production has been found characteristic of many strains associated with food poisoning outbreaks. Simkovicova and Gilbert (12) found 81% of the food poisoning strains produced penicillinase, and Gilbert and Wieneke (2) detected 83% of their strains resistant to penicillin. Out of the five enterotoxigenic strains tested in this study, four were found to produce penicillinase. The penicillin-sensitive strain, which was coagulase-negative, was also sensitive to erythromycin, lincomycin, methicillin and tetracycline.

Bacteriological evidence such as the isolation of large numbers of *S. aureus* from suspected food, faeces, or vomitus, is essential for confirmation of outbreaks of staphylococcal food poisoning. Phage-typing of cultures and enterotoxin production tests are valuable epidemiological aids in the investigation. The demonstration of enterotoxin in the suspected food itself is even better proof of staphylococcal involvement especially when cooking or other processing treatment has killed the organisms but left the heat-stable enterotoxin still active. However, the techniques are tedious as they involve the extraction, purification and concentration of a small amount of enterotoxin (2, 12).

The most important factor in the prevention of staphylococcal food poisoning is the education of the food handler in personal hygiene. Rapid and effective refrigeration of food will prevent the multiplication of staphylococci and the production of enterotoxin in the food. The standards of hygiene in processing plants, during transportation, and by distributors, retailers and consumers must be maintained which should include thorough and regular cleaning of surfaces and equipment.

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