

A COMPARISON OF SIX SELECTIVE MEDIA FOR THE ENUMERATION OF STAPHYLOCOCCUS AUREUS IN FOODS

YS LIM MSc*. M JEGATHESAN MBBS, MRCPath**
AS KOAY AIMHLT* ** AND SH KANG BSc (Hons)****

Summary

The relative effectiveness of six selective media in recovering coagulase-positive, enterotoxigenic *Staphylococcus aureus* from foods was studied. The overall colony counts of *S. aureus* were higher from the inoculated frozen cooked prawn samples than those from the inoculated meat samples. Baird-Parker agar was the most superior medium for the enumeration of the organism from both the food groups. The four other media, namely mannitol salt agar, KRANEP agar, *Staphylococcus* medium no. 110 and milk salt agar, showed comparable recoveries. Vogel and Johnson agar, however, was found to have an inhibitory effect considerably greater than all these media tested.

INTRODUCTION

Staphylococcus aureus is one of the most common food poisoning bacteria. The majority of enterotoxigenic staphylococci are also coagulase-positive, although the isolation of enterotoxigenic coagulase-negative strains has been reported.⁴ The presence of coagulase-positive staphylococci in food indicates a potential risk of food poisoning. Thus, it is very essential to recognise accurately the number of coagulase-positive staphylococci present in a food lot in order to determine its acceptance or rejection.

A large number of media has been developed for the selective enumeration of *S. aureus* in foods. The selectivity of these media is based on salt tolerance, ability to reduce tellurites to metallic tellurium, production of yellow pigment, mannitol fermentation, egg yolk reaction, etc.^{5,6} These properties, either alone or in combination, cannot provide precise identification; therefore, additional tests are still needed.

This report presents data on the efficacy of six selective media for the enumeration of coagulase-positive enterotoxigenic *S. aureus* strains from a variety of meats and samples of frozen cooked prawns which had been experimentally contaminated.

MATERIALS AND METHODS

Cultures: The *S. aureus* strains used in this study were isolated from food samples received for routine bacteriological examinations. They were all coagulase-positive and enterotoxigenic.

Of the 20 strains examined, 8 produced enterotoxin C, 6 produced enterotoxins A + E, 2 produced enterotoxin B and 1 each produced enterotoxin D, enterotoxins B + D, enterotoxins A + B + E and enterotoxins A + C + E.

Food samples: The food samples used consisted of 20 samples each of meat and frozen cooked prawn. The meat samples included raw meats (beef, mutton and pork) and meat products (beef burgers, beef sausages, pork sausages and ham).

Media: Six isolation media were used in the experiments. Baird-Parker agar (BP, Oxoid), Vogel and Johnson agar (VJ, Difco), mannitol salt agar (MS, Difco), kalium - rhodanid - actidione - natriumazid - eigelb - pyruvate (KRANEP) agar (KR, Oxoid) were prepared from commercial dry preparations. *Staphylococcus* medium no. 110 (SM) was prepared according to the manufacturer's direction. Milk salt agar (MKS) was prepared by following the formula of Nefedjeva.⁷

Enumeration procedure: A 50 gm amount of each food sample was weighed into a wide-mouth jar, cut into smaller pieces, and sterilised in an autoclave at 121°C for 15 min. A single strain of *S. aureus* was grown in brain heart infusion (Difco) broth for 18 h at 37°C. One millilitre of a 1 : 10,000,000 dilution of the broth culture in quarter-strength Ringer solution was inoculated into the sterile food and incubated at 37°C for 18 hr, after which 0.1 ml volumes of a suitable dilution of the food sample in quarter-strength Ringer solution were surface-spread on duplicate plates of each

* Bacteriologist, Institute for Medical Research, Kuala Lumpur. (Address for reprint requests)

** Senior Bacteriologist, Institute for Medical Research, Kuala Lumpur.

*** Medical Laboratory Technologist, Institute for Medical Research, Kuala Lumpur.

**** Microbiologist, Chemistry Department, Petaling Jaya, Selangor.

of the six media and incubated at 37°C. The seeded plates were counted after 24 and 48 h using a colony counter (Gallenkamp).

The procedure was repeated for each of the 20 meat and frozen cooked prawn samples included in this study, using the 20 coagulase-positive, enterotoxigenic strains of *S. aureus*.

RESULTS

The typical colony appearance of *S. aureus* on the six selective media are described as shown in Table 1. All the 20 strains used in the experiments exhibited typical reactions on these media.

The mean colony counts on the two food groups are shown in Table 2. It was found that the overall counts obtained with the inoculated frozen cooked prawn samples were higher than those with the inoculated meat samples. When recovery studies were performed on meat samples, BP agar supported more luxuriant growth of *S. aureus* than did any of the other media tested. Recoveries of *S. aureus* were comparable when the organism was grown on KR and MS agars. MKS agar was next in superiority, followed by SM agar. VJ agar, however, was found to have an inhibitory effect greater than all these media tested.

From the frozen cooked prawn samples, the best recovery was again found on BP agar. This value, however, was only slightly higher than those obtained when MKS and SM agars were used. MS and KR agars gave comparable results which were considerably lower than those obtained on the above three media. VJ agar was again found to be highly inhibitory to the *S. aureus* strains tested.

DISCUSSION

The results of this study showed that the enterotoxigenic *S. aureus* strains behaved very differently in various agar media. The overall values of colony-forming units of *S. aureus* from frozen cooked prawns were higher than those obtained from meat samples on all the media tested.

The best recovery in the testing of pure cultures of *S. aureus* from both the food groups was found for BP agar. When an international comparative study was undertaken by six laboratories in the United Kingdom, New Zealand, France, U.S.A., West Germany and Canada in 1978 to assess the performance of four selective media commonly used for the enumeration of *S. aureus* in foods,¹ it was found that BP agar performed most satisfactorily. Niskanen and Aalto⁹ in Finland

concluded that BP agar was the best medium when they compared five selective agars for the detection of enterotoxigenic *S. aureus* from inoculated minced meat. BP agar has also been shown to give the best recovery values for both "thermally stressed cells"¹⁰ and unheated cells¹¹. Our results, thus, are in agreement with these findings.

From the meat samples, KR agar was next in superiority to BP agar. The value obtained on KR agar was, however, almost equivalent to that obtained on MS agar. Only very slight variations in the recovery of *S. aureus* was observed when MKS and SM agars were used. Colony counts obtained when inoculated frozen cooked prawns were used showed different recoveries when MS, KR, SM and MKS agars were tested. In decreasing order of the relative effectiveness, MKS agar was followed by SM, MS and KR agars. The recoveries on MKS and SM agars were comparable and they did not differ greatly from that obtained on BP agar.

The medium which showed the greatest inhibitory effect when both the food groups were used to grow the *S. aureus* strains was VJ agar. In a study conducted by Jay,¹² it was found that VJ agar gave the lowest percentage of recovery of *S. aureus* from meats when six selective media were compared. Similarly, Niskanen and Aalto⁹ observed that VJ agar had an inhibitory effect considerably greater than the other four media when they studied the recovery of enterotoxigenic *S. aureus* from minced meat samples.

BP agar is a highly specific diagnostic and selective medium developed by Baird-Parker³ for the isolation and enumeration of coagulase-positive staphylococci from food. Staphylococcal colonies are black, shiny and convex on this medium with white entire margins surrounded by a clear zone. On VJ and MS agars, the colonies are black and yellow, respectively, with bright yellow zones due to mannitol fermentation. SM agar is a selective medium on the basis of salt tolerance, pigmentation, mannitol fermentation and gelatin liquefaction. Colonies growing on this medium show a deep yellow colour. KR and MKS agars both exhibit yellow convex colonies with opalescent and opaque zones due to the egg yolk and milk in the media, respectively.

For the detection and enumeration of *S. aureus* in frozen cooked prawns, the Ministry of Health in Malaysia⁴ recommends the use of BP agar in its code of practice for the export of frozen cooked prawns. A surface-plating procedure with BP agar is recommended

by the American Public Health Association¹⁵ and The International Commission on Microbiological Specifications for Foods¹⁶ as the standard method for the detection of *S. aureus*. Our data presented here show that BP agar is the medium of choice for the enumeration of *S. aureus* in foods and this agrees favourably with the above recommendations.

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TABLE 1
TYPICAL COLONY APPEARANCE OF *S. AUREUS* ON SIX SELECTIVE MEDIA

Medium	Typical colony appearance
Baird-Parker agar	Black, shiny, convex colonies, 1.0 to 1.5 mm in diameter with narrow white entire margins surrounded by a clear zone 2 to 5 mm in width.
Vogel and Johnson agar	Small, black colonies with yellow zones.
Mannitol salt agar	Yellow colonies with bright yellow zones.
KRANEP agar	Yellow, shiny, convex colonies 1.0 to 1.5 mm in diameter surrounded by opalescent zones.
<i>Staphylococcus</i> medium no. 110	Colonies with deep yellow colour.
Milk salt agar	Yellow, smooth, round colonies with entire margins surrounded by opaque zones.

TABLE 2
COMPARISON OF COLONY COUNTS OF *S. AUREUS* ON MEAT AND PRAWN SAMPLES
USING SIX SELECTIVE MEDIA

Medium	Colony count* on	
	Meats (X 10 ⁰ /ml)	Prawns (X 10 ⁴ /ml)
Baird-Parker agar	239	497
Vogel and Johnson agar	112	56
Mannitol salt agar	186	253
KRANEP agar	189	215
<i>Staphylococcus</i> medium no. 110	158	441
Milk salt agar	162	479

*Mean of 40 plates.