Staphylococcus aureus and food borne disease

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Staphylococcal food poisoning, which usually presents clinically at 2-6 hours post exposure, is attributed to the ingestion of food containing pre-formed staphylococcal enterotoxins (SEs) which are quite resistant to acid and proteolysis. Most cases of staphylococcal intoxication resolve within 24-48 hours, although they may be serious [1, 2].

Staphylococcus aureus is largely responsible for reported cases of food poisoning and is the target of food safety managers. The main sources of *S. aureus* in food are the people who prepare it and the equipment used in that preparation. Contamination from infected animals (such as mastitic cows) is not considered to be a problem with good husbandry practice.

As humans are the main reservoir for the organism, the primary management of food safety lies in ensuring that food is handled minimally (and appropriately) and that foods are stored under refrigeration temperatures that will prevent bacterial multiplication and enterotoxin production. Dirty equipment can also provide foci for bacterial multiplication. A milk storage tank valve was implicated as the source of contaminated skim milk in a staphylococcal outbreak in Japan that affected more than 10,000 people [3]. Other *Staphylococcus* species *(S. intermedius, S. hyicus, S. chromogenes* and some coagulase-negative Staphylococcus) are also capable of toxin production [4-7]. *S. aureus* is a poor competitor but has the capacity to grow in the presence of high salt levels over a wide pH range. Consequently, it grows best in cooked/processed food where there are few, if any, other flora [8].

The term coagulase-positive staphylococci or CPS is used by food microbiologists. It reflects on the fact that the Australian Standard method recognises the tube coagulase test for confirmation of the target bacterium. While this test will also include other species of staphylococci, such as *S. intermedius* (from dogs), *S. delphini* (from dolphins), *S. lutrae* (from otters) and *S. hyicus* (from pigs) [9], they are unlikely to be present in food. At least 20 enterotoxins and/or enterotoxin-like types have been identified [10], with the majority of human disease being due to SEA. These toxins are proteins which can act as superantigens.

The amount of staphylococcal enterotoxin required to cause illness varies with individual susceptibility. Through primate feeding trials using SEA, SEB and SEC [8], SEA dose estimates from outbreaks range from 20-100μg from skim milk to 200μg or less in chocolate milk [11, 12]. Food microbiologists have traditionally worked on the assumption that the enterotoxigenic dose is less than 1μg, an amount of toxin which is usually achieved when the *S. aureus* populations exceed 100,000 per gram of food [13]. This knowledge, coupled with the fact that contamination during food handling is almost unavoidable, mean that low level contamination in food is generally tolerated because it poses little risk. Standard 1.6.1 of the *Australian Food Standards Code* [14] lists limits for CPS for some foods but there is no legislated requirement to test.

It is important to note that SEs are resistant to food preservation processes such as heat and freezing [8] and it is possible for SEs to be present in poorly handled foods even after the *S. aureus* is destroyed in processing. Outbreaks linked to canned champignons in the late 1980s [15] highlight the need to interpret staphylococcal counts in the context of the handling and processing history of the food. It should also be noted that purified SEB has been proposed as a potential biological weapon; it is stable and it can be easily aerosolised. SEB can elicit a systemic and sometimes fatal response in humans when inhaled [16].

Detection of SEs in food have progressed over time from bioassays (monkey-feeding and kitten-intraperitoneal tests) to

serological tests based on gel diffusion such as the Ouchterlony double diffusion plate method [17] to the current use of commercial kits based on enzyme linked immunosorbent assay (ELISA), enzyme linked fluorescent assay (ELFA) and reverse passive latex agglutination (RPLA). These kits rely on the use of polyclonal or monoclonal antibodies to the SEs, usually A-E, which are thought to account for about 95% of staphylococcal food poisoning outbreaks [18]. Care is required in the interpretation of test results as current test kits do not cover the entire range of SEs. Non-specific reactions and the possible presence of enzymes in the food can lead to false-positive or false-negative results [8, 10, 19]. Detection of toxin genes may be useful for the characterisation of *S. aureus* isolated from food, and techniques have also been described for direct detection of toxin genes from food and clinical specimens [20]. SE detection can also be achieved, at levels of approximately 0.6 to 6 pg SEB and SEA respectively, using quantitative real-time immuno-PCR [21].

Reasons for examining food include to confirm the cause of an outbreak, to confirm the source of the outbreak or to detect post processing contamination. Clinical specimens (vomitus or stool) are examined from patients with a compatible illness, to either elicit the cause of disease or to epidemiologically link cases and source. Testing may thus be for isolates, for the various SE toxins or their genes. Detecting toxin in epidemiologically implicated food eaten by affected patients (nausea, vomiting, retching, abdominal pain and diarrhoea), particularly if toxin is also found in patient specimens, provides a sound basis for diagnosis. Finding indistinguishable toxin-producing or toxin-gene carrying strains in an implicated food source (especially if present at $\geq 10^5$ per g of food) and ill patients may also be compelling, as may finding these same strains in implicated food handlers. The place of testing for SE genes alone when testing food or investigating disease is not yet established.

There are relatively few reports of staphylococcal food poisoning in Australia. Possible reasons for this include under-reporting and limited testing of cases (symptoms are short-lived), better awareness of food safety and handling with readily available information on websites [22] and limited food testing opportunities.

Although testing for CPS is routinely conducted in food testing laboratories, implicated food is often unavailable in cases of gastroenteritis and clinical labs do not usually test for *S. aureus* or its enterotoxins in this setting. Thus, there is usually no evidence to implicate the organism as an outbreak cause.

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Agnes Tan oversees primary investigations and the quality program of the MDU. The MDU is a public health microbiology laboratory which integrates the detection and characterisation of bacteria and fungi of clinical and public health importance across all fields, including food and water.

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