A Review on Staphylococcal Food Poisoning

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Abstract
Foodborne diseases are of major concern worldwide. Staphylococcal food poisoning is one of the most common foodborne diseases in both humans and animals globally, resulting from the ingestion of staphylococcal enterotoxins preformed in food by enterotoxigenic strains of coagulase-positive staphylococci, mainly S. aureus. Staphylococci survive desiccation and tolerate high levels of salt. Staphylococcal cells are destroyed by heat but if they have already produced enterotoxins in a food, the toxins will survive approved doses of irradiation and some thermal processes, including pasteurization. Any food that provides a good medium for the growth of staphylococci may be implicated in this type of foodborne illness. The foods involved in different countries vary with the diet as well as the local conditions. Staphylococcal toxins could be used as a biological agent (bioterrorist attack). Humans and animals are the primary reservoirs. The most common symptoms are nausea, vomiting, retching, Diarrhea, abdominal cramping, and prostration and in more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur. The enterotoxins are identified by specific antibodies, which are the basis of the detection methods. There is no effective long term decolonization therapy for S. aureus carrier.

Keywords: Enterotoxin, Foodborne, Staphylococci, Food poisoning, S. aureus

1. INTRODUCTION
Foodborne diseases are of major concern worldwide. There have been around 250 different foodborne diseases described, and bacteria are the causative agent of two third of foodborne diseases outbreaks (Loir et al., 2003).

The battle against bacterial foodborne diseases is facing new challenges due to rapidly changing patterns of human consumption, the globalization of the food market and climate change. Today, consumers want more natural food products that are less processed, without preservatives, with low salt, sugar or fat contents, but with an extended shelf-life and high quality (Zink, 1997). The demand for convenient, ready-to-eat food has also increased, and the food industry has developed new food processing techniques such as semi-prepared, minimally processed, chilled food in response to these demands (Hedberg et al., 1994 and WHO, 2010).

Staphylococcal food poisoning is one of the most common food-borne diseases worldwide (Hennekinne et al., 2009), resulting from the ingestion of staphylococcal enterotoxins preformed in food by enterotoxigenic strains of coagulase-positive staphylococci, mainly S. aureus. As staphylococcal enterotoxins are heat stable, they may be present in food when S. aureus are absent (Balaban and Rasooly, 2000).

Of the various metabolites produced by the staphylococci, the enterotoxins pose the greatest risk to consumer health. Enterotoxins are proteins produced by some strains of staphylococci (Bergdoll, 1972), which, if allowed to grow in foods, may produce enough enterotoxin to cause illness when the contaminated food is consumed. These structurally related, toxicologically similar proteins are produced primarily by S. aureus, although S. intermedius and S. hyicus have also been shown to be enterotoxigenic (Adesiyun et al., 1984).

There is no effective long term decolonization therapy for S. aureus carrier. Even with the use of antibiotics, S. aureus can only be removed from the nose over a few weeks, but relapses are common within several months (Coates et al., 2009).

Prevention of staphylococcal food poisoning from the infected food handlers may be difficult as carriers are asymptomatic (Schmid et al., 2007).

Staphylococcal food poisoning (SFP) is usually self-limiting and typically resolves within 24-48hr after onset. Occasionally it can be severe enough to warrant hospitalization, particularly when infants, elderly or debilitated people are concerned (Murray, 2005).

Staphylococcal food poisoning is a common disease whose real incidence is probably underestimated for a number of reasons, which include misdiagnosis, unreported minor outbreaks, improper sample collection and improper laboratory examination. The control of this disease is of social and economic importance. In fact, it represents a considerable burden in terms of loss of working days and productivity, hospital expenses, and
economical losses in food industries, catering companies and restaurants (Anonymous, 2007; Chiang et al., 2008).

Hence, the objective of this paper was: To review the staphylococcal foodborne intoxications.

2. Literature Review
2.1 Historical Background
There is no record of when illnesses similar to staphylococcal food poisoning were first observed, but it is likely that humans have been afflicted with this illness as long as they have been consuming foods in which staphylococci could grow. There are records of illnesses of this type as early as 1830, although the organisms themselves were not recognized until 1878 and 1880 by Koch and Pasteur, respectively. Although Ogston is credited with applying the name ‘Staphylococcus’ to these organisms in 1881 because of the grapelike clusters of cocci he observed in cultures, it was Rosenbach who in 1884 obtained pure cultures of the microorganisms on solid media and accepted the name Staphylococcus (Bergdoll, and Lee Wong, 2006).

Dack (1956), in his book Food Poisoning, relates several descriptions of foodborne illnesses similar to staphylococcal food poisoning. The examination of cheese that had been implicated in food-poisoning outbreaks in Michigan was related to staphylococci. Sternberg stated: ‘It seems not improbable that the poisonous principle is a ptoamine developed in the cheese as a result of the vital activity of the above mentioned Micrococcus, or some other microorganisms which had preceded it, and had perhaps been killed by its own poisonous products.’ In 1894 Denys concluded that the illness of a family who had consumed meat from a cow dead of ‘vitellary fever’ was due to the presence of pyogenic staphylococci, and in 1907 Owen recovered staphylococci from dried beef implicated in a foodborne illness characteristic of staphylococcal food poisoning (Dack, 1956).

In 1914, Barber was the first investigator actually to relate staphylococcal food poisoning to a toxic substance produced by the staphylococci. He discovered that milk from a mastitic cow caused illness when left unrefrigerated, and showed that the illness was due to growth in the milk of the staphylococci isolated from the mastitis.

In 1929, Dack rediscovered the role of staphylococci in food poisoning with his classical work on two Christmas cakes that were responsible for the illness of 11 people. These three-layer sponge cakes with thick cream fillings were baked possibly 1 day before delivery and eaten 2 days later. They were presumably refrigerated at the bakery but not after delivery. Dack and his associates showed, with the aid of human volunteers, that the sponge cake substance was responsible for the illness. Staphylococci isolated from this part of the cake produced a substance that caused typical food poisoning symptoms in human volunteers. In essence, this was the beginning of the research on staphylococcal food poisoning (Dack et al., 1930).

2.2 Characteristics
2.2.1 Organism
Classification of Staphylococcus
The name Staphylococcus (staphyle = bunch of grapes in Greece) was introduced in 1883 by Ogston. One year later, Rosenbach used the term in a taxonomic sense and provided the first description of the genus Staphylococcus (Todar, 2008).

Taxonomically the staphylococci have been placed in the Family Micrococcaceae (Shah, 2003 and Todar, 2008). Baird-Parker (1963) proposed a system of classification of the micrococci and staphylococci based on certain physiological and biochemical tests. He divided the Family Micrococcaceae in to Group I (Staphylococcus Rosenbach emend. Evans) and Group II (Micrococcus Cohn emend. Evans). These groups were then divided in to subgroups on the basis of pigment production, coagulase and phosphatase reactions, acetone production, and formation of acid from glucose (both aerobically and anaerobically) and other sugars.

Staphylococcus can be differentiated from the other three members in the family, Micrococcus, Stomatococcus, and Planococcus, on the basis of the guanine plus cytosine content of the DNA, cell wall composition, and the ability to grow and ferment glucose anaerobically. Only three species of Staphylococcus (S. aureus, S. epidermidis and S. saprophyticus) were included in the genus in 1974 (Buchanan and Gibbons, 1974). They were differentiated primarily on the basis of the ability to produce coagulase, ferment mannitol (both aerobically and anaerobically) and produce heat-stable endonuclease, and by the cell wall composition (Baird-Parker, 1974).

Kloos and Schleifer (1975) outlined a simplified scheme for the routine identification of human Staphylococcus species. They divided these into 11 species on the basis of coagulase activity, hemolysis, nitrate reduction, and aerobic acid production from several sugars. Since then the number of species and sub-species had increased to 32 as of 1994 (Holt et al., 1994). This increase included the elevation of two of the S. aureus biotypes to species status, biotype E (from dogs) to S. intermedius and biotype F (from swine) to S. hyicus. An additional coagulase-positive species, S. delphini from dolphins, has been added (Bergdoll and Lee Wong, 2006).
Characteristics of *S. aureus*

*Staphylococcus aureus* is a non-motile, facultative anaerobic, Gram-positive coccus. Cells are spherical single and often form grape-like clusters (Jay, 2000; Shah, 2003). The organism produces catalase and coagulase. Staphylococci survive desiccation and tolerate high levels of salt. The cell wall of staphylococci is resistant to lysozyme and sensitive to lysostaphin, which specifically cleaves the pentaglycin bridges of *Staphylococcus spp.* The organisms are able to grow in a wide range of temperatures (7°C to 48°C with an optimum of 30°C to 37°C), pH (4.2 to 9.3, with an optimum of 7.0 to 7.5); and sodium chloride concentrations (up to 15% NaCl). These characteristics enable the bacteria to survive in a wide variety of foods, especially those require manipulation during processing, and including fermented food products like cheeses (Loir et al., 2003; Aycicek et al., 2005).

Other pathogenic *staphylococci*

Coagulase-positive staphylococci, other than *S. aureus*, can cause infections in humans and animals. Some veterinary isolates of coagulase-positive staphylococci are classified in the *S. intermedius* group (SIG). *S. intermedius* was originally described in 1976 and appeared to be part of the normal micro-flora of the skin and mucosal membranes of dogs and cats. It has also been detected in a variety of other animals, including horses, mink, goats, foxes, raccoons, and pigeons but is not commonly present in humans. Recent molecular analyses demonstrated that isolates of *S. intermedius* detected in a large number of different animals and geographic locations have some significant differences and the species can best be reclassified into three clusters: *S. intermedius, S. pseudintermedius,* and *S. delphini* A and B. These three species constitute the *S. intermedius* group (SIG) (Sasaki et al., 2007).

*Staphylococcus pseudintermedius* is the most frequently encountered pathogen in the SIG and was first identified as a novel species in 2005 by examination of rRNA gene sequences in clinical staphylococcal isolates from several animals (Devriese et al., 2005). The majority of isolates from dogs are now classified as *S. pseudintermedius* although earlier research papers identified them as *S. intermedius*. *S. delphini* was originally isolated from a dolphin but some isolates from horses, pigeons and mink, previously identified as *S. intermedius*, are now classified as *S. delphini* (Sledge et al., 2010).

*Staphylococcus intermedius* has been isolated from pet owners and veterinarians (Morris et al., 2010) and occasionally causes infections in humans exposed to dogs carrying these bacteria (Chuang et al., 2010; Stegmann et al., 2010). Invasive infections have occurred in persons bitten by dogs (Fitzgerald, 2009) and two recent articles reported *S. intermedius* as the cause of skin abscesses in an injecting drug user (Kelesidis and Tsiodras, 2010) and meningitis in an infant (Durdik et al., 2010).

*Staphylococcus intermedius* group pathogens produce a number of virulence factors (coagulase, hemolysins, exfoliative toxin and others) similar to those associated with *S. aureus* (Fitzgerald, 2009; Iyori et al., 2010). When animals are injured, sick, or otherwise weakened, these bacteria may cause skin, ear, and wound infections (Weese and Van Duijkeren, 2010). Some SIG isolates also produce enterotoxins and could potentially cause foodborne intoxication (Becker et al., 2001). One foodborne outbreak in southwestern U.S. in 1991 affecting over 265 people was traced to *S. intermedius* producing type A enterotoxin in a butter blend (Khabbaty et al., 1994).

Compared to coagulase-positive staphylococci, coagulase-negative staphylococci are rarely pathogenic and are often considered to be opportunistic pathogens, such as *S. epidermidis* is for humans (Cheung and Otto, 2010). However, occasionally coagulase-negative staphylococci produce enterotoxins and have been associated with foodborne outbreaks (Veras et al., 2008).

Certain coagulase-negative staphylococci are important components of meat starter cultures (Fadda et al., 2010). Recent investigations found that genes coding for staphylococcal virulence factors were rare in coagulase-negative staphylococci isolated from sausage and cheese. Of 129 strains tested, only one contained a gene coding for an enterotoxin and none were capable of producing toxic shock syndrome toxin. Some strains did have genetic information coding for hemolysins and some were capable of producing biogenic amines. Of somewhat greater potential concern was the presence of antibiotic resistance genes in 71% of isolates, with nearly half the strains resistant to more than one antibiotic (Even et al., 2010).

**Hosts and reservoirs**

The staphylococci are ubiquitous in nature, with humans and animals as the primary reservoirs. They are present in the nasal passages and throat, in the hair, and on the skin of probably 50% or more of healthy individuals. The prevalence is usually higher in individuals associated with hospital environments because many infections and diseases are caused by the staphylococci. These organisms are associated with sore throats and colds, and are found in abundance in postnasal drip following colds. Staphylococci can be isolated from animals, with the bovine being the most important because of the involvement of staphylococci in mastitis. Although animals and humans are the major source, staphylococci also can be found in the air, dust, water, and human and animal wastes (Bergdoll, and Lee Wong, 2006).
2.2.2 Enterotoxins and its characteristics

Staphylococcal enterotoxins are exoproteins produced in food and ingested by humans give rise to symptoms of acute gastroenteritis (responsible for SFP). The toxins have been shown to be proteins of low molecular weight, approximately 27-31 kDa, consisting only of amino acids and are usually produced by CPS species (Chiang et al., 2008).

The SEs are short proteins belonging to a large family of pyrogenic toxin super antigens encoded by phage, chromosome or plasmid genes with a disulphide bridge secreted in the medium and soluble in water and saline solutions. They are rich in lysine, aspartic acid, glutamic acid, and tyrosine residues. Most of them possess a cystine loop required for proper conformation and which is probably involved in the emetic activity (Loir et al., 2003; Salandra et al., 2008).

Staphylococcal enterotoxins are highly stable, resist most proteolytic enzymes, such as pepsin, or trypsin, and thus keep their activity in the digestive tract after ingestion. They are highly heat resistant as well, which can resist 100°C for at least 30 minutes and probably longer. Although pasteurization and cooking kills staphylococci cells which are heat labile, thermo-stable SEs generally retain their biological activity. Thus, cases of illness might occur although no viable bacteria can be isolated from the suspected foodstuff and since SEs are more heat stable than the staphylococci bacteria, it is possible to test a food product and obtain negative staphylococci culture results and positive SEs tests (Soejima et al., 2007).

The effect of gamma irradiation on SEB and SEA has been reported. A dose of 50 kGy (cobalt-60 source) was required to reduce the concentration of SEB in 0.04-M Veronal buffer (pH 7.2) from 31µg/ml to less than 0.7µg/ml. In milk, a dose of 200 kGy was needed to reduce the concentration from 30µg/ml to less than 0.5µg/ml. The authors concluded that irradiation processes used for pasteurization or sterilization of foods would not inactivate the enterotoxin (Read and Bradshaw, 1967). A dose of 8kGy was insufficient to inactivate all of 111.1ng/ml SEA (27-34% remained) in lean miniced-beef slurries, although SEA was denatured in gelatin phosphate buffer (Modi et al., 1990).

The amount of enterotoxins produced is determined by factors such as the composition of the food, competition from other microorganisms (the presence of other bacteria affects the production of enterotoxin apparently by limiting the multiplication of the staphylococci), temperature and time (Salyers and Whitt, 2002).

A family of 14 different SE types has been identified, which share structure and sequence similarities, of which the antigenic types (named SE-A, B, C, D, and E) are most commonly encountered in SFP (Kerouanton et al., 2007). In general, SE-A is recovered from food poisoning outbreaks more often than any of the others, with SE-D being second most frequent and the fewest number of outbreaks are associated with SE-E (Jay, 2000; Shah, 2003).

Recently, additional SEs has been identified: SEG, SEH, SEI, SEJ, SEK, SEI, SEI, SEI, SEI, SEI, SEI, SEI, SEI, SEI, SEI, SEI, SEI. Many of these newly discovered enterotoxins are structurally similar to the classic enterotoxins, which suggest that they also may illicit foodborne illness when consumed in large enough doses. The significance of these SEs in causing foodborne intoxication remains largely unknown and requires both future research and increased surveillance (Rall et al., 2008; Ono et al., 2008).

There is no enterotoxin F (SEF) because toxic shock syndrome toxin was misidentified as SEF when it was first isolated. Two SEKs were described independently by two different groups at about the same time however they are different proteins based on their deduced amino acid sequences (Orwin et al., 2001).

The toxins act on the emetic receptors on the abdominal viscera causing stimulation of the emetic center of the brain via vagus and sympathetic nerves. The nerve stimulation ultimately results in causing diarrhoea and vomiting (Walderhaug, 2007).

Staphylococcal toxins could be used as a biological agent (bioterrorist attack) either by contamination of food/water or by aerosolization and inhalation. Breathing in low doses of staphylococcal enterotoxin B may cause fever, cough, difficulty breathing, headache, and some vomiting and nausea. High doses of the toxin have a much more serious effect (CDC, 2010).

Because of the importance of these toxins in the public health and food sectors, an efficient screening to detect the prevalence of enterotoxic strains in foods is required. Indeed, not all staphylococci produce SEs, and SEs production may be insufficient for food intoxication (Turutoglu et al., 2005; Morandi et al., 2007).

2.3 Staphylococcal intoxication in human and animals

Staphylococcal intoxication is among the most significant pathogens causing a wide spectrum of diseases in both humans and animals (Johnson et al., 2006; Salandra et al., 2008).

2.3.1 Disease in food animals

In food producing animal reservoirs, such as ruminants, Staphylococcus aureus presents on the skin and mucosa. In animals, Staphylococcus aureus can cause pustular inflammation of the skin and other organs, mastitis being the most serious. It is frequently associated to subclinical mastitis becoming responsible of contamination of milk and dairy products and is of great economic importance to the dairy industry worldwide (Jones, 1998;
Salandra et al., 2008). Its large capsule protects the organism from attack by the cow's immunological defenses (Hein et al., 2005). The infection occurs through the teat canal with the organisms derived from contaminated environment especially from the skin of the udder and teat (Anderson and Pritchard, 2008).

Wash cloths, teat cup liners and flies mechanically transmit the infection from cow to cow. Cattle are often infected by humans and the infection is carried from one cow to another by the milkers' hands. There are estimates that 80-100% of all herds have at least some staphylococcal mastitis, with 5 to 10% of cows infected (Anderson and Pritchard, 2008). Herds with excellent milking hygiene practices and management have lower levels of staphylococcal intramammary infections as compared to those herds with poor hygiene or management (Kaloreu et al., 2007). The bacterium produces toxins that destroy cell membranes and can directly damage milk-producing tissues (Jones, 1998). Staphylococcal infections also develop in to metritis, enteritis, ear infections and conjunctivitis (Anderson and Pritchard, 2008).

2.3.2 Disease in humans

Staphylococcal infection presents with a wide range of syndromes in human beings affecting many tissues and caused by three mechanisms: local destruction (abscess), blood spread and toxin production (Loir et al., 2003; Soejima et al., 2007). They cause superficial skin lesions like boils (furuncles), pimples, impetigo, carbuncles and localized abscesses in other sites, deep-seated infections such as osteomyelitis and endocarditis and more serious skin infections such as staphylococcal scalded skin syndrome (SSSS) or furunculosis, hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices. Also result in food poisoning by releasing enterotoxins into food, toxic shock syndrome (TSS) by release of super antigens into the blood stream and urinary tract infections (Loir et al., 2003; Shah, 2003; Todar, 2008).

Staphylococcal food poisoning occurs with the ingestion of contaminated food in which the enterotoxigenic strains of S. aureus can multiply reaching about $10^5$ CFU/g of food; this bacterial load allows the production of an amount 20ng to 1µg of SE sufficient to determine symptoms in human beings (Quinn et al., 1999; Salandra et al., 2008).

The hazard to public health by ingestion of foods contaminated with S. aureus is particularly linked to the ability of 50% of these strains to produce thermo-stable SEs associated with food poisoning (Quinn et al., 1999; Miwa et al., 2001; Kerouanton et al., 2007).

Staphylococcus aureus is extremely prevalent in atopic dermatitis patients, who are less resistant to it than other people are. It often causes complications. The disease most likely found in fertile active places including, the armpits, hair and scalp. The large pimples that appear in those areas may cause the worst of the infection if popped. This can lead to Scalded skin syndrome (SSSS) (Todar, 2008).

2.3.3 Symptoms

All people are believed to be susceptible to this type of bacterial intoxication. However, The onset of symptoms in staphylococcal food poisoning is usually rapid (2–6 hours) and in many cases acute, depending on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested, and the general health of the victim. The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration. Some individuals do not demonstrate all the symptoms associated with the illness. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur (Jay, 2000; Aycicek et al., 2001; Kerouanton et al., 2007).

The variation in severity of staphylococcal food poisoning symptoms experienced by individuals suggests development of resistance to previous exposure to the enterotoxin, but there is no evidence to support this. One unreported attempt to check individuals involved in an outbreak for antibodies was unsuccessful (Bergdoll and Lee Wong, 2006).

2.3.4 Emetic dose

A toxin dose of less than 1.0µg in contaminated food will produce symptoms of staphylococcal intoxication. This toxin level is reached when S. aureus populations exceed $10^6$cfu. However, in highly sensitive people a dose of 100-200ng is sufficient to cause illness (Bergdoll, 1990).

2.3.5 Diagnosis

Any foodborne illness with the symptoms outlined here, particularly if it involves more than one person, is suspected of being staphylococcal food poisoning. A list of foods consumed at the previous meal or meals is needed to aid in the diagnosis, as there are certain foods that support the growth of staphylococci and are frequently involved in this type of illness. Any suspected food should be examined for the presence of staphylococci; if large numbers are present, it can be concluded with some degree of certainty that the illness is staphylococcal food poisoning. Additional information that can remove any doubt is whether the staphylococci are enterotoxigenic and/or whether enterotoxin can be detected in the suspected food. Although the latter is definite proof of the cause, often an insufficient quantity of food (10g can be used, but larger amounts are better)
is available for examination. The presence of enterotoxigenic staphylococci in the food is reasonable assurance that these organisms were the cause of the illness (Johnson et al., 2006; Bergdoll and Lee Wong, 2006).

2.4 Food products commonly implicated with staphylococcal food poisonings
Any food that provides a good medium for the growth of staphylococci may be involved in this type of foodborne illness. The foods involved in different countries vary with the diet as well as the local conditions (Bergdoll and Lee Wong, 2006). Foods that are frequently implicated in staphylococcal food poisoning include meat and meat products, poultry and egg products, salads such as egg, tuna, chicken, potato, and macaroni, bakery products such as cream-filled pastries, cream pies, and chocolate éclairs, sandwich fillings, and milk and dairy products. Foods that require considerable handling during preparation and that are kept at slightly elevated temperatures after preparation are frequently involved in staphylococcal food poisoning (Tatini and Bennett, 1999; Bennett and Monday, 2003).

2.5 Vehicles of transmission
Staphylococci exist in air, dust, sewage, water, milk, food, or on food equipment, environmental surfaces, humans, and animals (Maria et al., 2010; Bennett and Monday, 2003). Staphylococci are present in the nasal passages and throats and on the hair and skin of 50% or more of healthy individuals. This incidence is even higher for those who associate with or who come in contact with sick individuals and hospital environments. Although food handlers are usually the main source of food contamination in food-poisoning outbreaks, equipment and environmental surfaces can also be sources of contamination with S. aureus. Human intoxication is caused by ingesting enterotoxins produced in food by some strains of S. aureus, usually because the food has not been kept hot enough (60°C, 140°F, or above) or cold enough (7.2°C, 45°F, or below) (Acco et al., 2003; Bennett and Monday, 2003).

2.6 Public health and economic importance
Staphylococcal Food Poisoning (SFP) is one of the most common Foodborne diseases (FBD) and is of major concern in public health programs globally (Hennekinne et al., 2012). Staphylococcal infections are frequent but are usually contained by immune mechanisms to the site of entry. The highest incidence of disease usually occurs in people with poor personal hygiene, overcrowding and in children (Rho and Schaffner, 2007). In developing countries, the surveillance system of FBD hardly exists and it is therefore, difficult to estimate the real magnitude of the problem (Boschi-Pinto et al., 2008). Even in countries where surveillance services are very efficient, the precise incidence of food poisoning is not known, as outbreaks are often not reported to public health authorities. Hence, the incidence of FBD caused by staphylococci is thought to be much higher than reported since many cases remain undeclared (Walderhaug, 2007).

Food borne diseases are a serious and growing problem in the world (Baron, 2007). World Health Organization and the US Centers for Disease Control and Prevention (CDC) report every year a large number of people affected by foodborne illnesses (Busani et al., 2006). Globally, an estimated 2 million people died from diarrheal diseases in 2005; approximately 70% of diarrheal diseases are foodborne. It is estimated that up to 30% of the population suffer from foodborne illnesses each year in some industrialized countries (WHO, 2011). According to the estimation by CDC in 1999, around 76 million foodborne illnesses occur annually, resulting in 325,000 hospitalizations and 5200 deaths and costs annually 5-6 billion USD in the United States each year (Buzby & Roberts, 2009). However, a decrease in the incidence rates of notified foodborne illness was noticed from 1996 to 2005, but these rates have remained static since 2005 (Anderson et al., 2011). Identified pathogens account for an estimated 14 million illnesses, 60,000 hospitalizations, and 1800 deaths. Salmonella, Listeria, and SFP organisms are responsible for 1500 deaths. Unidentified pathogens account for the remaining 62 million illnesses, 265,000 hospitalizations, and 3200 deaths. Overall, FBD appear to cause more illnesses but fewer deaths than previously estimated (Baron, 2007).

Among FBD, SFP is of major concern in global public health programmes. Staphylococcal organisms alone have found to cause hospitalization rates as high as 14%. Although not considered especially lethal, death can ensue if large amounts of SE are ingested: fatality rates range from 0.03% in the general population to as high as 4.4% for highly sensitive persons such as immunocompromised persons, elderly persons and children (Kerouanton et al., 2007).

In the United States, latest available data on foodborne disease outbreaks reported by the Centre for Disease Control and Prevention showed that S. aureus, together with Shiga toxin-producing Escherichia coli, ranked as the third commonest bacterial causative agents (9.8%), following Salmonella (39.7%) and Clostridium perfringens (11.5%)(CDC, 2009). The disease burden attributed by S. aureus seemed to become smaller when compared with the mean annual total for the previous 5 years (15.0%), though it was similar to that for 1998 to 2002 (8.5%)(CDC, 2006).

The latest report produced by European Food Safety Authority, which received data from 27 European
Union Member States, showed that *S. aureus* was the fourth most common causative agent for the reported foodborne outbreaks in 2008, following *Salmonella*, foodborne viruses and *Campylobacter*. *S. aureus* caused 291 foodborne outbreaks which constituted 5.5% of total number of reported outbreaks in the European Union (EFSA, 2010).

2.7 Isolation and identification of staphylococci

Symptoms of FBD associated with staphylococci are not suggestive and have little importance to warrant diagnosis (Baron, 2007). In the diagnosis of SFP, proper interviews with the victims and gathering and analyzing epidemiologic data are essential (Hobbs and Gilbert, 1981). Incriminated foods should be collected and examined for staphylococci or the SEs produced. The latter is especially important when foods that have been heated before consumption are implicated in the outbreak. Food handlers are also tested to ensure whether they are carriers of the strain responsible (Rho and Schaffner, 2007).

Incorrect identification of an isolate can impact on the implementation of effective treatment and/or control measures (Smith, 2007). In the diagnosis of SFP, detailed history, including the duration of the disease, characteristics and frequency of bowel movements, and associated abdominal and systemic symptoms, may provide a clue to the underlying cause. The presence of a common source, types of specific food, travel history, and use of antimicrobials always should be investigated. Diagnosis is confirmed by isolation of the organism or SE from relevant specimens (Baron, 2007; Walderhaug, 2007).

The presence of relatively large numbers of enterotoxigenic staphylococci is a good circumstantial evidence that the food contains SEs. The most conclusive test is the linking of an illness with a specific food or in cases where multiple vehicles exist, the detection of the toxin in the food samples (Chiang et al., 2008). In cases where the food may have been treated to kill the staphylococci, as in pasteurization or heating, direct microscopic observation of the food may be an aid in the diagnosis (Walderhaug, 2007).

The isolation and identification of *Staphylococcus* species is conducted on the basis of colony morphology, haemolytic properties, Gram-stain, catalase production, coagulase production and biochemical profile or sugar fermentation (Quinn et al., 2002; Aycicek et al., 2005). Samples were inoculated aseptically on the surface of the BAP medium by spreading with a sterile loop in such a way that bacteria are ultimately deposited singly because when the bacteria are at a sufficient distance from each other, the whole progeny of each accumulates locally during growth to form a discrete mass or colony which is readily visible to the naked eye. Each colony was presumed to be a pure culture, consisting exclusively of the descendants of a single cell (Todar, 2008).

On agar plates, staphylococcal colonies appear opaque to golden yellow in colour, glistening, smooth and in circular form. Blood agar is the medium of choice for isolation of the organism from specimens, and on 24 hours incubation staphylococci give good growth of creamy, often deeply pigmented colonies that is surrounded by the narrow zones of clear haemolysis, a broader zone of incomplete haemolysis or none depending on the species (Bendahou et al., 2008). Some species of *Staphylococcus* synthesize the enzyme haemolysin. Haemolysin is an exoenzyme that lyses red blood cells. If a colony of bacterial cells is producing haemolysin and secreting it into the medium, there will be a round, clear zone surrounding the colony because the red blood cells in that area have been lysed. The presence or absence of haemolytic properties, therefore, cannot be used as a definitive identification of *Staphylococcus* species as some species and strains of *Staphylococcus* species may not cause haemolysis (Quinn et al., 2002; Salandra et al., 2008).

Preparation and examination of Gram stained smears from typical colonies shows Gram positive spherical bacterium (cocccus), which on microscopic examination appears in pairs, short chains, or bunched, grape like clusters (Aycicek et al., 2005; Todar, 2008).

Catalase test is important to distinguish streptococci (catalase-negative) from staphylococci, which are catalase-positive. The catalase test determines if the organism produces the enzyme catalase that breaks down hydrogen peroxide ($H_2O_2$) to water and oxygen (Shah, 2003; Sandel and McKilip, 2004). When mixed with 3% $H_2O_2$, catalase-positive organisms will generate bubbles of oxygen, which are visible to the naked eye while catalase negative organisms do not. This enzyme allows organisms to breakdown harmful metabolites of aerobic respiration and may be seen in aerobic and facultatively anaerobic organisms. It is preferable to test colonies for catalase production from media without blood since erythrocytes possess catalase activities (Quinn et al., 2002; Todar, 2008).

\[ H_2O_2 \xrightarrow{\text{catalase}} H_2O + O_2 \] (gas bubbles)

Pathogenic organisms require mechanisms to help them overcome host defense mechanisms. One mechanism involves coating the bacterial cells in a body substance, such as fibrin, to fool the immune system. The coating of a natural body substance will not trigger an immune response and this is accomplished through the production of coagulase. Coagulase is an exoenzyme that causes fibrin of blood plasma to be deposited on bacterial cells resulting in clot formation. Pathogenic staphylococci produce coagulase, while non-pathogenic strains are coagulase negative (Turutoglu et al., 2005; Morrison, 2008).
The ability of *Staphylococcus* to produce coagulase, an enzyme capable of clotting plasma, was first reported by Loeb in 1903 (Morrison, 2008). The most widely used and generally accepted criterion for identification of pathogenic staphylococci is usually by their ability to produce coagulase (the coagulase test correlates well with pathogenicity). The presence of this highly specific enzyme was a fairly certain indication that a *Staphylococcus* was pathogenic. A pathogenic *Staphylococcus* has become an organism which produces coagulase and *vice versa*. Fresh or reconstituted commercial freeze dried rabbit plasma is the reagent used. Rabbit plasma contains fibrinogen that is converted to fibrin by the staphylococcal coagulase enzyme (Quinn et al., 2002; Lamprell et al., 2004).

A range of selective and diagnostic media have been developed to assist in the detection and enumeration of staphylococci in routine food surveillance programmes and food poisoning investigations (Baird and Lee Wong, 1995). Selective bacteriological media containing one or more agents that are inhibitory to microorganisms other than the target pathogen (staphylococci) can be applied. The microorganism of interest is not inhibited by the presence of these components in the medium, and will therefore, form visible colonies during incubation (Quinn et al., 2002). The two selective agents most commonly used for these pathogens are sodium chloride (NaCl) and potassium tellurite (Baird and Lee Wong, 1995; Pal, 2007).

A common medium used for the isolation of pathogenic staphylococci is the mannitol salt agar (MSA). Some organisms cannot tolerate a high osmotic pressure. Media containing higher than normal salt concentrations such as MSA inhibit the growth of these non-tolerant organisms other than the salt tolerant staphylococci (Baird and Lee Wong, 1995). Mannitol salt agar contains a high salt concentration so only salt tolerant staphylococci will grow on it (high salt concentration of this medium inhibits the growth of most other organisms). Additionally, MSA contains the sugar mannitol. Staphylococcal organisms can utilize mannitol as a fermentable carbohydrate (food source) and will produce acid end products from this metabolism. Since this process is invisible and indicator is added to the media to detect changes in pH. Phenol red is the indicator used in MSA. It is red at a neutral pH but turns yellow if conditions in the media become acidic. Pathogenic staphylococci not only grow on the medium, but they also produce acid from it. This acid production turns the pH indicator from red to yellow. Non-pathogenic staphylococci can grow on the medium but produce no acid from it and the medium remains pink (Quinn et al., 2002).

### 2.8 Principles of detection of the enterotoxins

It was not possible to develop specific methods for the detection of the enterotoxins before Bergdoll et al. (1959a) identified and purified the first enterotoxin. Until that time, the only means of detecting the presence of the enterotoxins was by the use of animals that gave emetic reactions to the toxin, either intragastrically or intravenously. Fortunately, at the time Surgalla and Bergdoll began their research to identify the enterotoxin; immunological methods were being developed for the specific detection of individual proteins. These investigators were able to show that specific antibodies could be produced to the enterotoxin when the emetic reaction in monkeys was neutralized by antisera produced against the crude toxin (Bergdoll et al., 1959b; Surgalla et al., 1954). Subsequently, all laboratory methods for the enterotoxins have been based on the use of specific antibodies to each of the enterotoxins for their detection, because it is almost impossible to detect individual proteins by chemical methods (Bergdoll and Lee Wong, 2006).

The most conclusive test is the linking of an illness with a specific food or in cases where multiple vehicles exist, the detection of the toxin in the food samples (Chiang et al., 2008).

#### 2.8.1 Biological methods

Before the first enterotoxin was purified, many types of animals (such as pigs, dogs, cats and skitters, and monkeys) were tested in the search for an inexpensive specific test method. All of these animals, with the exception of monkeys, were relatively insensitive to the enterotoxins, unless the toxin was injected intraperitoneally or intravenously. Emesis is the most readily observable reaction to enterotoxins; hence animals without a vomiting mechanism, such as rodents, were of little value as test subjects (Schantz et al., 1965; Hammon, 1941; Bergdoll and Lee Wong, 2006).

Because antibodies are specific for each enterotoxin, it is necessary to continue the use of animal testing until each new enterotoxin has been purified and antibodies produced against it. Animal testing is also necessary for assessing the effect of various treatments, such as heat, on the enterotoxins (Bergdoll and Lee Wong, 2006).

#### 2.8.2 Immunological methods

The most specific and sensitive tests for the enterotoxins are based on their reactions with specific antibodies. The first tests developed were based on the reaction of the enterotoxin with the specific antibodies in gels to give a precipitin reaction. These were the only laboratory methods available until radioimmunoassay (RIA) was applied, and later the enzyme-linked immunosorbent assay (ELISA) and the reversed passive latex agglutination (RPLA) method were developed. The gel-diffusion methods have been used primarily for the detection of enterotoxin production by staphylococcal strains, although the RPLA method is used for testing strains for low production of enterotoxin. The RIA method was used for testing for enterotoxin in foods until the ELISA and
2.8.3 Detection in foods

The detection of enterotoxin in foods requires methods that are sensitive to less than 1 ng/g of food. The quantity of enterotoxin present in foods involved in food-poisoning outbreaks may vary from less than 1 ng/g to greater than 50 ng/g. Although little difficulty is usually encountered in detecting the enterotoxin in foods involved in food poisoning outbreaks, outbreaks do occur in which the amount of enterotoxin is less than 1 ng/g such as the case of the 2% chocolate milk. In such instances, the enterotoxin can be detected only by the most sensitive methods. Another situation in which it is essential to use a very sensitive method is in determining the safety of a food for consumption, where it is necessary to use the most sensitive methods available in order to show that no enterotoxin is present. The most important methods used to detect enterotoxins in foods are ELISA method, the RPLA method and screening methods (Hein et al., 2005; Bergdoll and Lee Wong, 2006).

2.9 Prevention and Control measures

Staphylococci are ubiquitous and are impossible to eliminate from the environment. The total destruction or significant reduction in the bacterial load in foods during growth, harvesting, processing, packaging, and storage prior to consumption has always been a general goal. However, the wide array of parameters for proliferation of foodborne pathogens is staggering. Some of the same methods for the control of organisms in the food supply are used separately or in combination in the preservation of foods. Staphylococci may be totally destroyed or injured when subjected to lethal or sublethal doses, respectively, of heat, cold, drying, irradiation, or chemicals. While total destruction of these organisms might be ideal, sublethal injury may occur, thus providing the organism an opportunity to recover and proliferate, if conditions are conductive (Martin and Myers, 1994; Bennett and Monday, 2003).

There is no effective long term decolonization therapy for S. aureus carrier. Even with the use of antibiotics, S. aureus can only be removed from the nose over a few weeks, but relapses are common within several months (Coates et al., 2009). Although post treatment eradication may be initially high, sustained decolonization drops to half of cases 6 to 8 months after treatment (Preston, 2010). Only pre-employment or routine medical and laboratory examinations of food handling personnel are of no value in the prevention of foodborne diseases but In addition, providing education and training in good hygienic practices to all food handling personnel have high importance (WHO, 1989; CDC, 2011).

Prevention of staphylococcal infections/intoxication requires strategies to interrupt various modes of transmission. Essentially these control programs include improvements in personal hygiene practices among healthcare workers and food handlers, decontamination of equipment, surfaces, and clothing, judicious use of antibiotics, proper cooking and storage of foods, and screening programs (Doyle et al., 2011).

To prevent food-poisoning outbreaks, it is necessary to keep foods either refrigerated (-10°C) or hot (45°C) to prevent proliferation of the organism to such numbers (10^5 cells/g) necessary for detectable toxin formation. Additionally, foods should be refrigerated in shallow layers or small portions to facilitate quick cooling (Bennett and Monday, 2003).

3. Conclusion and recommendations

Staphylococcal food poisoning is of major concern in public health programs worldwide. Among Staphylococcal species S. aureus is an important causative agent for food poisoning outbreaks globally. High proportion of the intestinal disorders frequently observed in developing countries is caused by staphylococcal food poisoning. Anyone who consumes S. aureus toxin can become ill. However, severity of the illness will depend on the age, sex, health status, amount of contaminated food and toxin consumed. Once Staphylococcal toxin has been produced, it cannot be destroyed by cooking and even in approved dose of irradiation. Outbreak of staphylococcal food poisoning is most frequently associated with inadequate refrigeration, preparing foods far in advance of planned service, infected person's practicing poor personal hygiene and inadequate cooking or heat processing.

Based on above conclusion, the following recommendations are forwarded:

- Special care should be given for risk groups such as young, infants, elder, pregnant and immunocompromised individuals.
- Do not allow cooked food to sit out at room temperature for prolonged periods of time.
- Proper sanitation by Food handlers and should be encouraged to join the education and training to ensure good hygiene practices.
- Considerable studying effort is still required for better understanding of the interactions between Staphylococcus and food products, and of the mechanisms of SEs production in foodstuffs.

4. References


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