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REVIEW ARTICLES

Staphylococcus Aureus Enterotoxins: A Review

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ABSTRACT

Food-borne diseases are of major concern worldwide. To date, 250 different food-borne diseases have been described and bacteria are the causative agents of two thirds of food-borne disease outbreaks. Food poisoning is a term used to express any type of disease, illness or malaffect after consuming food. The most serious type of food poisoning is bacterial food poisoning, which may be due to bacterial infection or food intoxication. Among the predominant bacteria involved in these diseases, Staphylococcus aureus is a leading cause of gastroenteritis resulting from the consumption of a food in which enterotoxigenic staphylococci have grown and produced toxins. As these toxins are excreted from the organism, they are referred to as exotoxins; however, they normally exert their effects on the gastrointestinal tract and therefore are called enterotoxins. While not considered a highly lethal agent due to the low mortality associated with the illness, staphylococcal enterotoxins are considered a potential biological threat because of their stability at high temperatures (100˚C for 1 h) and ability to incapacitate individuals for several days to two weeks. Here, a brief review on Staphylococcal enterotoxins is given.

Key Words: Staphylococcus aureus, Food poisoning, Enterotoxins

Introduction

Staphylococcus aureus is one of the most common causes of food borne infections in most of the countries of the world [1],[2] Especially in India, rate of infection is still higher because of warm and humid climate. Staphylococcus aureus is a facultative anaerobic gram-positive coccus, non-motile, catalase and coagulase positive of the micrococcaceae family. In 1884, Rosenbach described the two pigmented colony types of staphylococci and proposed the appropriate nomenclature: Staphylococcus aureus (yellow) and Staphylococcus albus (white). The latter species is now named Staphylococcus epidermidis. S. aureus colonizes mainly the nasal passages, but it may be found regularly in most other anatomical locales. S epidermidis is an inhabitant of the skin. In humans S. aureus is present on external sites, such as the nostrils [3],[4] or the skin [5] and also transiently in the oropharynx [6]and faeces [7]. Up to 30-50% of the human populations are carriers. Staphylococcus aureus is able to grow in a wide range of temperatures (7° to 48.5°C with an optimum of 30 to 37°C), pH (4.2 to 9.3, with an optimum of 7 to 7.5) and sodium chloride concentrations up to 15% NaCl [8]. The staphylococcal cell wall is resistant to

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lysozyme and sensitive to lysostaphin, which specifically cleaves the pentaglycin bridges of Staphylococcus spp. The various isolates of S. aureus show different characteristics features in their susceptibility/resistance towards antibiotics. Some S. aureus strains are able to produce staphylococcal enterotoxins (SEs) and are the causative agents of staphylococcal food poisonings. Staphylococcus aureus is able to grow in a wide range of temperatures (7° to 48.5°C with an optimum of 30 to 37°C), pH (4.2 to 9.3, with an optimum of 7 to 7.5) and sodium chloride concentrations up to 15% NaCl [8]. These characteristics enable S. aureus to grow in a wide variety of foods. Staphylococcus aureus strains can be classified into biotypes according to their human or animal origin like human, non--hemolytic human, avian, bovine, ovine, and nonspecific. [9]

Pathogenesis of S. aureus infections

The pathogenicity of Staphylococcus aureus is due to the toxins, invasiveness and antibiotic resistance. S.aureus is major cause of nosocomial and community acquired infections [10],[11], [12]. It is present as a normal flora of human beings and colonizes skin, but may become pathogenic and result in minor skin infections and abscesses, to life-threatening diseases such as pneumonia, meningitis, endocarditis, toxic shock syndrome (TSS), septicemia, mastitis, phlebitis, urinary tract infections, osteomyelitis and endocarditis.

Adherence to host cell proteins

S. aureus cells attach thought surface proteins to host cell's surface proteins e.g., laminin and fibronectin. In addition, most strains express a fibrin/fibrinogen binding protein (clumping factor) which promotes attachment to blood clots and traumatized tissue. Most strains of S. aureus express both fibronectin, fibrinogen-binding proteins and collagen binding proteins on surface.

Invasion

The invasion of host tissues by staphylococci apparently involves the production of a huge array of extra cellular, proteins, toxins, enzymes, proteins having affinity for Igs [13].

Toxins

α, ß, δ and leukocidins are the common toxins found in S.aureus. α̃ toxin most potent membrane-damaging toxin of S. aureus is a monomer and binds to the membrane of susceptible cells through receptors. Subunits then oligomerize to form heptameric rings with a central pore through which cellular contents leak. In humans, platelets and monocytes are particularly sensitive to α-toxin. The binding to the cells cause small pores through which monovalent cations can pass and the mode of action of α̃–hemolysin is likely by osmotic lysis. ß-toxin is a sphingomyelinase which damages membranes rich in lipid. The majority of human isolates of S. aureus lack ß-toxin. A lysogenic bacteriophage is known to encode the toxin. δ -toxin is a very small peptide toxin produced by most strains of S. aureus. The role of δ-toxin in disease is unknown. Leukocidin is a multicomponent protein toxin whichis an important factor in skin infections which kills leukocytes. It forms a hetero-oliogmeric transmembrane pore composed of four LukF and four LukS subunits, thereby forming an octameric pore in the affected membrane. Leukocidin is hemolytic, but less so than alpha hemolysin. Though 2% of S. aureus isolates express leukocidin, but nearly 90% of the isolates from severe dermonecrotic lesions express this toxin. Besides, the above given toxins, S.aureus releases certain extracellular enzymes like Coagulase, Staphylokinase, a lipase, a deoxyribonuclease (DNase) and a fatty acid modifying enzyme (FAME). In addition, S. aureus expresses a number of factors that have the potential to interfere with host defense mechanisms e.g., Capsular Polysaccharide, Protein A and antibiotic resistance. S. aureus secretes two types of toxin with superantigen activity, enterotoxins (SE's) and toxic shock syndrome toxin (TSST-1). Besides this, the exfoliatin toxin, associated with scalded skin syndrome, causes separation within the epidermis, between the living layers and the superficial dead layers.

Staphylococcal enterotoxins characteristics

Staphylococcal enterotoxins are a group of single-chain, low-molecular weight (27,000-34,000) proteins produced by some species of staphylococci, primarily Staphylococcus aureus, but also by S. intermedius, S. hyicus, S. xylosus and S. epidermidis. To date, 14 distinct enterotoxins have been identified based on their antigenicity and they have sequentially been assigned a letter of the alphabet in order of their discovery (SEA to SEO).

There is no enterotoxin F as this letter was assigned to a protein that proved not to be an enterotoxin. Also, several SECs have been recognized and, while they all react with the same antibody, three to five residue differences in their amino acid sequences differentiate them.

Physical symptoms

The appearance of symptoms of food poisoining depends upon quantity, type and toxicity of the toxin. The gastrointestinal symptoms typically appear after 1-6 hours whereas the other effects may appear after a longer time like neurological and haemotological effects. Symptoms can range from mild, moderate to severe and include abdominal cramps, nausea, vomiting, diarrhea, fever and dehydration and in severe cases, death may be the result. Like any other organism, S.aureus need temperature, moisture, nutrients and time to grow. The presence or absence of oxygen, salt, sugar, acidity and the microflora in the environment are other important factors for the growth of bacteria in the gastrointestinal tract. In the appropiate conditions, one bacterium may multiply by binary fission to become four million in eight hours. Since bacteria and toxins can neither be smelled nor seen with the naked eye, the best way to ensure that food is safe is to follow principles of good food hygiene [14].

Biological symptoms

Staphylococcal food poisoning is defined by a characteristic set of histological abnormalities in the gastrointestinal tract, chief among them being neutrophil infiltration and blood accumulation in the epitheliums and basement membranes of the stomach, upper part of the small intestine, and jejunum. In the lumen of the duodenum, oozing of mucus and pus is also noted [14].

Toxicity

Cell numbers greater than 10^5 S. aureus per g of food are needed to produce sufficient amounts of staphylococcal enterotoxins to cause illness. Compared with most exotoxins acquired through contaminated food, the amount of staphylococcal enterotoxin required to induce symptoms following oral exposure is large.

Epidemiologic features

Large outbreaks of Staphylococcal food poisoning (SFP) are relatively rare in developed countries. In Finland, France, Japan, Korea, and the Netherlands, staphylococci account for an estimated [10],[30],[5], [15], and 5% of total foodborne disease outbreaks, respectively [15],[16],[17],[18]. Moreover, SFP accounts in the United States for only 1.3% of the total estimated cases of foodborne illnesses caused by known agents [19],[20],[21]. In contrast, in the early 1980's, SFP was reported to account for 14% of total foodborne outbreaks in the United States. Similar decreases in frequency have been reported in Japan. Before 1984, 25-35% of all cases of bacterial foodborne illness in Japan involved SFP, whereas in the late 1990's, only 2-5% of incidents involved SFP. Many types of foods have been implicated as vehicles of SFP, but typically such foods are high in protein, sugar, or salt which provide a good medium for the growth of staphylococci. In a study of food poisoning in England, the most frequent products contaminated were meat (ham), poultry (chicken) or their products (75%) followed by fish/shellfish (7%) and milk products (8%) such as cream, cheese, and custards. In surveys conducted on food handlers, S. aureus types A, B, C, D, and E have been isolated with varying frequency. In Kuwait, the majority of S. aureus isolates obtained from hands of food workers was type B, whereas those isolated from the nose were predominantly of types A and B (28 and 28.5% of S. aureus isolated, respectively) followed by types C and D (16.4% and 3.5% of total S. aureus isolates, respectively) [22]. Enterotoxigenic S. aureus isolates from the nose, throat, hands and nails of food handlers in cafeterias of a Chilean restaurant were predominantly SEB and SED producers [23]. In this same study, male food handlers had a higher frequency of contamination (83%) than female food handlers (57%). Unfortunately, washing hands and skin surfaces has minimal effect on reducting S. aureus cell numbers on humans, largely because S. aureus is part of the resident flora of skin. Humans should not be considered the only source of S. aureus. For example, of 910 rats captured at restaurants in downtown Tokyo, 18% were positive for enterotoxigenic S. aureus and expressed predominantly SEA and SEB followed by SEC and SED [24].

The foods that are most often involved in staphylococcal food poisoning differ widely from one country to another. In the United Kingdom, for

example, 53% of the staphylococcal food poisonings reported between 1969 and 1990 were due to meat products, meat-based dishes, and especially ham; 22% of the cases were due to poultry, and poultry-based meals, 8% were due to milk products, 7% to fish and shellfish and 3.5% to eggs [25]. In France, things are different. Among the staphylococcal food poisonings reported in a two-year period (1999-2000), among the cases in which the food involved had been identified, milk products and especially cheeses were responsible for 32% of the cases, meats for 22%, sausages and pies for 15%, fish and seafood for 11%, eggs and egg products for 11% and poultry for 9.5% [26]. In the United States, among the staphylococcal food poisoning cases reported between 1975 and 1982, 36% were due to red meat, 12.3% to salads, 11.3% to poultry, 5.1% to pastries and only 1.4% to milk products and seafoods. In 17.1% of the cases, the food involved was unknown [27]. Thus, the origins of staphylococcal food poisoning differ widely among countries; this may be due to differences in the consumption and food habits in each of the countries. In France, for example, the consumption of raw milk cheeses is much higher than in Anglo-Saxon countries. This may explain the relative importance of milk products involved in staphylococcal food poisoning in France.

Foods Incriminated

Foods that are frequently incriminated 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 eclairs; 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. Staphylococci exist in air, dust, sewage, water, milk, and food or on food equipment, environmental surfaces, humans, and animals. Humans and animals are the primary reservoirs. Staphylococci are present in the nasal passages and throats and on the hair and skin of 50 percent 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 [28],[29],[30].

Molecular and structural features of enterotoxins

Studies on SEs started from the analysis of S. aureus strains involved in staphylococcal food poisoning. In the first SEs identified, the peptide sequence was available before the nucleo tide sequence. This was the case for SEA [31] SEB [32]and SEC [33] The abundance of literature on SEs varies considerably among the types, according to the chronology of their identification and their importance in staphylococcal food poisoning. To date, 14 different SE types have been identified, which share structure and sequence similarities. The major Characteristics of Staplylococcal enterotoxins (SE) and the genetic Support of staphylococcal enterotoxin (SE) genes are listed in [Table /fig 1],[Table/fig 2].

Subsequent translation leads to the generation of a precursor protein, containing a N-terminal leader sequence that is cleaved during export from the cell to form the mature enterotoxin protein. Slight variations in processing or post-translational modification may occur as evidenced by the existence of three SEA isoforms with three different isoelectric points. 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. They are highly stable, resist most proteolytic enzymes, such as pepsin or trypsin, and thus keep their activity in the digestive tract after ingestion. They also resist chymotrypsine, rennin and papain. Nevertheless, SEB and SEC1 have been cut in the cystine loop by mild trypsin digestion. Staphylococcal enterotoxin B can be destroyed by pepsin digestion at pH 2 but it is pepsin resistant at higher pHs, which are normal conditions in the stomach after food ingestion [54].Overall, 15% of the amino acid residues are entirely conserved in staphylococcal enterotoxins and these occur in four stretches of primary sequence located either centrally or at the C terminus. The two properties of enterotoxins mitogenicity and emetic activity are located on different sections of the protein. Whereas the toxin's mitogenic activity is postulated to be on

Table/fig. 1

SE Type	*ORF length (bp)*	*Precursor length (aa)*	*Mature SE length (aa)*	*Molecular mass (Kda)*	*pI*	*Reference*
A	774	257	233	27,100	7.3	34, 35
B	801	266	239	28,336	8.6	36
C1	801	266	239	27,531	8.6	37
C2	801	266	239	27,531	7.8	38
C3	801	266	239	27,563	8.1	39
C (bovine)				27,618	7.6	40
C (sheep)				27,517	7.6	40
C (goat)				27,600	7.0	40
D	777	258	228	26,360	7.4	41, 42
E	774	257	230	26,425	7.0	43
G	776	258	233	27,043	5.7	44
H	726	241	218	25,210	Nd	45
I	729	242	218	24,928	Nd	44
J	806	268	245	28,565	8.65	46
K	729	242	219	25,539	6.5	47
L	723	240	215	24,593	8.66	48
M	722	239	217	24,842	6.24	49
N	720	258	227	26,067	6.97	49
O	783	260	232	26,777	6.55	49

Major Characteristics of Staplylococcal enterotoxins (SE)

Table/fig. 2

Gene	*Genetic location*	*Reference*
Sea	prophage	34, 50
Seb	chromosome, transposon, plasmid	51, 52, 53
Sec1	plasmid	53
Sec $_{bov}$	pathogenecity island	48
Sed	plasmid (pIB485)	42
See	defective phage	43
Seg	Enterotoxin gene cluster (egc), chromosome	49
Sei	egc, chromosome	49
Sej	plasmid (pIB485)	46
Sek	pathogenecity island	47
Sel	pathogenecity island	48
Sem	egc, chromosome	49
Sen	egc, chromosome	49
Seo	egc, chromosome	49

Genetic Support of staphylococcal enterotoxin (SE) genes

the N-terminal segment (approx. 6000 MW), the C-terminal and the central portion of the molecule contains the site for emetic activity.

Assays for enterotoxins

There are several procedures like animal assays, immunological, molecular biological, biosensors etc to detect staphylococcal enterotoxins. The production of enterotoxin needs long incubation time (20 h). Some factors, which affect the incubation period, are the pH, the water activity and the used substrates. Numerous methods are based on the evidence of the enterotoxins directly in the food (ELISA, reversal passive latex agglutination and others), with a possibility to detect nanogram amounts of enterotoxins in one gram or in one mili-liter of food [55],[56],[57],[58] The advantage of these methods is that enterotoxins are detected even if the producer Staphylococcus aureus should not be identified by the classical bacteriological procedure, because it is usually devitalized by temperature. DNA amplification methods (polymerase chain reaction, PCR) can show the presence of enterotoxigenic strains of Staphylococcus aureus before the expression of enterotoxins on the base of specific gene sequences and in this way detect the potential source of contamination. The advantage of the PCR methods is that it is able to detect genes which code the production of staphylococcal enterotoxins also from heat treatment of food, because the DNA remains unchanged [59],[60]Several ELISA-based diagnostic kits for detection of staphylococcal enterotoxins are commercially available. All kits are capable of detecting SEA, SEB, SEC, SED, and SEE at levels of less than 1 ng/g of food. Slight variations between the kits exist with some (i.e. SET-EIA and RIDASCREEN) capable of differentiating between the enterotoxin serotypes, whereas others (i.e. TECRA and TRANSIA) not having this capability. An alternative diagnostic kit that is not ELISA-based is the reversed-passive latex agglutination assay (RPLA). It uses latex particles coated with enterotoxin antibodies that agglutinate in the presence of staphylococcal enterotoxins. The major problem with this kit is that the food extract must be perfectly clear or else a false-coagulation reaction can occur [61].

Biosensors are currently being developed to provide real-time detection of staphylococcal enterotoxins [62].In the case of SEA, it has been possible to detect, in less than 4 min, with little or no background interference, the enterotoxin in complex food matrices such as hot dogs, potato salad, milk, and mushrooms at levels of sensitivity of 10-100 ng/g. Since there are instances where non-specific interactions of food components with the sensor chip surface may occur, a biosensor assay has been enhanced by incorporating surface plasmon resonance detection with subsequent matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Using this two-step approach, the presence of SEB has been verified in milk and mushrooms at levels of 1 ng/ml.

Inactivation of enterotoxins

Thermal Inactivation

Staphylococcal enterotoxins are recognized for their heat resistance, with z values (the temperature in °C required for the thermal destruction curve to traverse one log cycle) ranging from 25 to 33°C, D121°C values (time in minutes at 121°C for 90% destruction) from 8.3 to 34 min, and F120°C values (equivalent time in minutes at 120°C for destruction) up to 30 min. Differences in stability do exist among the toxins [63],[64],[65],[66],[67],[68].

Irradiation

External factors affecting the inactivation of staphylococcal enterotoxins by irradiation are similar to those that influence inactivation by heating. For example, irradiation is more effective in inactivating enterotoxins that are dispersed in buffers than when they are dispersed in complex media. Considering the unusually high doses required for inactivation of staphylococcal enterotoxins, irradiation should not be considered an effective treatment for elimination of enterotoxins from intentionally contaminated food [69],[70].

Chemical Inactivation

A small number of treatments involving chemicals are available for inactivation of enterotoxins. On surfaces, application of soap or 0.5% hypochlorite (HOCl) for 10-15 min is recommended for inactivation of enterotoxins. Similarly in solutions, exposure of SEA (70 ng in phosphate-buffered saline) to 9 μg of HOCl causes a loss of enterotoxin immunoreactivity. In the presence of organic matter, however, increased levels of chlorine will be required [71],[72].

Biological Inactivation

Staphylococcal enterotoxins are resistant to proteolytic enzymes such as trypsin, chymotrypsin, rennin, papain, and pepsin. Hence, enterotoxins are not inactivated to any great extent in the digestive tract. Pepsin destroys the activity of SEB at a pH of about 2, but the pH of the stomach would be this low only after starvation. Comparatively, SEI is more susceptible to stomach proteases than SEA due to slight differences in structure. When SEI was exposed to monkey stomach fluid for greater than 40 min at 37°C, degradation occurred by 1 h, whereas SEA was stable for more than 1 h [73].Based on the poor ability of proteolytic enzymes to affect the biological activity of staphylococcal enterotoxins, it is not surprising that enterotoxin levels are unaffected by proteolytic or enteric bacteria. Lactic acid bacteria, however, do decrease enterotoxin concentrations. Decreases in enterotoxin concentrations could not be accounted for by addition of lactic acid alone, suggesting the involvement of specific enzymes or other metabolites. Alternatively, selective physical adsorption of toxin to the lactic acid bacteria may have occurred during removal of cells to obtain supernatants for toxin assays.

Clinical methods to prevent staphylococcal infection

Since Staphylococcus infection is mainly spread as a nosocomial infection through fomites touched already by the infectious organism carrying secretions like nasal secretions etc, consumption of contaminated foods and through healthy carriers. Though, there is no 100% prevention for a Staphylococcus infection but several easy steps can be taken to lower the risk of a “minor infection” or “minor cut” from becoming a “major infection.”

These include:

- Wash hands and wounds with soap and water after treating a “minor wound.” Proper washing technique is to scrub aggressively for 30 seconds or more. Anti-bacterial soaps offer no more cleaning power than other soaps. The time spent washing is more important.
- Wash hands after treating another person’s wound.
- Towels used for drying hands after contact with your own wound or another's should be used only once and laundered.
- Disposable gloves should be worn when treating another individual to prevent colonization spread from the caregiver to the patient and vice versa.
- Bed linens and clothing should be changed and washed if wounds are oozing through protective bandages.
- Treatment areas, such as sinks and counter tops should be cleaned immediately after use.
- The patient’s environment should be cleaned routinely and when soiled with body fluids.
- Notify physicians and other healthcare personnel who may care for the affected individual that they may be infected with a antimicrobial resistant bacteria.
- Keep cuts and abrasions clean and covered with proper dressings until healed.
- Avoid contact with another person’s wounds or any material that may have been contaminated from the wound.
- Hospital kitchens should be regularly checked for the presence of Staphylococcus aureus.

Conclusion

Staphylococcal food poisoining is of major concern in public health programs worldwide. Predictive models for S. aureus growth and SE production would be powerful tools for microbial risk assessment in food industries. However, many factors affect S. aureus growth and SE production in foodstuffs and further studies are still necessary in order to develop such predictive tools. Proper storage of food is an important part of reducing the risk of food poisoning. Foods must be stored in the refrigerator and eaten within a short period of time, other foods, such as flour, pulses, canned foods and many others last much longer and can be stored at room temperature. Dried foods too have limited shelf life. The microbes on our food that can cause poisoning are usually temperature controlled by heating (cooking) and/or chilling (refrigerating) the food. While reusing the refrigerated food, one should heat it properly if need to be restored or eaten after sometime. Because simply

warming at temperature not very high (20 ^{o} C-45 ^{o} C) activates the spores which start reproducing and reuse of the same food gets contaminated with active bacteria. Not only this, the quantity of bacteria also rises due to bacterial multiplication. Though bacteria are limited to the foods but given the chance they can easily spread around the kitchen – via our hands, chopping boards, cloths, knives and other utensils and even through fomites also. They may cross-contaminate other foods – especially cooked and ready-to-eat foods. Good kitchen and personal hygiene practices as well cooked foods are important to help control the consumption of contaminated foods and hence food poisoning. Considerable research effort is still required for better understanding of the interactions between S. aureus and the food matrix and of the mechanisms of SE production in foodstuffs. Research is also needed for the identification of new SEs and of new enterotoxigenic staphylococci. Much effort is being applied towards the development of new and more sensitive methods for SE detection in foodstuffs. Taken together, these studies should lead to better control and a subsequent reduction of staphylococcal food poisoning outbreaks.

Conflict of Interest: None declared

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