10 *Staphylococcus aureus*

Introduction

There are more than 30 species in the genus *Staphylococcus*, and the species with the greatest impact on human health is *Staphylococcus aureus*. While *S. aureus* is a natural inhabitant (commensal) of human and animal skin, nares, and respiratory and genital tracts, as an opportunistic pathogen, it can cause invasive and fatal infections that affect many organs. Of particular public health concern is the emergence of drug-resistant strains of *S. aureus*, which are now one of the most frequently isolated pathogens from hospital-associated (nosocomial) infections. In the USA, about half a million people annually acquire skin and soft tissue infections by *S. aureus*. The bacterium is also involved in foodborne outbreaks associated with food poisoning, leading to approximately 241,000 annual illnesses, an estimate, which probably does not account for sporadic cases.

Staphylococci form clusters when grown in liquid or solid media, a characteristic, which led to the name staphylococcus (*staphyle* means a bunch of grapes and *kokkos* means a grain or a berry in Greek) (Fig. 10.1). In 1871, Von Recklinghausen, a German scientist, observed cocci in a diseased kidney and called them "micrococci." Later, based on cell arrangements, Billroth (1874) classified them as "monococcus," "diplococcus," "streptococcus," and "gliacoccus." In 1880, Sir Alexander Ogston, a Scottish surgeon, and Louis Pasteur, a French scientist, confirmed that cocci-forming organisms are capable of causing disease. Later, Ogston coined the name "Staphylococcus," and he was given the credit for the discovery of the pathogen. In 1914, Barber discovered that a toxin produced by staphylococci was responsible for staphylococcal food poisoning.

Staphylococci are mostly associated with community-acquired and nosocomial infections and may be life-threatening in immunodeficient conditions. *Staphylococcus aureus* infections are traditionally treated with the β-lactam antibiotic penicillin, but bacteria frequently develop resistance by producing penicillinase (β-lactamase). To overcome resistance to penicillin, the β-lactamase-resistant drug methicillin was synthesized; however, some strains developed resistance to methicillin and are called methicillin-resistant *S. aureus* (MRSA). Several strains are also resistant to vancomycin (VRSA) and multiple other antibiotics and are routinely isolated from hospital settings. Antibiotic resistance and high virulence potential make this organism a very dangerous pathogen, and infection may be fatal because of lack of alternative antibiotics. MRSA may be either hospital associated (HA-MRSA) or community associated (CA-MRSA); however, in recent years MRSA has been associated with livestock (LA-MRSA). This later group may be responsible for the

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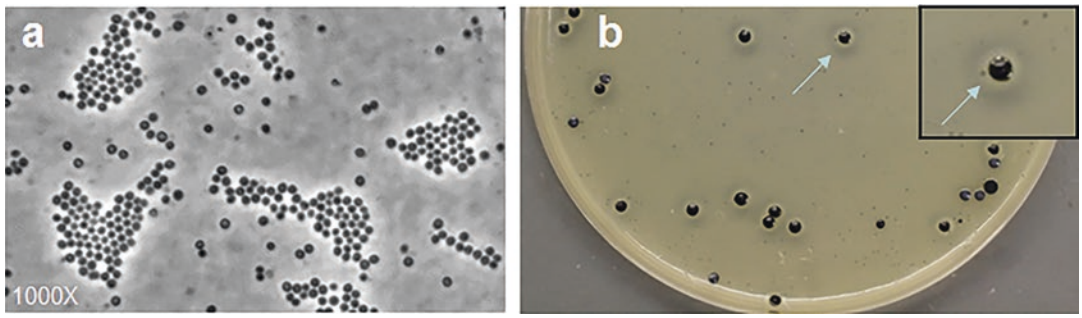

Fig. 10.1 (**a**) Phase contrast microscopic photograph of *Staphylococcus aureus* cells. Cells appear as clusters (magnification 1000×); (**b**) typical *S. aureus* colonies on Baird-Parker agar showing the characteristic black appearance surrounded by halo

transfer of the pathogen from animal to animal or animal to human and thus may have serious implication as a zoonotic pathogen. Most staphylococci are responsible for skin infections such as abscesses (boil, carbuncle, and furuncle), but some may cause life-threatening endocarditis, toxic shock syndrome, sepsis, and pneumonia. *S. aureus* also causes food poisoning, resulting in severe vomiting and cramping with or without diarrhea. Staphylococci also cause mastitis in cows and joint infection in humans, in animals, and in poultry, leading to edema and arthritis.

Classification

In the *Bergey's Manual of Determinative Bacteriology*, *Staphylococcus* has been placed in the family of *Micrococcaceae*. DNA–ribosomal RNA hybridization and comparative oligonucleotide analysis of 16S rRNA gene have demonstrated that staphylococci form a coherent group at the genus level. Staphylococci are differentiated from other close members of the family by their low G + C content of DNA, ranging from 30 to 40 mol%. The genus *Staphylococcus* has been further classified into more than 30 species and subspecies by biochemical analysis and by DNA–DNA hybridization. *Staphylococcus aureus* is the primary species in the genus *Staphylococcus* and is responsible for food poisoning and nosocomial and hospital-acquired infections. Other species which belong to this genus include *S. intermedius*, *S. chromogenes*, *S. cohnii*, *S. caprae*, *S. caseolyticus*, *S. delphini*, *S. epidermidis*, *S. felis*, *S. gallinarum*, *S. haemolyticus*, *S. hyicus*, *S. lentus*, *S. saprophyticus*, *S. sciuri*, *S. simulans*, *S. succinus*, *S. warneri*, and *S. xylosus*. A majority of them produce enterotoxins.

Morphology

Staphylococcus aureus is a Gram-positive coccus (1 μm in diameter) appearing microscopically as grape-like clusters due to three incomplete planar divisions (Fig. 10.1). They are nonsporeforming, are nonmotile, and produce golden yellow-pigmented colonies. This pigmentation is alluded to in the microbe's name, as aureus means golden, i.e., a gold coin of Rome. The cell wall of *S. aureus* contains three main components: the peptidoglycan comprising repeating units of *N*-acetylglucosamine β-1,4 linked to *N*-acetylmuramic acid; a ribitol teichoic acid bound via *N*-acetyl mannosaminyl-β-1,4-*N*-acetylglucosamine to a muramyl-6-phosphate; and protein A, which is covalently linked to the peptidoglycan. Protein A is characterized by its ability to bind to the Fc component of mammalian immunoglobulin molecules, which results in autoagglutination of mammalian plasma. Most of the other species of staphylococci lack protein A in their cell wall and therefore do not exhibit autoagglutination properties.

Cultural and Biochemical Characteristics

Staphylococcus aureus (Table 10.1) is a catalase-positive, facultative anaerobe, and grows abundantly under aerobic conditions, except for *S. saccharolyticus*, which is a true anaerobe. Under aerobic condition, it produces acetoin as the end product of glucose metabolism. It ferments mannitol, causes coagulation of rabbit plasma (coagulase positive), produces thermonuclease, and is sensitive to lysostaphin, a metalloendopeptidase. *S. aureus* produces hemolysins and causes an α-, β-, and α + β (double) hemolysis on blood agar plates. *S. aureus* can grow in a wide range of temperatures (7–48 °C; optimum 30–37 °C), pH (4–10; optimum 6–7), and water activity (A_w 0.83–0.99, optimum 0.98). *S. aureus* is highly salt-tolerant (up to 20% NaCl) and relatively resistant to drying and heat. The enterotoxins are produced at temperature 10–46 °C (optimum, 37–45 °C), pH 4–9.6 (optimum pH 7–8), A_w 0.85–0.99 (optimum 0.98), and NaCl 0–10% (optimum 0%). *S. aureus* coagulates rabbit plasma relatively quickly, while *S. intermedius* and *S. hyicus* subsp. *hyicus* cause delayed coagulation. *S. epidermidis* is coagulase negative and does not ferment mannitol. Many growth media have been developed for selective isolation of *Staphylococcus* species: mannitol salt agar (MSA) contains mannitol, a phenol red indicator, and 7.5% sodium chloride producing small colonies surrounded by yellow zones indicating mannitol fermentation. Baird-Parker media containing tellurite and egg yolk is suitable for isolation of coagulase-positive *S. aureus*, which produce black, shiny colonies surrounded by clear zones (Fig. 10.1).

Virulence Factors

Staphylococcus aureus produces a family of virulence factors such as adhesion proteins, enterotoxins, superantigens, toxic shock syndrome toxins (TSST), exfoliative toxins (ET), pore-forming hemolysins, ADP-ribosylating toxins, and proteases (Table 10.2).

Adhesion Proteins

Staphylococci possess multiple adhesion molecules, which are collectively known as MSCRAMM (microbial surface components recognizing adhesive matrix molecules). Internalization of the organism by host cells is triggered by MSCRAMM. Adhesion proteins include Bap (biofilm-associated proteins), which is responsible for biofilm formation and colonization in the mammary gland during mastitis. The C-terminus of Bap contains typical cell wall anchoring domain comprising of LPXTG motif, a transmembrane sequence, and a positively charged C-terminus. The fibronectin-binding protein (Fbp) binds to host fibronectin. Bacterial binding to fibronectin also facilitates internalization into nonprofessional phagocytes, such as

Table 10.1 Typical characteristics of *S. aureus*, *S. epidermidis*, *S. intermedius*, and *S. hyicus*

Characteristic	*S. aureus*	*S. epidermidis*	*S. intermedius*	*S. hyicus*
Catalase activity	+	+	+	+
Hemolysis	+	+/–	+	–
Coagulase production	+	–	+/–	+/–
Thermonuclease production	+	–	+	+/–
Hyaluronidase production	+	–	–	+
Lysostaphin sensitivity	+	+/–	+	+
Anaerobic utilization of				
Glucose	+	+	–	–
Mannitol	+	–	–	–

+, Most (90% or more) strains are positive
–, most (90% or more) strains are negative

Table 10.2 Virulence factors and enzymes produced by *Staphylococcus aureus*

Virulence factors	Receptors
Adhesin proteins	
Spa (protein A)	Fc part of IgG
Bap (biofilm-associated proteins)	Unknown
Fbp (fibronectin-binding protein)	Fibronectin, fibrinogen, elastin
ClfA (fibrinogen-binding protein)	Fibrinogen
Cna (collagen adhesin)	Collagen
IsdA, IsdB, IsdC, IsdH (iron-regulated surface proteins)	Hemoglobin, transferrin, hemin
Pls (plasmin-sensitive cell wall protein)	Cellular lipid called ganglioside GM_3
Atl (autolysin amidase)-bacteriolytic action	Fibronectin, fibrinogen, vitronectin
Enolase	Laminin
Teichoic acid	Unknown – binds epithelial cells
Enterotoxins (24)	
Staphylococcal enterotoxin SEA–SElX, except SEF	Glycosphingolipid
Pore-forming hemolysins	
Hemolysins α, β, γ, δ	Cholesterol
Superantigens	
TSST (toxic shock syndrome toxin)	MHC class II
Enterotoxins	Glycosphingolipid, MHC class II
Exfoliative toxins (ETA, ETB)	Desmoglein-1
ADP-ribosylating toxins	
Panton-Valentine Leukocidin, pyrogenic exotoxin	Complement receptor, C5aR
Proteases	
Metalloprotease, collagenase, hyaluronidase, endopeptidase, elastase	
Others	
Nuclease, lysozyme, phospholipases, coagulase	

keratinocyte, epithelial cell, endothelial cell, and osteoblast. Staphylococci also produce other adhesion factors including ClfA, a fibrinogen-binding protein that activates platelets aggregation and plays a role in staphylococcal arthritis; Pls, a plasmin-sensitive cell wall protein that binds to ganglioside GM_3 of host cells and promotes adhesion to nasal epithelial cells and; Cna, a collagen adhesin binds to collagenous tissues, i.e., cartilages.

Toxic Shock Syndrome Toxin-1 (TSST-1)

TSST-1 is a 22 kDa protein and acts as a superantigen, which generates a strong immune response in the host. The toxin stimulates the release of IL-2, TNF-α, and other proinflammatory cytokines (IL-1, IFN-γ, IL-12). In the1980s, TSST-1 was mostly recognized for its role in the outbreaks of toxic shock syndrome associated with tampon use. Only a small number (<10%) of *S. aureus* strains produce TSST-1, and toxic shock syndrome (TSS) is a rare but severe and potentially fatal disease. TSST is responsible for acute illness, high fever, erythematous lesion (rash), hypotension, and septic shock. It can cause organ failure and DIC (disseminated intravascular coagulation). Unlike the enterotoxin superantigens, TSST does not cause emesis.

Exfoliative Toxin

The exfoliative toxin (ET) is a serine protease of 30 kDa and has two serotypes: ETA and ETB. Strains can produce one or the both and dependent on the isolates of different geographical origins. ET is responsible for a severe skin disease that primarily affects infants, called staphylococcal scalded skin syndrome (SSSS), which is characterized by a bright red rash, blisters, and severe skin lesions. The ET binds to

receptor desmoglein-1, a desmosomal glycoprotein and selectively hydrolyzes the desmosomal cadherins of the superficial skin layer (stratum granulosum). This results in the destruction of the superficial skin layers causing dehydration and increased susceptibility to secondary skin infections.

Miscellaneous Enzymes and Toxins

Staphylococcus aureus produces coagulase, which contributes to the fibrin clot formation and accumulation on the bacterial cell surface, and aids in bacterial evasion of phagocytosis. The bacterium also secretes four types of hemolysins (α, β, γ, and δ); membrane active lipase such as phospholipase C (PLC); Panton-Valentine leukocidin, which destroys leukocytes; collagenase, which hydrolyzes collagen; hyaluronidase, which hydrolyzes hyaluronic acid component of the cellular basement membrane; metalloprotease; pyrogenic exotoxin; and staphylokinase, which degrades fibrin clots.

Enterotoxins

Staphylococcus aureus produces a large number of extracellular proteins and toxins. The most important toxins are called staphylococcal enterotoxins (SEs) and SE-like toxins (SEls) (Table 10.3), which share four common properties: (i) structural similarity, (ii) resistance to heat and proteolytic enzymes, (iii) superantigenicity, and (iv) emetic activity. Twenty-four major serologically distinct SEs are reported: SEA through SElX with no SEF. The SEF is similar to other SEs, but rather than inducing emesis, it causes toxic shock syndrome (TSS), hence designated TSST-1. One of the characteristics of SEs is the induction of emesis. If a SE is structurally similar to other SEs, but has not been tested for induction of emesis or negative for emesis in a primate model (monkey), the International Nomenclature Committee for Staphylococcal Superantigens (INCSS) designates them as SE-like toxins (SEls). The first five well-characterized SEs are SEA to SEE, and they all cause emesis. The SEC has three antigenically distinct subtypes: SEC1, SEC2, and SEC3. The SEG–SEI and SER–SET also have strong emetic activity, while SElL and SElQ are not emetic. The other SE1s including SElJ, SElK, SElM, SElN, SElO, and SElP have been tested in primate models and have shown emetic activity but much lower than the SEA or SEB. SElU–SElX have not been tested in a primate model. SEs are responsible for food poisoning, acute illness, fever, erythematous lesions, and hypotension.

SEs are a heterogeneous group of water-soluble, single-chain globular proteins of 168–257 amino acids with molecular weight of about 19.3–29 kDa. The SE polypeptide chain contains relatively a large number of lysine, aspartic acid, glutamic acid, and tyrosine residues. The SEs are generally heat-resistant (121 °C for 10 min), and a heat-denatured enterotoxin can be renatured by prolonged storage or in the presence of urea. Toxins remain active even after boiling for 30 min. In food, such as in mushrooms, they are stable at 121 °C for 28 min. The SEs also are resistant to proteolytic digestion such as trypsin and pepsin thus retain activity in the gastrointestinal tract to cause food poisoning.

SEA is the most common serotype found in *S. aureus*. It is the most common SE responsible for about 78% of food poisoning outbreak followed by SED and SEB. SEA is a 27.1 kDa toxin, and its production is not regulated by *agr* (accessory gene regulator). SEB is a 28.4 kDa toxin and is the most heat-resistant (stable at 60 °C for 16 h) among all the toxins. SEB also is resistant to gastrointestinal proteolytic enzymes such as trypsin and pepsin. SECs are a group of highly conserved proteins, and there are three antigenically distinct subtypes: SEC1, SEC2, and SEC3. Staphylococcal isolates from different animal species produce host-specific SECs. SED (24 kDa) is the second most serotypes responsible for food poisoning. SED has the ability to form a homodimer in the presence of Zn^{2+} which facilitates its binding to MHC class II molecule on antigen-presenting cells and enables it to serve as a superantigen (see below).

Table 10.3 Biological characteristics of staphylococcal enterotoxins and staphylococcal enterotoxin-like toxins

Enterotoxin	Genetic element	Molecular weight (kDa)	Superantigenic activity	Emetic activity in monkey[a]
1. SEA	Prophage	27.1	+	25
2. SEB	Chromosome, plasmid	28.4	+	100
3. SEC1	SaPI	27.5	+	5
4. SEC2	SaPI	27.6	+	NT
5. SEC3	SaPI	27.6	+	<50
6. SED	Plasmid	26.9	+	NT
7. SEE	Prophage	26.4	+	NT
8. SEG	Egc, chromosome	27	+	160–320
9. SEH	Transposon	25.1	+	30
10. SEI	Egc, chromosome	24.9	+	300–600
11. SElJ	Plasmid	28.6	+	NT
12. SElK	SaPI	25.3	+	NT
13. SElL	SaPI	24.7	+	Not emetic
14. SElM	Egc, chromosome	24.8	+	NT
15. SElN	Egc, chromosome	26.1	+	NT
16. SElO	Egc, chromosome	26.8	+	NT
17. SElP	Prophage	26.7	+	NT
18. SElQ	SaPI	25.2	+	Not emetic
19. SER	Plasmid	27	+	<100
20. SES	Plasmid	26.2	+	<100
21. SET	Plasmid	22.6	+	<100
22. SElU	Egc, chromosome	27.2	+	NT
23. SElV	Egc, chromosome	27.6	+	NT
24. SElX	Chromosome	19.3	+	NT

Adapted from Hu and Nakane (2014)
Egc enterotoxin gene cluster, *SaPI S. aureus* pathogenicity island, *NT* not tested
[a]μg/animal after oral administration

The genes for enterotoxin production are located either in a bacteriophage, chromosome, transposon, or in plasmids. The *sea* and *sep* are located in a bacteriophage; *seb*, *seh*, and family of *sec* are in the chromosome; and *sed*, *sej*, and *ser* are located in a plasmid. The *sed* and *sej* genes are colocalized and the same strain always produces these two toxins (SED and SEJ) together. Enterotoxin gene cluster (*egc*) can encode for several SEs such as SEG, SEI, SEM, SEN, and SEO. Certain enterotoxin genes are also located in the pathogenicity islands (PAI). There are five staphylococcal PAI: SaPI-1, SaPI-2, SaPI-3, SaPI-4, and SaPI-bov. SaPI-1 contains genes for TSST-1 and SEK and SEQ. SE genes located on mobile elements can result in horizontal gene transfer. Production of enterotoxins is not restricted to *S. aureus* alone, as other non-*aureus* staphylococci are reported to produce enterotoxin. The accessory gene regulator system (*agr*) is a main regulatory mechanism controlling the expression of virulence factors in *S. aureus*; however, not all SEs or SEls are regulated by *agr*.

Enterotoxins are expressed differentially, and the toxin production depends on the growth phase of bacteria, bacterial density, pH, and CO_2 levels. SEA and SEJ are synthesized mostly during the exponential phase, while SEB, SEC, and SED are produced during the transition from exponential to the stationary phase of growth.

Molecular Regulation of Virulence Gene Expression

The pathogenic potential of staphylococci in humans can be attributed to the expression of a wide array of virulence factors, most of which are

governed by Staphylococcal virulence regulators. Four loci have been implicated in the regulation of expression of virulence factors: accessory gene regulator, *agr*; the staphylococcal accessory regulator, *sar*; *S. aureus* exoprotein expression, *sae*; and exoprotein regulator, *xpr*.

Enterotoxin production is regulated by the two-component regulatory system, *agrAC*. In the two-component system, one protein serves as a sensor and transfers signal by phosphorylating the intracellular activator, while another regulates genes to provide response called response regulator. The *agr* locus comprises the global regulatory system, which regulates virulence gene expression in the post-exponential phase of growth. It consists of two divergent units driven by promoters P_2 and P_3. The P_2 transcript includes four open reading frames (ORFs) referred to as *agrA*, *agrB*, *agrC*, and *agrD*. The P_3 transcript RNA III is the actual effector molecule and activates secretion of enterotoxins and other exoproteins, primarily at the transcriptional level. In the *agr* locus, P2 operon includes the genes for response regulator, *agrA*; histidine kinase, *agrC*; autoinducing peptide, *agrD*; and autoinducing ligand inducer (AIP), *agrB*. The strains that produce high amounts of enterotoxin have a high concentration of RNA III. An intact *agr* locus is necessary for maximum SEC expression. The *agr* locus regulates SEC expression at the posttranscriptional level, presumably at the level of translation or secretion. RNA III is produced at lower levels under alkaline pH and SEC production decreases at low pH. In addition, Sar family of proteins also regulates virulence gene expression. SarA is a DNA-binding protein and binds to *agr* promoter stimulating the transcription of RNA II. Activation of RNA II and subsequently RNA III leads to alternating target gene expression. Expression of SEB is positively controlled by *sarA*. Xpr represents an additional genetic element involved in regulation of some *agr*-regulated proteins. Low levels of SEs production were observed in *xpr* mutants. Reduced levels of RNA II and III were also observed in these mutants.

Food Association and Enterotoxin Production

Staphylococcal food poisoning is one of the most common foodborne illnesses reported worldwide. Nearly one-third of all the food poisoning cases in the USA were caused by staphylococci during the 1970s and 1980s, which in general has decreased over the past two decades. However, it remains the main reported cause of food poisoning in a number of countries including Brazil, Egypt, Taiwan, Japan, and most of the other developing countries. About 50–80% of *S. aureus* isolates are positive for at least one superantigen gene. MRSA strains isolated from hospital patients tend to harbor more superantigen genes than the methicillin-sensitive strains. Intoxication occurs due to the ingestion of one or more preformed SEs or SE- SEls in contaminated food.

Staphylococcal foodborne illness is often associated with creamy food prepared with milk and milk products, cheese, custard (pudding), cream-filled pastries, cakes, salad dressing, shellfish, fish, meat, and hams, as well as foods that require greater hand preparation such as pasta and chicken salads, deli foods, and sandwiches. Staphylococci can be transmitted to food through meat grinder's knives and food handlers. The bacteria replicate in foods subject to temperature abuse, such as foods left at room temperature for a long period.

Generally, staphylococcal enterotoxins are produced at temperatures between 10 °C and 46 °C (optimum, 37–45 °C), pH 4–9.6 (optimum pH 7–8), A_w 0.85–0.99 (optimum 0.98), and NaCl 0–10% (optimum 0%). At temperature 60 °C or higher, the organism will not grow; however, below 60 °C, the organism will grow and produce toxins.

Mechanism of Pathogenesis

Emesis and Diarrhea

The infectious dose of *S. aureus* is 10^5–10^8 cfu g^{-1} of food and a toxin concentration of 1 ng g^{-1} of food. The emetic dose (ED_{50}) of SEA toxin in

Rhesus monkey is 5–20 μg kg^{-1}, while 200 ng kg^{-1} may be needed to show intoxication syndrome in human. SEB is highly toxic and a dose of 400 ng kg^{-1} is required for humans. Following consumption of *S. aureus* enterotoxin contaminated food, toxins are absorbed and cause typical gastroenteritis, while bacteria pass through the intestine without causing any adverse effects on the host. Studies conducted with SEA and SEB have shown that SE binds to submucosal mast cells and induces degranulation and release of 5-HT (5-hydroxytryptamine), a neurotransmitter (also known as serotonin). 5-HT interacts with the 5-HT_3 receptor on adjacent vagal afferent neurons in the stomach lining and stimulates medullary vomiting center (medulla oblongata) to induce a violent emetic reflex (Fig. 10.2). 5-HT synthesis inhibitors or 5-HT_3 receptor antagonist (ondansetron hydrochloride (Zofran)) drugs can prevent SE-induced vomiting. Although the mast cell receptor for SE is unknown, studies in kidney cells indicate that the putative receptor for SE is a glycosphingolipid. SE also activates Ca^{2+} signaling pathway in the intestinal epithelial cells. Enterotoxins elicit damage to the intestinal epithelial cells resulting in villus destruction, villi distension, crypt elongation, and lymphoid hyperplasia.

Superantigen Activity

The superantigenic property of staphylococcal enterotoxins distinguishes them from other bacterial toxins. Superantigens are the molecules that have the ability to stimulate an exceptionally high percentage of T cells ($CD4^+$ and $CD8^+$ cells), massive cytokine release, and systemic shock (Fig. 10.3). Enterotoxins cross the epithelial barrier, enter blood circulation, and bind to the α-chain of the MHC class II molecules on the surface of macrophages. The toxin is presented to the T cells that carry TCR (T-cell receptor) made with β-chain, also called Vβ carrying T cells. T cells proliferate and produce large quantities of IL-2 and IFN-γ. Elevated levels of IFN-γ also induce increased MHC class II expression in macrophages and other cells, which in turn bind more superantigens, and activate more T cells. Inflammatory cytokines such as IL-1 and TNF-α are produced by activated macrophages, and the cytokines initiate typical toxic shock syndrome with disseminated intravascular coagulation (DIC), high fever, low blood pressure, massive shock, and death (Fig. 10.3).

Superantigens exert immunopathological response in the GI mucosa. The intestinal mucosa harbors all of the major Th-cell subsets (Th1,

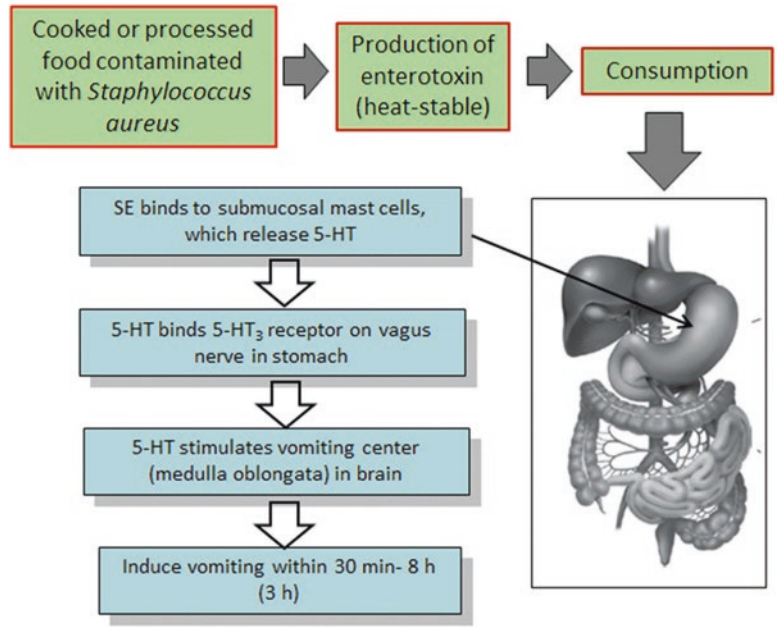

Fig. 10.2 The pathogenic mechanism of intoxication with enterotoxin from *Staphylococcus aureus*. 5-HT, 5-hydroxytryptomine

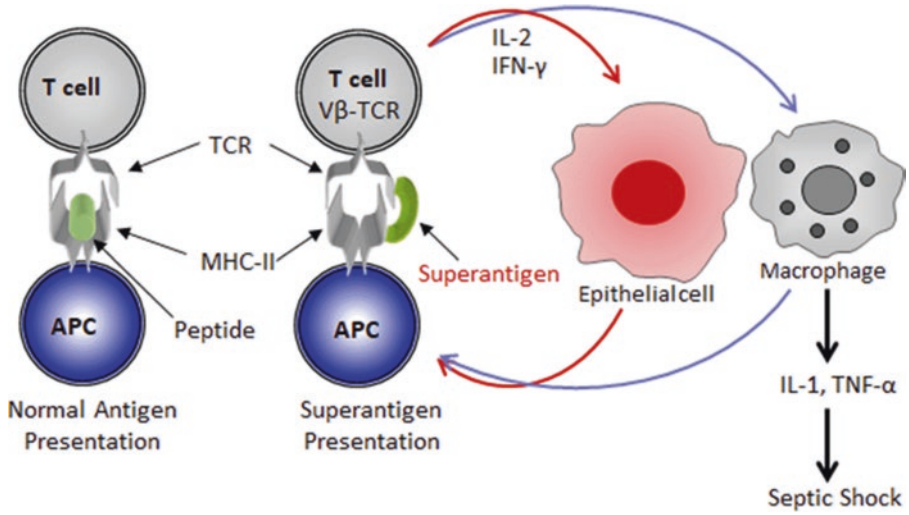

Fig. 10.3 Mechanism of superantigen action of staphylococcal enterotoxin

Th2, Th17). Superantigens, unlike others, do not stimulate T cells indiscriminately, as only specific Vβ sequences in the TCR are recognized. SE increases epithelial permeability and intestinal secretion by reducing TJ and AJ protein expression in epithelial cell–cell junctions. Epithelial permeability is mediated by enhanced secretion of IFN-γ and TNF-α from lymphocytes (*see* Chap. 4). SEs activate gastrointestinal T cells and provoke a "cytokine storm," in which Th1 releases IL-2 and IFN-γ, Th2 releases IL-4, and Th17 secrets IL-17, and activated macrophages and other cell types secrete TNFα, IL-1β, IL-6, and IL-8. These cytokines may act as chemoattractants and induce expression of adhesion molecules, favoring localization of diverse immune cells responding to SE. Cytokines also affect epithelial cell functions primarily the ion and water transport.

Animal and Cell Culture Models

Animal models have been developed to study emetic effect after oral administration. Nonhuman primate (monkey, i.e., *Macaca mulatta*), pigs, piglets, and dogs are sensitive to *S. aureus* emetic effects, while rodents (mice, rats, and rabbits) are less susceptible. The house musk shrew (*Suncus murinus*), a rodent, has been used as a small animal model which shows an emetic response after oral and intraperitoneal administration.

Staphylococcal enterotoxins act as superantigens. Examination of these toxins for the ability to cause mammalian cell damage provides a means to assay these toxins. A bioassay for superantigen on a T-lymphocyte cell line (Raji) has been developed. In this assay, the SEA-induced cytotoxic action was detected colorimetrically using the CytoTox 96-well lysis detection kit. This system can detect SEA at picomolar concentrations. *Staphylococcus aureus* enterotoxins are also detected in Madin-Darby bovine kidney (MDBK), bovine embryo lung (PEB), and dog carcinoma cell line (A-72) for possible cytopathic effects. The PEB cell line is the most susceptible, and the cytopathic effect is observed after 2 h of incubation.

Symptoms

Staphylococcal infection may cause skin infections such as abscesses (boil, carbuncle, and furuncle), staphylococcal scalded skin syndrome, impetigo, and cellulitis. Severe infections may cause life-threatening endocarditis, osteomyelitis, toxic shock syndrome, sepsis, high fever, pneumonia, and sudden infant death syndrome.

Symptoms of staphylococcal gastrointestinal intoxication appear within 30 min–8 h (average of 3 h) and include hypersalivation, nausea, violent vomiting in spurts, abdominal cramping with or without diarrhea, headache, dizziness, shivering, and general weakness. The significant fluid loss will cause dehydration and hypotension. In severe cases, headache, prostration, low blood pressure, and anaphylactic shock may happen. The mortality rate is very low, 0.02% occurring in the most susceptible persons, infants, and the elderly. The disease is self-limiting and may resolve within 24–48 h, but infants and elderly may require hospitalization. In the case of aerosol exposure of enterotoxin, sudden onset of fever, chills, headache, and cough occur. Fever may last for several days and the cough can last for 10–14 days.

Prevention and Control

For treatment of skin infection or systemic infection, antibiotics and other supportive therapies are needed. The staphylococcal food intoxication is mostly self-limiting; therefore, only fluid therapy and bed rest are recommended without any antibiotic therapy.

To prevent *S. aureus*-related food poisoning, cooking food thoroughly is important, but preventing contamination and cross-contamination is critical. After cooking, food should not be left at room temperature for longer than 2 h, because the permissive temperature for bacterial growth and toxin production is between 10 °C and 46 °C. Thus, food should be held at above 60 °C or cooled rapidly to below 5 °C or stored refrigerated to prevent toxin production. Since one of the major sources of *S. aureus* is human skin, handwashing and the use of protective gloves, masks, and hairnets before food handling should reduce the chance of food contamination. Maintaining cold chain during food preparation and processing will prevent bacterial growth. Ensuring quality of raw ingredients, processing methods, adequate cleaning, and disinfection of equipment should be a routine part of food production practices to prevent pathogen contamination and growth in food. Strict hygienic practices are crucial in preventing staphylococcal food poisoning. Implementation of Hazard Analysis and Critical Control Points (HACCP), good manufacturing practices (GMP), good hygienic practices (GHPs), and rapid microbiological analysis will aid in preventing pathogens in food manufacturing facility. Ondansetron hydrochloride (Zofran) is the choice of drug for controlling nausea and vomiting.

Detection

Culture Methods

Conventional culture methods allow isolation of bacteria on mannitol salt agar or Baird-Parker agar plates. The colonies on Baird-Parker appear black, shiny, circular, convex, and smooth with the entire margin forming a clear zone with an opaque zone (lecithinase halo) around the colonies (Fig. 10.1).

Nucleic Acid-Based Methods

Nucleic acid-based detection systems offer a very good alternative to conventional culture methods for detection of *Staphylococcus* in food. Nucleic acid probe-based methods have been developed for the detection and enumeration of staphylococci. PCR-based detection of enterotoxin genes including *egc* (enterotoxin gene cluster: SEA to SEE; SEG, SEH, SEI, SEM, SEJ, SEN, and SEO), TSST-1, exfoliative toxins A and B (*etA* and *etB*), methicillin-resistant (*mecA*) gene, and 16S rRNA from *S. aureus* has been reported. Fluorescence-based real-time PCR (TaqMan-PCR) has been demonstrated for enterotoxins A to D and *mecA* for rapid analysis of a large number of samples. A DNA microarray was developed for detection and identification of 17 staphylococcal enterotoxin (*ent*) genes simultaneously. The assay is based on PCR amplification of the target region of the *ent* genes with degenerate primers, followed by characterization of the PCR products by microchip hybridization with oligonucleotide probes specific for each *ent* gene. The use of degenerate primers allowed the

simultaneous amplification and identification of as many as nine different *ent* genes in one *S. aureus* strain.

Quantitative detection of staphylococci or their toxin genes was achieved through the use of quantitative real-time PCR (qRT-PCR). Commercial rapid assay kits that detect *S. aureus* 23S rRNA is available. A commercial array chip called Staphychips also is available for identification of five different staphylococci in an array format.

Immunoassays

Immunoassays based on ELISA are widely used for detection of enterotoxins. Automated commercial detection systems are available. The detection limit is <0.5–1 ng g^{-1} of enterotoxin. Enzyme immunoassay is available for the detection of superantigens: SEA, SEB, SEC, TSST-1, and streptococcal pyrogenic exotoxin A (SPEA) in the blood serum. A fluorescence immunoassay has been developed for SEB with a detection limit of 100 pg per well and is more sensitive than the conventional ELISA assay. Magnetic bead-based immunoassay has been used to detect SEs in a sandwich format. Rapid latex agglutination tests for SEA to SEE with a detection limit of 0.5 ng/ml are available.

Other Rapid Methods

Although above methods are widely applied for staphylococcal detection, some of the other methods which directly detect the whole cell or their metabolites include, direct epifluorescence technique (DEFT), flow cytometry, impedimetry, ATP-bioluminescence, are commonly used for the routine analysis of milk. Toxins are also detected by mass spectrometry.

Summary

Staphylococcus aureus, a natural inhabitant of the human and animal body, is mostly associated with community-acquired and nosocomial infections, which can be fatal in immunodeficient patients. Methicillin and vancomycin-resistant *S. aureus* can cause serious nosocomial infections in humans. *S. aureus* also causes mastitis in the cows, and joint infection in humans, animals and poultry. Staphylococci are also responsible for food poisoning characterized by severe vomiting and cramping with or without diarrhea. *S. aureus* produces a large number of toxins and enzymes, of which the enterotoxins (24 serotypes of toxins are identified) are most important in the production of gastroenteritis (vomiting and diarrhea) and superantigen-associated illness. Enterotoxins are heat-stable and are produced when the temperature of food is at or above 46 °C. Consumption of preformed toxins induces vomiting with or without diarrhea within 30 min–8 h (average 3 h). The enterotoxin induces the release of 5-HT (5-hydroxytryptamine) from mast cells, which stimulates vagal nerves in the stomach lining and induces vomiting. Enterotoxins are also called superantigens, because they form a complex with MHC class II molecules on the surface of antigen-presenting cells, activating and proliferating T cells to produce massive amounts of cytokines (IL-2, IFNγ, IL-1, TNF-α) that contribute to fatal toxic shock syndrome. The genes for enterotoxin production are present in pathogenicity islands in the chromosome, in plasmids, in transposons, and in temperate bacteriophages. Toxin production is regulated by a two-component regulatory system called *agrAC* (accessory gene regulator). Strict hygienic practices are crucial in preventing staphylococcal food poisoning. Skin infection or systemic infection requires antibiotic therapy, while the foodborne intoxication does not require antibiotic therapy since the disease is caused by the toxin and it is mostly self-limiting.

Further Readings

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