Food Microbiology

4th year Biology

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Staphylococcus:

The genus *Staphylococcus* belongs to family *Staphylococcacae*. They are well adapted to survive on the skin as normal flora. Species of the genus *Staphylococcus* divided in multiple planes at random & tend to form cluster (Greek: "Staphyle"= bunch of grapes, coccus= round). At this time, there are ~ 40 recognized species of this genus, and divided into coagulase positive *Staphylococci* (*S.aureus*, only the most virulent and best known member of the genus) and coagulase negative *Staphylococi* (CoNS) which include the rest of species but two of them are more important and associated with clinical infections such as *S.epidermidis* and *S. saprophyticus*. They are nonmotile, non-spore forming, facultative anaerobes. Grow most rapidly at 37° C but form pigment best at room temperature of 20-25°C. Are hardy organisms and are able to withstand a considerable amount of heat and drying as well as high salt concentrations. Therefore Mannitol Salt agar with 7.5% of NaCl is used as a selective media for isolation of this genus.

Members of this genus are catalase -positive and oxidase-negative, distinguishing them from the genus *Streptococci*, which are catalase-negative, and have a different cell wall composition to *Staphylococci*.

Staphylococcus aureus:

Is the most pathogenic specie of the genus *Staphylococcus*, being implicated in both community-acquired and nosocomial infections, is a major pathogen of increasing importance due to the rise in antibiotic resistance. The species named *aureus*, refers to the fact that colonies have a golden color when grown on solid media, whilst CoNS form pale, translucent, white colonies.

Can be found as normal commensals on the skin, the nasopharynx and anterior nares of some of the population. *S. aureus* is an opportunistic pathogen but significant host compromise is required for *S. aureus* infections such as:

- ➤ Break in skin defenses.
- ➤ Various immune deficits.
- > Previous viral infection.
- ➤ Foreign bodies.

Is the most common cause of skin and Soft - tissue infections, as well as of invasive infections acquired in hospitals.

Are Gram-positive cocci, occur generally in grape-like clusters, aerobic & facultative anaerobic, non-motile, non-capsular, and non-spore-forming; catalase-positive, relatively heat resistant, resistant to high concentrations of salt, can survive long periods on dry inanimate objects.

Transmission: Colonization with *S. aureus* can occur any time after birth, and carriage may be transient or persistent, are usually transmitted by direct contact, often via the hands with colonized or infected people. Humans remain infectious as long as the carrier state persists or the clinical lesions remain active. *S. aureus* can be transmitted from the mother to her neonate during delivery. Skin scales, sneezing, dust and other inanimate objects may play a role but are much less important. It can also be disseminated on food that has been contaminated by human carriers and in aerosols. *S. aureus* is important because it has the ability to make several types of toxins, many of which are responsible for food poisoning.

Pathogenesis:

The virulence of the pathogen is due to the combined actions of several virulence factors not only one factor:

- ➤ Cell wall virulence factors
 - ➤ Protein A(major component of the *S.aureus* cell wall),it bind with FC portion of IgG that inhibit phagocytic engulfment
 - ➤ Fibronectin-binding protein which binds to mucosal cells and tissues.

- ➤ Cytolytic exotoxins (*membrane-damaging toxins*) that lyses eucaryotic cell membranes (hemolysins which rupture RBCs and leukocidin which kill WBCs)
- ➤ Super antigen exotoxins: that damage host tissues or otherwise provoke symptoms of disease (Toxic shock toxin, Exfoliative toxin) and Enterotoxins.
- ➤ Enzymes: Coagulase, Penicillinase, Catalase, Coagulase, Hyaluronidase, proteinase, lipase, staphylokinase, fibrinolysin, DNase and Nuclease

What is Staphylococcal food poisoning?

Staphylococcal food poisoning is a gastrointestinal illness, is caused by eating foods contaminated with toxins produced by S. aureus. The organism produces five serologically different enterotoxins (A, B, C, D & E). Individual strains of S. aureus may produce one or more of enterotoxins types while growing in food. Enterotoxin A, B, and D molecules are heat-stable proteins capable of withstanding boiling for 30 min and produced by 30% to 50% of all S. aureus strains. Synthesis is plasmid or chromosomally mediated. Enterotoxin A and D are responsible for Staphylococcal food poisoning by inhibiting water absorption from the intestinal lumen and inducing diarrhea. Enterotoxin B damages the intestinal epithelium and produces colitis. Staphylococcal food poisoning is characterized by severe vomiting, diarrhea, and abdominal pain. The absence of fever is an important observation in the differential diagnosis of Staphylococcal food poisoning. Food workers who carry Staphylococcus and then handle food without washing their hands contaminate foods by direct contact. The bacterium can also be found in un-pasteurized milk and cheese products. Staphylococcus is salt tolerant and can grow in salty foods like ham.

The foods may not smell bad or look spoiled in order to produce the toxins.

How do I know if I have Staphylococcal food poisoning?

Toxin-producing *Staphylococcus aureus* can be identified in stool or vomit using specialized techniques and toxins can be detected in food items. Diagnosis of Staphylococcal food poisoning in an individual is generally based only on the signs and symptoms of the patient. Testing for the toxin-producing bacteria or

the toxin is not usually done in individual patients. Testing is usually reserved for outbreaks involving several persons.

Symptoms:

The intoxication dose of SE is less than 1.0 μ g. This toxin level is reached when *S. aureus* populations exceed 100,000 organisms / g in food. When ingested, the enterotoxin may rapidly produce symptoms, which commonly include nausea, abdominal cramping, vomiting, and diarrhea. In more severe cases, dehydration, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur.

The onset of symptoms usually is rapid (1 to 7 hours) and in many cases acute, depending on individual susceptibility to the toxin, amount of toxin ingested, and general health. Staphylococcal enterotoxins are stable in the gastrointestinal tract and indirectly stimulate the emetic reflex center by way of undetermined molecular events. It is thought that the vagus nerve is involved in the sequence of events that produce the emetic response.

Treatment:

For most patients, staphylococcal food poisoning will cause a brief illness of one to three days. The best treatments for these patients are rest, plenty of fluids, and medicines to calm their stomachs. Highly susceptible patients, such as the young and the elderly, are more likely to have severe illness requiring intravenous therapy and care in a hospital. Antibiotics are not useful in treating this illness because the toxin is not affected by antibiotics.

Prevention:

It is important to prevent the contamination of food with *Staphylococcus* before the toxin can be produced:

- Wash hands and under fingernails vigorously with soap and water before handling and preparing food.
- Do not prepare food if you have a nose or eye infection.
- Do not prepare or serve food for others if you have wounds or skin infections on your hands or wrists.
- Keep kitchens and food-serving areas clean and sanitized.
- If food is to be stored longer than two hours, keep hot foods hot (over 60°C) and cold foods cold (5°C or below).

• Store cooked food in a wide, shallow container and refrigerate as soon as possible.

Bacillus:

Members of the genus Bacillus are ubiquitous present in soil, air, dust, & water. Frequently isolated as "contaminants" in bacteriological culture media. Important Bacillus species: *Bacillus anthracis*, *Bacillus cereus* (*Bacillus stearothermophilus* it was reclassified as a member of the genus *Geobacillus*).

B. anthracis, the causative agent of "anthrax", is the most important pathogen. B. cereus can cause "food poisoning". All members are generally "motile" except B. anthracis, which is "non-motile".

Bacillus cereus:

Gram-positive, facultative anaerobic, endo-spore-forming, large rod. The spores are formed in a central or para-central position without swelling the sporangium. *B.cereus* has a wide distribution in nature, frequently isolated from soil and growing plants, but it is also well adapted for growth in the intestinal tract of insects and mammals. It is also present in the stools of 14 to 15% of healthy humans. It is frequently isolated from milk and dairy products. In milk, *B.cereus* causes a defect known as 'bitty' cream or sweet curdling. It is found in rice, rice products, oriental dishes and ingredients. *B.cereus* and *Lactobacillus* are used as probiotics in animal feed . Some spores survive cooking & germinate into vegetative bacilli which produce enterotoxins that cause food poisoning.

Virulence factors of B.cereus:

- Spores can survive in soil.
- Enterotoxins: heat-stable enterotoxin & heat-labile enterotoxin
- Enzymes: hemolysins, phoshpolipase C as well as many enzymes such as β-lactamases, proteases and collagenases are known as potential virulence factors of *B. cereus*.

- A special surface structure of *B.cereus* cells, the S-layer, has a significant role in the adhesion to host cells, in phagocytosis and in increased radiation resistance.

Pathogenesis:

Bacillus food-borne illnesses occur due to survival of the bacterial endo-spores when food is improperly cooked. Cooking temperatures less than or equal to 100 °C allows some *B.cereus* spores to survive. This problem is compounded when food is then improperly refrigerated, allowing the endo-spores to germinate. Cooked foods not meant for either immediate consumption or rapid cooling and refrigeration should be kept at temperatures above 60 °C. Germination and growth generally occurs between 10–50 °C, though some strains are psychrotrophic. Bacterial growth results in production of enterotoxins, one of which is highly resistant to heat and to pH between 2 &11; ingestion leads to two types of illness, diarrheal and emetic (vomiting) syndrome.

Symptoms:

B.cereus causes two types of food-borne intoxications:

1-"short-incubation" or emetic form of the disease:

Is characterized by nausea and vomiting and abdominal cramps and has an I.P (1 to 6 hours). It resembles *S.aureus* food poisoning in its symptoms and incubation period is caused by a preformed heat-stable enterotoxin (molecular weight less than 5,000 Daltons) infective dose 10^5 - 10^8 cells / g. The mechanism and site of action of this toxin are unknown. It is usually associated with consumption of cooked rice, usually fried rice from Chinese restaurants and other starchy foods such as potato, pasta, and cheese products.

2-"long-incubation" or diarrheal form of the disease:

Is manifested primarily by abdominal cramps and diarrhea with an I.P (8 to 16 hours) after consumption of contaminated food (Meat, soups, vegetables, puddings, sauces and milk). Diarrhea may be a small volume or profuse and watery and it resembles more food poisoning caused by *Clostridium perfringens*. In either type, the illness usually lasts less than 24 hours after onset. In a few patients symptoms may last longer. Illness is mediated by a heat-labile

enterotoxin (molecular weight of approximately 50,000 Daltons) which activates intestinal adenylate cyclase and causes intestinal fluid secretion. Infective $dose10^5$ - 10^7 cells / g.

Treatment

Treatment disease is mild and self limiting, requiring no specific treatment. Rehydration and antibiotics in systemic infections.

How can Bacillus cereus food poisoning are prevented??

- (a) The heat treatment normally used in food preparation, except for pressure cooking, may not destroy *B.cereus* spores. The most important control measure is to keep food at a temperature at which the spores do not germinate and cells do not grow by quick chilling of the food to 4-5°C or holding the food above 60°C.
- (b) Because *B.cereus* cells, given sufficient time, can grow and produce toxins at refrigerated temperature ($\sim 4^{\circ}$ C), a food should not be stored at low temperatures for long periods of time.
- (c) Because cells can get in a food through cross-contamination, proper sanitary measures should be adopted while handling a food.
- (d) Finally, as live cells are necessary for the symptoms, there should be reheating of a suspected food to above 75°C before serving. However, heating may not destroy heat-stable toxins associated with emetic symptoms.