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Review

Bacterial Toxins

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ABSTRACT

Poisonous molecules produced by bacteria inside or outside the organisms are generally called “toxins”. The ability of bacteria to synthesize toxins is called “toxicity”. Toxigenicity is an important factor that increases the virulence of bacteria. The most important structure of bacteria in damage formation is toxin. Toxins created by bacteria are mainly described as two groups, exotoxins and endotoxins. Exotoxins are extracellular structures that bacteria secrete into the external environment. Endotoxins are found in the cell wall structure and released when bacteria are broken down. Exotoxins cause damage in several different ways. Some of them cause paralysis by affecting the neuromuscular system, some cause oedema by breaking down blood cells, some cause food poisoning and some cause enterotoxaemia. Endotoxins, on the other hand, cause inflammation through indirect ways. These inflammations can lead to septic shock. In this review, information about the toxins formed by *Escherichia coli Campylobacter* sp., *Shigella* sp., *Staphylococcus aureus*, *Bacillus* sp., *Clostridium* sp. are given briefly.

Key words: Enterotoxin, exotoxin, endotoxin, bacteria, intoxication

Bakteriyel Toksinler

ÖZET

Bakteriler tarafından vücut içinde ya da vücut dışında meydana getirilen zehirli moleküllere genel olarak “toksin” denmektedir. Bakterinin toksin sentezleme yeteneğine ise “toksijenite” adı verilmektedir. Toksijenite bakterinin virulensini arttıran önemli bir faktördür. Bakterinin hasar oluşumunda en önemli yapısı toksinleridir. Bakteriler tarafından oluşturulan toksinler ekzotoksinler ve endotoksinler olmak üzere başlıca iki gruptur. Ekzotoksinler bakterilerin dış ortama salgıladıkları ekstraselüler yapılardır. Endotoksinler ise hücre duvarı yapısında bulunmaktadırlar ve bakteri parçalandığında açığa çıkarlar. Ekzotoksinler birkaç farklı yolla hasar oluştururlar. Bazıları sinir- kas sistemini etkileyerek felçler oluşturur, bazıları kan hücrelerini parçalayarak ödemlere sebep olur, bazıları gıda zehirlenmelerine, bazıları da enterotoksemilere sebep olmaktadır. Endotoksinler ise indirekt yollarla yangı oluşturur. Bu yangısal olaylar, septik şoka sebep olabilmektedir. Bu derlemede *Escherichia coli*, *Campylobacter* sp., *Shigella* sp., *Staphylococcus aureus*, *Bacillus* sp ve *Clostridium* sp., etkenlerinin oluşturduğu toksinler hakkında bilgi verilmektedir

Anahtar kelimeler: Enterotoksin, ekzotoksin, endotoksin, bakteri, intoksikasyon

Introduction

The microorganisms can multiply in the digestive system and cause disease when they are ingested in high amounts. The food poisoning caused by *Salmonella* sp. agents can be given as an example. Enteric diseases related to food poisoning are arising due to the production of exotoxins and endotoxins. In some types of food poisoning (*Clostridium perfringens*) living organisms must be digested for the disease to occur. But the microorganism does not reproduce in the digestive system. After digestion, vegetative cells sporulate and enterotoxins are released into the environment when the spore cells break down. Because living cells are required for this type of food poisoning to occur. The process of enterotoxin release into the environment after vegetative cells become spore cells can be considered as an infection with food. However, not all infections that occur in this way cause enteritis. Ingested living organisms pass through the digestive system mucosa and reach the vascular system and invade other body tissues (Tayfur, 2009).

After the foodstuff is contaminated with a pathogenic microorganism and this microorganism multiplies and releases the toxin, the poisoning caused by the consumption of this food item is called "Foodborne Poisoning". In other words, the disease does not occur after not only ingestion of the pathogenic microorganism with food, but also after taking the toxin secreted by the microorganism (Tunail, 2000; Karapınar and Aktuğ, 1998). Foodborne diseases generally show clinical pictures that occur with the consumption of foods contaminated with pathogenic microorganisms or microbial toxins and progress with gastrointestinal symptoms (CDC, 2007; Carrique-Mas and Bryant, 2013). These diseases spread by food and they can occur in the form of foodborne infections and foodborne microbial intoxications (Scallan et al., 2011). Among these diseases, enteritis caused by *Salmonella* and *Campylobacter* species take the first place (Scallan et al., 2011; Håstein et al., 2014). Although there are many bacteria that cause foodborne infections and intoxications, the most important and common ones are known as *Escherichia coli*, *Campylobacter* sp., *Shigella* sp., *Staphylococcus aureus*, *Bacillus* sp. and *Clostridium* sp. (CDC, 2007; Carrique Mas and Bryant, 2013).

Escherichia coli

In developing countries, ETEC (Enterotoxigenic *Escherichia coli*) is an important cause of diarrhoea, especially among young children. The most obvious symptoms of this disease are diarrheal and abdominal cramps, sometimes accompanied by nausea and headache. However, vomiting and fever are rare symptoms. While ETEC generally causes diarrhoea, some recent ETEC outbreaks have manifested themselves in chronic duration (Murray et al., 2009). Enterotoxigenic *E. coli* strains bind to the small intestine mucosa via adhesins called colonization factor antigens (CFA). The small intestine is not actually a region where *E. coli* is found in large numbers. These structures, historically called CFA or *E. coli* surface antigens, consist mainly of bacterial pilus. After such attachment to small intestinal mucosa cells, ETEC strains have two important toxins encoded by plasmids and they cause diarrhoea by releasing heat-labile toxin: LT and heat-stable toxin (ST). ETEC strains secrete at least one of two different toxin groups, thermolabile (LT) and thermostable (ST) (Nataro et al., 1987).

ETEC origins mostly secrete ST (35%), some secrete both ST and LT, and a lesser part only LT (Orskov, 1984; Puente and Finlay, 2001; Donnenberg et al., 2005; Forbes et al., 2007). Labile toxin is closely related to cholera toxin biologically and antigenically. These toxins consist of an A subunit with catalytic properties and a subunit of B (A-B toxins) that bind receptors in a pentamer structure. LT binds to the host cell with the B subunit, and the A subunit enters the enterocyte, activating the adenyl cyclase enzyme. The increase in intracellular cAMP (cyclic adenosine monophosphate) activates protein kinase A, which in turn enables a molecule called "cystic fibrosis transmembrane transition regulator (CFTR)" to be phosphorylated and activated. Thus, with the activation of a highly complex cascade, there is active secretion of chlorine, passive secretion of sodium and water into the intestinal lumen. Stable toxin is a completely different molecule, a small peptide and similar to a mammalian hormone called guaniline. ST also binds to GTP (Guanosine triphosphate), which is located in the enterocyte apical membrane, which is a guaniline receptor. As a result, intracellular GMP (Guanosine monophosphate) level increases and this time protein kinase G is activated, in conclusion CFTR phosphorylation, electrolyte and fluid secretion increases into the intestinal lumen (Orskov, 1984; Clarke, 2001; Puente and Finlay, 2001; Donnenberg et al., 2005; Forbes et al., 2007). Stable toxin, unlike labile toxin, causes diarrhoea in the intestine similar to labile toxin by affecting not adenylate cyclase, but guanylate cyclase (Töreci, 2002). Labile toxin is mostly synthesized in calf and bovine strains, while stable toxin synthesis differs according to species (Gülhan, 2009). Two different exotoxins produced by ETEC affect the intestinal mucosa, causing fluid loss and watery diarrhoea. Although two toxins have different molecular mechanisms, they both interfere with the passage of sodium and chloride ions across the cytoplasmic membrane. Thus, abundant watery diarrhoea is observed (Batzing, 2002; Gülhan, 2009; Altındiş, 2010).

Enterohemorrhagic, Vero or Shiga toxin-producing *Escherichia coli* (VTEC/STEC/EHEC)

Shiga toxin-producing *E. coli* was first discovered in 1977. It is one of the pathotypes of *E. coli* that cause disease in humans and animals. While the role of STEC has been determined only in endemic disease of pigs, its roles in disease formation in lambs, calves and dogs are not clear. The reason *E. coli* is named as STEC is that the cytotoxin it secretes is largely similar in genetics and protein structure to the Shiga toxin produced by *Shigella dysenteriae*. There are different other synonyms used for STEC. These are VTEC (Verotoxin producing *E. coli*) or EHEC (Enterohemorrhagic *E. coli*). Shiga / Vero toxin (Stx) is the main virulence factor and identifies STEC strains. This potent cytotoxin is the cause of many symptoms and deaths in STEC infections. There are two immunoreactive subgroups in Stx toxin subgroup. These are Stx1 and Stx2. A STEC strain can produce only Stx1 or only Stx2 or both toxins. STEC's Stx1 toxin is the same as *Sh. dysenteriae* 1 Shiga toxin (Olivier et al., 2013).

Enteropathogenic *Escherichia coli*

Enteropathogenic *E. coli* virulence factors are under the control of genes on chromosomes and plasmids. In order for EPEC to show all the features of its pathogenicity, it must have two genetic elements: first; EPEC carries plasmids that encode plasmids called adherence factor (EAF) plasmid type IV fimbria or bundle forming pilus (bfp). The adhesion and disruption effect, called as the "enterocyte effacement" (LEE) is governed by T3SS (Type 3 secretion system: an island of pathogenicity encoding the type 3 secretion system), the outer membrane adhesion structure, the translocated intimin receptor (Tir) and other proteins. The typical EPEC refers to both the EAF plasmid and the LEE pathogenicity island of the organism. These strains are attributed to enterocytes by showing local adherence. Atypical EPEC strains do not carry EAF plasmids, therefore they do not

have bfp and they are less pathogenic (Puente and Finlay, 2001; Donnenberg et al., 2005).

Enteroinvasive *Escherichia coli*

Enteroinvasive *E. coli* strains are usually dormant and invade colon cells, causing inflammation and often watery diarrhoea. Rarely, it can cause bloody diarrhoea with a pathogenic mechanism similar to that of *Shigella* sp. It carries a large invasion plasmid encoding T3SS. It invades the epithelium using T3SS, escapes from the phagosome and moves with actin flagella in the cytoplasm of the cell, finally reaches and enters the neighbouring cell. In the same way, it reaches the submucosa by being transported and apoptosis mechanism of the macrophages. The release of interleukin 1 from the macrophages initiates the inflammatory response. Leukocyte migration occurs, leukocytes come to the region by passing between cells, and they open a second path for bacteria to pass. After bacteria cross the epithelium in both ways, they easily infect epithelial cells (Salyers and Whitt 1994; Nataro and Kaper, 1998; Donnenberg et al., 2005; Forbes et al., 2007; Murray et al., 2009).

Enteroaggregative *Escherichia coli*

EAEC, which is typically defined by its specific aggregative adherence feature in Hep-2 cell cultures, is known to cause microvilli loss and cell death by adhering to the human colon mucosa by aggregation. EAEC have plasmid dependent piluses known as aggregative adherence fimbriae (AAF / I and AAF / II). Some EAEC strains are known to secrete cytotoxins. This toxin is called as enteroaggregative cytotoxin (EAST). This toxin is also encoded by the plasmid. This toxin has been shown to cause damage in cells, cell rupture in cell cultures, dilatation and cell destruction in crypts in the human intestinal model (Clarke, 2001; Campos, 2004; Donnenberg et al., 2005; Forbes et al., 2007; Murray et al., 2009). The pathogenicity of enteroaggregative *E. coli* has been confirmed by the diarrhoea outbreaks reported to be due to EAEC. While the relationship between paediatric diarrhoea and EAEC has not been shown in previous studies, this relationship has been shown with the development of molecular techniques that can distinguish the real pathogen with aggregative properties (Doğancı and Vidinlisan, 1988; Murray et al., 2009). Enteroaggregative *E. coli* is responsible for diarrhoea-related conditions such as endemic, epidemic diarrhoea in children in both poor and industrialized countries, travel diarrhoea in developing countries, and persistent diarrhoea observed among HIV-infected or AIDS patients (Murray et al., 2009).

Campylobacter jejuni

C. jejuni has the ability to produce cytotoxin and enterotoxin. *C. jejuni* toxin (CJT), which is an enterotoxin, has been defined as cholera-like toxin (CLT) because it resembles cholera toxin structurally and functionally and is inactivated by cholera antitoxin (Ruiz-Palacios, 1983). The interaction between *C. jejuni* and host intestinal epithelial cells occurs in four stages: In the first stage, the agent can adhere to the host cell by passing through the mucus layer with its mobility feature. Here, adhesin receptor interactions [(major OMP, lipo-oligosaccharide, capsular oligosaccharide, fibronectin binding protein (CadF), surface lipoprotein A (JlpA) and polyoma antigenic protein (PEB1)] play an important role. After that, the cytolethal distending toxin (CDT) with DNase enzyme, which is secreted by bacteria, inhibits the development of host cells. Although the release time of CDT is not known exactly, it is thought that it begins to be released from the intestines following ingestion by the host. It is observed that the cytotoxin produced by *C. jejuni* has different properties in terms of cell selectivity and is different from *Shigella*-like toxin, CDT and hemolysin. It has been found that this toxin is not related to cholera toxin genetically but causes watery diarrhoea similar to cholera toxin (Wassenaar, 1997). It has been reported that cdt ABC genes, commonly known as cytotoxins and produced by *E. coli*, are also encoded by *C. jejuni* (Pickett, 1996).

***Shigella* sp.**

Shigella strains secrete 3 different enterotoxins: *Shigella* enterotoxin 1 (shET1), *Shigella* enterotoxin 2 (shET2) and Shiga toxin (Stx). The shET1 encoded by the chromosome is secreted by all *Sh. flexneri* 2a strains. This toxin is rare in other Shigella species. shET2 is located on the large plasmid responsible for *Shigella* virulence and is encoded by many different *Shigella* serotypes (Fasano et al., 1995). Stx is a neurotoxic, cytotoxic and enterotoxic toxin and is encoded by genes located in the chromosome. Only *Sh. dysenteriae* serotype 1 produces this toxin. It consists of Stx A and B subunits. It binds with the Shiga toxin B subunit to a glycolipid receptor found in target cells. Subunit A inhibits protein synthesis by irreversibly binding to the 60S subunit of the ribosome in the host cell and causes cell death (Niyogi, 2005). Basically, as in *E. coli* (STEC) that produces Shiga toxin, except for bacterial strains causing oedema, Stx genes in *Sh. dysenteriae* are carried on chromosome. However, unlike STEC, *Sh. dysenteriae* serotype 1 does not carry intact Stx transforming phages. This is thought to be the result of the loss of essential phage genes caused by transposition and recombination events. Stx causes severe vascular lesions in the colon mucosa, renal glomeruli and other organs resulting in haemorrhagic colitis and in some cases haemolytic uremic syndrome (HUS) (McVey, 2013).

Staphylococcus aureus

S. aureus enterotoxins are heat-resistant, antigenic extracellular proteins, some of which have been held responsible for staphylococcal food poisoning for many years (Argudin et al., 2010). Five types of enterotoxins, which are also considered to be superantigens, were first defined as A, B, C (C1, C2, C3), D and E, and then F, G, H, I, J, K, L, M, N, O, P, Q, R and U types were added, and finally, S and T types were named. Among these, staphylococcal enterotoxins A and D are the most common in food poisoning (Ortega et al., 2010). Among the enterotoxin-producing staphylococci species, the most important species for foods is *S. aureus* (Roberts, 1990). Staphylococcal enterotoxins are a water-soluble heterogeneous group of simple proteins with molecular weights ranging from 28-35 kDa weight. Among the serologically separable toxin types (A, B, C1, C2, C3, D, E), staphylococcal enterotoxin A (SEA) and SED mostly in food poisoning, SEB in clinical isolates, SEC and SED types in mastitis milk (Halpin-Dahnalek et al., 1989). Since SEA is produced in the first period of the logarithmic reproduction phase of *S. aureus*, it can reach the level of intoxication even if the environmental conditions are not optimal. Therefore, SEA is the most common type of toxin in food poisoning. It has been reported that staphylococcal enterotoxins with thermostable character are not destroyed at pasteurization temperatures, and SEC is more resistant to high temperature than SEA and SEB. In a study, it was determined that SEA could not be detected serologically after 3 minutes at 80 °C and 1 minute at 100 °C (Bergdoll, 1989). Baird Parker (Baird-Parker, 1990) reported that staphylococcal enterotoxins are not degraded at 121 °C in 3-8 minutes and are resistant to drying, cooling and freezing processes.

Almost all strains of *S. aureus* produce a group of enzymes and cytokines. Among these secreted enzymes and cytokines are four haemolysins (alpha, beta, gamma, and delta), nucleases, proteases, lipases, hyaluronidase and collagenase. The main task of these proteins is to make the host tissues suitable for the growth of bacteria. Some other strains also have additional exoproteins such as toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEA, SEB, SEC, SED, SEE, SEH, and SEI), exfoliative toxin, and leukocidin. Among these, haemolysins and leukocidin, exfoliative toxin, TSST-1, staphylococcal enterotoxins are the toxins of *S. aureus*. Among these toxins, TSST-1 and staphylococcal enterotoxins are also known as pyrogenic toxin superantigens (PTSAGs) (Dinges et al., 2000).

Due to the stimulation of toxins local nerve receptors in the intestine, the impulses passing through the vagus nerve and sympathetic nerves reach the subcortical vomiting centre of the brain resulting in an emetic response (Sutherland and Varnam, 2002). The second most common symptom in staphylococcal food poisoning is diarrhoea. The direct effect of staphylococcal enterotoxins on intestinal cells is unclear and they are apart from classical enterotoxins such as cholera toxin or *E. coli* enterotoxins (Halpin-Dohnalek and Marth, 1989; Sutherland and Varnam, 2002).

The development phases with enterotoxigenic *Staphylococcus* sp. cells have an important effect on the formation of staphylococcal enterotoxin types. In relation to this, SEA is formed in the logarithmic phase of the cell, SEB, SEC and SED in the late logarithmic phase or early stationary phase (Bergdoll, 1989). In addition, secondary metabolites can also have an inhibitory effect on the bacteria. In this context, it has been determined that the *S. aureus* strains that constitute SEB are suppressed by the toxin during the development cycle (Halpin Dohnalek and Marth, 1989). The agr (accessory gene regulator) gene plays an important role in the regulation of virulence factors of *S. aureus*. Mutations in the agr locus result in reduced formation of many SE and other exoproteins. The gene can be transcriptionally and translationally regulated by the agr. It has been reported that agr regulates SEB and SEC at the transcriptional level, and the activation of agr and the formation of SEB and SEC and SED coincide with the bacterial growth cycle. Not all SEs are regulated by agr gene. For example, SEA is not affected by agr mutations (Tremaine et al., 1993). Agr is most highly activated at neutral pH values. It has been reported that genes targeted by agr are negatively regulated and exoprotein formation is reduced or not formed at all in glucose containing low pH media (Jablonski and Bohach, 1997). However, in environments where the bacterial density is low, agr cannot be activated, so that the formation of toxins and enzymes is prevented by the upregulation of adhesins. It has been reported that toxins and enzymes can be released by regulating agr up and adhesins down in cases where the bacterial density is high (Wisell, 2000).

Bacillus cereus

Food poisoning can be seen in individuals with taking large numbers of *B. cereus*. Especially when foods contaminated with *B. cereus* are not cooled sufficiently and quickly after cooking, or when the time between the preparation and consumption of food is prolonged, the microorganism can multiply and form a toxin that can cause food poisoning as a result of germination of live and heat-resistant spores. Food poisoning occurs when the number of bacteria in the food is higher than 10^6 cfu / g (Pichhardt, 2004).

B. cereus produces two different toxins. One of them is enterotoxin, a heat-resistant protein (Stable Toxin; ST) weighing 40 kDa. This toxin, which causes food poisoning, becomes inactive in 90 minutes at 126 °C. The other toxin is a peptide with a weight of 5–7 kDa, resistant to heat (Labil Toxin; LT). It is destroyed in a few minutes at 60oC. It is known that all strains of *Bacillus cereus* do not produce toxins and only certain serotypes produce toxins. It is not possible to say for sure that this bacterium causes disease through intoxication. It has also been shown that the bacterium known to develop in the intestine and form toxins creates toxins in anaerobic conditions *in vitro* (Tunail, 2000). The extracellular toxin that causes *B. cereus* food poisoning, can be detected after the number of bacteria in the environment reaches a certain level. *B. cereus* also secretes lecithinase C enzyme such as *C. perfringens*, but it has been reported that toxic activity is not involved in the same molecule. It is known that the toxin formation of *B. cereus* depends on the presence of some nutrients in the environment. Bacteria synthesize and release the toxin during the logarithmic phase. Studies have found that this bacterium synthesizes toxins between 18-44 °C and stops at 45 °C. The toxin of *B. cereus* is sensitive to pronase enzymes and becomes inactive with a dose of 0.01% of these enzymes at 37 °C in 60 minutes. The toxin does not lose its activity in 30 minutes at 45 °C, but becomes inactive at 56 °C (Pichhardt, 2004). *B. cereus* synthesizes two different types of enterotoxins, emetic and diarrheal enterotoxin, and causes two different types of poisoning. One of these is the "acute onset vomiting type syndrome" and is mostly associated with the toxin produced in cooked rice and rice foods. This toxin, called emetic toxin, is resistant to heat and low pH as well as trypsin and pepsin enzymes. The other type of disease is known as "prolonged diarrheal syndrome" and is associated with a wider food group. Among these foods; cereal-containing foods, especially corn and corn starch, mashed potatoes, vegetables, minced meat, pudding and soups. This toxin, known as diarrheal toxin, is in protein structure and is sensitive to heat with trypsin and pronase enzymes (Kaleli and *Özkaya*, 2000).

Bacillus anthracis

Anthrax has two important virulence factors. One is the antiphagocytic PGA capsule encoded by pX02 plasmid and the other is two exotoxins encoded by pX01 plasmid. The pX01 plasmid encodes three biologically inactive components: protective antigen (PA), oedema factor (EF) and lethal factor (LF). When PA and EF come together, it creates oedema toxin. Oedema toxin increases intracellular AMP level by showing adenylate cyclase activity. This causes fluid and electrolyte loss and impairs innate and acquired immune functions, including neutrophil chemotaxis. Together with PA, LF forms lethal toxin. This toxin is in the calmodulin-dependent zinc metalloprotease structure, inactivating protein kinases activated by mitogens and disrupting signal transduction (Baldari et al., 2006; Sherer et al., 2007).

Clostridium difficile

C. difficile strains, an anaerobic, sporulated Gram-positive bacterium, were first shown in 1977 to cause antibiotic-associated diarrhoea (Bartlett, 2008). According to classical knowledge, toxin A (enterotoxin) and toxin B (cytotoxin) encoded by the tcdA and tcdB genes located in the pathogenicity locus (PaLoc) of the *C. difficile* chromosome are responsible for the pathogenesis of the disease. Toxigenic strains of *C. difficile* often produce both toxins together. In recent years, differences in toxin A and toxin B production or variants in toxin genes (such as toxin A- / B +) have been identified. Also, in some countries, origins producing binary toxin different from toxins A and B,

causing hospital outbreaks have been reported (Rupnik, 2001). *C. difficile* origins that cause antibiotic-associated diarrhoea produce two different toxins called Toxin-A (Tox-A) and Toxin-B (Tox-B). These toxins, whose mechanisms of action are similar, enter the intestinal epithelial cell by endocytosis and cause cell death by affecting the actin skeleton in the cell. It has also been found that toxins lead to the secretion of certain cytokines, thereby the development of inflammatory response and the formation of pseudomembranes. In the past, it was thought that Tox-A caused damage to the intestinal epithelium, then Tox-B showed its effect, so Tox B could not be active alone, with the development of molecular techniques, it was understood that Tox-B without Tox-A had a cytotoxic effect (Poxton et al., 2001). In recent studies, strains producing only Tox-B have been identified, as well as some other variant strains have been reported. It has been found that some strains secrete large amounts of Tox-A and Tox-B, and there is a defect in the tcdC gene that negatively regulates toxin expression. It has been determined that the highly virulent 027 / NAP1 / BI origin, which causes hospital outbreaks in various countries, has a deletion in the tcdC gene and therefore secretes high levels of Tox-A and Tox-B. The 027 / NAP1 / BI origin has also been shown to produce a third toxin identified as binary toxin (B-Tox) (O'Connor et al., 2009).

Clostridium botulinum

C. botulinum exotoxin, commonly used as botulinum toxin (BTX), was shown by Van Ermengem in 1897 to be a spore-forming Gram-positive, anaerobic neurotoxin produced by *C. botulinum* bacteria (Van Ermengem, 1979) For the release of acetylcholine (Ach) BTX, denervation in muscles by binding necessary proteins, is the most effective biological toxin known. After BTX is synthesized as a single chain polypeptide, it takes the form of double chain with the help of endogenous bacterial proteases. These two chains, which are separated as "heavy" weighing 100 kDa and "light" weighing 50 kDa, are held together by disulphide bond (Simpson, 1981). There are 7 different serotypes of BTX (A, B, C, D, E, F, G). Of these, A is the strongest and the first form that entered medical use. Serotypes A, B, E are responsible for food poisoning in humans (Dertzbaugh, 1996).

Botulinum toxin is injected into the submandibular and parotid glands for hypersalivation treatment, and is also used in postoperative salivary fistulas (Rohrbach and Laskawi, 2003). In 2001, another serotype of neurotoxin, botulinum toxin type B (Btx B) Myobloc, was licensed in America and European countries for its use for cervical dystonia. Acetylcholine needs many transport proteins for release from nerve endings to the synaptic space. BTX-A irreversibly cuts the SNAP-25 protein, while BTX-B irreversibly cuts the VAMP protein (Moore and Naumann, 2003).

C. botulinum produces several proteins with "toxic" activity (botulinum toxin, C2 toxin and C3 exoenzyme), but only botulinum toxin plays a central role in the production of botulism. Although new "mosaic" toxins have been recently identified, there are seven types of botulinum toxin (BoNT for botulinum neuroxin) that differ by antigenic differences. Letters from A to G indicate the types. The type of neurotoxin characterizes the *C. botulinum* species that produces it. Therefore, the *C. botulinum* strain producing a type A BoNT will be identified as *C. botulinum* A type. All seven BoNT types are zinc endopeptidases with the same activity, i.e. the hydrolysis of the insertion proteins required for the fusion of neurotransmitter-containing vesicles. Although the result is the same with the presynaptic membrane (inhibition of neurotransmitter release), various BoNT types hydrolyze different docking proteins. Types A and E hydrolyse SNAP (synaptosomal associated protein); Types B, D, F and G hydrolyse VAMP (vesicle-associated membrane protein, also known as synaptobrevin) and type C hydrolyses SNAP and syntaxin. BoNT is a "di-chain" molecule (binding to nerve cells) consisting of a light chain (with zinc endopeptidase activity), a heavy chain consisting of a translocation domain (responsible for a pore through which the light chain passes), and a binding domain. Several "accessory" proteins thought to help the toxin survival in the gastrointestinal tract are secreted by BoNT. BoNT binds to cholinergic nerve cells of BoNT, each of which binds to a different receptor. Once bound, the toxin is internalized through receptor mediated endocytosis. BoNT-containing vesicles remain at the neuromuscular junction. After a cleavage event, the light chain (zinc endopeptidase) passes to the cytosol of the nerve cell where it hydrolyses docking proteins across the vesicle membrane. Both C2 toxin and C3 exoenzyme are ADP ribosyl transferases, C2 toxin and C3 exoenzyme ribosylate G-actin and Rho, respectively, and cause cytoskeleton degradation. Both enzymes do not appear to play a role in the disease process (McVey et al., 2013).

Conclusion

Symptoms and disorders about the digestive system such as nausea, vomiting, severe abdominal pain, weakness, muscle pain, are caused by eating foods contaminated with microorganisms and toxins produced by microorganisms. The most common food poisoning is caused by *Staphylococcus* sp. The bacterium is not the main disease factor itself, but the toxin of the bacteria causes disease. Exotoxins, one of the most important virulence factors of bacteria, cause damage in the body by several different mechanisms. Some exotoxins are produced by bacteria that contaminate feed and are taken into the body through digestion. This is a form of food poisoning. Food poisoning can occur in the same way in humans. Botulism disease, which occurs with feeds that produce toxins in animals, and ingestion of such foods in humans, is the best example for this disease. Botulinum toxin produced by *C. botulinum* is a very powerful toxin, even a small number of bacteria is enough for disease formation. Some exotoxins are synthesized by bacteria that grow in body wounds or abscesses and spread from these damaged tissues to the entire organism. Bacteria can also produce exotoxins while they are on mucosal surfaces in the body. For example, *E. coli* causes diarrhoea by producing enterotoxins in the intestine. Exotoxin-producing bacteria can cause systemic shock and death in animals by indirectly stimulating excessive cytokine production as in Anthrax disease.

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