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IN THIS ISSUE

163 Clinically Important Toxins in Bacterial Infection: Utility of Laboratory Detection

Clinically Important Toxins in Bacterial Infection: Utility of Laboratory Detection

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Abstract

The elaboration of proteins that damage host cells is fundamental to the pathogenesis of many bacterial pathogens. The clinical significance of many bacterial toxins is well recognized, and routine detection is necessary to confirm definitive diagnosis for some types of infectious diseases. Determining the clinical significance of a toxin involves many factors, including the toxin's prevalence, virulence, and role in disease pathogenesis. While essential from a diagnostic perspective, toxin detection has the potential to be important for patient management decision making, as well as infection prevention and control measures. This review focuses on the history, epidemiology, pathogenesis, clinical presentation, and management of infections associated with well-defined, clinically important toxins (such as Shiga toxin-producing *Escherichia coli*), as well as those that are less well defined (such as *Staphylococcus aureus*' Panton-Valentine leukocidin) where detection may yield clinically important information.

Introduction

In infectious diseases and clinical microbiology, the term "toxin" typically refers to molecules that are produced by microorganisms that may affect cells in the infected host. Knowledge surrounding toxin production and release, interaction with and entry into host target cells, mechanisms of action and of relevance, and clinical significance historically has been limited. With increasing utilization of genomics and proteomics techniques, however, there is a greater understanding of microbial pathogenesis and their role in clinical disease.

There is a spectrum of importance in detecting bacterial toxins for diagnostic purposes. Definitive diagnosis of some diseases requires toxin detection. Alternatively, for some diseases, the identification of an organism's toxin may help guide decision making, and for others, detection of a toxin provides little benefit. Toxins from many clinically relevant bacteria exist that clinical microbiology laboratories do not routinely screen for, either because their detection would not yield actionable information or because their importance is not well understood; detection of these toxins in microbiology laboratories may be important for diagnostic purposes, patient management, infection prevention and control, or public health. This article provides an overview of clinically important bacterial toxins, highlighting their history, disease epidemiology, pathogenesis, and clinical presentation and the management of toxin-associated infections, and finally, it discusses the usefulness of toxin detection. A summary of key toxins is provided in Table 1.

Toxins Produced by Frequently Isolated Bacteria

Many bacteria produce toxins, and in some cases, more than one toxin is produced by a given organism. *Staphylococcus aureus* strains, for example, are capable of secreting hemolysins, leukotoxins, exfoliative toxins, enterotoxins, and toxic shock syndrome toxin 1 (TSST-1). *Clostridium*

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difficile is an example of an organism for which two toxins (TcdA and TcdB) or their genes are regularly detected. Determining whether there is value in routine toxin detection considers prevalence, associated genotypes, virulence potential, and disease pathogenesis, as well as downstream implications. Here, we discuss selected toxins produced by bacteria commonly isolated in the clinical microbiology laboratory.

Panton-Valentine leukocidin

S. aureus is a major human pathogen responsible for significant morbidity and mortality worldwide. The virulence and pathogenicity of *S. aureus* is largely attributed to an impressive arsenal of cell surface proteins and secreted virulence determinants. For example, *S. aureus* is capable of producing pore-forming cytotoxins. This activity allows bacteria to survive inside host cells and

Table 1. Summary information for select bacterial toxins

Organism	Toxin name	Toxin type	Clinical significance	Routinely detected for definitive diagnosis
Bacillus anthracis	Edema toxin (ET) plus protective antigen (PA)	Adenylate cyclase	Edema and skin necrosis	Yes
	Lethal toxin (LT) plus PA	Metalloprotease		Yes
Bacteroides fragilis	*Bacteroides fragilis* enterotoxin	Metalloprotease	Unknown; implicated in diarrhea	No
Bordetella pertussis	Pertussis toxin (PT)	ADP-ribosylation	Tracheobronchitis	Yes
	Adenylate cyclase toxin (ACT)	Adenylate cyclase		No
Clostridium botulinum	Botulinum neurotoxin (BoNT)	Metalloprotease	Muscle paralysis, botulism	Yes
Clostridium difficile	Toxins A and B	Glucosylating toxins	Diarrhea	Yes
Clostridium perfringens	Perfringens enterotoxin	Adenylate cyclase	Diarrhea	No
	Perfringiolysin O	Pore-forming toxin	Unknown; may be involved in gas gangrene	No
Clostridium tetani	Tetanus toxin (TeNT)	Metalloprotease	Muscle spasms and rigidity	No
Corynebacterium diphtheriae group	Diphtheria toxin (DT)	ADP-ribosylation	Respiratory infection; cutaneous ulcers	Yes
Escherichia coli	Heat-labile toxin (LT)	ADP-ribosylation	Diarrhea	No
	Cytotoxic necrotizing factors (CNF1/CNF2)	Deamidating toxins	Not specific	No
	Shiga-like toxin	RNA glycosidase	Diarrhea (often bloody); hemolytic uremic syndrome	Yes
Listeria monocytogenes	Listeriolysin	Pore-forming toxin	Systemic infection, neonatal, meningitis	No
Pseudomonas aeruginosa	ExoY	Adenylate cyclase	Unknown	No
Shigella dysenteriae	Shiga-toxin	RNA glycosidase	Dysentery	Yes
Staphylococcus aureus	Panton-Valentine leukocidin	Pore-forming toxin	Necrotizing pneumonia; skin and soft tissue infections	No
	Exfoliatin toxin (ETA)	T-cell activator	Scalded skin syndrome; bullous impetigo	No
	Staphylococcal enterotoxins	T-cell activator	Diarrhea (watery)	No
	Toxic shock syndrome toxin (TSST-1)	T-cell activator	Inflammation, fever, shock	No
Streptococcus pneumoniae	Pneumolysin	Pore-forming toxin	Pneumonia, meningitis, sinusitis, otitis media, others	No
Streptococcus pyogenes	Streptococcal pyrogenic exotoxins (Spe)	T-cell activator	Localized erythematous reactions (i.e., scarlet fever) or systemic (inflammation, fever, shock)	No
Vibrio cholerae	Cholera toxin (CT)	ADP-ribosylation	Diarrhea (watery)	Yes

therefore prevents detection by the host innate immune response and ultimately causes host cell lysis by forming pores in the cytoplasmic membrane. Panton-Valentine leukocidin (PVL) is perhaps the best-recognized cytotoxin produced by *S. aureus*.

PVL was first described in 1894 as a toxin of *S. aureus* capable of leukocyte destruction. PVL was subsequently differentiated from other staphylococcal toxins (leukocidins, hemolysins, necrotoxins, and lethal toxin) in 1932, and further, correlations between PVL and severe skin and soft tissue infections (SSTIs) were first identified.

PVL is a two-component toxin encoded by two contiguous and co-transcribed genes that encode two separately secreted proteins: LukS-PV and LukF-PV. The proteins assemble into a pore-forming heptamer on leukocyte (i.e., neutrophil, monocyte, and macrophage) membranes, leading to efflux of the cell content and ultimately cell lysis. Interestingly, PVL exhibits concentration-dependent activities. For example, *in vitro* studies have shown that sublytic PVL concentrations lead to activation of internal apoptosis pathways whereas higher concentrations induce polymorphonuclear leukocyte necrosis [1]. Thus, cell lysis *in vitro* has been shown to manifest within a range of 1 to 6 hours, depending on the concentration of PVL [2]. *In vivo*, however, it is assumed that PVL in sublytic concentrations is capable of strengthening the host innate immune response by promoting the production of pro-inflammatory cytokines [3].

The toxin has been reported worldwide and, in addition, has been observed in diverse patient populations, including pediatric patients and adults. PVL-producing strains with particular disease phenotypes, however, exhibit a predilection for young, immunocompetent patients, with high case fatality rates [4]. The prevalence of PVL^+ *S. aureus* strains is relatively low (<5%). In addition, there is a strong epidemiological link between PVL and community-acquired methicillin-resistant *S. aureus* (CA-MRSA), including specific strains, such as USA300; less frequently, PVL^+ hospital-acquired MRSA and methicillin-susceptible *S. aureus* have been reported. Clinically, PVL^+ *S. aureus* manifests most frequently as SSTIs (abscesses, furuncles, and carbuncles) and necrotizing pneumonia [5]. The latter, interestingly, has occurred following influenza virus respiratory infections and, more recently, post-COVID-19 [6]. Septic arthritis, bacteremia, and other invasive infections are uncommon but have also been reported [7].

Despite increased understanding in recent years, the role of PVL in *S. aureus* virulence remains controversial. It has been suggested that the presence of PVL is not predominantly important to the severity of disease or clinical outcomes and instead may be influenced by factors that up-regulate toxin synthesis *in vivo*. For example, a multicenter observational study evaluated 109 patients with MRSA health care-associated pneumonia or ventilator-associated pneumonia [8]; their APACHE II (acute physiology and chronic health evaluation, a disease severity classification) scores at the time of diagnosis were 21 ± 8 and 20 ± 6 for PVL^+ and PVL^- MRSA, respectively, and mortality rates were similar at 10%. Therapeutic strategies therefore may not be useful in improving patient outcomes. Given the association with CA-MRSA, the toxin may increase a strain's virulence and, importantly, enhance transmission.

From a patient management perspective, it has been shown that infections with PVL^+ *S. aureus* strains were more susceptible to treatment than PVL^- *S. aureus* [9]. Vancomycin, clindamycin, linezolid, trimethoprim-sulfamethoxazole, and rifampicin have been used in various combinations and doses for treatment with variable success [10]. Current guidelines recommend clindamycin for the treatment of such toxin-mediated infections. Accordingly, Hodille et al. [11] showed that sub-MICs of clindamycin reduce expression of PVL, TSST-1, and alpha-hemolysin (Hla) among susceptible and inducible clindamycin-resistant *S. aureus* strains. In the context of active immunization, it was recently shown that rabbits vaccinated with Hla toxoid alone or PVL components alone were only partially protected against lethal pneumonia [12].

The potential for the development of resistance to commonly used antimicrobials in cases of severe SSTIs and necrotizing pneumonia caused by PVL^+ *S. aureus* isolates is an area of concern. Combined with increased transmissibility, strains producing PVL may present a public health risk. In this regard, several outbreaks have been reported worldwide [13,14]. While PVL detection in clinical microbiology laboratories is not routinely performed, there is some evidence to suggest that the information may be useful to clinicians in determining patient management. However, it could also be suggested that the combination of PVL's correlation with CA-MRSA (i.e., rapid MRSA testing) and clinical suspicion may be sufficient. Although not routinely used in clinical practice, lateral-flow assays [15], matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) [16] and reverse transcription (RT)-PCR assays [17], all of which exhibit reasonably high analytical sensitivity and specificity, have all been used for rapid detection of PVL^+ *S. aureus*.

Streptococcal pyrogenic exotoxins

Streptococcus pyogenes (group A *Streptococcus* [GAS]) produces numerous cell surface and secreted virulence factors. Included among them, are the streptococcal super antigen exotoxins which broadly function by activating T cells and, importantly, are recognized as one of the most potent T cell activators. Streptococcal superantigens were first identified in *S. pyogenes* in 1924. Interestingly, while the term "super antigen" was first used to describe the host T cell response [18], these superantigens are also widely known as erythrogenic toxins, since they play a role in causing a rash in the context of scarlet fever. The designation "streptococcal pyrogenic exotoxins" (Spe) was proposed in 1960. At least 14 genetically distinct superantigens have been reported in *S. pyogenes*; however, a given strain generally contains distinct genes for 3 to 6 of them. It is important to acknowledge that not all *S. pyogenes* strains harbor and/or release exotoxins with super antigen activity. Further, many superantigens are encoded within lysogenic bacteriophage elements [19].

Streptococcal superantigens contribute to disease pathogenesis by avoiding antigen presentation processes. For example, T cell

stimulation is conventionally mediated by major histocompatibility complex class II (MHC-II) antigen processing and presentation to T cell receptors (TCR) to ultimately trigger cytokine production. Superantigens, however, allow less specific binding to MHC-II and TCR, thereby stimulating a higher number of T cells and a significant amount of cytokine production. The cytokine release is assumed to be responsible for clinical manifestations of streptococcal toxic shock syndrome (STSS). While it is widely recognized that STSS predominantly involves streptococcal superantigens, interplay among other enzymes and toxins, as well as the host response, is similarly important.

Clinically, *S. pyogenes* infections are frequent and wide ranging; pharyngitis, bacteremia, pneumonia, meningitis, and soft tissue infections are a few examples. STSS represents a severe complication of (predominantly) invasive GAS infections. While invasive GAS infections have been widely recognized for some time, it was not until the 1990s that shock and multiorgan failure were found to be associated with *S. pyogenes* infections. At present, infants and the elderly are at the highest risk for development of an invasive infection [20]. Other risk factors include pre-existing skin lesions, alcohol abuse, chronic lung disease, and immunosuppression; however, as many as a third of cases occur in persons with no risk factors. According to the CDC case definition, there are many clinical criteria that must be met in order to establish a diagnosis of STSS; microbiologically, it is required that *S. pyogenes* be isolated from a sterile site; these strains generally harbor specific M proteins, as well as secreting SpeA, SpeB, or both.

STSS occurs globally and has been observed in all age groups. The majority of infections occur sporadically, though outbreaks in several settings have occurred. STSS is a relatively rare disease with a mortality of roughly 30%, occurring in up to approximately 15% of patients who present with invasive GAS infection [21]. It is also worth acknowledging that global epidemiological studies evaluating invasive GAS have not been performed in recent years.

Overall, management of STSS requires a multidisciplinary team. *S. pyogenes* is universally susceptible to beta-lactam antibiotics *in vitro*, though additional antimicrobials, such as clindamycin, are often used in combination therapy. The rationale for clindamycin use in this setting is that it decreases super antigen production in animal models [22]. Penicillin and clindamycin have been shown to differentially inhibit *in vitro* SpeA and SpeB production, with greater inhibition occurring with clindamycin use [23].

Given the widespread occurrence of streptococcal superantigens among invasive *S. pyogenes* strains, direct detection methods are likely not needed outside of public health and epidemiological purposes. There are reports of amplifying *speB* to detect *S. pyogenes* via PCR [24] and case reports utilizing 16S rRNA gene targeted amplicon sequencing capable of detecting *speA* and *speB* [25], however, they are not routinely performed. Alternatively, as some *emm* types are exclusively isolated from invasive GAS, identification of the M type (also known as *emm* typing) may be utilized to identify invasive strains [26].

Shiga toxin

Shigella dysenteriae was first described in 1898, and while early research may have alluded to endotoxic activity associated with the organism, it was the cumulative work of many decades that led to the definitive identification of a distinct endotoxic protein, Shiga toxin. Shiga toxins are named after the discoverer of *S. dysenteriae*, Kiyoshi Shiga. In 1977, the Shiga toxin was identified in *Escherichia coli* and subsequently renamed verotoxin due to its ability to kill Vero cells in culture; the toxin was thereafter renamed again to Shiga-like toxin 1 (Stx1) and Stx2, as they differ by at most one amino acid from Shiga toxin [27].

The pathogenicity of Shiga toxin-producing *E. coli* (STEC) is largely mediated by Shiga toxin genes that are located on the pathogenicity island of the locus of enterocyte effacement (LEE). The LEE (which encodes many virulence factors) mediates bacterial attachment via mechanisms described previously [28], which is followed by Shiga toxin production. Specifically, the STEC group is characterized by the presence of *stx1* and/or *stx2*; there are 10 *stx* subtypes, some of which are preferentially associated with more severe disease. The Shiga toxin, which is made up of one subunit A and five subunit B moieties, is internalized in the host cell and transported to the Golgi apparatus and endoplasmic reticulum and ultimately inhibits protein synthesis, causing cell death [29].

There are many STEC serogroups that are capable of causing human disease, with the O157 serogroup the most common. Transmission occurs via consumption of contaminated foods, the fecal-oral route, or cross-contamination, with an incubation period ranging from 3.5 to 8.1 days [30]. STEC causes approximately 3 million cases and 200 deaths annually [31]. Most cases of STEC O157 infection are sporadic; however, large outbreaks have occurred. Historically, fewer cases of non-O157 STEC infection have been reported, though with changing diagnostic practices (discussed below), cases (and outbreaks) are now more commonly identified. STEC strains have the potential to cause severe human disease. Commonly reported symptoms, which generally last between 5 and 7 days, include diarrhea (profuse and/or bloody), abdominal pain, vomiting, fever, and fatigue. Approximately 5 to 10% of patients develop severe complications, such as hemolytic uremic syndrome (HUS) [32].

STEC testing has historically focused exclusively on *E. coli* O157:H7 using culture-dependent methods. In particular, sorbitol-MacConkey agar (or other differentiating/selective agar) is often used to screen (stool) specimens for the O157 serogroup's distinct phenotype as sorbitol non-fermenters. While culture is valuable from a public health perspective, it creates a significant diagnostic issue, since other STEC serogroups are able to ferment sorbitol and would thus be overlooked if culture were the only diagnostic method employed. Therefore, appropriate clinical management and public health measures are heavily dependent on detection of *stx1* and *stx2*. Several assays to detect STEC—regardless of the serogroup—are commercially available. Broadly, they include enzyme immunoassays (EIAs), which detect Stx1 and Stx2 antigens, and PCR, which detects *stx1* and *stx2*. A recent meta-analysis of 43 articles and over 25,000 specimens

evaluated the performance characteristics of EIA and PCR [33]. The STEC EIA pooled sensitivity and specificity were 0.68 and 1.00, respectively, whereas PCR demonstrated improved sensitivity (1.00) and similar specificity (0.99). Another, alternative test to detect STEC includes the use of chromogenic selective agar (CHROMagar STEC), which is also capable of detecting non-O157 serogroups [34].

The Infectious Diseases Society of America (IDSA) guidelines recommend against the use of antibiotics in Stx2-producing STEC infections due to the association with HUS complications, underscoring the importance of toxin detection [35]. Moreover, the guidelines also indicate that there is a lack of evidence for similar recommendations in Stx1-producing STEC. A recent review evaluated the link between antibiotics and HUS [36]. The authors noted that several studies reported an increased risk of HUS with the administration of antibiotics, whereas other studies reported no or reduced risk of HUS development. Moreover, the particular STEC strain, timing, and type of antibiotics were found to be important. Interestingly, beta-lactams and trimethoprim-sulfamethoxazole were found to be detrimental, whereas other antibiotics, such as fosfomycin and fluoroquinolones, have shown positive effects.

Toxin Detection in Infrequently Isolated Bacteria

The toxins discussed below have been well studied and are essential components of clinical disease, without which the organisms are attenuated or rendered unable to cause disease. Many of the bacteria are infrequently isolated pathogens; thus, toxin detection is typically performed at reference laboratories. However, identification of the toxins remains important, as definitive diagnosis is often dependent on toxin detection through toxigenicity assays.

Diphtheria toxin and pertussis toxin

The causative organisms of diphtheria and pertussis are *Corynebacterium diphtheriae* and *Bordetella pertussis*, which express toxins (diphtheria toxin [DT] and pertussis toxin [PT], respectively). They are ADP-ribosylating toxins that contribute to the pathogenesis of clinical disease. Globally, there are regions of endemicity for both diphtheria and pertussis, and factors such as age and vaccination status influence prevalence. Currently, in industrialized countries with high vaccination coverage, the incidence of these infections is relatively low, though it appears to be increasing [37, 38]. In the case of diphtheria, DT is responsible for the clinical manifestations of disease, though several additional toxins, including adenylate cyclase and tracheal cytotoxin, are also involved in diphtheria disease.

Interestingly, until the 1980s, it was assumed that only *C. diphtheriae* possessed the gene (*tox*) encoding DT. However, *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* (which encompass the *C. diphtheriae* group) have now been shown to express DT, as well. In addition, non-toxigenic *tox*-bearing *C. diphtheriae* and *C. ulcerans* strains have been reported [39]. While not common in frontline clinical microbiology laboratories, toxigenic assays are routinely performed at the reference laboratory level. They include immunodiffusion (e.g., the Elek test), EIAs, agglutination assays, and, importantly, PCR to detect the *tox* gene [40]. RT-PCR assays that target *rpoB* and *tox* allow rapid species level differentiation, as well as identification of toxigenic versus non-toxigenic strains [41]. Further confirmatory tests, such as the Elek test, are also needed to confirm *tox* expression.

Assays to detect the insertion sequence IS*481*, found in several *Bordetella* spp., and the gene encoding the PT promoter (*ptxP*), which is specific to *B. pertussis*, have been described [42]. PCR has superior sensitivity over culture for the identification of *Bordetella* spp. [43] and has replaced culture in many laboratories. Similar to *Corynebacterium* spp., though rare, *B. pertussis* strains lacking PT have been identified [44].

Anthrax toxins

Bacillus anthracis, the causative agent of anthrax, was the first bacterial pathogen to be discovered. Early studies of *B. anthracis* helped to support Koch's postulates, and additionally, the first anti-bacterial vaccine was created by Pasteur using attenuated *B. anthracis* strains and challenging sheep with virulent strains [45]. There are three (major) forms of disease that are recognized—cutaneous, pulmonary, and gastrointestinal—where *B. anthracis* endospores gain entry to the host via distinct mechanisms. Other, less common forms of disease include injection related infection, which is primarily associated with intravenous drug use, and meningeal, which is a relatively common sequela of inhalational and gastrointestinal anthrax. Spores germinate and rapidly divide while encoding toxins, enabling the organism to evade the host immune response.

Toxin and capsule production require the presence of two plasmids, pXO1 and pXO2, the latter encoding protective antigen (PA), lethal factor (LF), and edema factor (EF). *B. anthracis* virulence is dependent upon these three toxin components (i.e., tripartite), which assemble into two toxins (PA and EF forming edema toxin and PA and LF forming lethal toxin). Essentially, PA binds to the anthrax toxin receptors TEM8 (tumor endothelial marker 8) and CMG2 (capillary morphogenesis protein 2), which facilitates translocation of LF and EF to the host cell cytosol, where they mediate cellular damage via different mechanisms [46]. LF is a calmodulin-dependent zinc metalloprotease that interferes with signal transduction processes by the cleavage and inactivation of mitogen-activated protein kinases. EF is an adenylate cyclase that interferes with cell wall function (i.e., promoting fluid accumulation and edema) by increasing cAMP concentrations.

Confirmation of *B. anthracis* is often performed via PCR, which detects pXO1 and pXO2 [47], though this poses problems, since the plasmids can be lost or transferred to other species. Accordingly, reference-based testing (i.e., the Laboratory Response Network and Canada's National Microbiology Laboratory) utilize PCR approaches that target both plasmids, in addition to a *B. anthracis*-specific chromosomal target. Additional methods, such as MALDI-TOF MS targeting LF, have also been described but have not been adopted for use in clinical laboratories [48].

Tetanus toxin and botulinum toxin

Clostridial neurotoxins produced by *Clostridium tetani* (TeNT) and *Clostridium botulinum* (BoNT) are among the most potent toxins known; the 50% lethal toxin dose of BoNT is 0.001 g/kg body weight. These toxins are metalloproteases that target the SNARE (soluble *N*-ethylmaleimide-sensitive fusion protein attachment receptor) complex [49], a complex that is essential for synaptic transmission. Several serotypes of these neurotoxins exist, resulting in slightly distinct clinical presentations. While the diagnosis of clostridial botulism is largely clinical and based on patient history and presenting symptoms, microbiology techniques are required for a laboratory-confirmed diagnosis. A definitive diagnosis is dependent on detection of the toxin rather than isolation of the organism. Accordingly, methods employed for toxin detection include mouse neutralization assays (which are superior), EIAs, and PCR [50]. BoNT can be detected from a variety of biological specimen types. They include serum, feces, vomitus or gastric contents, wounds, and autopsy specimens, as well as food samples. It is recommended that fluid and wound specimens be shipped (to reference laboratories) in an anaerobic transport system, and the latter in the absence of a refrigerant. All other specimens submitted for BoNT testing should be shipped with a refrigerant. Similarly, toxigenic assays are required for confirmation of toxigenic *C. tetani*.

Cholera toxin

Vibrio cholerae, particularly serogroups O1 and O139, is responsible for causing diarrheal disease. In countries where cholera is endemic, the incidence of disease follows a seasonal distribution, while the occurrence of *V. cholerae* in regions of non-endemicity tends to result in rapid spread of disease. Importantly, there are two major virulence factors of *V. cholerae* related to toxin production that have been recognized for over 60 years. They are cholera toxin (CT) and the toxin-co-regulated pilus. CT is encoded by the *ctxAB* operon, which corresponds to the genome of CTXΦ (integrated into the *V. cholerae* chromosome) [51]. CT in particular functions by activating adenylyl cyclase, thereby increasing cAMP, and subsequently leads to several metabolic changes, such as ion channel activation and intestinal lumen electrolyte imbalances (secretory diarrhea) [52]. *V. cholerae* can be identified by isolation of the organism from clinical (primarily stool) and environmental specimens, and this method still represents the gold standard. Accordingly, selective media, such as thiosulfate citrate bile salt or chromID *Vibrio*, have proven to be useful in identification. Ramamurthy et al. [53] recently described various methods for the detection of *V. cholerae*, including PCR, enzyme-linked immunosorbent assays, fluorescence assays, and coagulation tests. Reference-based laboratories in Canada and the United States largely utilize PCR to detect *ctx*, and in some cases *vhlyA* and *rtx*, which are accessory toxins.

Other toxins

Many additional toxins not described above exist and play pivotal functions in disease but are outside the scope of this review. Some examples are ExoY (*Pseudomonas aeruginosa*), cytotoxic necrotizing factors (*E. coli*), *Bacillus fragilis* enterotoxin, and pneumolysin (*Streptococcus pneumoniae*). For many toxins, molecular detection methods, such as PCR, have been now described. However in many cases, there is little clinical benefit to knowing whether a strain is toxigenic. For some organisms though, toxin genes represent targets for molecular assays that can are important for establishing a definitive diagnosis.

Emerging Bacterial Toxins

Advances in sequencing and bioinformatics abilities provide the capacity to detect emerging organisms and identify novel toxins. This is particularly true of whole-genome sequencing, and also of metagenomics. As an example, it was recently shown that botulinum-like toxins were found in *Enterococcus faecium*, *Chryseobacterium piperi*, and *Weissella oryzae* [54]. The use of metagenomics is exceptionally promising, as it provides an avenue for direct sequencing from specimens, with the potential to identify any and all toxins present. The limitation, however, is the inability to determine toxin expression, and therefore, confirmatory toxigenicity testing would be required if clinical metagenomics were applied.

Summary

Toxins produced by bacteria are integral to infectious-disease processes, as they are predominantly responsible for clinical manifestations. Whether this role is significant enough from a diagnostic standpoint to warrant toxin detection is debatable. Routine toxin detection occurs in both frontline clinical microbiology and reference laboratories and depends on the pathogen involved, the assay performed, and also the patient population (and corresponding prevalence of disease).

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