Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
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 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.

**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*
**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.

---

**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      |                                                            |                                             |
| *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    |                                                            |                                             |
| *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                                          |
|                           | Perfringolysin 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                                         |

---

**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...
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 $21 \pm 8$ and $20 \pm 6$ 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, Hodil 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
strectococal 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 toxoid and pertussis toxoid

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 IS481, 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 meningitis, 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), ELAs, 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 adenyl 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 detectCtx, and in some cases *vibA* 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 be 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 *Weissellaoryzae* [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).

**References**

[1] Graves SF, Kobayashi SD, Braughton KR, Whitney AR, Sturdevant DE, Rasmussen DL, et al. Sublytic concentrations of *Staphylococcus aureus* Panton-Valentine leukocidin alter human PMN gene expression and enhance bactericidal capacity. J Leukoc Biol 2012;92:661-74.

[2] Genestier A-L, Michallet M-C, Prévost G, Bellot G, Chalabreysse L, Peyrol S, et al. *Staphylococcus aureus* Panton-Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. J Clin Invest 2005;115:3117-27.

[3] Rasigade J-P, Laurent F, Lina G, Meugnier H, Bes M, Vandenesch F, et al. Global distribution and evolution of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus*, 1981-2007. J Infect Dis 2010;201:1589-97.

[4] Gillet Y, Dumitrescu O, Tristan A, Dauwalder O, Javouhey E, Floret D, et al. Pragmatic management of Panton-Valentine leukocidin-associated staphylococcal diseases. Int J Antimicrob Agents 2011;38:457-64.

[5] Hoppe P-A, Holzhauer S, Lala B, Bührer C, Gratoft H, Manisch LG, et al. Severe infections of Panton-Valentine leukocidin-positive *Staphylococcus aureus* in children. Medicine (Baltimore) 2019;98:e17185.

[6] Duployez C, Le Guern R, Tinez C, Lejeune A, Robinquet L, Six S, et al. Panton-Valentine leukocidin-secreting *Staphylococcus aureus* pneumonia complicating COVID-19. Emerg Infect Dis 2020;26:1939-41.

[7] Shallercross LJ, Fragaszy E, Johnson AM, Hayward AC. The role of the Panton-Valentine leukocidin toxin in staphylococcal disease: a systematic review and meta-analysis. Lancet Infect Dis 2013;13:43-54.
[8] Peyrani P, Allen M, Wiemken TL, Haque NZ, Zervos MJ, Ford KD, et al. Severity of disease and clinical outcomes in patients with hospital-acquired pneumonia due to methicillin-resistant Staphylococcus aureus strains not influenced by the presence of the Panton-Valentine leukocidin gene. Clin Infect Dis 2011;53:766-71.

[9] Bae I-G, Tinhath GT, Styrewsky ME, Rudy TI, Reilly LF, Barriere SL, et al. Presence of genes encoding the Panton-Valentine leukocidin exotoxin is not the primary determinant of outcome in patients with complicated skin and skin structure infections due to methicillin-resistant Staphylococcus aureus: results of a multinational trial. J Clin Microbiol 2009;47:3952-7.

[10] Morgan MS. Diagnosis and treatment of Panton-Valentine leukocidin (PVL)-associated staphylococcal pneumonia. Int J Antimicrob Agents 2007;30:289-96.

[11] Hodille E, Badiou C, Bouveyron C, Bes M, Tristan A, Vandenesch F, et al. Clindamycin suppresses virulence expression in inducible clindamycin-resistant Staphylococcus aureus strains. Ann Clin Microbiol Antimicrob 2018;17:1-6.

[12] Tran VG, Venkatasubramaniam A, Adhikari RP, Krishnan S, Wang X, Le VT, et al. Efficacy of active immunization with attenuated α-hemolysin and Panton-Valentine leukocidin in a rabbit model of Staphylococcus aureus necrotizing pneumonia. J Infect Dis 2020;221:267-75.

[13] Gopal Rao G, Batura R, Nicholl R, Coogan F, Patel B, Bassett P, et al. Outbreak report of investigation and control of an outbreak of Panton-Valentine leukocidin-positive methicillin-sensitive Staphylococcus aureus necrotizing pneumonia. J Infect Dis 2020;221:267-75.

[14] Abu Al-Soud W. Detection of the Panton-Valentine leukocidin gene in a hospital. J Infection Chemother 2020;26:76-81.

[15] Monecke S, Müller E, Buechler J, Rejman J, Stieber B, Akpaka PE, et al. Rapid detection of Panton-Valentine leukocidin in Staphylococcus aureus cultures by use of a lateral flow assay based on monoclonal antibodies. J Clin Microbiol 2013;51:487-95.

[16] Bittar F, Ouchenane Z, Smati F, Raoul D, Rolain J-M. MALDI-TOF-MS for rapid detection of staphylococcal Panton-Valentine leukocidin. J Infect Dis 2012;206:1817-21.

[17] Abu Al-Soud W. Detection of the Panton-Valentine leukocidin gene in Swedish isolates of methicillin-resistant Staphylococcus aureus. USA300 clone among hospitalized patients and nursing staff in a tertiary care university hospital. J Infection Chemother 2020;26:76-81.

[18] White J, Herman A, Pullen AM, Kubo R, Kappler JW, Marrack P. The Vβ-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cell's and clonal deletion in neonatal mice. Cell 1989;56:27-35.

[19] McShan WM, McCallor KA, Nguyen SV. The bacteriophages of Streptococcus pyogenes. In: Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA, Braunitz M, Rood JJ, editors. Gram-Positive Pathogens. New York: John Wiley & Sons, Ltd; 2019, p. 158-76.

[20] Davies HD, McGee A, Schwartz B, Green K, Cann D, Simor AE, et al. Invasive group A streptococcal infections in Ontario, Canada. N Engl J Med 1996;335:547-54.

[21] O'Loughlin RE, Roberson A, Cieslak PR, Lynfield R, Gandhi KM, Craig A, et al. The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000-2004. Clin Infect Dis 2007;45:853-62.

[22] Skrickson D, Mckee A, Hall L, Cohen J. Comparative effects of clindamycin and ampicillin on superantigenic activity of Streptococcus pyogenes. J Antimicrob Chemother 1997;40:275-7.

[23] Coyle EA, Cha R, Rybak MJ. Influences of linezolid, penicillin, and clindamycin, alone and in combination, on streptococcal pyrogenic exotoxin A release. Antimicrob Agents Chemother 2003;47:1752-5.

[24] Louie L, Simor AE, Louie M, McGeer A, Low DE. Diagnosis of group A streptococcal necrotizing fasciitis by using PCR to amplify the streptococcal pyrogenic exotoxin B gene. J Clin Microbiol 1998;36:1769-71.

[25] Muldrew KL, Simpson JF, Stratton CW, Tang Y-W. Molecular diagnosis of necrotizing fasciitis by 16S rRNA gene sequencing and superantigen gene detection. J Mol Diagn 2003;5:641-5.

[26] Khan RMA, Anwar S, Pirzada ZA. Streptococcus pyogenes strains associated with invasive and non-invasive infections present possible links with emm types and superantigens. Indian J Basic Med Sci 2020;23:133-9.

[27] Takao T, Tanabe T, Hong Y-M, Shimonomi H, Kurazono H, Yutsudo T, et al. Identification of molecular structure of Shiga-like toxin 1 (VT1) from Escherichia coli O157:H7 with that of Shiga toxin. Microb Pathog 1988;5:357-69.

[28] Castro VS, Carvalho RCT, Conte-Junior CA, Figuiredo EES. Shiga-toxin producing Escherichia coli: pathogenicity, supershedding, diagnostic methods, occurrence, and foodborne outbreaks. Comp Rev Food Sci Food Safety 2017;16:1269-80.

[29] Melton-Celsa AR. Shiga toxin (Stx) classification, structure, and function. Microbiol Spectr 2014;2:EHEC-0024-2013.

[30] Awofisayo-Okuyelu A, Brainard J, Hall I, McCarthy N. Incubation period of Shiga toxin-producing Escherichia coli. Epidemiol Rev 2019;41:121-9.

[31] Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Anderson FJ, et al. Global incidence of human Shiga toxin-producing Escherichia coli infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathog Dis 2014;11:447-55.

[32] E. coli (Escherichia coli) | E. coli | CDC 2020. http://www.cdc.gov/ecoli/index.html (accessed 8 May 2020).

[33] Tarr GAM, Lin CY, Vandermeer B, Lorenzetti DL, Tarr PI, Chui L, et al. Diagnostic test accuracy of commercial tests for detection of Shiga toxin-producing Escherichia coli: a systematic review and meta-analysis. Clin Chem 2020;66:302-15.

[34] Jenkins C, Perry NT, Godbole G, Gharbia S. Evaluation of chromogenic selective agar (CHROMagar STEC) for the direct detection of Shiga toxin-producing Escherichia coli from faecal specimens. J Med Microbiol 2020;69:487-91.

[35] Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, et al. 2017 Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. Clin Infect Dis 2017;65:e45-80.

[36] Kakoulis L, Papachristodoulou E, Chra P, Panos G. Shiga toxin-induced haemolytic uraemic syndrome and the role of antibiotics: a global overview. J Infect 2019;79:75-94.

[37] Clarke KEN, MacNeil A, Hadler S, Scott C, Tiwari TSP, Cherian T. Global epidemiology of diphtheria, 2000-2017. Emerg Infect Dis 2017;23:133-9.

[38] Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ, et al. Global incidence of human Shiga toxin-producing Escherichia coli infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathog Dis 2014;11:447-55.

[39] Kakoulis L, Papachristodoulou E, Chra P, Panos G. Shiga toxin-induced haemolytic uraemic syndrome and the role of antibiotics: a global overview. J Infect 2019;79:75-94.

[40] Clarke KEN, MacNeil A, Hadler S, Scott C, Tiwari TSP, Cherian T. Global epidemiology of diphtheria, 2000-2017. Emerg Infect Dis 2017;23:133-9.

[41] Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ, et al. Global incidence of human Shiga toxin-producing Escherichia coli infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathog Dis 2014;11:447-55.

[42] CDC 2020. http://www.cdc.gov/ecoli/index.html (accessed 8 May 2020).

[43] Rieti GL, Barba RP, Klein J, Cesarini CR, Ferreira CM, Andrade MA, et al. Diagnosis of necrotizing fasciitis by 16S rRNA gene sequencing and potential vaccine implications: United States, 2000-2004. Clin Infect Dis 2007;45:853-62.

[44] Srisandan S, McKea A, Hall L, Cohen J. Comparative effects of clindamycin and ampicillin on superantigenic activity of Streptococcus pyogenes. J Antimicrob Chemother 1997;40:275-7.

[45] Coyle EA, Cha R, Rybak MJ. Influences of linezolid, penicillin, and clindamycin, alone and in combination, on streptococcal pyrogenic exotoxin A release. Antimicrob Agents Chemother 2003;47:1752-5.
[42] Leber AL, Lisby JG, Hansen G, Relich RF, Schneider UV, Granato P, et al. Multicenter evaluation of the QIAstat-Dx respiratory panel for detection of viruses and bacteria in nasopharyngeal swab specimens. J Clin Microbiol 2020;58:e00155-20.

[43] Relich RF, Leber A, Young S, Schutzbank T, Dunn R, Farhang J, et al. Multicenter clinical evaluation of the automated Aries Bordetella assay. J Clin Microbiol 2019;57:e01471-18.

[44] Williams MM, Sen K, Weigand MR, Skoff TH, Cunningham VA, Halse TA, et al. Bordetella pertussis strain lacking pertactin and pertussis toxin. Emerg Infect Dis 2016;22:319-22.

[45] Turnbull PCB. Introduction: anthrax history, disease and ecology. In: Koehler TM, editor. Anthrax. Berlin; Springer; 2002, p. 1-19.

[46] Bhunia AK. Bacillus cereus and Bacillus anthracis. In: Bhunia AK, editor. Foodborne microbial pathogens: mechanisms and pathogenesis. New York: Springer; 2018, p. 193-207.

[47] Banada PP, Deshpande S, Russo R, Singleton E, Shah D, Patel B, et al. Rapid detection of Bacillus anthracis bloodstream infections by use of a novel assay in the GeneXpert system. J Clin Microbiol 2017;55:2964-71.

[48] Gallegos-Candela M, Boyer AE, Woolfitt AR, Brumlow J, Lins RC, Quinn CP, et al. Validated MALDI-TOF-MS method for anthrax lethal factor provides early diagnosis and evaluation of therapeutics. Anal Biochem 2018;543:97-107.

[49] Dong M, Masuyer G, Stenmark P. Botulinum and tetanus neurotoxins. Annu Rev Biochem 2019;88:811-37.

[50] Santos RP, George M. Tetanus, diphtheria, and botulism. In: Domanchowske J, editor. Introduction to clinical infectious diseases: a problem-based approach. Cham: Springer International Publishing; 2019, p. 285-300.

[51] Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 1996;272:1910-4.

[52] Rivera-Chávez F, Mekalanos JJ. Cholera toxin promotes pathogen acquisition of host-derived nutrients. Nature 2019;572:244-8.

[53] Ramamurthy T, Das B, Chakraborty S, Mukhopadhyay AK, Sack DA. Diagnostic techniques for rapid detection of Vibrio cholerae O1/O139. Vaccine 2020;38:A73-82.

[54] Doxey AC, Mansfield MJ, Montecucco C. Discovery of novel bacterial toxins by genomics and computational biology. Toxicon 2018;147:2-12.