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Review Article

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Virtuous aspects of vicious bacterial toxins

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ABSTRACT

Pathogenic bacteria exert their harmful effects in host through a cascade of virulence factors: exotoxins, endotoxin, invasions proteins and the like. Various toxins secreted by different bacteria not only subvert the host intracellular signaling pathways but also have unique affinity for specific host cells. Detailed information has been accumulated over the years regarding the purification, chemical characterization, enzymic action and architecture of various bacterial toxins. Toxins ability to bind to the specific target cells makes them good candidate for drug delivery and in cancer therapeutics. A number of immunotoxins; hybrid molecules of bacterial toxins and antibodies, have wide applications in cancer and are currently under clinical trial. Advances in genomics and proteomics have created a wealth of information related to the nucleotide and protein sequences of numerous toxins and different databases (DBETH, VFDB, BETAWRAP, RASTA) are available with elaborate information thereof. In present review we have provided a brief account of therapeutic aspect of bacterial toxins and an effort has been made to facilitate the reader's understanding as to how we can turn the vicious toxin into virtuous toxin for human benefits.

Key words: .Bacterial Toxin, Immunotoxins, AB_5, A_2B_5, Toxin databases, Therapeutic toxin.

Bacterial toxins; the soluble antigens, secreted by a number of pathogenic bacteria has a standing reputation of being a poison secreted during the course of pathogenesis. Toxins can modulate the cellular functions by selectively targeting a number of signaling pathways within the host cell in order to tilt the balance in bacteria favor. With the enhanced understanding of the toxin purifications methods, gene cloning, protein sequences and three dimensional crystallographic structures today, we can comprehend the unique behavior of toxins such as binding ability to specific target cell and several enzymic functions. Scientific community is investing efforts to exploits the exquisite features of bacterial toxins for a number of therapeutic purposes in clinical settings.

1. Historical perspective

Bacterial pathogens have been causing various diseases in humans for centuries and even today these pathogenic bacteria are coexisting and evolving with us, despite having different means for their containment in clinical practices (namely antibiotics, vaccines and phage therapy). Most of these bacteria exert their detrimental effects in infected host through toxins. Since the discovery of the first diphtheria toxin by Emile Roux and Alexandre Yersin in 1888 from the bacteria-*Corynebacterium diphtheriae* [1], more than 500 bacterial toxins are known till date and improved understanding of detailed structure, mechanism of action at cellular and organ level is available in a number of publications [2]. Toxins can be classified in two broad categories: exotoxin and endotoxin, and can be produced by both Gram-positive and Gram-negative bacterium (Table-1) [3].

2. Toxins: unique enzymes with complex architecture

Exotoxins produced by different bacterium like *botulinum*, *clostridium*, *diptheria* toxins exhibiting enzyme activities like ADP-ribosylation, phospholipase, adenylate cyclase, metalloprotease, deamidase, protease and deoxyribonuclease activity (Table-2). Toxins showed preferential binding for specific cells depending on the carbohydrate moieties present on cell membrane [4]. Accumulated wealth of information obtained in last 127 years, facilitated deep insights into the genetics, molecular sequences and complex architecture of various toxins. Specific binding, internalization and subsequent hijacking of cellular machinery have been the hallmark of toxins mode of action. Detailed knowledge of toxin structure may facilitate our understanding regarding the uniqueness of toxin action. Remarkable structural similarities were observed in bacterial toxin architecture and hormones as both of these bio-molecules are composed of two subunits: Subunit A and Subunit B. On the basis of two sub-units (A & B), toxins can be divided into four categories namely AB, AB_5, A_2B_5, and A2B7 (Figure-1). Subunit-A of holotoxin in different categories (such as AB, AB_5, A_2B_5 and A_2B_7) acts as enzyme (namely ADP-ribosyltransferase, Metalloprotease and the like) whereas subunit-B helps in binding to target cells. Botulinum toxin is an example of AB-type toxin containing light-A chain (50 kDa) and heavy-B chain (100 kDa) both chain-A and Chain-B are nicked but held together by a sulfide bond (Figure) in Botulinum toxin. AB_5 category is usually composed of most clinically relevant pathogens namely Cholera toxin, *E. coli* (LT), Shiga toxin, Pertussis toxin and Anthrax toxin. Subunit-B in AB_5 category is composed of five identical polypeptide chains in each type [5]. A_2B_5 represents the latest category of typhoid toxin from *Salmonella* Typhi where two covalently linked subunit-A found attached to non-covalently [6]

A_2B_5 type typhoid toxin is unique as it is composed of subunit A (enzymatic PltA and CdtB) and receptor binding pentameric subunit B (PltB) (Figure-2) The related AB_5 toxin has two subunits PltA and PltB, whereas cytolethal distending toxin (CDT) has three subunits, namely CdtA, CdtB and CdtC. Genotoxic CDT exerts cytotoxic effect through its three subunits [7] CdtB protein of subunit A is absent in AB_5 type toxins and CdtB may contribute to the acute symptoms of typhoid fever as a catalytic mutant of CdtB failed to produce detectable symptoms in animals.

In A_2B_7 type category, Anthrax toxin has two subunits-A and seven subunits-B. Anthrax toxin is usually composed of three proteins: the protective antigen (PA) and the lethal factor (LF) and the edema factor (EF) [8].

3. Toxin Databases

Phenomenal growth has been witnessed in the field of genomics and proteomics in last one decade and details of complete genome sequences of various organisms including pathogenic bacteria, is available to understand the disease pathogenesis with greater depth. With the discovery of new toxins and other virulence effectors among bacteria, a number of databases related to bacterial toxins have been created and freely available for scientific community. The major aim of toxin databases is to understand the structures, functions and mode of action of different bacterial toxin with a view to exploit the same for therapeutic use in serious illness like cancer. Toxin database like DBETH, VFDB, BETAWRAP, BTBD, RASTA-Bacteria ,*Ec*MLST , and MvirDB , provide excellent compilation of gene sequences, virulence factors, toxin-antitoxin system, toxin-protein and enzymes.

DBETH (Database of Bacterial Exotoxins for Human) for example, contains 229 toxins from twenty six bacterial genus, 31,769 toxin sequence and 360 three- dimensional protein structure of toxins. DBETH server can predict the potential toxin sequence and works on Hidden Markov Model system, http://www.hpppi.iicb.res.in/btox/ [9].

VFDB (virulence factor database) provides comprehensive, updated and experimentally validated bacterial virulence factors including enzymes, toxins, secreted effectors, outer membrane protein and capsular polysaccharides. VFDB provides detailed information regarding 5,955 virulence factor genes from 75 bacterial genera, http://www.mgc.ac.cn/VFs/v3index.h.tm [10].

MvirDB (Microbial virulence database) is a comprehensive repository protein and DNA sequence of toxin, virulent factors and antibiotic resistance genes for rapid detection of any pathogen having bio-weapon potential, http://www.hsls.pitt.edu/obrc/index.php?page=URL1174507980 [11].

BETAWRAP is a computational program that recognizes parallel β-helix secondary motifs based on the spatial pair wise correlation, usually present in toxins and virulence factors produced by bacterial and fungal pathogen. This computation program identifies 2,448 sequences in NCBI protein database, NCBI; http://www.ncbi.nlm.nih.gov/). [12]

BTBD; a biotoxin database harbors approximately 447 toxin proteins from various species of snake, spider and scorpions. Three dimensional structures of these toxins were predicted by homology modeling and their functions were annotated subsequently. BTBD provides toxin protein sequence, 3D-structure and annotated functions in one place is user friendly. [13]
http://www.b-u.ac.in/btdb/home.html

RASTA-Bacteria; a web based tool that provides detailed information regarding toxin/antitoxin (TA) system found in bacteria and archaea group. TA system is known to control growth and programmed cell death in bacteria and thus can be instrumental in developing new class of antibiotics, http://genoweb1.irisa.fr/duals/RASTA-Bacteria/index.php?page=form **[14].**

*Ec*MLST database provides useful typing system for pathogenic *Escherichia coli* strain based on the multi-locus sequence typing. The database is based on XML and Perl modules, creates a dynamic web page for searching any query related to pathogenic *E. coli*, http://www.shigatox.net/ecmlst/cgi-bin/index [15]

4. Toxin Therapeutics

4.1 Bacterial protein toxins as toxoid vaccine

A number of pathogenic bacterium secretes protein exotoxins which mediate processes resulting in to the characteristics symptoms of the diseases like diphtheria, tetanus, pertussis and anthrax (Table-3). Toxoid vaccines (such as diphtheria and tetanus) have saved millions of life since the first usage in 1900 Toxoids are prepared from the growth of pathogenic bacterium on semi-synthetic medium and subsequently treating the secreted exotoxin with formaldehyde. Diphtheria toxin (DT) is produced by a *tox* gene in *Corynebacterium diphtheriae* and biochemically is an enzyme having a mass of 58 kDa with 535 amino acid residues [16]. Likewise tetanus (caused by *Clostridium tetani*) and pertussis (caused by *Bordetella pertussis*) toxins are having a mass of 150 kDa and 105 kDa respectively, and toxins are encoded by the genes *text* and *ptx/ptl* respectively [17].

Anthrax toxin is a unique binary toxin composed of tripartite structure: protective antigen (PA), lethal factor (LF- a zinc-Metalloproteases) and edema factor (EF- an adenylyl cyclase) Protective antigen, central component of tripartite system is used for vaccine purpose and required for entry LF and EF into host cells [18].

4.2 Role of toxin as Immunotoxins in treatment of cancer

Immunotoxins are the hybrid molecules that combined the binding specificities of antibodies and the cytotoxicity effects of toxins for the target cells (Cancer tumors) [19]. On the basis of complete structure of toxin, immunotoxins can be divided into three generations; first generation immunotoxins are conjugate of antibodies/ligand to intact bacterial toxin (Enzymic domain and binding domain) whereas in second generation immunotoxins, bacterial toxin is lacking binding domain. To reduce the overall size of immunotoxin and better penetrability into solid cancer tumors, binding domain of toxin part has been replaced by Fv portion of antibody in the third generation of immunotoxins [20].

4.3 Diphtheria toxin (DT) based immunotoxin

DT represents one of the most exploited ADP-ribosylating toxins that has two subunits: subunit A (Catalytic domain) and subunit B (Binding domain) [21]. DT binds to heparin binding epidermal growth factor on the cell membrane in target cell and undergoes cleavage by furin-like protease. After the delivery of catalytic domain into host cell cytoplasm DT exerts its cytotoxic effect (apoptosis) by inhibiting eukaryotic translation elongation factor (eEF2) [22].

Tumor cells specifically express a number of receptors such as IL-2 receptor (different leukemia and lymphomas), GM-CSF receptor (acute myeloid leukemia) Transferrin receptor (malignant brain tumor) IL-4 receptor (breast, ovarian and renal carcinoma) IL-13 receptor (Glioblastoma multiforme-GBM) [23], EGFR receptor (breast, lung, prostrate, colorectal, brain and ovarian carcinoma). Receptor binding domain of DT toxin can be replaced by a number of cytokine like IL-2, IL-13, GM-CSF, EGF and protein like transferrin to produce therapeutic immunotoxins which binds to respective receptors in growing tumors and result in extensive cell death. A number of therapeutic DT immunotoxins; DAB389 IL-2 (Ontak), DT388-GM-CSF, Tf-CRM107 are already under clinical trials [24].

4.4 *Pseudomonas* Exotoxin A-based immunotoxins

Pseudomonas exotoxin-A (PE) is initially synthesized as proenzyme (with 638 amino acid long polypeptide) and mature toxin has only 613 amino acids after the removal of 25 amino acids from N-terminal of the polypeptide chain. PE toxin has three major functional domains: domain I (1-252 amino acids long), domain II (253-364 amino acids long) and domain III (405-613 amino acids long) Domain I is involved in receptor binding and subdivided into Ia (1-252 amino acids) and Ib (395-404 length) whereas domain II is involved in intracellular trafficking of toxin. Domain III along with stretch of domain Ib mediates the ADP-ribosylation and inhibition of EF2 which ultimately result in cell death. [25].

Secreted PE binds to LRP1 or LRP1B, CD25, CD22 cell surface receptor. A number of PE based therapeutic immunotoxins such as LMB-2 (for leukemia and lymphoma), BL22 (for human B-cell lymphoma) and LMB-1 (for human epithelial carcinomas), SS1P (for mesothelioma, lung cancer), MR1-1(for brain tumor), Cervene (for brain and CNS tumors) have been tested in laboratory and currently under clinical trial [26].

4.5 Anthrax toxin based immunotoxins

The tripartite anthrax toxin is composed of three subunits: protective antigen (PE), lethal factor (LF) and edema factor (ED [27]. Protective antigen (83 kDa) has been used for tumor therapy after proteolytic activation. Cleavage of PA by cell surface proteases like furin, results into two fragments: N-terminal PA63 (63 kDa) and a C-terminal PA20 (20 kDa) fragment. TEM8 (Tumor endothelial marker) and CMG2 (capillary morphogenesis protein 2) are among the major membrane receptors that binds with PA and ultimately forms homo heptamer which further facilitates the entry of LF and EF into cell cytoplasm [28].

LF; a zinc metalloprotease, can cleave mitogen activated protein kinases (MAPKK) which can be activated by Ras oncoprotein. [29]. Moreover, LT can also reduce the neovascularization which is a prerequisite for tumor growth

Anthrax toxin has an inherent advantage over other toxins such as diphtheria and Pseudomonas toxin as most of the cancer patients have pre-existing antibodies in their blood that may neutralize anti-cancer immunotoxins.

5. Botulinum Toxin Based Therapeutics

5.1 Dermatological use of Botulinum toxin:

Since the 1970 when Alan Scott introduced botulinum neurotoxin A as a therapeutic agent, the number of different uses of this drug has increased exponentially. FDA has approved Botox not only for facial aesthetics but also for neurogenic hyperactive bladder function [30]. Injectable botulinum toxin type A has been extensively used for range of medical disorders like Dyshidrotic hand eczema pompholyx, Pitted keratolysis, Brachioradial pruritus, strabismus, blepharospasm, focal dystonia, spasticity associated with juvenile cerebral palsy and various cosmetic treatments.

Botulinum toxin has been experimentally and clinically shown to be effective in chronic migraine patients. Toxin exerts its therapeutic effect by cleavage of soluble N –ethylmaleimide- sensitive factor attachment protein receptor SNARE proteins when delivered in nasal cavity. The releases of inflammatory mediators such as CGRP, glutamate, and others from its effect on SNARE proteins reduce sever migraine pain [31].

Each serotype of Botulinum bacterium demonstrates its own diverse mechanisms of action and duration of effect. Like Incobotulinumtoxin A is a commercially available botulinum toxin preparation widely used to cure glabellar frown lines [32]. Similarly Neuronox (neu-BoNT/A) used as aesthetic medicine [33], Chinese type A botulinum toxin (CBTX-A) for neurological and musculoskeletal disorders [34], NABOTA (DWP450) and Botulinum toxin type A topical gel (RT001) 150-kDa toxin with a novel peptide that facilitates transcutaneous flux of the toxin [35] . Botulinum toxin bind and block the unmyelinated C-fibers and partially myelinated A-delta fibers of the sensory nervous system which block acetylcholine release that can therapeutically improve conditions such as hyperhidrosis, sialorrhea, sphincter spasms, and rhinitis [36].

5.2 Non-dermatological use of Botulinum toxin:

Non-dermatological uses of Botulinum toxin in opthalmology- include blepharospasm, apraxia of lid opening, intermittent exotropia, congenital nystagmus, lacrimal hypersecretion, pain relief in acute angle closure glaucoma, Whereas in neurology and gastrointestinal it is used for hemifacial spasm, oromandibular dystonia, spasmodic torticollis, gustatory sweating, achalasia, anal fissure [37].

5.3 Nanopores, Toxins in biosensing and polymer transport

Nanopores devices may have sensitivity up to single molecule detection level by using a nanoscale pore. Bacterial toxin pores were promisingly used to probe mechanisms of molecular detection and macromolecule transport. Biological nanopores, including the channel-forming bacterial toxins such as *Gramicidin A* of *Bacillus Brevis* (GrA), Alpha-hemolysin (αHL), and component of Anthrax Toxin of *Bacillus Anthracis* (PA63) are natural single-molecule biosensors Alpha hemolysin (αHL) plays major virulence factor in the pathogenesis of *Staphylococcus aureus* and its cytotoxicity is mediated through transmembrane pore formation. Biosensing properties of αHL's can be mediated through its ability to reversibly bind with drug delivery β-cyclodextrin-based (βCD) molecular adapters. GrA is used to study for probing enzyme activity, protein-protein interactions and for fabricating components of drug delivery systems in nanomedicine [38].

5.4 Immunotoxins based on ribosome inactivating proteins

Ribosome inactivating proteins (RIPs) are toxins that are able to inhibit protein translation irreversibly [39]. They are the group of glycosylated and non glycosylated enzyme with N-glycosidase activity found predominantly in higher plants, bacteria, algae and fungi. Ribosome inactivating proteins has shown antimicrobial activities in vitro, antibacterial, antifungal and broad spectrum anti-viral activities [40]. A number of ribosomal inactivating proteins based immunotoxins have been constructed namely; RFT5-dgA (IMTOX25), ki-4 dgA (anti-CD30 immunotoxin), RFB4-dgA (IMTOX22), Ber-H2-SO6 (anti-CD30 monoclonal antibody), H65-RTA, XOMAZYME-MEL, and HD37-dgA. Ber-H2-SO6 anti-CD30 monoclonal antibody rapidly and substantial reduce tumor mass. The anti-CD30 immunotoxin Ki-4-dgA constructed by linking the monoclonal antibodies RFT5 and Ki-4 to deglycosylated ricin A-chain (dgA) eradicate residual tumor cells. RFT5-dgA (IMTOX25) finds certain cancer cells and kills them without harming normal cells. Whereas immunoconjugate XOMAZYME-MEL cures metastatic malignant melanoma [41].

Figure 1

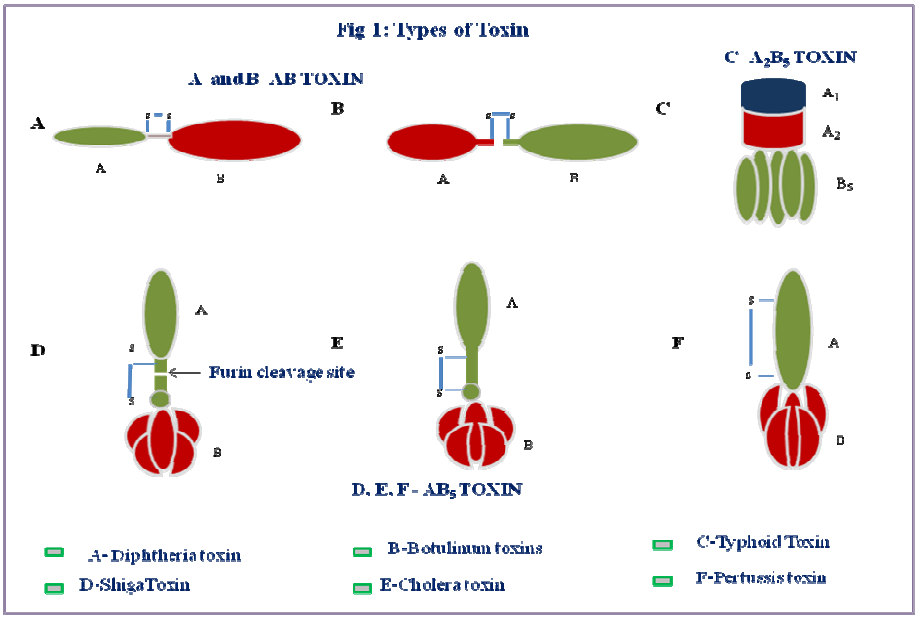


Figure 2

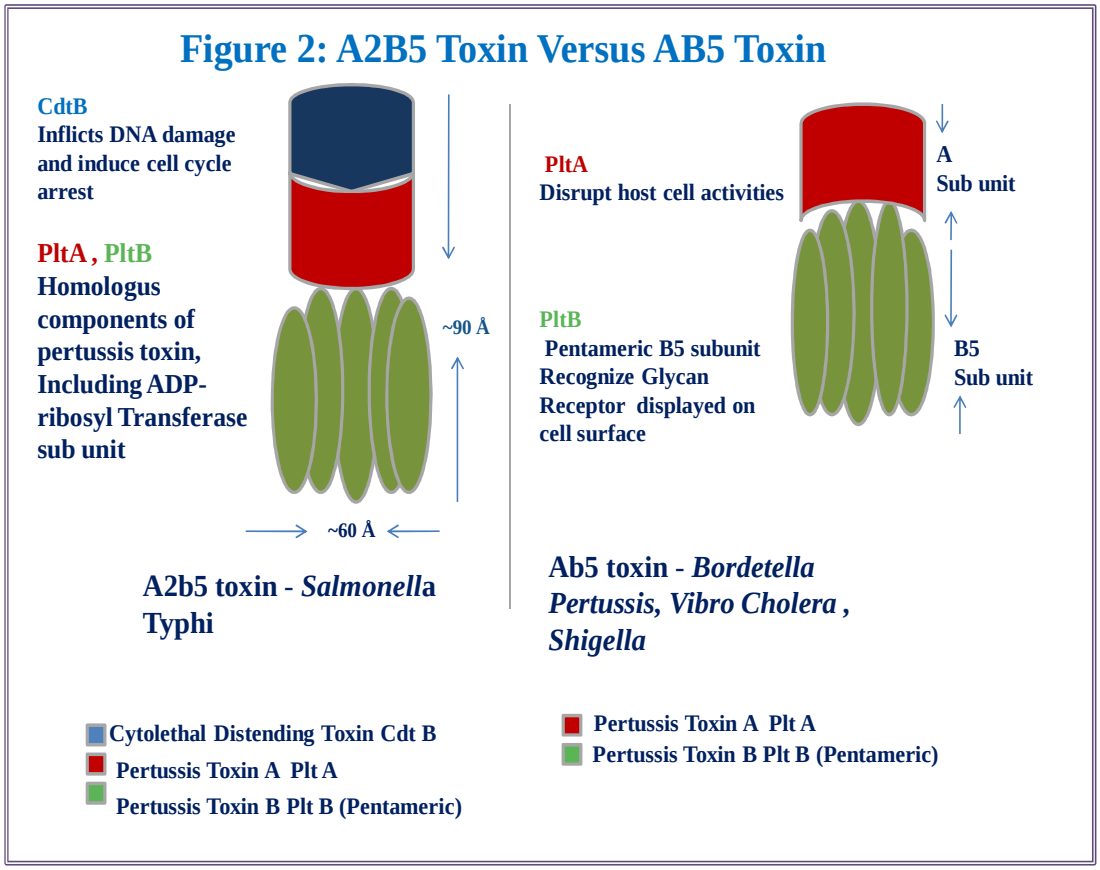
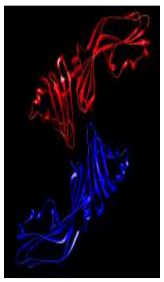
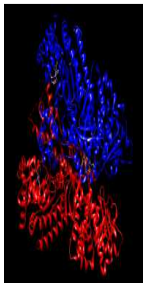
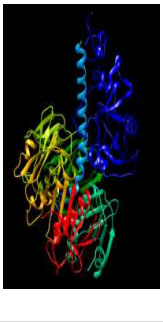


Table 1

Toxin	PDB Structure	Mode of Action	Cell Surface Receptor	Disease	Reference
Gram Positive Bacteria					
Botulinum toxin *C. botulinum*	 **PDBID: 3MPP**	Protease, specific for synaptobrevin, VAMP or SNAP25	Polysialogangliosides+ synaptotagmin I and II (Botulinum A, B and G)	Botulism	A Rummel; et al 2004, M Dong; *et al* 2003
Staphylococcal enterotoxins *S. aureus*	 **PDBID: 3BL6**	Superantigen, 5-HT release stimulation of 5-HT3 receptor (emesis)	histocompatibility complex class II molecules	Abcessesc	JG Naglich; *et al* 1992

Enterotoxin *C. perfringens*	 **PDBID: 2YHJ**	pore-forming activity	Phospholipid	Gas gangrene	H Moreau; *et al* 1988
Cereulide *B. cereus*	 **PDBID: 1X7F**	K+ ionophore	5HT3 receptor	food borne illness	R mikkola; *et al* 199
Anthrax lethal toxin *Bacillus Anthracis*	 **PDBID:1J7F**	Cell surface receptor for PA component of anthrax toxin is tumor endothelial marker 8	Protease specific for mitogen activated protein kinase kinase	Anthrax	G.J.A Rainey; *et al* 2005
Gram Negative Bacteria					
Cholera toxin *V. cholerae*	 **PDBID:1XTC**	inactivation of Gsα and activation of adenylate cyclase	ganglioside GM1	Diarrhea. Cholera	V Heyningen; 1974

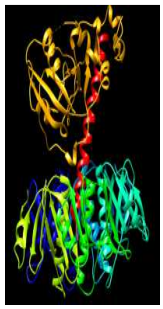
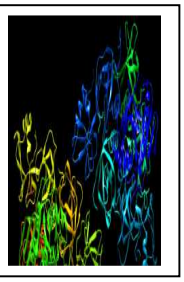
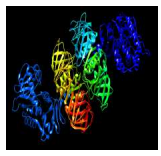
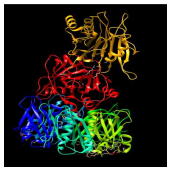
Heat labile enterotoxin *E. coli*	 **PDBID:1TII**	inactivation of Gsα and activation of adenylate cyclase	ganglioside GM1	UTI	E Cheng *et al* 1999
Pertussis-toxin *Bordetella pertussis*	 **PDBID:1PRT**	ADP ribosylation of G proteins	Sialic acid-galactose glycoproteins	Whooping Cough	J Moss *et al* 1983
Shiga-toxin *Shigella*	 **PDBID: 1R4Q**	Endoglycosidase	Globotriaosylceramide/glycoprotein P1	Shigellosis	M Jacewicz; *et al* 1986
***Salmonella* enterica** *Salmonella Typhi*	 **PDBID:4K6L Typhoid Toxin**	ADP ribosylation	**Podxyl** podocalyxin-like protein 1 Erythrocyte receptor tyrosine phosphatase C in a range of cells including T and B cells, macrophages, haematopoietic cells, red blood cells and platelets.	Typhoid	TD Merdol *et al* 2001

Table: 2 Toxin Receptor and Their Enzymatic Activity

Toxins	Reported toxin Receptor	Enzyme Activity	Reference
Diphtheria toxin *Salmonella spp*	Diphthamide of eEF2 (eukaryotic elongation factor 2) Interleukin-12 Receptor Toll-Like Receptor 4	ADP-Ribosylating Toxins Transferase and NADase activity	R J Collier; *et al* 1975. V Masignani; *et al* 2004.
E. coli	GM_1 monosialotetrahexosylganglioside	Deamidase activity	G Flateau; *et al* 1997
Vibrio Cholera *Rickettsia* *S. aureus*	GM_1 monosialotetrahexosylganglioside FLIPr- Formyl peptide receptor−like 1 inhibitor	Phospholipases	J Hinnebusch; *et al* 2000.
Tetanus *Botulinum* *Bacillus anthracis*	Sialidasesensitive disialosyl (GDlb) and trisialoganglioside (GTlb). GTlb, GQlb, and GDlb. Anthrax toxin receptors (ATRs)	Metalloproteases	M Jepson; R Titball; *et al* 2000.
Bordetella pertussis, *Pseudomonas aeruginosa exotoxin Y*	Cysteine residue of Gαi subfamily (Gαi , Gαo, and Gαt) except Gαz) eEF2 Target Receptor Cyclic AMP Receptor Lipoprotein receptor-α_2M-macroglobulin (α2M). Carbohydrate receptor: β-D-GalNAc (1-4)- β-D-Gal	Adenylate cyclases	S Lory; *et al* 2004.
Clostridium *difficile* Clostridium *sordellii*	Host Cell Receptor LSR Scavenger receptor AI/II(SR-AI/II)(participates in macrophage)	Glucosyl transferases	V E Streiber C; *et al* 1996, K Aktories; 2003.
Cytolethal distending toxins	GM_1 and GM_2 monosialotetrahexosylganglioside (G protein coupled receptor)	Deoxyribonuclease activity	LA Dreyfus; 2003, M Thelestam; T Frisan; 2004.

Table: 3 Old toxin vaccines and their novel therapeutic uses

S. No	Conventional use of toxin as vaccines	Novel therapeutic Uses of toxin/toxoids	References
A	Tetanus Diphtheria Pertussis (Tdap)	Combined form truncated Diphtheria toxin	C Lapenta; *et al* 2005
B	Whole cell pertussis vaccine (DTP)		N Guiso; 2015**.**
C	Botulinum toxoid (BT) vaccine	**Profound symptomatic relief from:** Strabismus, dystonia, Anismus, Spasmodic dysphonia. **Botulinum toxin type A:** Clinical benefit for patients with Parkinson's disease.	E J Schantz; EA Johnson; e*t al* 1992
D	Shigella toxin - Under Development	**Shiga Toxin Receptor globotriaosylceramide Gb3Cer/CD77**: Promising Therapeutic Target in Pancreas and Colon Cancer.	S Ashkenazi; D Cohen; 2013. U distler; *et al* 2009
E **F** **G**	Live attenuated vaccine: (Ty21a) Parenteral capsular polysaccharide vaccines (ViCPS) Conjugate vaccine (Vi-TT)	**Protection against** typhoid fever caused by *Salmonella* Typhi. **Useful vector** system for presentation of heterologous antigens.	WHO 2014
H	Oral Typhoid Vaccine M01ZH09	**Vaccine** is prepared with a bicarbonate buffer solution, which provides gastric-acid neutralization at the time of vaccination.	BD Kirkpatrick ; *et al* 2005
I	StaphVax – Under trial phase	**Under Trial Phase**	T Jones; 2002.
J	Poly-*N*-acetyl glucosamine (PNAG)	**Broadly protective**: Antimicrobial vaccine.	Maira-Litran T *et al* 2002.

CONCLUSION

Pathogenic bacteria have been causing diseases in humans for centuries and, surprisingly, these pathogens are still coexisting and evolving with us. Despite the improved understanding of their pathogenesis and management, still there is an urgent need to address several unanswered questions like severe humoral response to immunotoxins by host and neutralization of immunotoxins by preexisting antibodies in host. Therapeutic potential of various toxins have been realized in last few decades but further research is required to tap the full potential of the bacterial lethal weapon. Different toxin databases have been created, and are freely available to research community; these databases can enhance our knowledge and understanding in a big way in near future.

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