1 **Metagenomic screening of global microbiomes identifies**
2 **pathogen-enriched environments**

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10 **Abstract**

11 **Background** Human pathogens are widespread in the environment, and examination of pathogen-enriched
 12 environments in a rapid and high-throughput fashion is important for development of pathogen-risk
 13 precautionary measures.

14 **Methods** In this study, a Local BLASTP procedure for metagenomic screening of pathogens in the environment
 15 was developed using a toxin-centered database. A total of 27 microbiomes derived from ocean water, freshwater,
 16 soil, feces, and wastewater were screened using the Local BLASTP procedure. Bioinformatic analysis and
 17 Canonical Correspondence Analysis were conducted to examine whether the toxins included in the database
 18 were taxonomically associated.

19 **Results** The specificity of the Local BLASTP method was tested with known and unknown toxin sequences.
 20 Bioinformatic analysis indicated that most toxins were phylum-specific but not genus-specific. Canonical
 21 Correspondence Analysis implied that almost all of the toxins were associated with the phyla of *Proteobacteria*,
 22 *Nitrospirae* and *Firmicutes*. Local BLASTP screening of the global microbiomes showed that pore-forming
 23 RTX toxin and adenylate cyclase Cya were most prevalent globally in terms of relative abundance, while
 24 polluted water and feces samples were the most pathogen-enriched.

25 **Conclusions** A Local BLASTP procedure was established for rapid detection of toxins in environmental
 26 samples. Screening of global microbiomes in this study provided a quantitative estimate of the most prevalent
 27 toxins and most pathogen-enriched environment.

28 **Keywords**

29 Metagenomics, Microbiome, Local BLASTP, Toxins, Pathogens

30

31 Introduction

32 Rapid identification of pathogens in a particular environment is important for pathogen-risk management.
 33 Human pathogens are ubiquitous in the environment, and infections from particular environments have been
 34 reported worldwide. For example, soil-related infectious diseases are common [1, 2]. *Legionella longbeachae*
 35 infection has been reported in many cases, mainly due to potting mixes and composts [3]. Survival of enteric
 36 viruses and bacteria has also been detected in various water environments, including aquifers and lakes [4-7].

37 Examination of pathogens from infected individuals with a particular clinical syndrome has been a major
 38 achievement of modern medical microbiology [8]. Nevertheless, we still know little about the magnitude of the
 39 abundance and diversity of known common pathogens in various environments, which is very important to the
 40 development of appropriate precautions for individuals who work or play with certain environmental substrates.
 41 This can be realized through metagenomic detection of pathogenic factors in a time-efficient and high-
 42 throughput manner using next-generation sequencing methods.

43 Metagenomic detection of pathogens can be accomplished through different schemes. Li et al. examined
 44 the level and diversity of bacterial pathogens in sewage treatment plants using a 16S rRNA amplicon-based
 45 metagenomic procedure [9]. Quantitative PCR has also been applied for monitoring specific pathogens in
 46 wastewater [10]. More studies have applied the whole-genome-assembly scheme to detect one or multiple
 47 dominant pathogens, most of which were for viral detection in clinical samples [11-14]. Although metagenomic-
 48 based whole-genome-assembly for bacterial pathogen detection can be conducted at the single species level [15],
 49 its computational requirements are high if in a high-throughput fashion. In 2014, Baldwin et al. [16] designed
 50 the PathoChip for screening pathogens in human tissues by targeting unique sequences of viral and prokaryotic
 51 genomes with multiple probes in a microarray. This approach can screen virtually all pathogen-enriched samples
 52 in a high-throughput manner.

53 Despite the aforementioned progress in metagenomic tools for pathogen detection, metagenomic screening
 54 for bacterial pathogens in environments such as soil, where microbial diversity is tremendous, is still
 55 challenging. This is mostly due to difficulty in assembling short reads generated by next-generation sequencing
 56 [8]. The whole-genome-assembly approach is efficient at identifying viromes, but not at dealing with bacterial
 57 communities. Amplicon-based approaches are able to detect bacterial pathogens in a high-throughput manner;
 58 however, it is well known that phenotypic diversity exists widely across and within microbial species of a genus
 59 because of divergent evolution [17, 18]. This also holds true for pathogenic factors [19]. Moreover, toxin factors,
 60 such as the Shiga toxin (*stx*) of *Shigella*, are primarily transferable through lateral gene transfer, which leads to
 61 the continuous evolution of pathogen species [20]. Therefore, it is necessary to examine the pathogen diversity
 62 in environmental metagenomes using essential virulence genes as biomarkers.

63 In this study, a toxin-centered virulence factors database was established, and the well-developed Local
 64 BLASTP method was applied to detect virulent factors in various environments globally. This procedure is
 65 metagenome-based and can be conducted in a high-throughput fashion, which greatly simplifies development of
 66 precautions for pathogen-enriched environments.

67 **Methods and Materials**

68 **Environments and their metagenomes**

69 Twenty-seven metagenomes were selected and downloaded from the MG-RAST server (Table 1). These
70 metagenomes were derived from ocean water, freshwater, wastewater, natural soil, deserts and feces,
71 representing the major environmental media found worldwide. Sequencing methods of the metagenomes include
72 the Illumina, Ion Torrent and 454 platforms, and predicted proteins in the metagenomes ranged from 33,743
73 (fresh water, ID mgm4720261) to 1,966,121 (weedy garden soil, ID mgm4679254). The gene calling results
74 were used for toxin factor screening in this study. The taxonomic composition at the genus level was also
75 retrieved from the MG-RAST server for each metagenome.

76 **Toxin factor database**

77 A toxin-centered database was established for bacterial pathogen detection in metagenomes in this study.
78 Candidate toxin factors for pathogenic screening of environmental metagenomes were gathered based on well-
79 studied pathogens summarized in Wikipedia® under the entry of “pathogenic bacteria”, the Virulence Factor
80 Database [21], a soil borne pathogen report by Jeffery and van der Putten [2], and a manure pathogen report by
81 the United States Water Environment Federation [22]. Sequences of the toxin factors were then retrieved by
82 searching the UniProt database using the toxin plus pathogen names as an entry [23], while typical homologs at
83 a cutoff E value of 10^{-6} were gathered from GenBank based on BLAST results. Considering that virulence
84 process involves several essential factors including toxins, various pathogen-derived secretion proteins were
85 also included in the database, and it was tested that whether secretion proteins were as specific as toxin proteins
86 for pathogen detection. The disease relevance of all virulence factors was screened using the WikiGenes system
87 [24] and relevant publications (Table 2).

88 **Local BLASTP**

89 The Local BLASTP was applied following the procedure used in our previous study [58]. Basically, the gene
90 calling results of each metagenome were searched against the toxin factor database using BLASTP embedded in
91 BioEdit. The cutoff expectation E value was set as 10^{-6} . The results of the Local BLASTP in BioEdit were then
92 copied to an Excel worksheet, after which they were subjected to duplicate removal, quality control and
93 subtotaled according to database ID. Duplicate removal was based on the hypothesis that each sequence
94 contains one copy of a specific toxin factor, since the gene-calling results were used. For quality control of the
95 BLAST results, a cutoff value of 40% for amino acids identity and 20 aa (1/3 of the length of the shortest toxin
96 factors (e.g., the Heat-Stable Enterotoxin C)) for query alignment length were used to filter the records. The
97 toxins abundance matrix was formed for subsequent analyses.

98 **Specificity tests of the Local BLASTP method**

99 Sequences from the toxin database established in this study, as “known sequences” to the database, were
100 selected randomly and searched against the database using the BLASTP procedure. The genome of *Clostridium*

perfringens ATCC 13124 (NC_008261), as “unknown” sequences to the database, was subject the Local BLASTX procedure as well. Homologous proteins were searched exhaustively in the GenBank database using BLASTP, with the representative toxin factors in the toxins database as a query. Sequences were retrieved and aligned using ClustalW, and Maximum-likelihood phylogeny was conducted with MEGA 7 [59].

Data analysis

The toxin frequency in each metagenome was normalized to a total gene frequency of 1M to eliminate the effects of gene pool size. Toxin abundance in the 27 metagenomes was visualized using Circos [60]. The genus abundance of all metagenomes was calculated and sorted by genus name, followed by manual construction of a genus abundance matrix for subsequent biodiversity-toxin abundance Canonical Correspondence Analysis using R [61].

Results and Discussion

In this study, a toxin-centered database was established for bacterial pathogen screening in various microbiomes globally through a Local BLASTP procedure. The specificity of the procedure was tested, the relative abundance of toxins in the microbiomes was examined, and the toxin-taxonomic abundance correspondence analysis was performed.

Like the previously established Local BLASTN method for antibiotic and metal resistance genes screening [58, 62, 63], the Local BLASTP method using the toxin-centered pathogen database in this study was successful at accurately identifying toxin proteins from the database. For screening of the *Clostridium perfringens* ATCC 13124 genome, the methods successfully detected the pore-forming genes and multiple copies of the glucosyltransferase (*tox*B-like) and ADP-ribosyltransferase (*spv*B-like) genes, based on the raw data. These results are consistent with the virulence genetic features of *Clostridium* sp. [21], which have not been well detailed in the GenBank annotation record. Such a cross-validation positively indicated that the Local BLASTP procedure established here is useful in predicting toxin genes in unknown genomes. Yet for a semi-quantitative method to estimate toxin factors in metagenomes, a false positive analysis is required to examine to what level mismatch is included in the Local BLASTP results. Actually, the cutoff values of identity greatly impact the homolog virulence factor abundance returned. At cutoff values of 40% for identify and 20 aa for alignment length, only four records for *Clostridium perfringens* ATCC 13124 genome query were returned after duplication removal, one for 1-phosphatidylinositol phosphodiesterase, one for pore-forming alveolysin, one for Ornithine carbamoyltransferase and one for RNA interferase NdoA. At a cutoff identity value of 35%, one more record (Toxin secretion ATP binding protein) was returned. This means that the Local BLASTP procedure was able to detect the virulence factors in unknown genomic dataset at least semi-quantitatively, with proper cutoff values for data quality control. The accuracy of the BLASTP procedure in virulence factor detection was further tested using the genomes of *Bacillus thuringiensis* serovar *konkukian* str. 97-27 (AE017355.1) and *Helicobacter pylori* 26695 (AE000511.1) (results not shown).

As mentioned above, functional genes including toxin factors may partly evolve through lateral gene transfer, which makes their taxonomic affiliation difficult. It is thus interesting to explore how specific toxin

factors are associated with the taxonomic units of pathogens. Here, I explored this issue by investigating the taxonomic distribution of homologs of toxins retrieved from the GenBank database. Generally, at a lower expectation value, most toxins were associated with a specific group of pathogens. For example, at a cutoff E value of 10^{-6} (the default unless specified), 241 out of 242 returned records of *Mycobacterium tuberculosis* RelE homologs fell within the phylum *Actinobacteria*. Moreover, 89% of these homologs were from the genus *Mycobacterium*, while 99.7% of *Yersinia pestis* CdiA homologs and 92.7% of *Bordetella pertussis* cya homologs belonged to *Proteobacteria*, and homologs of *Aeromonas dhakensis* repeats-in toxin (RtxA) were mostly associated with the class *Gammaproteobacteria* (206 out of 242). However, no obvious genus-toxin association was identified. It is worth noting that these results largely depended on the availability of toxin sequences in each taxonomic unit. The lack of a genus-toxin association basically denied the possibility of detecting a specific pathogen using a specific toxin as a single signature [16].

It is still not clear whether virulence secretion proteins are specific for pathogen detection as signatures, through they are essential for virulence process [20]. For example, the contact-dependent toxin delivery protein CdiA was found to be widespread in bacteria [37]. The relative abundance of secretion proteins in the 27 microbiomes was determined as well as that of the toxins which are essential to virulence processes. The results of the present study showed that the abundance of secretion proteins selected in the database was strongly correlated with the toxin abundance ($R^2 = 0.80$, Figure 1). The most abundant secretion proteins included *L. waltersii* toxin secretion protein (LWT1SS), *L. pneumophila* toxin secretion protein ApxIB, and *Aeromonas hydrophila* RTX transporter (RtxB) (data not shown). Further exploration indicated that although *A. hydrophila* RtxB homologs from GenBank were found in all *Proteobacteria* classes, most of the RtxB-harboring species have been reported to be pathogens, including *Vibrio* spp. [64], *Pseudomonas* spp., *Neisseria meningitidis* [65], *Ralstonia* spp. [66], and *Yersinia* spp. [21]. This may imply the pathogen-specific nature of secretion proteins included in the database, and that toxin secretion proteins can be used as signatures for pathogen detection as well.

Toxin-phyla CCA results showed that all phyla can be clearly separated into two groups, and that almost all toxins were associated with *Proteobacteria*, *Nitrospirae* and *Firmicutes* (Figure 2). Considering the phylum-specificity of the toxins stated above, these results can be biased because of the taxonomic affiliation of toxins included in the Local BLASTP database. The taxonomic distribution proportion of currently available genomes of identified pathogens was reflected in the toxin database, with *Proteobacteria* and *Firmicutes* accounting for the majority of the genomes. However, the CCA results may also indicate, at least in part, a proportional lack of pathogens in some phyla, such as *Crenarchaeota*, *Euryarchaeota*, *Verrucomicrobia* and *Bacteroidetes* [67]. Archaea cannot easily absorb phage particles because of their extracellular structures, which differ from bacteria [68]. A recent study by Li et al. also found that the five most abundant bacterial pathogens were from either *Proteobacteria* or *Firmicutes* in wastewater microbiomes [9]. Taken together, these findings could indicate that *Proteobacteria* or *Firmicutes* were evolutionarily enriched with pathogens when they dominated most environmental microbiomes on the planet [69, 70].

Interestingly, there was a strong association between the phylum *Nitrospirae* and toxins of RNase inteferases (MvpA and MapC) and *Listeria monocytogenes* 1-phosphatidylinositol phosphodiesterase PLC.

Further searches against the UniProt database [71] revealed no homologous records of MvpA and PLC from *Nitrospirae*, and only 109 out of 15,574 bacterial records for VapC were from *Nitrospirae*. These findings imply that there are many more *Nitrospirae* pathogens harboring MvpA and PLC that have yet to be discovered.

The screening of toxins in the 27 global microbiomes revealed the most prevalent toxins and pathogen-enriched environment. Specifically, the results showed that the RTX toxin RtxA and adenylate cyclase Cya were most prevalent globally in terms of relative abundance. RTX toxins comprise a large family of pore-forming exotoxins. Known homologs in the GenBank database of *Aeromonas dhakensis* RtxA were mainly in the genera of *Aeromonas*, *Pseudomonas* (e.g., CP015992), *Vibrio* (e.g., CP002556) and *Legionella* (e.g., CP015953). These genera are well known to be associated with gastroenteritis, eye and wound infections, cholera and legionellosis, and RTX toxins are a key part of the virulence systems of each of these conditions [72-75]. Cya is an essential unit of *Bacillus anthracis* virulence that causes anthrax and may lead to mammalian death [76]. Known homologs in the GenBank database of *Bacillus anthracis* Cya were mainly from *Bacillus* spp., *Bordetella* spp., *Pseudomonas aeruginosa*, *Yersinia pseudotuberculosis*, and *Vibrio* spp. Their presence in the environment should be carefully examined and precautions should be taken to prevent infection by these organisms since many of them are associated with very common diseases such as whooping cough.

The main purpose of the Local BLASTP method established here was to screen pathogen-enriched environments to enable development of precautionary measures. Our results clearly indicated that contaminated lake water, feces and wastewater microbiomes were rich in pathogens (Figure 3). Although there was no detailed background information regarding these environments in this study, the results presented herein may provide important implications for pathogen-related risk control. Surprisingly, two lake water microbiomes from Nanjing, China contained the highest toxin factors among the 27 samples. Further investigation of the location and contamination status supported the sewage-nature of the lake water. In China, most polluted lakes receive sewage that includes feces materials [77]. According to an official survey conducted in 2015, Nanjing has 28 lakes with a total area of 14 km², among which 96.7% are classified as polluted (Class V of the national standard). Studies have documented that pathogens tend to be enriched in polluted waters [14]. It is not surprising to find that feces samples had very high abundance of toxins. Epidemical statistics have indicated that feces are the most important pathway for diarrheal diseases, which is a leading cause of childhood death globally [78]. Thus, the present study provides a method for obtaining quantitative estimates of pathogen enrichment of various environments, and polluted freshwater systems are found to be highly pathogen-enriched relative to safer environments such as ocean water and natural soils.

Conclusions

A Local BLASTP procedure was established for rapid detection of toxins in environmental samples. Screening of global microbiomes in this study provided a quantitative estimate of the most prevalent toxins and most pathogen-enriched environments.

Declarations

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Competing interests

The author declares no conflict of interest.

Availability of data and materials

The toxin database is available in the Supplementary Materials. All toxin abundance data in this study can be provided by the author upon request.

Authors’ contributions

XL initiated the study, analyzed the data and wrote the manuscript.

Ethics approval

Not applicable.

Consent for publication

Not applicable.

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Figure captions

Figure 1 Correlation between relative abundance of toxins and secretion proteins in the global microbiomes.

Figure 2 Canonical correspondence analysis of the associations between phyla and toxins.

Figure 3 Circular visualization of the toxin abundance in the microbiomes selected from locations worldwide.

The designated environment was prefixed with the first letters of the environment names and suffixed with the

398 last three numbers of their MG-RAST ID in Table 1.

Table 1 General information regarding the metagenomes retrieved from the MG-RAST server

MG-RAST ID	bp Count	Sequence Count	Material	Location	Country	Coordinates	Sequencing Method	Protein Predicted
mgm4713202	89,726,117	442,552	Water	Moorea	Pacific Ocean	17.538, -149.829	Illumina	254,139
mgm4713205	106,474,596	476,363	Water	Moorea	Pacific Ocean	17.538, -149.829	Illumina	235,777
mgm4713197	60,417,678	272,918	water	Moorea	Pacific Ocean	17.538, -149.829	Illumina	140,195
mgm4719940	360,335,259	1,425,556	Water	Irish Sea	Atlantic Ocean	53.225, -4.159	Illumina	1,023,445
mgm4533707	134,833,790	668,257	Brackish Water	Columbia River margin, OR & WA coastal	USA	46.265, -123.999	Illumina	508,217
mgm4720261	35,487,527	6,896	Fresh Water	Galway	Ireland	53.276, -9.060	Illumina	33,743
mgm4695622	114,430,648	111,889	Fresh Water	Nanjing, Jiangsu	China	32.600, 118.160	Illumina	148,833
mgm4695626	86,732,360	78,621	Fresh Water	Nanjing, Jiangsu	China	32.400, 118.140	Illumina	111,489
mgm4589537	337,068,782	2,099,471	Surface Water	West Virginia	USA	38.094, -81.959	Illumina	1,842,975
mgm4679248	603,919,746	3,365,512	Soil	Seoul	South Korea	37.460, 126.948	Illumina	1,361,948
mgm4679254	689,019,062	3,688,750	Soil	Seoul	South Korea	37.459, 126.948	Illumina	1,966,121
mgm4514299	322,114,449	242,0832	Saline Desert Soil	Gujarat of India	India	23.7925, 71.008	Ion Torrent	1,323,378
mgm4543019	282,578,916	2,016,127	Saline Desert Soil	Gujarat of India	India	23.908, 70.538	Ion Torrent	842,475
mgm4697397	143,214,978	397,067	Organic Soil	Beijing	China	32.054, 118.763	Illumina	299,940
N/A	507,124,889	1,552,234	Shrub Land Soil	Mt Isa	Australia	20.440, 139.300	Illumina	1,413,889
N/A	532,850,584	1,632,914	Red Soil	Mt Isa	Australia	20.440, 139.300	Illumina	1,408,943
N/A	433,386,397	1,338,665	Red Soil Polluted	Mt Isa	Australia	20.440, 139.300	Illumina	1,081,822
mgm4507016	163,648,718	227,551	Feces	Bologna	Italy	44.495, 11.343	Illumina	250347
mgm4718752	329,518,322	1,312,822	Feces	Upstate NY	USA	42.668, -76.528	Illumina	950489
mgm4568577	10,065,266	50,137	Mine Water	Guangdong	China	24.503, 113.710	454	34,287
mgm4568580	12,911,442	62,018	Mine Water	Guangdong	China	22.940, 112.050	454	36,461
mgm4620491	52,759,415	244,855	Biosolides	Guelph ON Canada	Canada	43.545, -80.248	Illumina	238630
mgm4546371	84,424,005	907,785	Wastewater	Universiti Teknologi Malaysia	Malaysia	2.558, 104.642	Illumina	803,682
mgm4620487	147,523,219	696,132	Wastewater	Guelph ON Canada	Canada	43.5448, -80.248	Illumina	640,283
mgm4620488	115,131,556	578,337	Wastewater	Guelph ON Canada	Canada	43.545, -80.248	Illumina	537,267
mgm4560423	22734940	73479	Feces	Lake Eyasi, Tanzania	Tanzania	-3.635, 35.083	Illumina	76,569

mgm4440281	35439683	334386	Mine drainage	Soudan Mine	United States of America	47.819, -92.243	454	227,038
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Table 2 Typical virulence factors investigated in this study and their disease–relevance

Toxin factor	ID in the databse	Typical pathogens and disease	Role of the toxin	Reference
Mono(ADP-ribosyl)transferase	spvB	<i>Salmonella dublin</i> (gastroenteritis)	ADP-ribosylating, destabilizing cytoskeleton	[25]
Adenylate cyclase	cyaA	<i>Sa. choleraesuis</i> (typhoid fever) <i>Bordetella pertussis</i> (whooping cough) <i>Bacillus anthracis</i> (anthrax)	Pore-forming with cAMP-elevating activity	[26]
1-phosphatidylinositol phosphodiesterase	PLC	<i>Listeria monocytogenes</i> (listeriosis)	Lysis of the phagolysosomal membrane	[27]
Chlamydia protein associating with death domains	CADD	<i>Chlamydia trachomatis</i> (trachoma, urethritis, etc.)	Inducing cell apoptosis	[28]
Listeriolysin O	hly	<i>Li. monocytogenes</i> (listeriosis)	Pore forming, hemolysin	[29, 30]
Alveolysin	alo	<i>Ba. anthracis</i>	Pore-forming	[31]
Perfringolysin O	pfo	<i>Clostridium perfringens</i> (food poisoning)	Pore-forming	[32]
Glucosyltransferase toxin B	toxB	<i>Cl. sordellii</i> <i>Cl. difficile</i> (diarrhea)	Cytopathic effects	[33]
Shiga toxin 1	stx1	<i>Escherichia coli</i> (diarrhea) <i>Shigella dysenteriae</i> (Shigellosis)	Haemolytic uraemic syndrome	[20]
Shiga-like toxin 2	stx2	<i>Enterobacteria phage 933W</i> <i>E. coli</i>	Haemolytic uraemic syndrome	[34]
Leucotoxin	luk	<i>Staphylococcus aureus</i> (sinusitis, skin abscess)	Lysis of leukocytes	[35]
Exfoliative toxin	ET	<i>St. aureus</i>	Proteolytic activity	[36]
Toxin CdiA	cdiA	<i>E. coli</i> <i>Yersinia pestis</i> (plaque)	Decreasing aerobic respiration	[37]

RNA interferase	mazF/pemK/ndoA/relE/relK/relG/yoeB/higB/mvpA	<i>Proteus vulgaris</i> (wound infections) <i>Mycobacterium tuberculosis</i> (tuberculosis) <i>E. coli</i>	and ATP levels Cleavage of cellular mRNAs, inhibiting growth tRNase activities	[38-43]
Endonuclease VapC	vapC	<i>Shigella flexneri</i> (diarrhea) <i>Sa. Dublin</i> <i>My. Tuberculosis</i> <i>Coxiella burnetii</i> (Q fever) <i>Pseudomonas savastanoi</i>		[40]
Ornithine carbamoyltransferase	argK		Promoting survival and pathogenicity	[44]
Exotoxin A	ETA	<i>Ps. aeruginosa</i> (eye and wound infections)	ADP-ribosylating eukaryotic elongation factor 2	[45]
Hemolytic phospholipase C	plcH	<i>Ps. Aeruginosa</i> <i>Clostridium perfringens</i> (food poisoning)	Membrane-damaging	[46]
ADP-ribosyltransferase toxin	exoS	<i>P. aeruginosa</i>	Inhibition of phagocytosis	[47]
Exoenzyme U	exoU	<i>P. aeruginosa</i>	Membrane-lytic and cytotoxic	[48]
Dermonecrotic toxin	dnt	<i>Bo. pertussis</i>	Stimulating the assembly of actin stress fibers and focal adhesions	[49, 50]
Pertussis toxin subunit 1	ptxA	<i>Bo. pertussis</i>	Causing disruption of host cellular regulation	[49]

Repeats-in toxin	rtxA	<i>Legionella pneumophila</i> (Legionnaires' disease) <i>Aeromonas dhakensis</i> (gastroenteritis, septicemia) <i>Aeromonas</i> spp.	Adherence and pore forming	[51, 52]
Aerolysin	aerA		Cytolytic pore-forming	[53]
Cholera toxin secretion protein EpsF	epsF	<i>Le. pneumophila</i>	Toxin secretion	[54]
Zeta toxin family protein	ZETA	<i>Coxiella</i> sp. DG_40	Inhibiting cell wall biosynthesis	[55]
Toxin secretion ATP binding protein	LwT1SS	<i>Le. waltersii</i>	Toxin secretion	[56]
Outer membrane channel protein CpnT	cpnT	<i>My. tuberculosis</i>	Nutrient uptake	[57]
Type IV secretion system protein Ptl	ptlCH	<i>Bo. pertussis</i>	Secretion of pertussis toxin	[49]

Fig 1

Fig 2

Fig 3