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Shiga Toxin: Emerging Producer Strains, Prophylactic Approaches, and Application in Cancer Therapy

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Shiga toxin-producing *Escherichia coli* is the most prevalent bacterial strain responsible for Shiga toxin-related infections. While Shiga toxin is inherently toxic, it has potential therapeutic applications as a component of anticancer drugs. Despite its association with infections and harmful effects on human health, Shiga toxin is being explored as a viable element in drug delivery systems targeting cancer cells. The findings indicate that the production of mutated bacteria containing Shiga toxin is an effective preventive strategy for immunization against these toxins. Furthermore, the B subunit of Shiga toxin shows promise for imaging cancer cells, opening new paths for therapeutic interventions.

Key Words Shiga toxin, *Escherichia coli*, Cancer

INTRODUCTION

Shiga toxins (Stxs) are type 2 ribosome-inactivating proteins primarily produced by Stx-producing *Escherichia coli* (STEC) and were first identified in *Shigella dysenteriae* [1,2]. They are classified into Stx1 and Stx2, with various subtypes, and their nomenclature has been inconsistent, causing confusion in identifying STEC strains [3]. A comparison of the nucleotide sequences of Stx from *S. dysenteriae* and STEC revealed that the genes encoding these toxins are almost identical [4]. Stx2 infections are more common than Stx1, and risk factors include dietary habits, environmental exposure, and socioeconomic status. The toxins are genetically phage-derived, and hybrid *E. coli* strains may increase pathogenicity [5]. Hybrid strains of *E. coli* have been described to be highly pathogenic. The development of these strains may be attributed to outbreaks when a mixture of different virulence genes is present in a single strain [6].

Stx infections can cause severe conditions like hemorrhagic colitis and hemolytic uremic syndrome (HUS), with symptom severity varying among individuals. Some gut microbiota can inhibit Stx production, suggesting probiotics may boost immune responses [7]. It has been shown that the commensal bacteria of the gut microbiota prevent Stx production by producing short-chain fatty acids such as acetic acid, lactic acid, and propionic acid, which can lower the intestinal tract pH. Furthermore, enhancing the levels of immunoglobulin A and promoting inflammatory responses against Stxs are associated with the presence of probiotic bacteria [8]. Additionally, high levels of Stxs' receptor Gb3, found in cancer tissues, suggests its potential therapeutic applications in cancer treatment [5]. The present review summarizes studies describing the different characteristics of Stx and its producing strains with focus on Stx's role in cancer prevention and treatment. In addition, especially this study will investigate Stx's role in cancer prevention and treatment.

COMPARISON OF TOXICITY OF STX1 AND STX2

In 1993, Tesh et al. [9] found that toxicity of Stx2a is considerably lower than that of Stx1a in mice when administered intravenously or intraperitoneally. Subsequent studies in primate models indicated that Stx1 caused more severe kidney histopathological damage compared to Stx2 [10]. However, epidemiological studies show that infections from STEC strains producing only Stx2a, or both Stx1a and Stx2a, are more likely to provoke life-threatening conditions such as HUS and thrombotic thrombocytopenic purpura than those from strains producing only Stx1a [11]. For example, intravenous doses

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of Stx2 induced HUS, while equivalent doses of Stx1 did not [12]. Additionally, Stx2a was found to be more potent than Stx1a in mice and had a significantly higher cytotoxicity to human renal microvascular endothelial cells. Notably, the presence of Stx1a in certain strains can reduce overall morbidity and Stx1a's B subunit could decrease the toxicity of Stx2a in vivo [13]. The induction of stx1 expression does not cause bacterial lysis, with Stx1 mainly residing within bacterial cells, whereas *stx*2 gene transcription is closely linked to the phage lytic cycle and is more often associated with severe disease [14]. Studies have shown that Stx2a is more prevalent among HUS patients and commonly coexists with other STEC virulence factors [15]. Understanding the mechanisms underlying the greater potency of Stx2 is crucial for developing strategies to prevent *E. coli* O157:H7 infections from progressing to HUS. Stx2 also shows superior binding to Gb3 compared to Stx1 [16].

ESCHERICHIA COLI AS A PROMINENT BACTERIUM THAT PRODUCES SHIGA TOXIN

Stxs are present in various STEC serotypes, including O26, O45, O55, O91, O103, O104, O111, O113, O121, O128, O145, O146, and O157 [17]. Notably, the serotype O157:H7 evolved from the non-STEC O55:H7 through the acquisition of two prophages that encode Stx [18]. Enterohaemorrhagic *E. coli* (EHEC), a subset of STEC, is associated with severe illnesses like bloody diarrhea and HUS [19]. EHEC strains also contain an additional *eae* gene found in the locus of enterocyte effacement (LEE) Pathogenicity Island, marking them as the "classical" subset of STEC [20]. While Stx and the LEE are significant virulence factors for classical EHEC, virulence levels among Stx-encoding *E. coli* strains are highly variable, and the LEE is not consistently present in all pathogenic strains [2].

HYBRID STRAINS AND NON-*E. COLI* BACTERIA THAT PRODUCE SHIGA TOXIN

Hybrid pathotypes of *E. coli* are prevalent in outbreaks and consist of diverse strains that can share genetic Stx components with non-Stx-producing strains. These hybrid strains, including STEC/enterotoxigenic *E. coli* (ETEC) and STEC/uropathogenic *E. coli*, have been identified in both epidemic and non-epidemic contexts [21]. The Bai et al. [22] study identified clinical hybrids of STEC and ETEC. Notably, some hybrids, such as O15:H16, O187:H28, O100:H30, and O136:H12 serotypes, have also been isolated from individuals without any reported illness, indicating that healthy contacts can harbor

Table 1. *Stx* gene(s)-positive non-*Escherichia coli* bacteria, type/subtype of *stx* harbored, *stx* carrier and origin

Bacteria	Stx type/ subtype	*stx* gene carrier	Origin of *stx* gene	Source	Reference
Shigella flexneri serotype *2a*	Stx1	ΦPOC-J13 phage	-	Clinical sample	[30]
S. flexneri serotype Y	Stx1	ΦPOC-J13 phage	-	Clinical sample	[31,32]
S. flexneri serotype 2	Stx1a	-	-	Clinical sample	[33]
Shigella dysenteriae serotype 1	Stx1	-	Chromosome	-	[34,35]
S. dysenteriae serotype 4	Stx1	Phage	-	Clinical sample	[31,32]
Shigella sonnei	Stx1	Phage	*S. dysenteriae* serotype 1	Clinical sample	[36]
S. sonnei	Stx1, Stx2a	Phage	*E. coli*	Clinical sample	[37]
S. sonnei	Stx1	ΦSs-VASD phage	-	Clinical sample	[38]
Listeria	Stx1a	-	-	Animal manure	[39]
Aeromonas hydrophila F-0050	Stx1, Stx2	Extracellular DNA in outer membrane vesicles	STEC O157:H7 CECT 4076	Clinical sample	[40]
A. hydrophila	Shiga like toxin1	Plasmid	STEC O157:H7	Clinical sample	[41]
Aeromonas caviae	Shiga like toxin1	Plasmid	STEC O157:H7	Clinical sample	[41]
Aeromonas	Stx1, Stx2	Phage	-	Clinical sample	[42]
Salmonella enterica	Stx2f	-	-	-	[39]
Acinetobacter hemolyticus	Stx2	Phage	-	Clinical sample	[43]
Enterobacter cloacae M12X01451 strain	Stx1e	Phage	-	Clinical sample	[44]
E. cloacae strain 13047	Stx1e, Stx1a	Phage	*E. cloacae* M12X01451, *E. coli* RM8530	Designed in laboratory	[18]
Citrobacter freundii	Shiga like toxin2	-	STEC O157	Clinical sample	[45]
C. freundii RIT669	Shiga like toxin	-	-	Turtles	[46]
Citrobacter rodentium DBS770	Stx2d	Phage	-	Designed in laboratory	[47]

Stx, Shiga toxin; -, not applicable.

these hybrids. This suggests a broader ecological presence of these serotypes beyond clinical settings [22]. Gati et al. [23] explored hybrid strains producing both diarrhea and urinary tract infections, suggesting a link to selective pressures within specific *E. coli* populations. Notably, hybrids like O104:H4, which emerged during the 2011 outbreak in Germany, exhibited a hybrid genome more similar to enteroaggregative *E. coli* than EHEC. Other serotypes, such as O137:H6, have also been identified as hybrids [24,25]. Stxs are also produced by non-*E. coli* bacteria, not just STEC strains. These toxins can be found in various bacterial genera through lysogenic phage transduction, leading to their insertion into bacterial genomes. For example, *Campylobacter jejuni* produces Shiga-like toxins at lower levels, which are antigenically different from those produced by STEC. Ultimately, the level of toxin production by non-STEC bacteria determines its classification as Stx [26-29]. Table 1 and 2 show different Stx types and subtypes in non-*E. coli* bacteria and virulence genes identified in STEC hybrid strains, respectively [30-53].

TREATMENT/PROPHYLAXIS APPROACHES

Hybrid STEC strains primarily derived from *E. coli* typically do not produce Stxs and are associated with severe complications, such as bloody diarrhea and HUS. The serotype O157 is the most common in HUS cases, and a study indicated that pediatric patients under two are particularly affected [54]. High levels of Stx can trigger the SOS response in the host, leading to increased toxin production and worsened infections [55]. Research has explored antibiotics that inhibit transcription and translation to reduce Stx production in EHEC, suggesting potential treatments for Stx-related infections. Additionally, dietary factors, such as the level of butyrate and fiber intake, have been shown to influence Stx binding and uptake, affecting the severity of infections in mouse models. High-fiber diets were linked to increased STEC colonization and to worse health outcomes compared to low-fiber diets [56].

Table 2. Virulence genes in the STEC hybrid strains

Hybrid strain	Serotype	Combined virulence gene	Reference
EPEC/EHEC	-	*eae*A[a] + *stx*2[b]	[48]
EPEC/EHEC	-	*eae*A + *bfp*[c] + *stx*1,2	[48]
EHEC/ETEC	-	*stx*2 + *sta*[d] *stx*1 + *sta* *stx*1 + *sta* + *lt*[e]	[48]
EAEC/EHEC/ETEC	-	*eagg*[f] + *stx*1 + *sta*	[48]
EPEC/EHEC/ETEC	-	*eae*A + *stx*1 + *sta* *eae*A + *stx*1,2 + *lt* + *sta*	[48]
STEC/ETEC strain SE572	O187:H28	*stx*2 + *sta*4,5	[49]
STEC/ETEC strain SE573	O15:H16	*stx*2 + *sta*4	[49]
STEC/ETEC strain SE574	O136:H12	*stx*2 + *sta*4,5	[49]
STEC/ETEC strain SE575	O100:H30	*stx*2 + *sta*1	[49]
STEC/ETEC strain MFDS1016416	O100:H30	*stx*2A + *stx*2B	[50]
STEC/ETEC strain MFDS1015939	O168:H8	-	[50]
STEC/ETEC strain MFDS1016233	O8:H9	-	[50]
STEC/ETEC strain MFDS1016229	O155:H21	-	[50]
STEC/ETEC strain MFDS1016200	O141:H29	-	[50]
STEC/ETEC strain MFDS1016224	O174:H2	-	[50]
STEC/ETEC strain MFDS1007784	O2:H27	-	[50]
STEC/ETEC strain MFDS1009736	O148:H7	-	[50]
STEC/ETEC strain MFDS1012367	O2:H25	-	[50]
UPEC/STEC	-	*hly*A[g] + *cnf*[h] + *stx*	[51]
STEC/UPEC	-	*hly*A + *stx* + EHEC-*hly*AC*	[51]
UPEC/STEC/EAEC	-	*hly*A + *stx* + *ast*A[i]	[51]
EAEC/EHEC strain LB226692	O104:H4	*stx*2 + rfb_{O104}[j] + $fliC_{H4}$[k]	[52]
STEC/ETEC strain FE95160	O2:H25	*stx*2A + *stx*2B + *ast*A + *sta*1 + *hly*A-D	[53]
STEC/ETEC strain IH57218	O2:H27	*stx*1A + *stx*1B + *sta*1 + *hly*A-D	[53]
STEC/ETEC strain IH53473	O101:H33	*stx*1A + *stx*1B + *hly*A-D	[53]
STEC/$UPEC_{HM}$ strain 2018C-3367	O75:H7	*stx*2[b] + *fyu*A[l] + *vat*[m] + *yfc*V[n]	[18]
STEC/$UPEC_{HM}$ strain 2013C-3244	O1:K22:H4	*stx*2[b] + *fyu*A + *vat* + *yfc*V	[18]

STEC, Shiga toxin-producing *Escherichia coli*; EPEC, enteropathogenic *Escherichia coli*; stx, Shiga toxin; EHEC, enterohaemorrhagic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; EAEC, enteroaggregative *Escherichia coli*; UPEC, uropathogenic *Escherichia coli*; -, not applicable. [a]*Escherichia coli* attaching and effacing, [b]Shiga toxin, [c]bundle-forming pili, [d]heat-stable enterotoxin, [e]heat-labile enterotoxin, [f]entero adherent aggregative, [g]hemolysin,[h]cytotoxic necrotizing factor, [i]aggregative heat-stable toxin, [j]lipopolysaccharide, [k]flagellin, [l]ferric yersiniabactin, [m]vacuolating autotransporter toxin, [n]fimbrial protein.

VACCINES FOR SHIGA TOXIN-RELATED INFECTIONS

Research is exploring vaccines as alternatives to antibiotics for preventing Stx-related infections, as antibiotics can increase the risk of progressing to HUS. Vaccination against stx2d-producing *Citrobacter rodentium* has shown efficacy, and a conjugate vaccine protected mice against STEC O157:H7 and O91:H21 [55]. Cattle, asymptomatic carriers of STEC O157, are targeted for vaccination to reduce zoonotic transmission [57]. Studies demonstrated that adjuvant vaccines in cattle elicited strong immune responses, while vaccination of pigs reduced mortality from STEC [58]. Transcutaneous immunization with the Stx 1 B subunit (StxB1) in rabbits showed promise, improving weight gain and eliciting specific antibody responses [59]. Additionally, oral administration of a *Vibrio cholerae* strain produced neutralizing antibodies, and a mutant *Salmonella* strain expressing the StxB1 induced significant immune responses [48,60]. Overall, these findings encourage continued development of vaccines for STEC infections.

USING STX FOR CANCER THERAPY

The Gb3 receptor for Stx is overexpressed in cancer tissues, suggesting a potential path for using Stx in combination with therapeutic compounds for cancer imaging and targeted therapy [61]. However, Farkas-Himsley et al. [62] have noted that increased Gb3 levels are not directly indicative of malignancy but rather serve as a marker of cellular growth. Elevated levels of Gb3 have been observed in various cancers, including B-cell lymphomas and certain solid tumors like, such as testicular, breast, and ovarian carcinomas. Consequently, several planned Stxs have been developed and are currently under evaluation as potential anticancer agents. Additionally, researchers are exploring the potential of the non-active binding subunit, StxB, as a delivery mechanism for the treatment or imaging of Gb3-positive tumors [63].The attachment of a molecule to StxB or StxA can change the original Stx's chemical and physical properties, affecting its stability, immunogenicity, and biodistribution, which could influence off-target toxicity and efficacy [63]. Moreover, the small size of StxB may hinder its ability to bind to Gb3 when connected to larger molecules. Thus, ensuring stability and optimal pharmacokinetics is essential in developing engineered Stxs [64,65]. Key points regarding the application of Stxs in biomedical contexts, focusing on their therapeutic potential, challenges, and innovations in engineering for cancer treatment are summarized as follows: Stxs specifically target Gb3-positive cells and efficiently deliver toxins to the cytosol, surpassing endosomal degradation. Stxs are stable in extreme pH and protease presence, and can cross tissue barriers, enhancing biodistribution. Gb3 is overexpressed in various tumors (e.g., breast, ovarian, pancreatic, etc.), including metastatic cancers, suggesting a potential link to chemoresistance. Stxs may possess antineoplastic (anti-cancer) and anti-angiogenic properties, effectively inducing cancer cell apoptosis [62-70]. Shiga and Shiga-like toxins show promise for treating Gb3-expressing cancers due to their high specificity and ability to induce apoptosis. This apoptotic process involves increased levels of pro-apoptotic proteins and a reduction in anti-apoptotic ones. By selectively targeting specific signaling pathways, these toxins may minimize damage to normal tissues and serve as a foundation for developing targeted antitumor therapies [71].

PREFERENCE FOR MODIFIED STX TOXIN

Intratumor injections of Stx have improved survival in mice with various cancers. Holotoxins have significant toxicity (e.g., damage to endothelial cells) and high immunogenicity, which limits their clinical applications [61]. Testing Stx in clinical trials is also constrained by its immunogenicity, primarily attributed to the A subunit [63,72,73]. Preference for modified toxins over holotoxins to reduce immunogenicity while preserving therapeutic effects are as follows:

The antibody-coupled A subunit for cancer treatment

Immunotoxins that are Linking link toxin subunits to antibodies (immunotoxins) enhances specificity and have been utilized in Food and Drug Administration-approved treatments, though few have been approved [63,73].

Engineered toxin bodies

Molecular templates have developed engineered toxin bodies (ETBs), where a modified Stx1 A subunit is fused to antibody fragments for targeted therapy (e.g., MT-3724 for diffuse large B-cell lymphoma). Early trials of MT-3724 show promising results with manageable side effects. In the second-generation ETBs the deimmunized StxA are employed and are showing potential in targeting other markers (e.g., HER2 with MT-5111). In the third-generation ETBs, further ETBs are engineered to deliver antigens for immune recognition, such as MT-6402, targeting PD-L1 and enhancing immune response against tumors [63,74-77]. Danielewicz et al. [78] created a novel noncanonical amino acid, azido lysine incorporated-StxB1 (STxAZK), which effectively recognizes and binds to the Gb3 receptor, as confirmed by flow cytometry. Gb3 was detected in human colorectal adenocarcinoma cell lines, particularly abundant in HT-29 cells, and present in lower amounts in LS-174 cells. Moreover, STxAZK was combined with the anti-cancer prodrug monomethyl auristatin E, resulting in a highly effective formulation capable of eliminating HT-29 cancer cells [78].

UNIQUE PROPERTIES OF STXB FOR CANCER THERAPY

StxB retains significantly more binding sites on cancer cells compared to antibodies, making StxB a more effective vector for targeted delivery of cytotoxic drugs to Gb3-positive cancers. It can transport poorly soluble molecules and has been successfully coupled with various chemotherapy agents like doxorubicin, auristatin F, and SN38, demonstrating impressive potency against cancer cell lines, particularly with StxB-SN38 showing over 100 times stronger cytotoxicity than irinotecan [63]. StxB has also enhanced the efficacy of photosensitizers used in photodynamic therapy, showing improved delivery and effectiveness in targeting cells [79-83]. Recently, StxB has been shown to facilitated the delivery of engineered monobodies that inhibit oncogenic proteins, and studies indicate its potential for tumor suppression, although most research has been in vitro [84]. Additionally, StxB has potential in cancer immunotherapy as it can promote antigen presentation and T-cell responses, highlighting its versatility as a carrier in both drug delivery and vaccine development. Vaccination of mice with StxB conjugated to E7, a protein derived from HPV16, has demonstrated the ability to inhibit the growth of tumors that express E7 [63,85]. Therefore, StxB represents a promising and competitive candidate as a carrier protein for cancer vaccines. The StxB effectively targets antigens to dendritic cells (DC) in vivo, which enhances anti-tumor immunity [85]. By facilitating the delivery of these antigens, StxB promotes the activation of T-cells, leading to a stronger immune response against cancer cells. The findings suggest that StxB could serve as a valuable tool in cancer immunotherapy by improving antigen presentation and boosting the body's ability to fight tumors [85].

Some in vivo studies, such as the N8A-StxB fusion showing tumor growth suppression in mice, suggest that further optimization of StxB conjugates is necessary for enhanced efficacy in clinical settings [86]. They concluded STxB is a potent and versatile carrier protein for immunization. Since STxB binds to the same glycolipid, Gb3, in both human and mouse DC, and this receptor's distribution is consistent across species; the preclinical findings can likely be translated to human applications [85]. Table 3 summarizes the investigated anti-cancer or anti-tumor activities of Stx, highlighting its targets and mechanisms or effects [87-94].

KEY CHALLENGE IN UTILIZING STXS AS ANTICANCER AGENTS

A significant challenge in using Stxs as anticancer agents is the economic and environmental feasibility of their large-scale production, which is typically done in *E. coli*. However, these recombinant proteins often aggregate in inclusion bodies, limiting yield and requiring extensive processing, leading to further losses. Moreover, endotoxins produced during this process must be removed before human use. This subject contributed to the discontinuation of the clinical use of some approved immunotoxins [63,95].

CONCLUSION

Stxs are prominent protein synthesis-inhibiting toxins produced by STEC and pose significant health risks. To combat infections caused by these toxins, innovative vaccine candidates are being developed, as conventional antibiotic treatment becomes less viable due to rising bacterial resistance. Stx shows potential for targeted cancer therapy by exploiting specific cellular receptors commonly overexpressed in tumors. Stxs have evolved to be highly effective carriers for delivering toxic proteins to cells by binding to specific surface receptors. They can be utilized as anticancer agents in both their native and engineered forms, with ETBs showing particular promise. Recent advancements in genetic engineering facilitate the creation of immunotoxins. Ongoing research is critical to further establish Stx the efficacy and safety profile of Stx across varied cancer types.

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Table 3. Anti-cancer activities of Stx

Target	Mechanism	Reference
Cancer cells	Stx induces apoptosis through activation of caspase-3, leading to programmed cell death	[87,88]
Endothelial cells	Stx inhibits angiogenesis by binds to Gb3 receptors on endothelial and tumor cells	[66]
Tumor microenvironment	Stx can be used in antigen presentation studies, to induce protective cell mediated immunity to improve the clearance of certain tumors Stx can deliver antigen directly to dendritic cells	[85]
Glycolipid receptors	Stx binds to Gb3 receptors on cancer cells, resulting in cell death	[90-92]
Cell cycle regulators	Stx induces G1 phase arrest in cancer cells, leading to inhibited proliferation	[93]
Metastatic spread	Stx may disrupt cellular adhesion and migration, potentially reducing metastasis	[94]

Stx, Shiga toxin.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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REFERENCES

1. Chan YS, Ng TB. Shiga toxins: from structure and mechanism to applications. Appl Microbiol Biotechnol 2016;100:1597-610.
2. Monteiro R, Ageorges V, Rojas-Lopez M, Schmidt H, Weiss A, Bertin Y, et al. A secretome view of colonisation factors in Shiga toxin-encoding *Escherichia coli* (STEC): from enterohaemorrhagic *E. coli* (EHEC) to related enteropathotypes. FEMS Microbiol Lett 2016;363:fnw179.
3. Scheutz F, Teel LD, Beutin L, Piérard D, Buvens G, Karch H, et al. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. J Clin Microbiol 2012;50:2951-63.
4. Vishram B, Jenkins C, Greig DR, Godbole G, Carroll K, Balasegaram S, et al. The emerging importance of Shiga toxin-producing *Escherichia coli* other than serogroup O157 in England. J Med Microbiol 2021;70:001375.
5. Karmali MA. Emerging public health challenges of shiga toxin-producing *Escherichia coli* related to changes in the pathogen, the population, and the environment. Clin Infect Dis 2017;64:371-6.
6. Santos ACM, Santos FF, Silva RM, Gomes TAT. Diversity of hybrid- and hetero-pathogenic *Escherichia coli* and their potential implication in more severe diseases. Front Cell Infect Microbiol 2020;10:339.
7. Baker S. Bacterial enteropathogens. In: Farrar J, Hotez PJ, Junghanss T, Kang G, Lalloo D, White NJ, et al. eds. Manson's Tropical Diseases. Elsevier, pp 365-380, 2024.
8. Liu D, Li C, Cao T, Lv X, Yue Y, Li S, et al. *Bifidobacterium longum* K5 prevents enterohaemorrhagic *Escherichia coli* O157:H7 infection in mice through the modulation of the gut microbiota. Nutrients 2024;16:1164.
9. Tesh VL, Burris JA, Owens JW, Gordon VM, Wadolkowski EA, O'Brien AD, et al. Comparison of the relative toxicities of Shiga-like toxins type I and type II for mice. Infect Immun 1993;61:3392-402.
10. Stearns-Kurosawa DJ, Oh SY, Cherla RP, Lee MS, Tesh VL, Papin J, et al. Distinct renal pathology and a chemotactic phenotype after enterohemorrhagic *Escherichia coli* Shiga toxins in non-human primate models of hemolytic uremic syndrome. Am J Pathol 2013;182:1227-38.
11. Carrillo CD, Koziol AG, Mathews A, Goji N, Lambert D, Huszczynski G, et al. Comparative evaluation of genomic and laboratory approaches for determination of Shiga toxin subtypes in *Escherichia coli*. J Food Prot 2016;79:2078-85.
12. Fuller CA, Pellino CA, Flagler MJ, Strasser JE, Weiss AA. Shiga toxin subtypes display dramatic differences in potency. Infect Immun 2011;79:1329-37.
13. Creydt VP, Silberstein C, Zotta E, Ibarra C. Cytotoxic effect of Shiga toxin-2 holotoxin and its B subunit on human renal tubular epithelial cells. Microbes Infect 2006;8:410-9.
14. Feng PC, Reddy S. Prevalences of Shiga toxin subtypes and selected other virulence factors among Shiga-toxigenic *Escherichia coli* strains isolated from fresh produce. Appl Environ Microbiol 2013;79:6917-23.
15. Flagler MJ. Determination of the molecular basis for the difference in potency between Shiga toxins 1 and 2 [thesis]. Cincinnati, OH: University of Cincinnati; 2010.
16. Karve SS, Weiss AA. Glycolipid binding preferences of Shiga toxin variants. PLoS One 2014;9:e101173.
17. EFSA BIOHAZ Panel; Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bover-Cid S, Chemaly M, et al. Pathogenicity assessment of Shiga toxin-producing Escherichia coli (STEC) and the public health risk posed by contamination of food with STEC. EFSA J 2020;18:5967.
18. Khalil RK, Skinner C, Patfield S, He X. Phage-mediated Shiga toxin (Stx) horizontal gene transfer and expression in non-Shiga toxigenic *Enterobacter* and *Escherichia coli* strains. Pathog Dis 2016;74:ftw037.
19. Caprioli A, Morabito S, Brugère H, Oswald E. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Vet Res 2005;36:289-311.
20. Hauswaldt S, Nitschke M, Sayk F, Solbach W, Knobloch JK. Lessons learned from outbreaks of Shiga toxin producing *Escherichia coli*. Curr Infect Dis Rep 2013;15:4-9.
21. Lee W, Kim MH, Sung S, Kim E, An ES, Kim SH, et al. Genome-based characterization of hybrid Shiga toxin-producing and enterotoxigenic *Escherichia coli* (STEC/ETEC) strains isolated in South Korea, 2016-2020. Microorganisms 2023;11:1285.
22. Bai X, Zhang J, Ambikan A, Jernberg C, Ehricht R, Scheutz F, et al. Molecular characterization and comparative genomics of clinical hybrid Shiga toxin-producing and enterotoxigenic *Escherichia coli* (STEC/ETEC) srains in Sweden. Sci Rep 2019;9:5619.
23. Gati NS, Middendorf-Bauchart B, Bletz S, Dobrindt U, Mellmann A. Origin and evolution of hybrid Shiga toxin-producing and uropathogenic *Escherichia coli* strains of sequence type 141. J Clin Microbiol 2019;58:e01309-19.
24. Mellmann A, Harmsen D, Cummings CA, Zentz EB, Leopold SR, Rico A, et al. Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology. PLoS One 2011;6:e22751.
25. Gioia-Di Chiacchio RM, Cunha MPV, de Sá LRM, Davies YM, Pereira CBP, Martins FH, et al. Novel hybrid of typical

enteropathogenic *Escherichia coli* and Shiga-toxin-producing *E. coli* (tEPEC/STEC) emerging from pet birds. Front Microbiol 2018;9:2975.
26. Moore MA, Blaser MJ, Perez-Perez GI, O'Brien AD. Production of a Shiga-like cytotoxin by Campylobacter. Microb Pathog 1988;4:455-62.
27. Bolukaoto JY, Singh A, Alfinete N, Barnard TG. Occurrence of hybrid diarrhoeagenic *Escherichia coli* associated with multidrug resistance in environmental water, Johannesburg, South Africa. Microorganisms 2021;9:2163.
28. Nyholm O, Halkilahti J, Wiklund G, Okeke U, Paulin L, Auvinen P, et al. Comparative genomics and characterization of hybrid shigatoxigenic and enterotoxigenic *Escherichia coli* (STEC/ETEC) strains. PLoS One 2015;10:e0135936.
29. Lindsey RL, Prasad A, Feldgarden M, Gonzalez-Escalona N, Kapsak C, Klimke W, et al. Identification and characterization of ten *Escherichia coli* strains encoding novel Shiga toxin 2 subtypes, Stx2n as well as Stx2j, Stx2m, and Stx2o, in the United States. Microorganisms 2023;11:2561.
30. Zhi S, Parsons BD, Szelewicki J, Yuen YTK, Fach P, Delannoy S, et al. Identification of Shiga-toxin-producing Shigella infections in travel and non-travel related cases in Alberta, Canada. Toxins (Basel) 2021;13:755.
31. Gray MD, Lacher DW, Leonard SR, Abbott J, Zhao S, Lampel KA, et al. Prevalence of Shiga toxin-producing *Shigella* species isolated from French travellers returning from the Caribbean: an emerging pathogen with international implications. Clin Microbiol Infect 2015;21:765.e9-14.
32. Gray MD, Lampel KA, Strockbine NA, Fernandez RE, Melton-Celsa AR, Maurelli AT. Clinical isolates of Shiga toxin 1a-producing *Shigella flexneri* with an epidemiological link to recent travel to Hispañiola. Emerg Infect Dis 2014;20:1669-77.
33. Strockbine NA, Jackson MP, Sung LM, Holmes RK, O'Brien AD. Cloning and sequencing of the genes for Shiga toxin from Shigella dysenteriae type 1. J Bacteriol 1988;170:1116-22.
34. Beutin L, Strauch E, Fischer I. Isolation of *Shigella sonnei* lysogenic for a bacteriophage encoding gene for production of Shiga toxin. Lancet 1999;353:1498.
35. Lamba K, Nelson JA, Kimura AC, Poe A, Collins J, Kao AS, et al. Shiga toxin 1-producing *Shigella sonnei* infections, California, United States, 2014-2015. Emerg Infect Dis 2016;22:679-86.
36. Adams C, Vose A, Edmond MB, Lyckholm L. *Shigella sonnei* and hemolytic uremic syndrome: a case report and literature review. IDCases 2017;8:6-8.
37. Carter CC, Fierer J, Chiu WW, Looney DJ, Strain M, Mehta SR. A novel Shiga toxin 1a-converting bacteriophage of *Shigella sonnei* with close relationship to Shiga toxin 2-converting pages of *Escherichia coli*. Open Forum Infect Dis 2016;3:ofw079.
38. Wang X, Yu D, Chui L, Zhou T, Feng Y, Cao Y, et al. A comprehensive review on Shiga toxin subtypes and their niche-related distribution characteristics in Shiga-toxin-producing *E. coli* and other bacterial hosts. Microorganisms 2024;12:687.
39. Palma-Martínez I, Guerrero-Mandujano A, Ruiz-Ruiz MJ, Hernández-Cortez C, Molina-López J, Bocanegra-García V, et al. Active Shiga-like toxin produced by some *Aeromonas* spp., isolated in Mexico City. Front Microbiol 2016;7:1522.
40. Haque QM, Sugiyama A, Iwade Y, Midorikawa Y, Yamauchi T. Diarrheal and environmental isolates of *Aeromonas* spp. produce a toxin similar to Shiga-like toxin 1. Curr Microbiol 1996;32:239-45.
41. Alperi A, Figueras MJ. Human isolates of *Aeromonas possess* Shiga toxin genes (*stx1* and *stx2*) highly similar to the most virulent gene variants of *Escherichia coli*. Clin Microbiol Infect 2010;16:1563-7.
42. Grotiuz G, Sirok A, Gadea P, Varela G, Schelotto F. Shiga toxin 2-producing *Acinetobacter haemolyticus* associated with a case of bloody diarrhea. J Clin Microbiol 2006;44:3838-41.
43. Probert WS, McQuaid C, Schrader K. Isolation and identification of an *Enterobacter cloacae* strain producing a novel subtype of Shiga toxin type 1. J Clin Microbiol 2014;52:2346-51.
44. Bai L, Xia S, Lan R, Liu L, Ye C, Wang Y, et al. Isolation and characterization of cytotoxic, aggregative *Citrobacter freundii*. PLoS One 2012;7:e33054.
45. Thomas SG, Glover MA, Parthasarathy A, Wong NH, Shipman PA, Hudson AO. Expression of a Shiga-like toxin during plastic colonization by two multidrug-resistant bacteria, *Aeromonas hydrophila* RIT668 and *Citrobacter freundii* RIT669, isolated from endangered turtles (*Clemmys guttata*). Microorganisms 2020;8:1172.
46. Kimmitt PT, Harwood CR, Barer MR. Toxin gene expression by shiga toxin-producing Escherichia coli: the role of antibiotics and the bacterial SOS response. Emerg Infect Dis 2000;6:458-65.
47. Ling H, Pannu NS, Boodhoo A, Armstrong GD, Clark CG, Brunton JL, et al. A mutant Shiga-like toxin IIe bound to its receptor Gb(3): structure of a group II Shiga-like toxin with altered binding specificity. Structure 2000;8:253-64.
48. Russo LM, Melton-Celsa AR, Smith MJ, O'Brien AD. Comparisons of native Shiga toxins (Stxs) type 1 and 2 with chimeric toxins indicate that the source of the binding subunit dictates degree of toxicity. PLoS One 2014;9:e93463.
49. Siegler RL, Obrig TG, Pysher TJ, Tesh VL, Denkers ND, Taylor FB. Response to Shiga toxin 1 and 2 in a baboon model of hemolytic uremic syndrome. Pediatr Nephrol 2003;18:92-6.
50. Louise CB, Obrig TG. Specific interaction of *Escherichia coli* O157:H7-derived Shiga-like toxin II with human renal endothelial cells. J Infect Dis 1995;172:1397-401.
51. Petro CD, Trojnar E, Sinclair J, Liu ZM, Smith M, O'Brien AD, et al. Shiga toxin type 1a (Stx1a) reduces the toxicity of the more potent Stx2a *in vivo* and *in vitro*. Infect Immun 2019;87:e00787-18.
52. Szczerba-Turek A, Chierchia F, Socha P, Szweda W. Shiga toxin-producing *Escherichia coli* in faecal samples from wild ruminants. Animals (Basel) 2023;13:901.
53. Berger M, Aijaz I, Berger P, Dobrindt U, Koudelka G. Transcriptional and translational inhibitors block SOS response and Shiga toxin expression in enterohemorrhagic *Escherichia coli*. Sci Rep 2019;9:18777.
54. Dierick M, Ongena R, Vanrompay D, Devriendt B, Cox E.

Exploring the modulatory role of bovine lactoferrin on the microbiome and the immune response in healthy and Shiga toxin-producing *E. coli* challenged weaned piglets. J Anim Sci Biotechnol 2024;15:39.
55. Bowser S, Melton-Celsa A, Chapartegui-González I, Torres AG. Further evaluation of enterohemorrhagic *Escherichia coli* gold nanoparticle vaccines utilizing citrobacter rodentium as the model organism. Vaccines (Basel) 2024;12:508.
56. Babolhavaeji K, Shokoohizadeh L, Yavari M, Moradi A, Alikhani MY. Prevalence of Shiga toxin-producing *Escherichia coli* O157 and non-O157 serogroups isolated from fresh raw beef meat samples in an industrial slaughterhouse. Int J Microbiol 2021;2021:1978952.
57. Schaut RG, Boggiatto PM, Loving CL, Sharma VK. Cellular and mucosal immune responses following vaccination with inactivated mutant of *Escherichia coli* O157:H7. Sci Rep 2019;9:6401.
58. Lee SI, Ntakiyisumba E, Won G. Systematic review and network meta-analysis to compare vaccine effectiveness against porcine edema disease caused by Shiga toxin-producing *Escherichia coli*. Sci Rep 2022;12:6460.
59. Zhu C, Yu J, Yang Z, Davis K, Rios H, Wang B, et al. Protection against Shiga toxin-producing *Escherichia coli* infection by transcutaneous immunization with Shiga toxin subunit B. Clin Vaccine Immunol 2008;15:359-66.
60. Mauro SA, Koudelka GB. Shiga toxin: expression, distribution, and its role in the environment. Toxins (Basel) 2011;3:608-25.
61. Engedal N, Skotland T, Torgersen ML, Sandvig K. Shiga toxin and its use in targeted cancer therapy and imaging. Microb Biotechnol 2011;4:32-46.
62. Farkas-Himsley H, Hill R, Rosen B, Arab S, Lingwood CA. The bacterial colicin active against tumor cells in vitro and in vivo is verotoxin 1. Proc Natl Acad Sci USA 1995;92:6996-7000.
63. Robert A, Wiels J. Shiga toxins as antitumor tools. Toxins (Basel) 2021;13:690.
64. Hadjerci J, Billet A, Kessler P, Mourier G, Ghazarian M, Gonzalez A, et al. Engineered synthetic STxB for enhanced cytosolic delivery. Cells 2023;12:1291.
65. Mohseni Z, Sedighian H, Halabian R, Amani J, Behzadi E, Imani Fooladi AA. Potent in vitro antitumor activity of B-subunit of Shiga toxin conjugated to the diphtheria toxin against breast cancer. Eur J Pharmacol 2021;899:174057.
66. Heath-Engel HM, Lingwood CA. Verotoxin sensitivity of ECV304 cells in vitro and in vivo in a xenograft tumour model: VT1 as a tumour neovascular marker. Angiogenesis 2003;6:129-41.
67. Johansson D, Andersson C, Moharer J, Johansson A, Behnam-Motlagh P. Cisplatin-induced expression of Gb3 enables verotoxin-1 treatment of cisplatin resistance in malignant pleural mesothelioma cells. Br J Cancer 2010;102:383-91.
68. Arab S, Rutka J, Lingwood C. Verotoxin induces apoptosis and the complete, rapid, long-term elimination of human astrocytoma xenografts in nude mice. Oncol Res 1999;11:33-9.
69. Salhia B, Rutka JT, Lingwood C, Nutikka A, Van Furth WR. The treatment of malignant meningioma with verotoxin. Neoplasia 2002;4:304-11.
70. Ishitoya S, Kurazono H, Nishiyama H, Nakamura E, Kamoto T, Habuchi T, et al. Verotoxin induces rapid elimination of human renal tumor xenografts in SCID mice. J Urol 2004;171:1309-13.
71. Pinatih KJP, Suardana IW, Widiasih DA, Suharsono H. Shiga-like toxin produced by local isolates of *Escherichia coli* O157:H7 induces apoptosis of the T47 breast cancer cell line. Breast Cancer (Auckl) 2021;15:11782234211010120.
72. Bast DJ, Sandhu J, Hozumi N, Barber B, Brunton J. Murine antibody responses to the verotoxin 1 B subunit: demonstration of major histocompatibility complex dependence and an immunodominant epitope involving phenylalanine 30. Infect Immun 1997;65:2978-82.
73. Levine MM, McEwen J, Losonsky G, Reymann M, Harari I, Brown JE, et al. Antibodies to Shiga holotoxin and to two synthetic peptides of the B subunit in sera of patients with Shigella dysenteriae 1 dysentery. J Clin Microbiol 1992;30:1636-41.
74. Diaz Duque AE, Perekhrestenko T, Musteata V, Zodelava M, Guthrie TH, Strack T, et al. A phase II study of MT-3724, a novel CD20-targeting engineered toxin body, to evaluate safety, pharmacodynamics, and efficacy in subjects with relapsed or refractory diffuse large B-cell lymphoma. J Clin Oncol 2020;38:TPS8074.
75. Tache J, Katz DA, Mazumder A, Peace D, Burnett C, Strack T, et al. Abstract PO-63: a phase 2a open-label study of MT-3724, a novel CD20-targeting engineered toxin body, in combination with lenalidomide (LEN) in subjects with relapsed or refractory B-cell non-Hodgkin lymphoma (NHL). Blood Cancer Discov 2020;1:PO-63.
76. Kumar SK, Cornell RF, Landgren O, Ailawadhi S, Higgins JP, Willert EK, et al. A phase 1 first-in-human study of the anti-CD38 dimeric fusion protein TAK-169 for the treatment of patients (pts) with relapsed or refractory multiple myeloma (RRMM) who are proteasome inhibitor (PI)- and immunomodulatory drug (IMiD)-refractory, including pts relapsed/refractory (R/R) or naïve to daratumumab (dara). Blood 2019;134:1867.
77. Waltzman RJ, Sarkar A, Williams ET, Iberg AT, Higgins JT, Willert EK. MT-5111: a novel HER2 targeting engineered toxin body in clinical development. J Clin Oncol 2020;38:433.
78. Danielewicz N, Rosato F, Tomisch J, Gräber J, Wiltschi B, Striedner G, et al. Clickable Shiga toxin B subunit for drug delivery in cancer therapy. ACS Omega 2023;8:15406-21.
79. El Alaoui A, Schmidt F, Sarr M, Decaudin D, Florent JC, Johannes L. Synthesis and properties of a mitochondrial peripheral benzodiazepine receptor conjugate. ChemMedChem 2008;3:1687-95.
80. Kostova V, Dransart E, Azoulay M, Brulle L, Bai SK, Florent JC, et al. Targeted Shiga toxin-drug conjugates prepared via Cu-free click chemistry. Bioorg Med Chem 2015;23:7150-7.
81. Geyer PE, Maak M, Nitsche U, Perl M, Novotny A, Slotta-Huspenina J, et al. Gastric adenocarcinomas express the glycosphingolipid Gb3/CD77: targeting of gastric cancer cells with Shiga toxin B-subunit. Mol Cancer Ther 2016;15:1008-17.
82. Maak M, Nitsche U, Keller L, Wolf P, Sarr M, Thiebaud M, et al.

Tumor-specific targeting of pancreatic cancer with Shiga toxin B-subunit. Mol Cancer Ther 2011;10:1918-28.
83. Tarragó-Trani MT, Jiang S, Harich KC, Storrie B. Shiga-like toxin subunit B (SLTB)-enhanced delivery of chlorin e6 (Ce6) improves cell killing. Photochem Photobiol 2006;82:527-37.
84. Schmit NE, Neopane K, Hantschel O. Targeted protein degradation through cytosolic delivery of monobody binders using bacterial toxins. ACS Chem Biol 2019;14:916-24.
85. Vingert B, Adotevi O, Patin D, Jung S, Shrikant P, Freyburger L, et al. The Shiga toxin B-subunit targets antigen in vivo to dendritic cells and elicits anti-tumor immunity. Eur J Immunol 2006;36:1124-35.
86. Ryou JH, Sohn YK, Hwang DE, Park WY, Kim N, Heo WD, et al. Engineering of bacterial exotoxins for highly efficient and receptor-specific intracellular delivery of diverse cargos. Biotechnol Bioeng 2016;113:1639-46.
87. Johansson D, Kosovac E, Moharer J, Ljuslinder I, Brännström T, Johansson A, et al. Expression of verotoxin-1 receptor Gb3 in breast cancer tissue and verotoxin-1 signal transduction to apoptosis. BMC Cancer 2009;9:67.
88. Fujii J, Matsui T, Heatherly DP, Schlegel KH, Lobo PI, Yutsudo T, et al. Rapid apoptosis induced by Shiga toxin in HeLa cells. Infect Immun 2003;71:2724-35.
89. Dekker JD, Khanna S, Saputra E, Dong W, Aschenbach L, Rabia LA, et al. Abstract 1628: engineered toxin bodies targeting PD-L1 to alter tumor immunophenotypes and deliver broad antigenic diversity and patient coverage. Cancer Res 2021;81:1628.
90. Arab S, Russel E, Chapman WB, Rosen B, Lingwood CA. Expression of the verotoxin receptor glycolipid, globotriaosylceramide, in ovarian hyperplasias. Oncol Res 1997;9:553-63.
91. Gupta V, Bhinge KN, Hosain SB, Xiong K, Gu X, Shi R, et al. Ceramide glycosylation by glucosylceramide synthase selectively maintains the properties of breast cancer stem cells. J Biol Chem 2012;287:37195-205.
92. Ohyama C, Fukushi Y, Satoh M, Saitoh S, Orikasa S, Nudelman E, et al. Changes in glycolipid expression in human testicular tumor. Int J Cancer 1990;45:1040-4.
93. Suardana IW, Swacita IBN, Pinatih KJP, Suharsono H, Widiasih DA. Assessment of cell cycle and induction of apoptosis by Shiga-like toxin produced by Escherichia coli O157:H7 in T47D breast cancer cells using flow cytometry. Asian Pac J Cancer Prev 2022;23:3247-52.
94. Tomisch J, Busse V, Rosato F, Makshakova ON, Salavei P, Kittel AS, et al. A Shiga toxin b-subunit-based lectibody boosts T cell cytotoxicity towards Gb3-positive cancer cells. Cells 2023;12:1896.
95. Shafiee F, Aucoin MG, Jahanian-Najafabadi A. Targeted diphtheria toxin-based therapy: a review article. Front Microbiol 2019;10:2340.