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Invited Review Article

The role of bacterial Type VI secretion system (T6SS) in microbial competition in the gut

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Abstract

India has continuously progressed from domestic poultry farming to the techno-commercial sector to meet the increased consumer preference for quality eggs, meat, and meat products. However, its growing demand is often associated with the injudicious use of antimicrobials and growth promoters for gainful poultry production leading to the emergence of multidrug-resistant organisms. Our recent study revealed a novel feature of *Campylobacter jejuni (C. jejuni)* in utilizing a syringe-shaped puncturing device known as the Type-VI secretion system (T6SS) to arbitrate "self-destruction" under environmental stress. An in-depth understanding of the unique attributes of bacterial T6SS may provide insight into developing novel antibiotic alternative approaches in mitigating gut colonization of potentially harmful zoonotic pathogens, such as *C. jejuni.*

Keywords: Antimicrobial alternatives, *Campylobacter jejuni*, Microbial competition, Type VI secretion system (T6SS)

Highlights

- Bacterial T6SS contributes to intracellular communication and self-survival in the complex gut.
- Environmental stress can perturb the target-driven activity of bacterial T6SS.
- The functional attributes of T6SS can be utilized for targeted dysbiosis of gut pathogens.

INTRODUCTION

Selective targeting of potentially harmful gut pathogens without affecting the state of gut "eubiosis" (balanced gut microbiota) can address many challenges commonly faced by animal husbandry practices. Poultry gut, in particular, can host a large number of potentially harmful zoonotic pathogens, including *Campylobacter jejuni* (*C. jejuni*) which can persistently colonize the gut with little or no pathogenicity (Pielsticker *et al.*, 2012; Humphrey *et al.*, 2014). Therefore, poultry serves as the most implicated source for human transmission via consuming and handling contaminated poultry or poultry products.

The epidemiological status of *C. jejuni* prevalent in chickens is largely unknown in India since we do not have a national surveillance program for campylobacteriosis (Magana *et al.*, 2017). Hence, we do not know whether all the poultry-associated *C. jejuni* strains are capable of causing human infections. Our recent small population prevalence study reveals that the chicken gut hosts a wide range of *C. jejuni* genotypes encoding major virulence repertoire genes, including the Type VI Secretion System (T6SS), a newly discovered secretion machinery of *Campylobacter* sp. (Singh and Mallick, 2019; Gupta *et al.*, 2021). *C. jejuni*-T6SS resembles an inverted phage-like-puncturing device composed of 13 core proteins, TssA to M, encoded by a group of tightly clustered genes (Bleumink-Pluym *et al.*, 2013). The Valine-Glycine Repeat G (Vgr G or TssI) and Hemolysin Co-regulated Protein (Hcp or TssD) are primarily considered hallmarks of functional T6SS (Fig. 1).

In general, nearly 25 % of Gram-negative pathogens, including *Pseudomonas aeruginosa*, *Vibrio cholera* (Miyata *et al.*, 2011), *Burkholderia mallei* (Schwarz *et al.*, 2010), *Salmonella enterica* (Amaya *et al.*, 2022), and *C. jejuni* (Lertpiriyapong *et al.*, 2012; Bleumink-Pluym *et al.*, 2013; Singh and Mallick, 2019; Gupta *et al.*, 2021) have been reported to harbour T6SS. Recent studies by our group supported by others suggest that T6SS secretes a vast repertoire of effector proteins targeting host cells or competing bacteria in a contact-dependent manner (Monjarás Feria and Valvano, 2020). A functional T6SS facilitates bacterial predation (Cianfanelli *et al.*, 2016; Drebes Dörr and Blokesch, 2018), host cell adherence, and invasion, induces inflammatory responses in host, conferring a selective advantage over the other bacteria in the same

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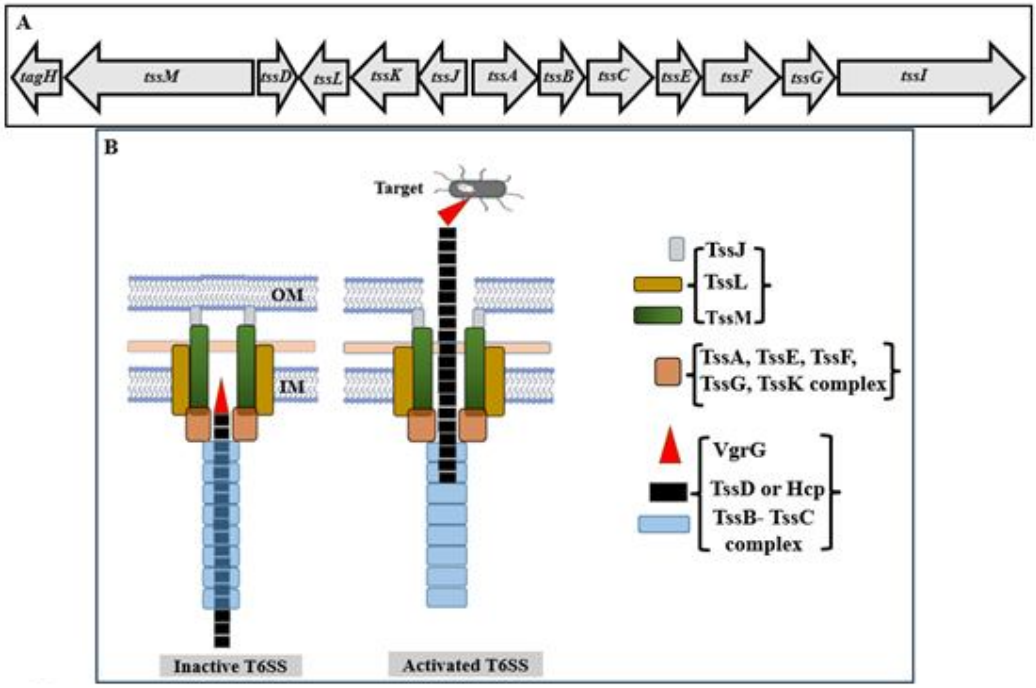
environmental niche (Basler and Mekalanos, 2012; Basler *et al*., 2013).

The first case of T6SS-positive *C. jejuni* was identified in 2011 in Canada from a human clinical sample (*C. jejuni* 43431), followed by the UK in 2012 (a bank vole isolates, *C. jejuni* 414). Subsequent studies in Egypt reported ~57.6% of *C. jejuni* tested positive for *tssD* or *hcp*. A contemporary study in Pakistan found that nearly 4.43% of chicken isolates, 1.64% of cow isolates, 15% of wildlife isolates, and 15.6% of sewage isolates were positive for the presence of *tssD* (Siddiqui *et al.*, 2015). In India, our small population prevalence study estimated that nearly 10% *C. jejuni* genotypes are positive for *tssD* or *hcp* T6SS (Singh and Mallick 2019; Gupta *et al.*, 2021). The whole genome analysis of one of these isolates confirms the presence of all 13 genes that encode a functional T6SS clustered in a single pathogenic locus (NCBI Reference Sequence: NZ_JAPCIC000000000.1) (Fig. 1) ("*Campylobacter jejuni* strain 18aM, whole genome shotgun sequencing project" 2022).

However, despite the proven role of *C. jejuni* T6SS in driving severe pathogenicity and cell cytotoxicity in humans, the functional characteristics of T6SS remain unclear (Gabbert *et al.*, 2023). With the availability of a complete genome sequence of *C. jejuni*, some novel perspectives on the T6SS function have been proposed, particularly toward its involvement in intra-/inter bacterial competition and host adaptation (Monjarás Feria and Valvano, 2020; Robinson *et al.*, 2021).

This brief review describes and summarises our current understanding of T6SS functionality in the complex gut environment. We also tried to cover a future perspective of T6SS on how further research can address the key challenges associated with animal health and production.



[Fig. 1.A: Diagaramatic representation of *C. jejuni* T6SS associated gene arrangement. A total of 13 genes (tssA to tssM) are represented by each box arrow. The direction of the arrows indicates the transcriptional orientation of each gene. The layout for the gene organization is prepared from the whole genome sequencing data of T6SS-positive *C. jejuni* (18aM) isolate (NZ_JAPCIC000000000.1) ("*Campylobacter jejuni* strain 18aM, whole genome shotgun sequencing project" 2022).

Fig. 1.B: Schematic of different subunits (colored differently) assembled to form complete T6SS of *C. jejuni* (active and inactive state). In the inactive state of T6SS, the membrane complex{TssJLM} remains tightly associated with the cytoplasmic baseplate {TssEFGK}. The TssD (Hcp) tube and extended TssBC sheath complex are assembled to form an extended sheath loaded with effector proteins. In the inactive state of T6SS, the unfolded syringe-shaped subunit {TssDBC-VgrG} is contained (retracted) within the periplasm-spanning channel. Following sensing the target, the activated T6SS transport the substrates/effectors from the cytoplasm to the outside of the bacterial cell in a Sec- or Tat-independent one-step process or deliver directly inside the target cells.]

Fig. 1(A, B). Linear layout of gene clusters encoding complete T6SS of *C. jejuni* and its structure

Bacterial T6SS in self-survival and fitness advantages in the gut environment

Commensal bacteria in the gut interact symbiotically with the hosts and play a major role in gut health and overall fitness (Wu and Wu, 2012; Belkaid and Hand, 2014). These effects are exhibited by modulating several host functions, including development, metabolism, and immunity (Carding *et al.*, 2015; Henry *et al.*, 2021). In contrast, common gut pathogens are equipped with several intrinsic mechanisms for subverting host defense, facilitating host pathogenicity, and enduring exposure to various environmental stressors (Tlaskalová-Hogenová *et al.*, 2011). The pathogenic gut microbiota often utilizes highly specialized secretion systems for self-survival, promoting subsequent disease progression and gaining competitive advantages (Kamada *et al.*, 2013; Chen *et al.*, 2019).

To this end, bacterial T6SS has emerged as a unique secretion machinery of Gram-negative bacteria that can function defensively or aggressively to facilitate self-survival and promote bacterial virulence (Basler and Mekalanos, 2012). Following sensing and recognizing a potential target, bacteria assemble the complete T6SS machinery as a "defensive tactic" known as 'tit-for-tat' (Basler *et al.*, 2013). In fact, some bacteria, such as *P. aeruginosa*, can determine the position of the initial assault and efficiently counterattack by firing the T6SS apparatus in the exact direction of the attacker (Basler *et al.*, 2013). In contrast, the T6SS of *V. cholerae* and *Serratia marcescens* are found to utilize T6SS in releasing effector molecules continuously and indiscriminately into the extracellular milieu (Basler *et al.*, 2012). The identification of the T6SS was first based on the fact that *V. cholerae* could kill *Dictyostelium discoideum* amoebae; the T6SS effector VasX was later shown to induce cytotoxicity by binding to host membrane lipids and interrupting host cell signaling in the amoebae (Pukatzki *et al.*, 2007). In line with these observations, our recent study found that T6SS-positive *C. jejuni* can effectively reduce the density of *E. coli* by restricting bacterial growth and alternating their morphology (elongation and polar shredding) (Gupta *et al.*, 2021). Interestingly, studies by another group of researchers showed that the T6SS-harboring gut pathogen, *Helicobacter pullorum*, can coexist with T6SS-positive *C. jejuni* but not T6SS-negative *C. jejuni* (Kanwal *et al.*, 2019).

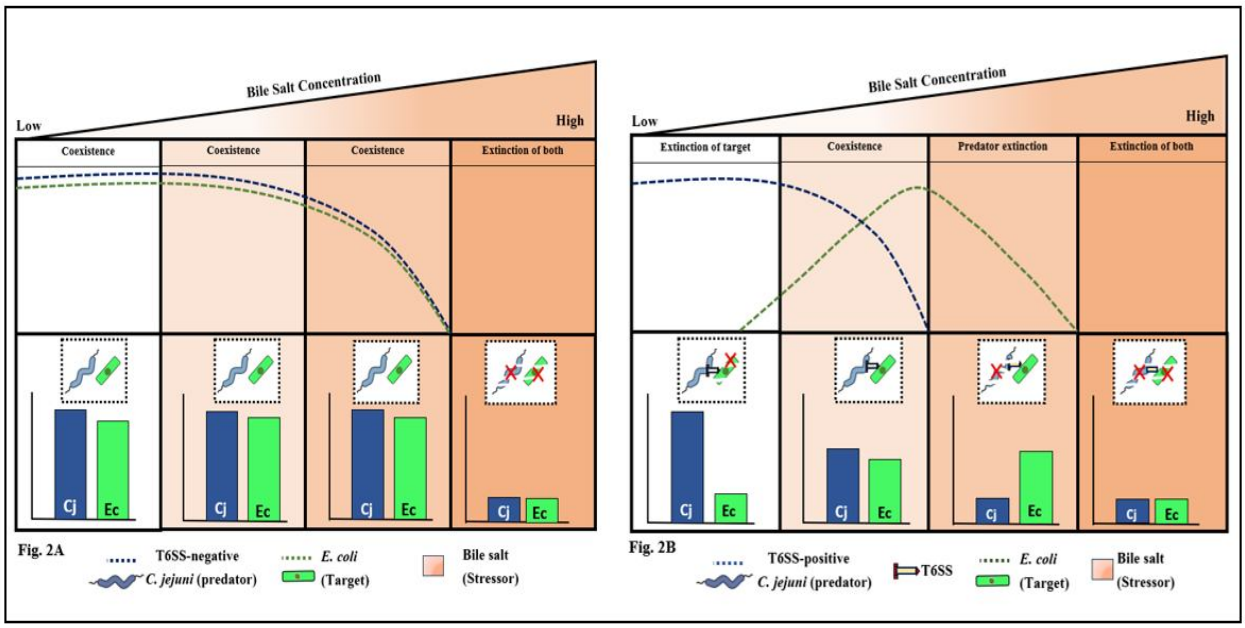
From the offensive perspective of T6SS functionality, it targets the prokaryotic and eukaryotic cells in a contact-dependent manner via dynamic cycles of assembly, contraction, and disassembly (Ho *et al.*, 2014; Hernandez *et al.*, 2020; Song *et al.*, 2021). Despite the competitive fitness advantage of the T6SS, recent studies suggest that its functionality can depend on the physico-chemical composition of the gut, such as pH, bile salt concentration, and other antimicrobial components (Chen *et al.*, 2019; Gupta *et al.*, 2021). However, an intriguing and open question is how such stressors/conditions affect the overall T6SS activity and the ultimate fate of T6SS-harboring pathogens. In particular, the question remains how T6SS-positive *C. jejuni* exhibits enteric commensalism in the chicken gut where many other bacteria coexist, including T6SS-negative *C. jejuni*.

Perturbation of T6SS functionality in the gut ecosystem

The gut microbial population forms a diverse ecosystem comprising many beneficial and harmful microbes, including bacteria, archaea, fungi, viruses, and bacteriophage. Our understanding of the ecological processes that lead to maintaining or disrupting gut homeostasis is being constantly updated. Recently, based on *in vitro* competition between T6SS-positive *C. jejuni* and non-pathogenic *E. coli* in the presence of bile salt, we showed the possibility of perturbing T6SS functionality, which can lead to self-destruction (Gupta *et al.*, 2021). As a dynamic and target-driven secretion machinery of *C. jejuni*, we hypothesized that functional T6SS in the presence of an appropriate stressor might exhibit two-way effector functions leading to amplifying the stress-induced self-intoxication (Fig. 2). In line with this presumption, a previous study (Van Alst and DiRita 2020; Crisan *et al.*, 2021) showed that by exploiting the glucose metabolism of some commensal bacteria, enteric colonization of multiple serotypes of *V. cholerae* could be restricted. This, in turn, suggests that a similar inhibitory effect can be achieved by careful tuning of the bacterial T6SS functionality. Specifically, this exciting feature of T6SS raises the possibility of applying them towards targeted dysbiosis of gut pathogens in food animals, including poultry.

Potential application of T6SS functionality toward improving gut health

Interference with the host responses or its intrinsic defense, when hijacked by gut pathogens for their benefit, results in traditional strategies failing to kill the pathogens or prevent their growth (Mues and Chu, 2020). The growing concern over the indiscriminate use of antibiotics in poultry and other food animals has left us with limited options (Martin *et al.*, 2015). Alternatives to antibiotics include nutritional growth promoter



[Model demonstrating how the stressor (bile salt) affects *C. jejuni* T6SS functionality (predation) and determines the ecological outcome. The T6SS-positive *C. jejuni* co-cultured with non-pathogenic *E. coli* in an optimal medium under varied bile salt concentrations showing differential upshots (target killing, co-existence, predator extinction or extinction of both competing bacteria). The color gradient panels (1-4) represent each possible outcome with the function of the stressor (bile-salt) concentration in the medium. The natural ability of T6SS-positive *C. jejuni* (grown under the physiological limit of bile salt) to exterminate their target (*E. coli*) can be perturbed under enhanced stress conditions (high bile salt concentration) (Fig. 2B). How the altered environmental conditions trigger the "predation cost" to T6SS-positive *C. jejuni,* leading to "self-destruction," remains unclear. However, stress-induced predation costs do not arise when T6SS-negative *C. jejuni* interacts with the targets (Fig. 2A), suggesting active involvement of functional T6SS during microbial interaction (Modified figures are adapted from (Gupta *et al.* 2021), with permissions)].

Fig. 2(A, B). Schematic of T6SS functionality during intra-species competition under environmental stress

supplementation, faecal microbiota transplantation, bacteriophage treatment, predatory bacteria, bacteriocins, and competitive pathogen exclusion (Allen *et al.*, 2014). Unfortunately, they cannot selectively exclude commensal and other beneficial gut microbial communities (Allen *et al.*, 2014). This is partly due to the dynamic and complicated nature of the gut environment, which covers an intricate setup comprising the epithelial barrier, the mucosal immune system, and a complex consortium of a diverse array of microbiota (Kho and Lal, 2018).

To this end, selective targeted "dysbiosis" of potentially harmful pathogens without affecting the state of gut "eubiosis" (balanced gut microbiota) should be ideal (Gagliardi *et al.*, 2018). This is, in particular, highly relevant for food animals, including poultry which serves as reservoirs for a range of beneficial as well as potentially harmful zoonotic pathogens. Moreover, for hosting pathogenic microbes in the gut, the host is expected to persuade immune tolerance, regulate inflammation, and compete with the resident microbiota for resource sharing, all of which incur an energy cost (Belkaid and Hand, 2014).

A growing body of evidence, including our ongoing research, suggests that *C. jejuni* T6SS, in addition to toxin delivery and cell-to-cell communication, also helps in the "predation" of other resident bacteria, mainly for competitive resource sharing and self-survival (Gupta *et al.*, 2021). Intriguingly, while the presence of T6SS in *C. jejuni* bids several advantages over T6SS-negative *C. jejuni* (Gupta *et al.*, 2021), bacterial T6SS activity under environmental stress can counter-intuitively mediates the 'self-destruction' of *C. jejuni* (Gupta *et al.*, 2021). To this end, exploring both the active and passive modes of T6SS interactions with the host and other microbial populations can help improve the existing frameworks for designing and predicting effective measures to control emerging infectious diseases of zoonotic importance.

Conclusion

The exclusive feature of T6SS functionality can be further explored to see whether the prey-driven activity of T6SS can be utilized as a " living therapeutic" to help clear the problem associated with persistent colonization of "hard-to-treat" gut pathogens such as

C. jejuni in poultry. Future research in this direction may also allow us to establish an exciting platform for addressing many challenging questions, including creating a road map for "One health approach".

Conflict of interest: The authors declare no conflict of interest.

Author's contribution: SG, AIM: Conceptualization, writing original draft; AIM: Interpretation of results; SG, AG, SD: Editing and reviewing.

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REFERENCES

Allen HK, Trachsel J, Looft T and Casey TA, 2014. Finding alternatives to antibiotics. Ann N Y Acad Sci, 1323(1): 91-100, doi: 10.1111/nyas.12468

Amaya FA, Blondel CJ, Barros-Infante MF, Rivera D, Moreno-Switt AI *et al*., 2022. Identification of Type VI secretion systems effector proteins that contribute to interbacterial competition in Salmonella Dublin. Front Microbiol, 13: 811932, doi: 10.3389/fmicb.2022.811932

Basler M and Mekalanos JJ, 2012. Type 6 secretion dynamics within and between bacterial cells. Science, 337(6096): 815, doi: 10.1126/science.1222901

Basler M, Pilhofer M, Henderson GP, Jensen GJ and Mekalanos JJ, 2012. Type VI secretion requires a dynamic contractile phage tail-like structure. Nature, 483(7388): 182-186, doi: 10.1038/nature10846

Basler M, Ho BT and Mekalanos JJ, 2013. Tit-for-tat: Type VI secretion system counterattack during bacterial cell-cell interactions. Cell, 152(4): 884-894, doi: 10.1016/j.cell.2013.01.042

Belkaid Y and Hand TW, 2014. Role of the microbiota in immunity and inflammation. Cell, 157(1): 121-141, doi: 10.1016/j.cell.2014.03.011

Bleumink-Pluym NMC, van Alphen LB van, Bouwman LI, Wösten MMSM and van Putten JPM van, 2013. Identification of a functional Type VI secretion system in *Campylobacter jejuni* conferring capsule polysaccharide sensitive cytotoxicity. PLOS Pathog, 9(5): e1003393, doi: 10.1371/journal.ppat.1003393

Campylobacter jejuni strain 18aM, whole genome shotgun sequencing project, 2022. http://www.ncbi.nlm.nih.gov/nuccore/NZ_JAPCIC000000000.1 [Accessed on 31 July 2023]

Carding S, Verbeke K, Vipond DT, Corfe BM and Owen LJ, 2015. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis, 26: 10.3402/mehd.v26.26191

Chen C, Yang X and Shen X, 2019. Confirmed and potential roles of bacterial T6SSs in the intestinal ecosystem. Front Microbiol, 10: 1484, doi: 10.3389/fmicb.2019.01484

Cianfanelli FR, Monlezun L and Coulthurst SJ, 2016. Aim, load, fire: The Type VI secretion system, a bacterial nanoweapon. Trends Microbiol, 24(1): 51-62, doi: 10.1016/j.tim.2015.10.005

Crisan CV, Nichols HL, Wiesenfeld S, Steinbach G, Yunker PJ *et al*., 2021. Glucose confers protection to *Escherichia coli* against contact killing by *Vibrio cholerae*. Sci Rep, 11(1): 2935, doi: 10.1038/s41598-021-81813-4

Drebes Dörr NC and Blokesch M, 2018. Bacterial type VI secretion system facilitates niche domination. Proc Natl Acad Sci USA, 115(36): 8855-8857, doi: 10.1073/pnas.1812776115

Gabbert AD, Mydosh JL, Talukdar PK, Gloss LM, McDermott JE *et al*., 2023. The missing pieces: The role of secretion systems in *Campylobacter jejuni* Virulence. Biomolecules, 13(1): 135, doi: 10.3390/biom13010135

Gagliardi A, Totino V, Cacciotti F, Iebba V, Neroni B *et al*., 2018. Rebuilding the gut microbiota ecosystem. Int J Environ Res Public Health, 15(8): 1679, doi: 10.3390/ijerph15081679

Gupta S, Ray S, Khan A, China A, Das D *et al*., 2021. The cost of bacterial predation via type VI secretion system leads to predator extinction under environmental stress. Science, 24(12): 103507, doi: 10.1016/j.isci.2021.103507

Henry LP, Bruijning M, Forsberg SKG and Ayroles JF, 2021. The microbiome extends host evolutionary potential. Nat Commun, 12(1): 5141, doi: 10.1038/s41467-021-25315-x

Hernandez RE, Gallegos-Monterrosa R and Coulthurst SJ, 2020. Type VI secretion system effector proteins: effective weapons for bacterial competitiveness. Cell Microbiol, 22(9): e13241, doi: 10.1111/cmi.13241

Ho BT, Dong TG and Mekalanos JJ, 2014. A view to a kill: The bacterial type VI secretion system. Cell Host Microbe, 15(1): 9-21, doi: 10.1016/j.chom.2013.11.008

Humphrey S, Chaloner G, Kemmett K, Davidson N, Williams N *et al*., 2014. *Campylobacter jejuni* is not merely a commensal in commercial broiler chickens and affects bird welfare. mBio, 5(4): e01364-14, doi: 10.1128/mBio.01364-14

Kamada N, Chen GY, Inohara N and Núñez G, 2013. Control of pathogens and pathobionts by the gut microbiota. Nat Immunol, 14(7): 685-690, doi: 10.1038/ni.2608

Kanwal S, Noreen Z, Aalam V, Akhtar J, Masood F *et al*., 2019. Variation in antibiotic susceptibility and presence of type VI secretion system (T6SS) in *Campylobacter jejuni* isolates from various sources. Comp Immunol Microbiol Infect Dis, 66: 101345, doi: 10.1016/j.cimid.2019.101345

Kho ZY and Lal SK, 2018. The human gut microbiome- A potential controller of wellness and disease. Front Microbiol, 9: 1835, doi: 10.3389/fmicb.2018.01835

Lertpiriyapong K, Gamazon ER, Feng Y, Park DS, Pang J *et al*., 2012. *Campylobacter jejuni* Type VI secretion system: roles in adaptation to deoxycholic acid, host cell adherence, invasion, and *in vivo* colonization. PLoS One, 7(8): e42842,

doi: 10.1371/journal.pone.0042842

Magana M, Chatzipanagiotou S, Burriel AR and Ioannidis A, 2017. Inquiring into the gaps of Campylobacter surveillance methods. Vet Sci, 4(3): 36, doi: 10.3390/vetsci4030036

Martin MJ, Thottathil SE, and Newman TB, 2015. Antibiotics overuse in animal agriculture: A call to action for health care providers. Am J Public Health, 105(12): 2409-2410, doi: 10.2105/AJPH.2015.302870

Miyata ST, Kitaoka M, Brooks TM, McAuley SB and Pukatzki S, 2011. *Vibrio cholerae* requires the Type VI secretion system virulence factor VasX to kill *Dictyostelium discoideum*. Infect Immun, 79(7): 2941-2949, doi: 10.1128/IAI.01266-10

Monjarás Feria J and Valvano MA, 2020. An overview of anti-eukaryotic T6SS effectors. Front Cell Infect Microbiol, 10: 584751, doi: 10.3389/fcimb.2020.584751

Mues N and Chu HW, 2020. Out-smarting the host: bacteria maneuvering the immune response to favor their survival. Front Immunol, 11: 819, doi: 10.3389/fimmu.2020.00819

Pielsticker C, Glünder G and Rautenschlein S, 2012. Colonization properties of *Campylobacter jejuni* in chickens. Eur J Microbiol Immunol (Bp), 2(1): 61-65, doi: 10.1556/EuJMI.2.2012.1.9

Pukatzki S, Ma AT, Revel AT, Sturtevant D and Mekalanos JJ, 2007. Type VI secretion system translocates a phage tail spike-like protein into target cells where it cross-links actin. Proc Natl Acad Sci USA, 104(39): 15508-15513, doi: 10.1073/pnas.0706532104

Robinson L, Liaw J, Omole Z, Xia D, van Vliet AHM *et al*., 2021. Bioinformatic analysis of the *Campylobacter jejuni* type VI secretion system and effector prediction. Front Microbiol, 12: 694824, doi: 10.3389/fmicb.2021.694824

Schwarz S, West TE, Boyer F, Chiang W-C, Carl MA *et al*., 2010. Burkholderia Type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions. PLoS Pathog, 6(8): e1001068, doi: 10.1371/journal.ppat.1001068

Siddiqui F, Champion O, Akram M, Studholme D, Eqani SAMA *et al*., 2015. Molecular detection identified a type six secretion system in *Campylobacter jejuni* from various sources but not from human cases. J Appl Microbiol, 118(5): 1191-1198, doi: 10.1111/jam.12748

Singh A and Mallick AI, 2019. Role of putative virulence traits of *Campylobacter jejuni* in regulating differential host immune responses. J Microbiol (Seoul, Korea), 57(4): 298-309, doi: 10.1007/s12275-019-8165-0

Song L, Pan J, Yang Y, Zhang Z, Cui R *et al*., 2021. Contact-independent killing mediated by a T6SS effector with intrinsic cell-entry properties. Nat Commun, 12: 423, doi: 10.1038/s41467-020-20726-8

Tlaskalová-Hogenová H, Štûpánková R, Kozáková H, Hudcovic T, Vannucci L *et al*., 2011. The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. Cell Mol Immunol, 8(2): 110-120, doi: 10.1038/cmi.2010.67

Van Alst AJ and DiRita VJ, 2020. Aerobic metabolism in *Vibrio cholerae* is required for population expansion during infection. mBio, 11(5): e01989-20, doi: 10.1128/mbio.01989-20

Wu H-J and Wu E, 2012. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes, 3(1): 4-14, doi: 10.4161/gmic.19320

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