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Current Opinion in Microbiology

Type VI secretion and anti-host effectors

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Secretion systems play a central role in infectious diseases by enabling pathogenic bacteria to deliver virulence factors into target cells. The type VI secretion system (T6SS) mediates bacterial antagonism in various environments including eukaryotic niches, such as the gut. This molecular machine injects lethal toxins directly in target bacterial cells. It provides an advantage to pathogens encountering the commensal flora of the host and indirectly contributes to colonization and persistence. Yet, the T6SS is not employed for the sole purpose of bacterial killing and several T6SS effectors are dedicated to the subversion of eukaryotic cells. As described for type III and type IV secretion systems, these effectors impede host cell functions and promote immune evasion, thereby enabling successful infection.

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Current Opinion in Microbiology 2016, **29**:81–93

This review comes from a themed issue on **Host-microbe interactions: bacteria**

Edited by **Elizabeth Hartland** and **Anthony Richardson**

For a complete overview see the Issue and the Editorial

Available online 24th December 2015

http://dx.doi.org/10.1016/j.mib.2015.11.006

1369-5274/Published by Elsevier Ltd.

Introduction

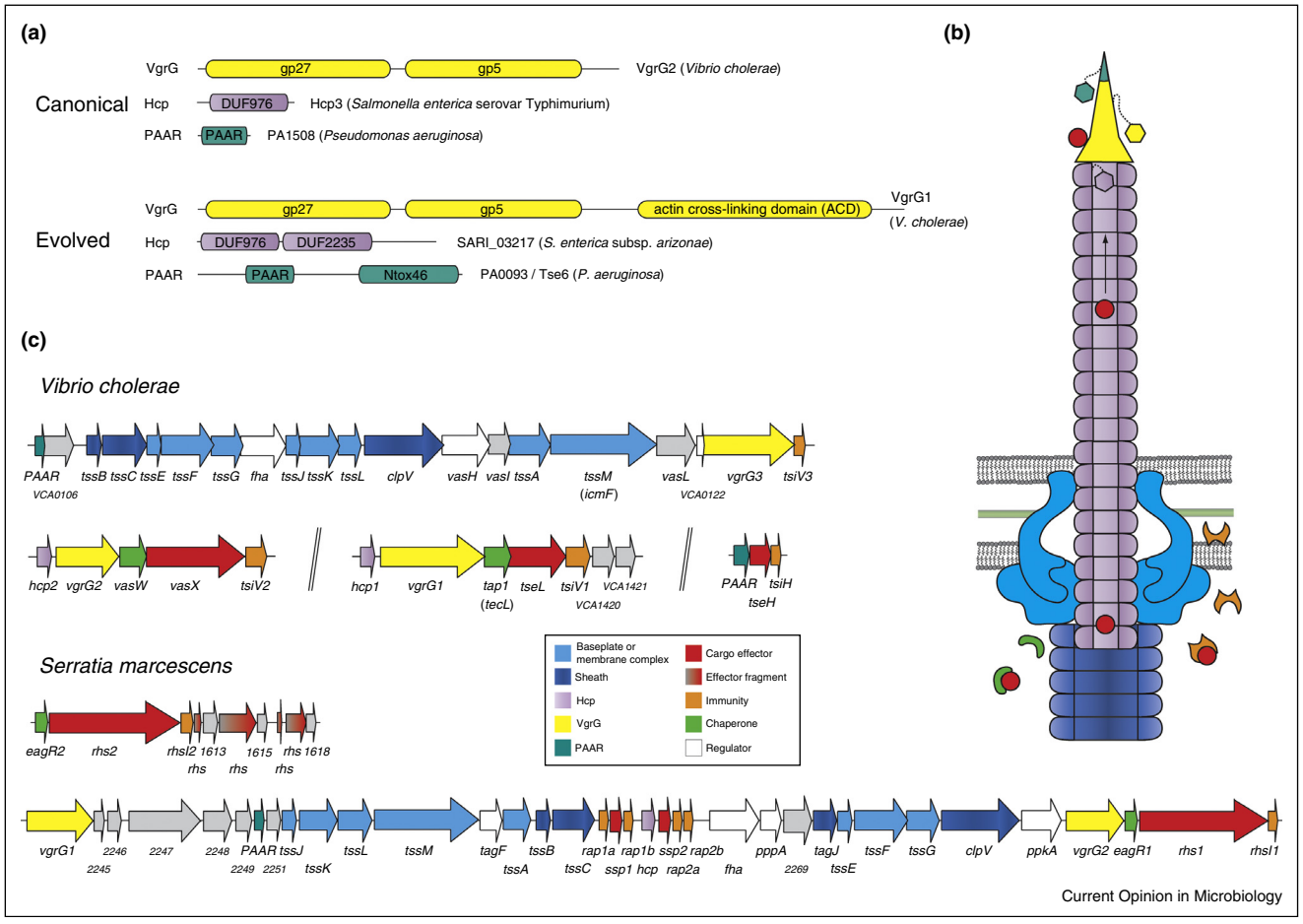
Bacteria have evolved several strategies and molecular mechanisms allowing colonization and survival within diverse hosts. Amongst them, protein secretion systems exert a critical role and deliver a plethora of enzymes/toxins/effectors into the environment, a competitor or a host. In Gram-negative bacteria, the type VI secretion system (T6SS) is a contact-dependent secretion mechanism. It is mainly present in proteobacteria and has been extensively described as a nano-machine targeting competing Gram-negative bacteria, thereby mediating bacterial antagonism in various environmental niches. T6SS-dependent bacterial killing operates via the injection of an array of toxins targeting various cellular processes of the prey. The attacker is protected owing to the presence of cognate immunity proteins that are absent from the target cells [1]. Strikingly, these toxins are crucial for optimal fitness during host colonization where the pathogen could displace host commensal bacteria (dysbiosis) [2•] or eliminate bacterial competitors in order to establish a niche [3•,4•].

Genes encoding components of the T6SS, which is depicted as an intracellular contractile and membrane bound molecular machine, are found in up to 25% of Gram-negative bacteria, including several human and animal pathogens [5]. Although most of these bacteria harbor a single T6SS cluster, multiple distinct copies have been identified within several species. For example *Burkholderia pseudomallei* and *Burkholderia thailandensis* display six and five T6SSs, respectively, and *Pseudomonas aeruginosa* possesses three T6SSs (H1-T6SS, H2-T6SS, and H3-T6SS) [6•,7]. Interestingly, these clusters do not seem functionally redundant, as in *B. thailandensis*, T6SS-1 is used to mediate bacterial antagonism, while T6SS-5 promotes intercellular spreading in infected macrophages [6•]. Similarly, in *P. aeruginosa* the H1-T6SS cluster is devoted to bacterial killing while the H2-T6SS has been shown to target both prokaryotic and eukaryotic cells [1,8,9••]. This versatility towards cell targets is also apparent in *Vibrio cholerae* as its single T6SS targets both cell types [10••,11]. Based on its distribution across bacterial phyla, three classes of T6SS have been described in the literature, namely T6SS^{I}, T6SS^{II} and T6SS^{III}, with the latter two classes only beginning to be characterized. Class III is restricted to the *Bacteroidetes* phylum and is involved in bacterial competition, while class II is found in one order of γ-proteobacteria that includes *Francisella* species, and is essential for their virulence [2•,12]. In this review, we will summarize the role and impact of identified T6SS^{I}s that contribute directly to host cell subversion and pathogenesis, and detail the activities of the best-characterized T6SS anti-host effectors.

The T6SS as a molecular syringe

A clear parallel between key components of the T6SS and elements of the contractile tail of the T4 bacteriophage has been established from the beginning of T6SS research [13]. Initially, a hallmark of this system was based on an ability to secrete two categories of proteins *in vitro*: Hcp (hemolysin co-regulated protein)

Figure 1



The T6SS secretion system. **(a)** T6SS spike/tube proteins can be canonical or evolved. Canonical proteins are thought to have solely a structural or mechanical purpose, while evolved components bear an additional C-terminal domain with catalytic activity. Examples of canonical VgrG, Hcp and PAAR proteins include *V. cholerae* O1 bv. El Tor str. N16961 VgrG2; *S. enterica* serovar Typhimurium LT2 Hcp3; and *P. aeruginosa* PAO1 PA1508, respectively. The first T6SS effector discovered was *V. cholerae* VgrG1, which carries an actin cross-linking domain (ACD) [35]. *S. enterica* subsp. *arizonae* (III) Hcp protein SARI_03217 appears to harbor DUF2235 domain through Phyre analysis [71]. This hydrolase domain belongs to the T6SS lipase superfamily Tle1, so SARI_03217 may exhibit phospholipase A_2 activity [8,72]. The *P. aeruginosa* anti-bacterial toxin PA0093/Tse6 is an evolved PAAR protein with a C-terminal Ntox46 domain. This domain is part of the Toxin_61 superfamily, and degrades NAD^+ and $NADP^+$ in targeted bacteria [22,73,74]. **(b)** Schematic of the T6SS in its contracted state. The tube/spike piece can be decorated with either effector domains from evolved VgrG, Hcp and PAAR proteins, and possibly cargo effectors as well, giving rise to a modular delivery mechanism [14,22]. Other cargo effectors may use Hcp as a chaperone, which would enable transit through the nanotube conduit [23], whereas other chaperone proteins such as Tap/Tec and EagR1 are essential for the secretion of their cognate effector [24–26]. Cognate immunity proteins protect the donor bacterium from the deleterious effects of the toxins. **(c)** Representations of the T6SS clusters of *V. cholerae* and *Serratia marcescens* subsp. *marcescens* Db10, used as examples of bacteria encoding a single T6SS apparatus and which also contain genomic islands encoding VgrG, Hcp, PAAR, chaperone and effector proteins.

and VgrG (Valine Glycine repeat protein) [10••]. The Hcp protein is thought to act as the counterpart of the gp19 protein forming the tail tube of the T4 bacteriophage [13]. This tube is capped by a tail spike, formed by VgrG proteins, which is further sharpened by a proline-alanine-alanine-arginine (PAAR) motif-containing proteins [14••]. The tube and spike are now considered to be the puncturing device of the machinery that allows the transport and subsequently the delivery of toxins/effectors into target cells. The tube/spike structure is propelled from the bacterium into the target cell by the membrane-associated T6SS machine, which is composed of at least 13 core components generically called Tss proteins (Figure 1). The contraction of the TssB/C sheath, which accommodates the tube/spike, appears to be the driving force of the T6SS dynamics [15]. However, the understanding of the assembly and dynamics of the machinery is still in its infancy, as several components of the apparatus are yet to be fully characterized [16,17].

A nascent and modular repertoire of T6SS effectors

VgrG proteins are formed by at least two domains similar to those of the phage spike: the N-terminal gp27-like and the C-terminal gp5-like domains, which constitute what we will call the canonical VgrG. However, some VgrGs present C-terminal extensions adjoining the gp5 region and which could bear catalytic domains; qualifying them as potential effectors (Figure 1a) [18,19]. The enzymatic activity of these 'evolved VgrGs' is dispensable for the puncturing and delivery functions of these proteins [20]. There are recent observations that such catalytic domains could be fused to other components of the tube/spike, thus suggesting the existence of evolved Hcp and PAAR proteins [14••,21]. Importantly, not all effectors are evolved structural proteins as in numerous cases, distinct cargo effectors are hypothesized to interact with VgrG, Hcp or PAAR proteins in an 'à la carte delivery' mechanism [14••,22,23]. The modularity of the T6SS spike can then be specified either by effectors directly binding VgrG proteins or possibly via the recently described adaptors and chaperones (Tec/Tap-1 in *V. cholerae* and *Aeromonas hydrophila;* and EagR in *Serratia marcescens*) which may load effectors onto the VgrG spike (Figure 1b) [22,24–26]. Effector-encoding genes are not necessarily linked to T6SS clusters, rendering their identification by genome mining rather cumbersome. The recent description of MIX motifs was proposed to enable the identification of proteins secreted by the T6SS [27], however the validity and stringency of these signatures are yet to be verified for most T6SS-positive strains. Nonetheless, it is now recognized that genetic islands, encoding VgrG, Hcp, PAAR, Tec or EagR proteins, scattered throughout bacterial genomes could pinpoint potential uncharacterized T6SS effectors (Figure 1c).

General impact of the T6SS on eukaryotic cells

Hypothesized as a virulence factor, much of the early work on the T6SS focused on its role during host–pathogen interactions, but this has been relegated by the strong antibacterial focus that has swept through the field. Several studies reported the induction of T6SS loci in infection models and using eukaryotic cell lines, suggesting that host subversion is a major function of the secretion system. Indeed, loss of a functional T6SS often impacts the pathogenicity of bacteria towards their hosts. Despite the significant number of these observations, which are recapitulated in Table 1, the characterization of effectors responsible for these phenotypes remains by and large elusive.

The impact on pathogenicity may also be indirectly related to T6SS activity as several groups have demonstrated that mutations in T6SS genes often result in the downregulation of other virulence factors including type I fimbriae and flagella [28–30] or could interfere with biofilm formation [6•,30,31], thereby obscuring the role of T6SS effectors during infection. Notwithstanding a possible indirect effect on pathogenicity, purified Hcp and VgrG proteins from pathogenic *Escherichia coli* strains and *A. hydrophila* can influence host functions including phagocytosis and the inflammatory response [30,32–34]. However, these isolated observations may not be generally applicable to these components from all T6SSs.

Identified T6SS effectors targeting host cell functions

From the substantial T6SS literature to date, several reports have provided evidence demonstrating the activities of *bona fide* T6SS effectors. These target diverse host cell processes encountered by pathogens during host infection (Figure 2).

Manipulation of the host cytoskeleton

Some bacteria may resist phagocytosis or promote their internalization in non-phagocytic cells by directly altering cytoskeleton components in a T6SS-dependent manner. So far, these phenotypes have been attributed to evolved VgrG proteins. Effectors that mediate phagocytosis resistance include VgrG1 from *V. cholerae* V52 ($VgrG1^{VC}$) which displays at its C terminus an actin cross-linking domain (ACD), that is homologous to those carried by some of the MARTX toxins [35–37]. The T6SS injects $VgrG1^{VC}$ into the eukaryotic cytosol after endocytosis, which is a prerequisite for effector translocation [20]. The ACD domain is then able to cross-link G-actin, leading to an accumulation of toxic actin oligomers [38] and ultimately host cell death [20]. The activity of the ACD is also observable *in vivo*, as it contributes significantly to T6SS-dependent inflammatory phenotypes observable in a mouse infection model [39]. Another effector mediating phagocytosis resistance is VgrG1 of *A. hydrophila* ($VgrG1^{AH}$), which has also been shown to target the actin cytoskeleton. This effector displays a vegetative insecticidal protein-2 (VIP-2) domain bearing an ADP-ribosyl transferase (ADPRT) signature [32]. Upon translocation, $VgrG1^{AH}$ ribosylates the barbed ends of G-actin monomers, inhibiting polymerization to F-actin [40]. Such activity then leads to cell rounding, cell detachment and caspase-3- and caspase-9-dependent cell death [32].

P. aeruginosa PAO1 has been shown to promote its entry into non-phagocytic cells via the H2-T6SS, with the evolved VgrG2b playing an important role in this process [41,42]. The C terminus of VgrG2b interacts *in vitro* with members of the γ-tubulin ring complex. Chemical inhibition of microtubule assembly prevented VgrG2b-dependent bacterial entry, whereas ectopic expression of VgrG2b restored productive internalization of a *vgrG2b* mutant [42]. This observation supports the decoupling of the puncturing and catalytic roles of evolved VgrGs. The mode of action and catalytic mechanism associated with VgrG2b are still unknown.

Table 1

T6SS-mediated phenotypes of bacterial interactions with a eukaryotic host. Impacts of T6SS-associated gene disruption on phenotypes (adhesion, internalization, host cytoskeletal and morphological changes, bacterial survival, strain cytotoxicity, immune response of the host and strain virulence) during infection and competition experiments with eukaryotic hosts. Please note that defects in virulence may be due to disruption of delivery of anti-bacterial toxins into competitors during colonization of the host. * Denotes a transposon insertion rather than a gene knock-out; CTD denotes C-terminal extension.

Phenotype	Bacterium	Gene(s) disrupted	Impact	Cell Lines/Species/ Animal Models	Reference
Adhesion	*Campylobacter jejuni* ATCC43431	*hcp1, tssM1*	Reduced adhesion	T84; RAW267.4	[76]
	Escherichia coli RS218	*hcp2*	Reduced adhesion	HBMEC	[34]
	E. coli SEPT362	*tssM*	Reduced adhesion	HeLa	[77]
	E. coli TW-XM	*vgrG1*	Reduced adhesion	DF-1; bEnd.3	[30]
		vgrG2	Reduced adhesion	bEnd.3	
	Helicobacter hepaticus ATCC51449	*hcp, tssM*	Increased adhesion	MODE-K	[78]
	Vibrio parahaemolyticus HZ	*hcp1, tssM1*	Reduced adhesion	Caco-2; HeLa	[79]
		hcp2, tssM2	Reduced adhesion	HeLa	
Internalization/ Invasion	*C. jejuni* ATCC43431	*hcp1, tssM1*	Reduced invasion	T84; RAW267.4	[76]
	E. coli RS218	*hcp2*	Reduced invasion	HBMEC	[34]
	E. coli SEPT362	*tssM*	Reduced invasion	HeLa	[77]
	E. coli TW-XM	*vgrG1*	Reduced invasion	DF-1; bEnd.3	[30]
		vgrG2	Reduced invasion	bEnd.3	
	Edwardsiella tarda EIB202	*evpP*	Reduced internalization	EPC	[80]
	H. hepaticus ATCC51449	*hcp, tssM*	Increased internalization	MODE-K	[78]
	P. aeruginosa PAO1	*clpV2, clpV2pldB, clpV3pldA, vgrG2a,2b*	Reduced internalization	HeLa	[9••,41,42]
	Salmonella enterica serovar Typhi GIFU100007	*clpV*	Increased invasion	HeLa	[81]
	S. enterica serovar Typhimurium LT2	*clpV* (over-expressed)	Reduced invasion	HEp-2	[82]
	Yersinia pseudotuberculosis IP32953	*clpV2* (over-expressed)	Reduced invasion	HEp-2	[82]
Morphological changes & cytoskeletal alterations	*Aeromonas hydrophila* SSU	*vasH, tssM*	Enhanced phagocytosis	RAW267.4	[83]
	Burkholderia cenocepacia J2315	*hcp*	No deamidation of RhoA GTPase	DC2.4	[50]
	B. cenocepacia K56-2	*hcp*	Diminished active Rac1 and Cdc42 GTPases and cofilin pools	ANA-1	[45]
		tssC	No membrane protrusions		[44]
	Burkholderia mallei ATCC23344	*tssE5*	No MNGC formation, actin polymerization defects	RAW267.4	[84]
	Burkholderia pseudomallei 340	*vgrG5*	No MNGC formation	HEK293	[58]
	B. pseudomallei E8	*tagAB5*	No MNGC formation	RAW267.4	[85]
	B. pseudomallei K96243	*hcp5*	No MNGC formation	RAW267.4	[86]
	B. pseudomallei KHW	*tssBC5*	No MNGC or actin-tail formation	RAW267.4	[59]
	Burkholderia thailandensis E264	*clpV5, vgrG5*	Reduced/no MNGC formation	HEK293	[58,87]
		tssK5, vgrG5 (CTD)	No MNGC formation	RAW267.4	[57]
	E. coli SEPT362	*hcp, clpV*	Induction of filopodia and ruffles	HeLa	[28]
	Vibrio cholerae V52	*hcp1hcp2, vgrG1, vgrG1 tssM*	No actin crosslinking	J774; RAW267.4	[35,64]
		tssM	No actin crosslinking	CHO-$F_c\gamma$RII	[20]
Fitness/intracellular survival	*Bordetella bronchiseptica* RB50	*clpV*	Increased intracellular replication	RAW267.4	[88]
	B. cenocepacia K56-2	*hcp*	Reduced resistance to amoebae	*Dictyostelium discoideum*	[44]
	B. mallei ATCC23344	*tssE5*	Reduced survival	RAW267.4	[84]

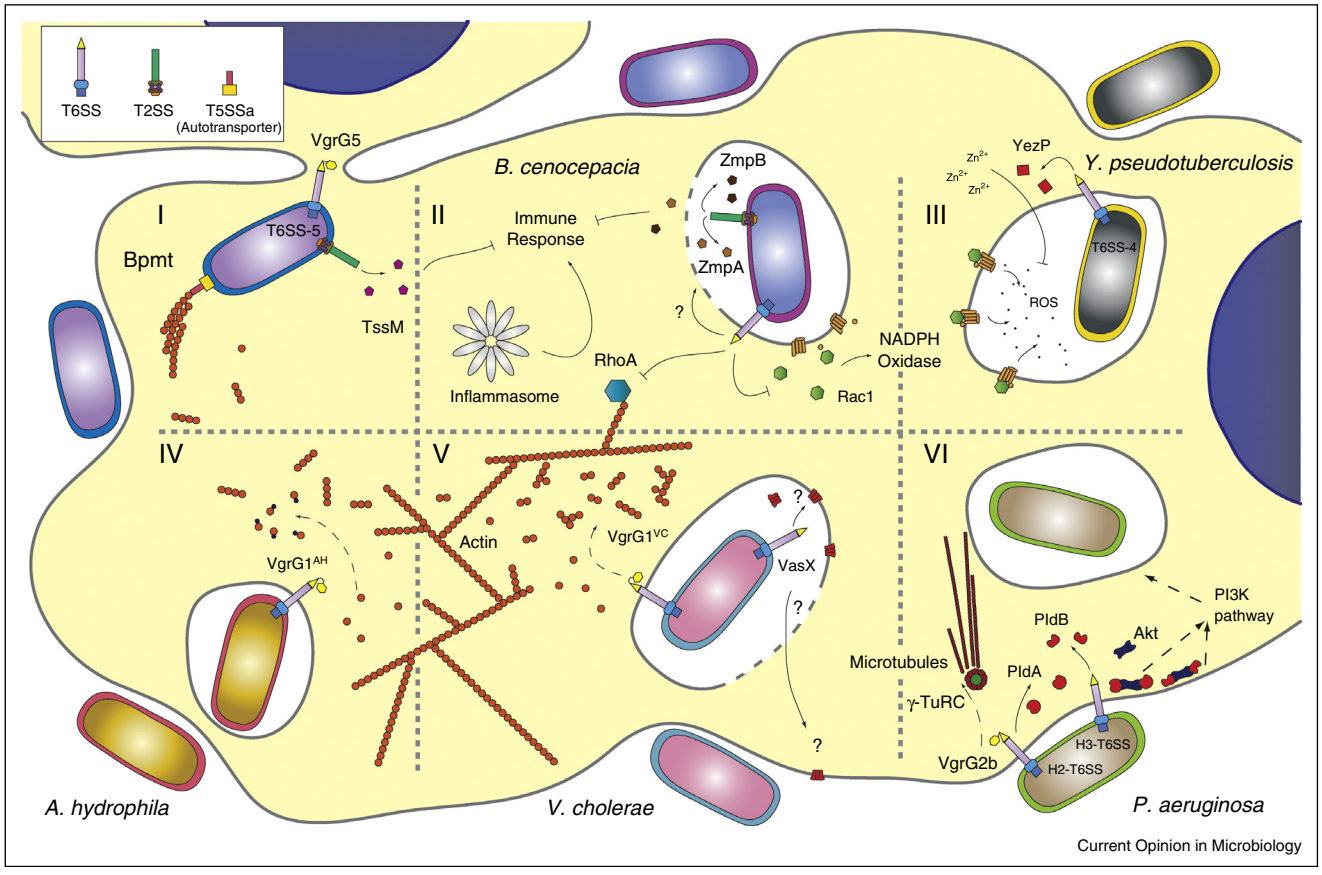
Table 1 (*Continued*)

Phenotype	Bacterium	Gene(s) disrupted	Impact	Cell Lines/Species/ Animal Models	Reference
	B. pseudomallei E8	*tagAB5*	Reduced survival	A549; Murine BMDM	[85]
		hcp5	Reduced survival	A549	
	B. pseudomallei K96243	*hcp5*	Reduced survival	RAW267.4	[86]
	B. thailandensis E264	*clpV5*	Increased intracellular replication	HEK293	[87]
	E. coli SEPT362	*tssM*	Reduced survival	J774	[77]
	E. tarda PPD130/91	*hcp*	Reduced intracellular replication	Blue gourami phagocytes	[89]
	H. hepaticus ATCC51449	*hcp, tssM*	Increased survival	MODE-K	[78]
	Pseudomonas syringae pathovar Tomato DC3000	*hcp1 hcp2*	Reduced fitness in competition with yeast; reduced amoebal resistance	*Cryptococcus carnescens*; *Acanthamoeba polyphaga*	[90]
	S. enterica serovar Gallinarum 287/91	*clpV*	Reduced survival	RAW267.4; HD11	[91]
	S. enterica serovar Typhimurium LT2	*tssM*	Increased survival	J774	[92]
	S. enterica serovar Typhimurium SL1344	*clpV, tssM*	Reduced intracellular replication	RAW267.4	[93]
	V. cholerae V52	*clpV, hcp, tseL fha, vasH,I,X, vgrG1, vgrG1*-(CTD), *tssA,B,C,E,F,G,J,K,L,M*	Reduced resistance to amoebae	*D. discoideum*	[10••,20,64, 65,94]
Cytotoxicity	*B. bronchiseptica* RB50	*clpV, hcp*	Reduced cytotoxicity and apoptosis	J774, RAW264.7	[88,95]
	B. cenocepacia K56-2	*tssM*	Reduced cytotoxicity	Human monocytes	[49]
	B. pseudomallei E8	*BPSS1504*	Reduced cytotoxicity	Murine *caspase-1*$^{-/-}$ BMDM	[85]
	B. pseudomallei K96243	*hcp5*	Reduced cytotoxicity	RAW267.4	[86]
	B. thailandensis E264	*clpV5*	Reduced cytotoxicity	HEK293	[87]
	C. jejuni 108	*hcp, tssM*	Reduced haemolysis	Horse red blood cells	[96]
	Citrobacter freundii CF74	*clpV, hcp*	Reduced cytotoxicity	HEp-2	[29]
	S. enterica serovar Typhi GIFU100007	*clpV*	Reduced cytotoxicity	HeLa	[81]
	V. cholerae V52	*tssM*	Reduced cytotoxicity	J774	[10••,20]
Host response	*B. bronchiseptica* RB50	*clpV*	Reduced IL-6, IL-1β, IL-17, IL-10 Increased IL-1β	J774; Mouse *Rag1*$^{-/-}$ mouse	[95] [88]
	B. cenocepacia J2315	*hcp*	Reduced IL-1β and caspase-1 activation	U937 + *Pyrin*^{human}; DC2.4 + *Pyrin*^{mouse}	[50]
	B. cenocepacia K56-2	*tssM*	Reduced IL-1β and caspase-1 activation	Human monocytes; THP-1	[49]
	B. pseudomallei K96243	*hcp5*	Reduced PMN infiltration and necrosis	Hamster	[86]
	H. hepaticus ATCC51449	*tssM*	Downregulates *TLR4, APC* and *IL-17RA*. Increased inflammation	MODE-K; T-cell reconstituted *Rag1*$^{-/-}$ mouse	[78]
		vgrG1	Reduced pathogenicity	T-cell reconstituted *Rag2*$^{-/-}$ mouse	[97]
	Klebsiella pneumoniae 521145	*KpST66_3367**	Reduced NF-κB signaling	A549	[98]
	Vibrio parahaemolyticus HZ	*hcp2, tssM2, vgrG2*	Lower LC3-II lipidation, autophagosome punctuation and cAMP levels	RAW267.4	[99]

Table 1 (*Continued*)

Phenotype	Bacterium	Gene(s) disrupted	Impact	Cell Lines/Species/ Animal Models	Reference
	S. enterica serovar Typhimurium 14028 s	*clpV (SPI-6)*	No necrosis	Chick	[100]
Virulence	*A. hydrophila* SSU	*hcp1,2, tssM, vasH, vgrG1,2,3*	Less virulent	Mouse	[83,101]
	Acinetobacter baumannii DSM30011	*tssM*	Less virulent	*Galleria mellonella*	[102]
	Agrobacterium tumefaciens C58	-	Increased fitness *in planta* than *in vitro*	Tobacco	[4•]
		hcp	Reduced tumorigenesis	Potato	[103]
	B. bronchiseptica RB50	*clpV*	Less persistent	Mouse	[95]
			Hypervirulent, higher bacterial burden in some tissues	*Rag1*$^{-/-}$ mouse	[88]
	B. pseudomallei E8	*tagAB5*	Less virulent	Mouse	[85]
	B. pseudomallei K96243	*hcp5*	Less virulent	Hamster	[86]
	B. thailandensis E264	*tssK5, vgrG5 (CTD)*	Less virulent	Mouse	[6•,57]
	C. jejuni ATCC43431	*tssM*	Less persistent	Mouse	[76]
	E. tarda PPD130/91	*clpV, evpP, hcp, vgrG,*			
	tssA,B,C,E,F,G,J,K,L,M	Less virulent	Blue gourami fish	[89,104]	
	E. coli SEPT362	*clpV, hcp*	Less virulent	Chick	[28]
	E. coli TW-XM	*vgrG1*	Less virulent, lower systemic load	Duck	[30]
		vgrG2	Delayed virulence, lower burden in brain	Duck	
	H. hepaticus ATCC51449	*hcp, tssM*	Hypercolonization	Germ-free mouse	[78]
	K. pneumoniae 521145	*pld1*	Less virulent	Mouse	[63]
	Pantoea ananatis LMG 2665^{T}	*hcp, tssA*	Less virulent	Onion	[105]
	Pectobacterium atrosepticum SCRI1043	*hcp1* (over-expression)	More virulent	Potato	[106]
	P. aeruginosa PAO1	*(clpV1, PA0098, tagT1, tle3, tli1b, tsi5, tssA1,F1,M1)**	Less virulent	Rat	[107]
		clpV2,3	Less virulent	*Caenorhabditis elegans*	[41,108]
		pldA	Less persistent	Rat	[61•]
	P. aeruginosa PA14	*stk1-rhsP2 (H2-T6SS), tssM3-PA14_33970 (H3-T6SS)*	Less virulent	*Arabidopsis thaliana*, Mouse	[109]
	Ralstonia solanacearum GMI1000	*tssC*	Lower bacterial load	Tomato	[110]
	Rhizobium leguminosarum biovar *trifolii* RCR5	*tssK**	Inhibits symbiotic pea nodulation	Pea	[111,112]
	S. enterica serovar Dublin CT_02021853	*SPI-6*	Less virulent	Mouse	[113]
		SPI-6 SPI-19	Less virulent	Chick	
	S. enterica serovar Gallinarum 287/91	*clpV*	Reduced *in vivo* fitness	Chick	[114]
	S. enterica serovar Typhimurium 14028 s	*clpV*	Less virulent	Chick	[100]
	S. enterica serovar Typhimurium LT2	*clpV*	Delayed death	Mouse	[93]
		tssM	More virulent; Delayed death	Mouse	[92,93]
	V. cholerae A1552	*hcp1 hcp2, vgrG3*	Reduced *in vivo* fitness	Mouse	[115]
	V. cholerae C7606	*tsiV3*	Reduced *in vivo* fitness	Rabbit	[3•]
		tsiV1,2,3		Mouse	[70••]
	V. cholerae V52	*vgrG1* (CTD)	Lower bacterial load in intestine	Mouse	[39]
	Y. pseudotuberculosis YPIII	*clpV4 znuCB, yezP znuCB*	Less virulent	Mouse	[46•]

Figure 2



T6SS-dependent mechanisms of host cell subversion. I. *Burkholderia mallei*, *Burkholderia pseudomallei* and *Burkholderia thailandensis* (grouped as Bpmt) encode five, six and five T6SSs respectively, of which the T6SS-5 is essential for full virulence during infection [6•,55,75]. The evolved VgrG5, with unknown catalytic activity, is responsible for the fusion of host cell membranes leading to multinucleated giant cell formation [57,58]. The T6SS-5 cluster also encodes an autotransporter, BimA, involved in actin-mediated propulsion; and TssM, a T2SS-dependent effector that dampens NF-κB and type I interferon signaling [51,53,55]. II. To date, no T6SS effectors of *Burkholderia cenocepacia* have been characterized, but several T6SS-dependent phenotypes have been described [44]. This secretion system causes the deregulation of cytoskeleton-associated Rho-GTPases including RhoA and Rac1 [45]. RhoA inactivation leads to a pyrin inflammasome-mediated immune response [49,50] while Rac1 inactivation inhibits NADPH oxidase assembly, thus protecting the bacterium from oxidative damage derived from the respiratory burst [45]. The T6SS is also thought to contribute to the release of T2SS substrates ZmpA and ZmpB from the vacuole, which subsequently hamper the antimicrobial responses of the host [47,48]. III. *Yersinia pseudotuberculosis* invades host cells, which then subject the pathogen to oxidative stress in the phagosome. The T6SS-4 secretes a zinc-binding effector, YezP, which protects the bacterium from the generated reactive oxygen species (ROS) [46•]. IV. VgrG1^{AH} is secreted by *Aeromonas hydrophila*, and its evolved catalytic domain possesses ADP-ribosyltransferase activity, inhibiting actin polymerization [32]. V. *Vibrio cholerae* utilizes a single T6SS to secrete VgrG1^{VC}, harboring a C-terminal actin cross-linking domain into the cytosol of macrophages and predatory amoebae [20,35]. VasX is another *V. cholerae* T6SS effector important in virulence, thought to form pores in lipid bilayers [64]. VI. *Pseudomonas aeruginosa* uses two distinct T6SSs to secrete effectors into epithelial cells. VgrG2b is H2-T6SS-dependent and modulates microtubule-mediated bacterial internalization through an interaction with the gamma-tubulin ring complex (γ-TuRC) [42]. PldA and PldB are H2-T6SS- and H3-T6SS-dependent effectors, respectively, which bind Akt to facilitate bacterial internalization using the PI3K pathway [9••].

Other T6SS-dependent phenotypes impacting the cytoskeleton have been described, but are yet to be linked to a specific effector (Table 1). For example, the T6SS of *B. cenocepacia* is essential for virulence *in vivo* and confers resistance to predation by *Dictyostelium* [43,44]. Deletion of *hcp* abrogates formation of actin-mediated membrane projections in infected macrophages, indicating that the T6SS manipulates the cytoskeleton [44]. This phenotype was further supported by studies showing a T6SS-mediated deregulation of Rho GTPase family members such as Cdc42, RhoA, and Rac1 [45].

Host defense evasion

Amongst the strategies deployed by phagocytic cells to eradicate pathogenic intruders, generation of reactive oxygen species (ROS) during the respiratory burst is one of the most efficient defense mechanisms. The *Yersinia pseudotuberculosis* T6SS-4 cluster has been shown

to respond to oxidative stress imposed by the host through secretion of YezP. This zinc-binding effector, which has been proposed to scavenge zinc ions and subsequently protect the pathogen from ROS. Moreover, the T6SS-4 and YezP are paramount for *in vivo* virulence [46•]. Such mechanisms may apply to other pathogens, as the T6SS-4 cluster present in *B. pseudomallei*, *B. mallei* and *B. thailandensis* (Bpmt) is homologous to that of *Y. pseudotuberculosis* and also encodes a YezP orthologue.

B. cenocepacia was also shown to suppress this microbicidal mechanism by decreasing the pool of GTP-bound Rac1 in a T6SS-dependent manner. As a result, this pathogen hinders NADPH oxidase assembly at the membrane of the *B. cenocepacia*-containing vacuole (BcCV), and thus prevents generation of the oxidative burst [45].

Modulation of the inflammatory response

In macrophages, *B. cenocepacia* stimulates the inflammatory response leading to the secretion of IL-1β [47]. The bacterium survives in the BcCV by avoiding fusion with lysosomes before escaping into the host cell cytosol. Although the T6SS is not directly involved in maturation arrest of the endocytic vacuole, it is required for its disruption to facilitate the release of the T2SS-dependent effectors ZmpA and ZmpB into the macrophage cytosol [47]. These proteases cleave antimicrobial peptides and could be involved in caspase-1 activation and IL-1β processing [47,48]. Furthermore, the pyrin inflammasome triggers IL-1β secretion through sensing the N41 deamidation of RhoA, which is abolished in the *B. cenocepacia hcp* mutant [49,50]. An unknown T6SS effector could be conceived as modulating RhoA activity to promote survival within macrophages, but not as far as to elicit an overt immune response [45,50]. Interference with host immunity pathways is achieved in Bpmt infection by the deubiquitinase TssM (unrelated to the T6SS structural component of the same name), which inhibits NF-κB and type I interferon signaling [51,52]. TssM is a T2SS-dependent protein but is in fact encoded within the *T6SS-5* cluster [53].

After T3SS-3-dependent phagosomal escape, *B. pseudomallei*, *B. mallei* and *B. thailandensis* propel themselves through the host cytosol using actin tail polymerization. This is mediated by the type Va secretion system (T5SSa) autotransporter BimA [54], whose gene, similarly to *tssM*, is genetically linked to the *T6SS-5* cluster. Several studies have established that T6SS-5 is required for intercellular dissemination and virulence in the acute mouse model [6•,55]. Bpmt spreads between cells by fusing the membranes of adjacent macrophages, resulting in the formation of multinucleated giant cells (MNGCs) [56]. The C-terminal domain of the evolved VgrG-5 is necessary for the fusion of host membranes [57,58]. The *T6SS-5* locus is regulated by a major virulence determinant, the two-component system VirAG, which upon sensing host intracellular glutathione activates the transcription of *bimA*, *tssM* and *T6SS-5* genes. This results in the coordination of propulsion, immune modulation and host cell fusion by Bpmt during infection [51,55,59,60].

Targeting membranes: the rise of trans-kingdom T6SS effectors

As demonstrated with VgrG-5 in Bpmt, T6SS effectors can alter host membrane architecture. However, this is not restricted to evolved VgrGs as other, non-evolved effectors targeting cell membranes have also been characterized. The first cargo effector shown to be important in pathogenicity was PldA (Tle5a) of *P. aeruginosa*, wherein a *pldA* mutant could not establish a persistent infection in rats [61•]. PldA, along with the effector PldB (Tle5b) exhibit phospholipase D (PLD) activity and are transported by the H2- and H3-T6SS, respectively [8,9••,61•]. These enzymes are encoded within two genetic islands encoding distinct canonical VgrG and cognate immunity proteins. Both enzymes target the phospholipid bilayers of competing bacteria and are also translocated into epithelial cells by *P. aeruginosa in vitro* [8,9••]. In the host cell, these PLDs induce PI3K activation by interacting with Akt1 and Akt2 [9••]. This is consistent with previous findings exposing the hijacking of the PI3-kinase pathway and the role of the H2-T6SS during the uptake of *P. aeruginosa* into non-phagocytic cells [41,62]. Remarkably, *P. aeruginosa* promotes its entry via two H2-T6SS effectors targeting different host pathways, but the potential interplay between PldA and VgrG2b is yet to be explored. A Tle5 homologue, Pld1, has recently been identified as essential for *Klebsiella pneumoniae* virulence in mice. This phospholipase is encoded within a T6SS core gene cluster, but the requirement of the T6SS for Pld1-mediated pathogenicity has not been investigated [63]. Although having no apparent role in antibacterial activity (notwithstanding apparent duplication of *pld1*) the *pld1* gene resides in a similar proximal genetic environment to *pldB* of *P. aeruginosa* (i.e. between a *vgrG* and three successive putative lipoprotein immunity genes), so may also exert its activity across kingdoms. VasX from *V. cholerae* is an anti-bacterial toxin but is also important in resistance to amoebal predation [64,65]. Rather than displaying evident catalytic activity, it has been proposed to form pores in phospholipid bilayers, thereby dissipating the membrane potential. It also binds to different phosphoinositide species *in vitro*, suggesting that VasX could alter lipid distribution within membranes, thereby influencing host signaling networks involved in infection [64].

Conclusions

Much evidence supports the role of the T6SS in the delivery of effectors targeting eukaryotic cells. Species including *V. cholerae* and *Y. pseudotuberculosis* appear to use the T6SS to avoid elimination by the host. Moreover, intracellular pathogens such as *Burkholderia* species use

the machinery to exploit signaling pathways to enable their spread during infection. Anti-eukaryotic effectors exist both as evolved VgrGs and cargo effectors, often targeting conserved structures such as the cytoskeleton or host membranes. Several effectors exert activity on both prokaryotic and eukaryotic cells. This challenges the assumed dichotomy expected from secretion machineries and their toxins, which has recently been supported by observations regarding the activity of the T4SS [66]. This emerging concept should be addressed upon future identification of T6SS effectors and applied to existing toxins where appropriate. For instance, the Tle5 lipase superfamily is prevalent in many bacterial genera but whether they exhibit anti-host or anti-bacterial activities will have to be determined. Heightened attention must be given to the regulatory networks governing the T6SS, as coordination between secretion systems (e.g. in *Burkholderia* species) and synergism with other virulence factors during infection is becoming increasingly evident. Finally, it has been reported that bacterial membrane disturbances, such as those inflicted in T6SS dueling, T4SS conjugation or exposure to antibiotics [67–69], induce and activate the T6SS. Pathogens are subjected to lethal attacks during infection, which could be then opposed by T6SS responses that may have co-evolved with the host defense mechanisms.

In these cases, focusing on the host response pathways may expose cues for T6SS activation in eukaryotic settings [70••]. Subsequent reproduction of such stimuli *in vitro* could then lead to the discovery of novel anti-host effectors that are otherwise difficult to identify. In all, this calls for a more integrative analysis of the molecular mechanisms of the T6SS within a host in order to expand our understanding of this versatile and fascinating bacterial weapon.

Acknowledgements

We apologize to all scientific colleagues whose work has not been mentioned in this manuscript due to space limitation. A.H. is supported by the H2020-MSCA-Global Fellowship-2014, grant number 657766; T.E.W. is supported by the Wellcome Trust Molecular and Cellular Basis of Infection PhD program, and A.F. is supported by the Medical Research Council Program Grant MR/K001930/1.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hood RD, Singh P, Hsu F, Güvener T, Carl MA, Trinidad RRS, Silverman JM, Ohlson BB, Hicks KG, Plemel RL *et al.*: **A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria**. *Cell Host Microbe* 2010, **7**:25-37.

2. • Russell AB, Wexler AG, Harding BN, Whitney JC, Bohn AJ, Goo YA, Tran BQ, Barry NA, Zheng H, Peterson SB *et al.*: **A type VI secretion-related pathway in *Bacteroidetes* mediates interbacterial antagonism**. *Cell Host Microbe* 2014, **16**:227-236.
Using bioinformatic and functional characterization, this study defined three classes of T6SS, and identified bacterial antagonism in bacteria, such as *Bacteroidetes* spp., present at high density in the human body. These observations suggest that the T6SS could be considered as a determinant of the human flora composition.

3. • Fu Y, Waldor MK, Mekalanos JJ: **Tn-seq analysis of *Vibrio cholerae* intestinal colonization reveals a role for T6SS-mediated antibacterial activity in the host**. *Cell Host Microbe* 2013, **14**:652-663.
Using high throughput analysis, this study underlined the importance of bacterial antagonism *in vivo* during infections.

4. • Ma LS, Hachani A, Lin JS, Filloux A, Lai EM: ***Agrobacterium tumefaciens* deploys a superfamily of type VI secretion DNase effectors as weapons for interbacterial competition *in planta***. *Cell Host Microbe* 2014, **16**:94-104.
The identification of a novel class of T6SS toxins that target the nucleic acids of bacterial competitors has highlighted the role of the T6SS in the establishment of bacterial niches.

5. Shrivastava S, Mande SS: **Identification and functional characterization of gene components of type VI secretion system in bacterial genomes**. *PLoS One* 2008, **3**:e2955.

6. • Schwarz S, West TE, Boyer F, Chiang W-C, Carl MA, Hood RD, Rohmer L, Tolker-Nielsen T, Skerrett SJ, Mougous JD: ***Burkholderia* type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions**. *PLoS Pathog* 2010, **6**:e1001068.
This very thorough and elegant work exposed the various functions of distinct T6SS encoded by *B. thailandensis*. Although highlighting the antibacterial activity of T6SS-1, the authors demonstrated that the T6SS-5 is a major determinant in the pathogenicity of *B. thailandensis*.

7. Filloux A, Hachani A, Bleves S: **The bacterial type VI secretion machine: yet another player for protein transport across membranes**. *Microbiology* 2008, **154**:1570-1583.

8. Russell AB, LeRoux M, Hathazi K, Agnello DM, Ishikawa T, Wiggins PA, Wai SN, Mougous JD: **Diverse type VI secretion phospholipases are functionally plastic antibacterial effectors**. *Nature* 2013, **496**:508-512.

9. •• Jiang F, Waterfield NR, Yang J, Yang G, Jin Q: **A *Pseudomonas aeruginosa* type VI secretion phospholipase D effector targets both prokaryotic and eukaryotic cells**. *Cell Host Microbe* 2014, **15**:600-610.
By unveiling PldB as the first cargo effector for the H3-T6SS in *P. aeruginosa*, this study has also demonstrated that this lipase and its H2-T6SS-dependent homologue, PldA, were able to target both bacterial and eukaryotic cells. Such unprecedented observation places the T6SS as one of the most versatile secretion systems at play in bacterial pathogenesis.

10. •• Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, Heidelberg JF, Mekalanos JJ: **Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the *Dictyostelium* host model system**. *Proc Natl Acad Sci U S A* 2006, **103**:1528-1533.
This seminal paper provided major grounds establishing the T6SS as a key player in the bacterial secretion field. Several phenotypes described in this study such as: (1) the secretion of Hcp and VgrG proteins; (2) the structural similarity between the T6SS and the T4 phage tail; and (3) beside their structural function for the puncturing, evolved VgrG proteins carry catalytic domains that could target eukaryotic cells. These observations are hallmarks of the T6SS and are considered as the 'ABC' for the identification and study of the T6SS.

11. MacIntyre DL, Miyata ST, Kitaoka M, Pukatzki S: **The *Vibrio cholerae* type VI secretion system displays antimicrobial properties**. *Proc Natl Acad Sci U S A* 2010, **107**:19520-19524.

12. de Bruin OM, Ludu JS, Nano FE: **The *Francisella* pathogenicity island protein IglA localizes to the bacterial cytoplasm and is needed for intracellular growth**. *BMC Microbiol* 2007, **7**:1.

13. Leiman PG, Basler M, Ramagopal UA, Bonanno JB, Sauder JM, Pukatzki S, Burley SK, Almo SC, Mekalanos JJ: **Type VI secretion apparatus and phage tail-associated protein complexes share a common evolutionary origin**. *Proc Natl Acad Sci U S A* 2009, **106**:4154-4159.

14. •• Shneider MM, Buth SA, Ho BT, Basler M, Mekalanos JJ, Leiman PG: **PAAR-repeat proteins sharpen and diversify the type VI secretion system spike**. *Nature* 2013, **500**:350-353.

By identifying PAAR containing proteins, this ground-breaking study refined the understanding of the tail/spike composition and also offered novel and refreshing perspectives on the modularity displayed by the T6SS spike for toxin/effector delivery in target cells.

15. Kudryashev M, Wang RY-R, Brackmann M, Scherer S, Maier T, Baker D, DiMaio F, Stahlberg H, Egelman EH, Basler M: **Structure of the Type VI secretion system contractile sheath**. *Cell* 2015, **160**:952-962.

16. Basler M, Pilhofer M, Henderson GP, Jensen GJ, Mekalanos JJ: **Type VI secretion requires a dynamic contractile phage tail-like structure**. *Nature* 2012, **483**:182-186.

17. Durand E, Nguyen VS, Zoued A, Logger L, Péhau-Arnaudet G, Aschtgen M-S, Spinelli S, Desmyter A, Bardiaux B, Dujeancourt A *et al.*: **Biogenesis and structure of a type VI secretion membrane core complex**. *Nature* 2015, **523**:555-560.

18. Pukatzki S, McAuley SB, Miyata ST: **The type VI secretion system: translocation of effectors and effector-domains**. *Curr Opin Microbiol* 2009, **12**:11-17.

19. Barret M, Egan F, Fargier E, Morrissey JP, O'Gara F: **Genomic analysis of the type VI secretion systems in *Pseudomonas* spp.: novel clusters and putative effectors uncovered**. *Microbiology* 2011, **157**:1726-1739.

20. Ma AT, McAuley SB, Pukatzki S, Mekalanos JJ: **Translocation of a *Vibrio cholerae* type VI secretion effector requires bacterial endocytosis by host cells**. *Cell Host Microbe* 2009, **5**:234-243.

21. Blondel CJ, Jiménez JC, Contreras I, Santiviago CA: **Comparative genomic analysis uncovers 3 novel loci encoding type six secretion systems differentially distributed in *Salmonella* serotypes**. *BMC Genomics* 2009, **10**:354.

22. Hachani A, Allsopp LP, Oduko Y, Filloux A: **The VgrG proteins are "à la carte" delivery systems for bacterial type VI effectors**. *J Biol Chem* 2014, **289**:17872-17884.

23. Silverman JM, Agnello DM, Zheng H, Andrews BT, Li M, Catalano CE, Gonen T, Mougous JD: **Haemolysin coregulated protein is an exported receptor and chaperone of type VI secretion substrates**. *Mol Cell* 2013, **51**:584-593.

24. Unterweger D, Kostiuk B, Ötjengerdes R, Wilton A, Diaz-Satizabal L, Pukatzki S: **Chimeric adaptor proteins translocate diverse type VI secretion system effectors in *Vibrio cholerae***. *EMBO J* 2015, **34**:2198-2210.

25. Liang X, Moore R, Wilton M, Wong MJQ, Lam L, Dong TG: **Identification of divergent type VI secretion effectors using a conserved chaperone domain**. *Proc Natl Acad Sci* 2015, **112**:9106-9111.

26. Diniz JA, Coulthurst SJ: **Intra-species competition in *Serratia marcescens* is mediated by type VI secretion Rhs effectors and a conserved effector-associated accessory protein**. *J Bacteriol* 2015, **197** JB. 00199-15.

27. Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA, Grishin NV, Mirzaei H, Orth K: **Marker for type VI secretion system effectors**. *Proc Natl Acad Sci U S A* 2014, **111**:9271-9276.

28. De Pace F, Nakazato G, Pacheco A, De Paiva JB, Sperandio V, Da Silveira WD: **The type VI secretion system plays a role in type 1 fimbria expression and pathogenesis of an avian pathogenic *Escherichia coli* strain**. *Infect Immun* 2010, **78**:4990-4998.

29. Liu L, Hao S, Lan R, Wang G, Xiao D, Sun H, Xu J: **The type VI secretion system modulates flagellar gene expression and secretion in *Citrobacter freundii* and contributes to the adhesion and cytotoxicity to host cells**. *Infect Immun* 2015, **83** IAI. 03071-14.

30. Ma J, Bao Y, Sun M, Dong W, Pan ZH, Zhang W, Lu C, Yao H: **Two functional type VI secretion systems in avian pathogenic *Escherichia coli* are involved in different pathogenic pathways**. *Infect Immun* 2014, **82**:3867-3879.

31. Aschtgen MS, Bernard CS, De Bentzmann S, Lloubès R, Cascales E: **SciN is an outer membrane lipoprotein required for type VI secretion in enteroaggregative *Escherichia coli***. *J Bacteriol* 2008, **190**:7523-7531.

32. Suarez G, Sierra JC, Erova TE, Sha J, Horneman AJ, Chopra AK: **A type VI secretion system effector protein, VgrG1, from *Aeromonas hydrophila* that induces host cell toxicity by ADP ribosylation of actin**. *J Bacteriol* 2010, **192**:155-168.

33. Suarez G, Sierra JC, Kirtley ML, Chopra AK: **Role of Hcp, a type 6 secretion system effector, of *Aeromonas hydrophila* in modulating activation of host immune cells**. *Microbiology* 2010, **156**:3678-3688.

34. Zhou Y, Tao J, Yu H, Ni J, Zeng L, Teng Q, Kim KS, Zhao GP, Guo X, Yao Y: **Hcp family proteins secreted via the type VI secretion system coordinately regulate *Escherichia coli* K1 interaction with human brain microvascular endothelial cells**. *Infect Immun* 2012, **80**:1243-1251.

35. Pukatzki S, Ma AT, Revel AT, Sturtevant D, Mekalanos JJ: **Type VI secretion system translocates a phage tail spike-like protein into target cells where it cross-links actin**. *Proc Natl Acad Sci U S A* 2007, **104**:15508-15513.

36. Durand E, Derrez E, Audoly G, Spinelli S, Ortiz-Lombardia M, Raoult D, Cascales E, Cambillau C: **Crystal structure of the VgrG1 actin cross-linking domain of the *Vibrio cholerae* type VI secretion system**. *J Biol Chem* 2012, **287**:38190-38199.

37. Satchell KJF: **Structure and function of MARTX toxins and other large repetitive RTX proteins**. *Annu Rev Microbiol* 2011, **65**:71-90.

38. Heisler DB, Kudryashova E, Suarez C, Winkelman JD, Birukov KG, Kotha SR, Parinandi NL, Vavylonis D, Kovar DR, Kudryashov DS: **ACD toxin-produced actin oligomers poison formin-controlled actin polymerization**. *Science (80-)* 2015, **349**:535-539.

39. Ma AT, Mekalanos JJ: ***In vivo* actin cross-linking induced by *Vibrio cholerae* type VI secretion system is associated with intestinal inflammation**. *Proc Natl Acad Sci U S A* 2010, **107**:4365-4370.

40. Simon NC, Aktories K, Barbieri JT: **Novel bacterial ADP-ribosylating toxins: structure and function**. *Nat Rev Microbiol* 2014, **12**:599-611.

41. Sana TG, Hachani A, Bucior I, Soscia C, Garvis S, Termine E, Engel JN, Filloux A, Bleves S: **The second type VI secretion system of *Pseudomonas aeruginosa* strain PAO1 is regulated by quorum sensing and *fur* and modulates internalization in epithelial cells**. *J Biol Chem* 2012, **287**:27095-27105.

42. Sana TG, Baumann C, Merdes A, Soscia C, Rattei T, Hachani A, Jones C, Bennett KL, Filloux A, Superti-Furga G *et al.*: **Internalization of *Pseudomonas aeruginosa* strain PAO1 into epithelial cells is promoted by interaction of a T6SS effector with the microtubule network**. *MBio* 2015, **6**:1-11.

43. Hunt TA, Kooi C, Sokol PA, Valvano MA: **Identification of *Burkholderia cenocepacia* genes required for bacterial survival *in vivo***. *Infect Immun* 2004, **72**:4010-4022.

44. Aubert DF, Flannagan RS, Valvano MA: **A novel sensor kinase-response regulator hybrid controls biofilm formation and type VI secretion system activity in *Burkholderia cenocepacia***. *Infect Immun* 2008, **76**:1979-1991.

45. Rosales-Reyes R, Skeldon AM, Aubert DF, Valvano MA: **The type VI secretion system of *Burkholderia cenocepacia* affects multiple Rho family GTPases disrupting the actin cytoskeleton and the assembly of NADPH oxidase complex in macrophages**. *Cell Microbiol* 2012, **14**:255-273.

46. • Wang T, Si M, Song Y, Zhu W, Gao F, Wang Y, Zhang L, Zhang W, Wei G, Luo Z-Q *et al.*: **Type VI secretion system transports Zn^{2+} to combat multiple stresses and host immunity**. *PLOS Pathog* 2015, **11**:e1005020.

Having determined that the T6SS-4 cluster of Y. pseudotuberculosis is transcriptionally upregulated under oxidative stress, this study proceeded to identify the T6SS-secreted protein YezP. This represents a novel type of effector with a defensive role, in order to protect the bacterium from oxidative environmental and host insults.

47. Rosales-Reyes R, Aubert DF, Tolman JS, Amer AO, Valvano MA: ***Burkholderia cenocepacia* type VI secretion system mediates escape of type II secreted proteins into the cytoplasm of infected macrophages**. *PLoS One* 2012, **7**:1-14.

48. Kooi C, Sokol PA: ***Burkholderia cenocepacia* zinc metalloproteases influence resistance to antimicrobial peptides**. *Microbiology* 2009, **155**:2818-2825.

49. Gavrilin MA, Abdelaziz DHA, Mostafa M, Abdulrahman BA, Grandhi J, Akhter A, Khweek AA, Aubert DF, Valvano MA, Wewers MD *et al.*: **Activation of the pyrin inflammasome by intracellular *Burkholderia cenocepacia***. *J Immunol* 2012, **188**:3469-3477.

50. Xu H, Yang J, Gao W, Li L, Li P, Zhang L, Gong Y-N, Peng X, Xi JJ, Chen S *et al.*: **Innate immune sensing of bacterial modifications of Rho GTPases by the pyrin inflammasome**. *Nature* 2014, **513**:237-241.

51. Shanks J, Burtnick MN, Brett PJ, Waag DM, Spurgers KB, Ribot WJ, Schell MA, Panchal RG, Gherardini FC, Wilkinson KD *et al.*: ***Burkholderia mallei tssM* encodes a putative deubiquitinase that is secreted and expressed inside infected RAW 264.7 murine macrophages**. *Infect Immun* 2009, **77**:1636-1648.

52. Tan KS, Chen Y, Lim Y-C, Tan G-YG, Liu Y, Lim Y-T, Macary P, Gan Y-H: **Suppression of host innate immune response by *Burkholderia pseudomallei* through the virulence factor TssM**. *J Immunol* 2010, **184**:5160-5171.

53. Burtnick MN, Brett PJ, Deshazer D: **Proteomic analysis of the *Burkholderia pseudomallei* type II secretome reveals hydrolytic enzymes, novel proteins, and the deubiqitinase TssM**. *Infect Immun* 2014, **82**:3214-3226.

54. Benanti EL, Nguyen CM, Welch MD: **Virulent *Burkholderia* species mimic host actin polymerases to drive actin-based motility**. *Cell* 2015, **161**:348-360.

55. Schell MA, Ulrich RL, Ribot WJ, Brueggemann EE, Hines HB, Chen D, Lipscomb L, Kim HS, Mrázek J, Nierman WC *et al.*: **Type VI secretion is a major virulence determinant in *Burkholderia mallei***. *Mol Microbiol* 2007, **64**:1466-1485.

56. Kespichayawattana W, Rattanachetkul S, Wanun T, Sirisinha S, Utaisincharoen P: ***Burkholderia pseudomallei* induces cell fusion and actin-associated membrane protrusion: a possible mechanism for cell-to-cell spreading**. *Infect Immun* 2000, **68**:5377-5384.

57. Schwarz S, Singh P, Robertson JD, LeRoux M, Skerrett SJ, Goodlett DR, Eoin West T, Mougous JD: **VgrG-5 is a *Burkholderia* type VI secretion system-exported protein required for multinucleated giant cell formation and virulence**. *Infect Immun* 2014, **82**:1445-1452.

58. Toesca IJ, French CT, Miller JF: **The type VI secretion system spike protein VgrG5 mediates membrane fusion during intercellular spread by *pseudomallei* group *Burkholderia* species**. *Infect Immun* 2014, **82**:1436-1444.

59. Chen Y, Wong J, Sun GW, Liu Y, Tan GYG, Gan YH: **Regulation of type VI secretion system during *Burkholderia pseudomallei* infection**. *Infect Immun* 2011, **79**:3064-3073.

60. Wong J, Chen Y, Gan YH: **Host cytosolic glutathione sensing by a membrane histidine kinase activates the type VI secretion system in an intracellular bacterium**. *Cell Host Microbe* 2015 http://dx.doi.org/10.1016/j.chom.2015.06.002.

61. • Wilderman PJ, Vasil AI, Johnson Z, Vasil ML: **Genetic and biochemical analyses of a eukaryotic-like phospholipase D of *Pseudomonas aeruginosa* suggest horizontal acquisition and a role for persistence in a chronic pulmonary infection model**. *Mol Microbiol* 2001, **39**:291-303.
This study was the first to identify, before the recognition of the T6SS, PldA as a virulence determinant, and to hint on its potential transport by VgrG proteins, which had not been characterized at that point.

62. Kierbel A, Mostov K, Engel JN: **The phosphoinositol-3-kinase — protein kinase B/Akt pathway is critical for *Pseudomonas aeruginosa* strain PAK internalization**. *Mol Biol Cell* 2005, **16**:2577-2585.

63. Lery LMS, Frangeul L, Tomas A, Passet V, Almeida AS, Bialek-Davenet S, Barbe V, Bengoechea JA, Sansonetti P, Brisse S *et al.*: **Comparative analysis of *Klebsiella pneumoniae* genomes identifies a phospholipase D family protein as a novel virulence factor**. *BMC Biol* 2014, **12**:41.

64. Miyata ST, Kitaoka M, Brooks TM, McAuley SB, Pukatzki S: ***Vibrio cholerae* requires the type VI secretion system virulence factor Vasx to kill *Dictyostelium discoideum***. *Infect Immun* 2011, **79**:2941-2949.

65. Zheng J, Ho BT, Mekalanos JJ: **Genetic analysis of anti-amoebae and anti-bacterial activities of the type VI secretion system in *Vibrio cholerae***. *PLoS One* 2011:6.

66. Souza DP, Oka GU, Alvarez-Martinez CE, Bisson-Filho AW, Dunger G, Hobeika L, Cavalcante NS, Alegria MC, Barbosa LRS, Salinas RK *et al.*: **Bacterial killing via a type IV secretion system**. *Nat Commun* 2015, **6**:6453.

67. Basler M, Ho BT, Mekalanos JJ: **Tit-for-tat: Type VI secretion system counterattack during bacterial cell–cell interactions**. *Cell* 2013, **152**:884-894.

68. Ho BT, Basler M, Mekalanos JJ: **Type 6 secretion system-mediated immunity to type 4 secretion system-mediated gene transfer**. *Science* 2013, **342**:250-253.

69. Dong TG, Dong S, Catalano C, Moore R, Liang X, Mekalanos JJ: **Generation of reactive oxygen species by lethal attacks from competing microbes**. *Proc Natl Acad Sci* 2015 http://dx.doi.org/10.1073/pnas.1425007112.

70. •• Bachmann V, Kostiuk B, Unterweger D, Diaz-Satizabal L, Ogg S, Pukatzki S: **Bile salts modulate the mucin-activated type VI secretion system of pandemic *Vibrio cholerae***. *PLoS Negl Trop Dis* 2015, **9**:e0004031.
This study explores the impact of the intestinal environment on the T6SS activity of *Vibrio cholerae*. The authors propose that intestinal mucins activates the bactericidal activity of *V. cholerae*. The latter is also controlled by the host bile salts, which, upon metabolization by members of the microbiota into bile acids, dampen the bactericidal activity of *V. cholera*.

71. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJE: **The Phyre2 web portal for protein modeling, prediction and analysis**. *Nat Protoc* 2015, **10**:845-858.

72. Hu H, Zhang H, Gao ZQ, Wang DQ, Liu GF, Xu JH, Lan K, Dong YH: **Structure of the type VI secretion phospholipase effector Tle1 provides insight into its hydrolysis and membrane targeting**. *Acta Crystallogr Sect D Biol Crystallogr* 2014, **70**:2175-2185.

73. Zhang D, de Souza RF, Anantharaman V, Iyer LM, Aravind L: **Polymorphic toxin systems: comprehensive characterization of trafficking modes, processing, mechanisms of action, immunity and ecology using comparative genomics**. *Biol Direct* 2012, **7**:18.

74. Whitney JC, Quentin D, Sawai S, Goodlett DR, Raunser S, Mougous JD: **An Interbacterial NAD(P)$^+$ glycohydrolase toxin requires elongation factor Tu for delivery to target cells**. *Cell* 2015, **163**:607-619.

75. Shalom G, Shaw JG, Thomas MS: ***In vivo* expression technology identifies a type VI secretion system locus in *Burkholderia pseudomallei* that is induced upon invasion of macrophages**. *Microbiology* 2007, **153**:2689-2699.

76. Lertpiriyapong K, Gamazon ER, Feng Y, Park DS, Pang J, Botka G, Graffam ME, Ge Z, Fox JG: ***Campylobacter jejuni* type VI secretion system: roles in adaptation to deoxycholic acid, host cell adherence, invasion, and *in vivo* colonization**. *PLoS One* 2012:7.

77. de Pace F, de Paiva JB, Nakazato G, Lancellotti M, Sirsili MP, Stehling EG, da Silveira WD, Sperandio V: **Characterization of *icmF* of the type VI secretion system in an avian pathogenic *Escherichia coli* (APEC) strain**. *Microbiology* 2011, **157**:2954-2962.

78. Chow J, Mazmanian SK: **A pathobiont of the microbiota balances host colonization and intestinal inflammation**. *Cell Host Microbe* 2010, **7**:265-276.

79. Yu Y, Yang H, Li J, Zhang P, Wu B, Zhu B, Zhang Y, Fang W: **Putative type VI secretion systems of *Vibrio parahaemolyticus* contribute to adhesion to cultured cell monolayers**. *Arch Microbiol* 2012, **194**:827-835.

80. Wang X, Wang Q, Xiao J, Liu Q, Wu H, Xu L, Zhang Y: ***Edwardsiella tarda* T6SS component *evpP* is regulated by *esrB***

and iron, and plays essential roles in the invasion of fish. *Fish Shellfish Immunol* 2009, **27**:469-477.

81. Wang M, Luo Z, Du H, Xu S, Ni B, Zhang H, Sheng X, Xu H, Huang X: **Molecular characterization of a functional type VI secretion system in *Salmonella enterica* serovar Typhi**. *Curr Microbiol* 2011, **63**:22-31.

82. Schlieker C, Zentgraf H, Dersch P, Mogk A: **ClpV, a unique Hsp100/Clp member of pathogenic proteobacteria**. *Biol Chem* 2005, **386**:1115-1127.

83. Suarez G, Sierra JC, Sha J, Wang S, Erova TE, Fadl AA, Foltz SM, Horneman AJ, Chopra AK: **Molecular characterization of a functional type VI secretion system from a clinical isolate of *Aeromonas hydrophila***. *Microb Pathog* 2008, **44**:344-361.

84. Burtnick MN, DeShazer D, Nair V, Gherardini FC, Brett PJ: ***Burkholderia mallei* cluster 1 type VI secretion mutants exhibit growth and actin polymerization defects in RAW 264.7 murine macrophages**. *Infect Immun* 2010, **78**:88-99.

85. Hopf V, Göhler A, Eske-Pogodda K, Bast A, Steinmetz I, Breitbach K: ***BPSS1504*, a cluster 1 Type VI secretion gene, is involved in intracellular survival and virulence of *Burkholderia pseudomallei***. *Infect Immun* 2014, **82**:2006-2015.

86. Burtnick MN, Brett PJ, Harding SV, Ngugi SA, Ribot WJ, Chantratita N, Scorpio A, Milne TS, Dean RE, Fritz DL *et al.*: **The cluster 1 type VI secretion system is a major virulence determinant in *Burkholderia pseudomallei***. *Infect Immun* 2011, **79**:1512-1525.

87. French CT, Toesca IJ, Wu T-H, Teslaa T, Beaty SM, Wong W, Liu M, Schröder I, Chiou P-Y, Teitell MA *et al.*: **Dissection of the *Burkholderia* intracellular life cycle using a photothermal nanoblade**. *Proc Natl Acad Sci U S A* 2011, **108**:12095-12100.

88. Bendor L, Weyrich LS, Linz B, Rolin OY, Taylor DL, Goodfield LL, Smallridge WE, Kennett MJ, Harvill ET: **Type six secretion system of *Bordetella bronchiseptica* and adaptive immune components limit intracellular survival during infection**. *PLoS One* 2015, **10**:e0140743.

89. Srinivasa Rao PS, Yamada Y, Yuen PT, Ka YL: **Use of proteomics to identify novel virulence determinants that are required for *Edwardsiella tarda* pathogenesis**. *Mol Microbiol* 2004, **53**:573-586.

90. Haapalainen M, Mosorin H, Dorati F, Wu RF, Roine E, Taira S, Nissinen R, Mattinen L, Jackson R, Pirhonen M *et al.*: **Hcp2, a secreted protein of the phytopathogen *Pseudomonas syringae* pv. tomato DC3000, is required for fitness for competition against bacteria and yeasts**. *J Bacteriol* 2012, **194**:4810-4822.

91. Blondel CJ, Jiménez JC, Leiva LE, Álvarez SA, Pinto BI, Contreras F, Pezoa D, Santiviago CA, Contreras I: **The type VI secretion system encoded in *Salmonella* pathogenicity island 19 is required for *Salmonella enterica* serotype Gallinarum survival within infected macrophages**. *Infect Immun* 2013, **81**:1207-1220.

92. Parsons DA, Heffron F: ***sciS*, an *icmF* homolog in *Salmonella enterica* serovar Typhimurium, limits intracellular replication and decreases virulence**. *Infect Immun* 2005, **73**:4338-4345.

93. Mulder DT, Cooper CA, Coombes BK: **Type VI secretion system-associated gene clusters contribute to pathogenesis of *Salmonella enterica* serovar Typhimurium**. *Infect Immun* 2012, **80**:1996-2007.

94. Dong TG, Ho BT, Yoder-Himes DR, Mekalanos JJ: **Identification of T6SS-dependent effector and immunity proteins by Tn-seq in *Vibrio cholerae***. *Proc Natl Acad Sci U S A* 2013, **110**:2623-2628.

95. Weyrich LS, Rolin OY, Muse SJ, Park J, Spidale N, Kennett MJ, Hester SE, Chen C, Dudley EG, Harvill ET: **A type VI secretion system encoding locus is required for *Bordetella bronchiseptica* immunomodulation and persistence *in vivo***. *PLoS One* 2012:7.

96. Bleumink-Pluym NMC, van Alphen LB, Bouwman LI, Wösten MMSM, van Putten JPM: **Identification of a functional type VI secretion system in *Campylobacter jejuni* conferring capsule polysaccharide sensitive cytotoxicity**. *PLoS Pathog* 2013, **9**:16-18.

97. Bartonickova L, Sterzenbach T, Nell S, Kops F, Schulze J, Venzke A, Brenneke B, Bader S, Gruber AD, Suerbaum S *et al.*: **Hcp and VgrG1 are secreted components of the *Helicobacter hepaticus* type VI secretion system and VgrG1 increases the bacterial colitogenic potential**. *Cell Microbiol* 2013, **15**:992-1011.

98. Tomás A, Lery L, Regueiro V, Pérez-Gutiérrez C, Martínez V, Moranta D, Llobet E, González-Nicolau M, Insua JL, Tomas JM *et al.*: **Functional genomic screen identifies *Klebsiella pneumoniae* factors implicated in blocking NF-κB signalling**. *J Biol Chem* 2015, **290** jbc.M114.621292.

99. Yu Y, Fang L, Zhang Y, Sheng H, Fang W: **VgrG2 of type VI secretion system 2 of *Vibrio parahaemolyticus* induces autophagy in macrophages**. *Front Microbiol* 2015, **6**:1-8.

100. Pezoa D, Yang HJ, Blondel CJ, Santiviago CA, Andrews-Polymenis HL, Contreras I: **The type VI secretion system encoded in SPI-6 plays a role in gastrointestinal colonization and systemic spread of *Salmonella enterica* serovar Typhimurium in the chicken**. *PLoS One* 2013:8.

101. Sha J, Rosenzweig JA, Kozlova EV, Wang S, Erova TE, Kirtley ML, van Lier CJ, Chopra AK: **Evaluation of the roles played by Hcp and VgrG type 6 secretion system effectors in *Aeromonas hydrophila* SSU pathogenesis**. *Microbiology* 2013, **159**:1120-1135.

102. Repizo GD, Gagné S, Foucault-Grunenwald M-L, Borges V, Charpentier X, Limansky AS, Gomes JP, Viale AM, Salcedo SP: **Differential role of the T6SS in *Acinetobacter baumannii* virulence**. *PLoS One* 2015, **10**:1-20.

103. Wu HY, Chung PC, Shih HW, Wen SR, Lai EM: **Secretome analysis uncovers an Hcp-family protein secreted via a type VI secretion system in *Agrobacterium tumefaciens***. *J Bacteriol* 2008, **190**:2841-2850.

104. Zheng J, Leung KY: **Dissection of a type VI secretion system in *Edwardsiella tarda***. *Mol Microbiol* 2007, **66**:1192-1206.

105. Shyntum DY, Theron J, Venter SN, Moleleki LN, Toth IK, Coutinho TA: ***Pantoea ananatis* utilizes a type VI secretion system for pathogenesis and bacterial competition**. *Mol Plant Microbe Interact* 2015, **28**:420-431.

106. Mattinen L, Nissinen R, Riipi T, Kalkkinen N, Pirhonen M: **Host-extract induced changes in the secretome of the plant pathogenic bacterium *Pectobacterium atrosepticum***. *Proteomics* 2007, **7**:3527-3537.

107. Potvin E, Lehoux DE, Kukavica-Ibrulj I, Richard KL, Sanschagrin F, Lau GW, Levesque RC: ***In vivo* functional genomics of *Pseudomonas aeruginosa* for high-throughput screening of new virulence factors and antibacterial targets**. *Environ Microbiol* 2003, **5**:1294-1308.

108. Sana TG, Soscia C, Tonglet CM, Garvis S, Bleves S: **Divergent control of two type VI secretion systems by RpoN in *Pseudomonas aeruginosa***. *PLoS One* 2013, **8**:1-10.

109. Lesic B, Starkey M, He J, Hazan R, Rahme LG: **Quorum sensing differentially regulates *Pseudomonas aeruginosa* type VI secretion locus I and homologous loci II and III, which are required for pathogenesis**. *Microbiology* 2009, **155**:2845-2855.

110. Zhang L, Xu J, Xu J, Zhang H, He L, Feng J: **TssB is essential for virulence and required for type VI secretion system in *Ralstonia solanacearum***. *Microb Pathog* 2014, **74**:1-7.

111. Bladergroen MR, Badelt K, Spaink HP: **Infection-blocking genes of a symbiotic *Rhizobium leguminosarum* strain that are involved in temperature-dependent protein secretion**. *Mol Plant Microbe Interact* 2003, **16**:53-64.

112. Roest HP, Mulders IH, Spaink HP, Wijffelman CA, Lugtenberg BJ: **A *Rhizobium leguminosarum* biovar *trifolii* locus not localized on the Sym plasmid hinders effective nodulation on plants of the pea cross-inoculation group**. *Mol Plant Microbe Interact* 1997, **10**:938-941.

113. Pezoa D, Blondel CJ, Silva CA, Yang HJ, Andrews-Polymenis HL, Santiviago CA, Contreras I: **Only one of the two type VI secretion systems encoded in the *Salmonella enterica* serotype Dublin genome is involved in colonization of the avian and murine hosts**. *Vet Res* 2014, **45**:1-9.

114. Blondel CJ, Yang HJ, Castro B, Chiang S, Toro CS, Zaldĭvar M, Contreras I, Andrews-Polymenis HL, Santiviago CA: **Contribution of the type VI secretion system encoded in SPI-19 to chicken colonization by *Salmonella enterica* serotypes Gallinarum and Enteritidis**. *PLoS One* 2010:5.

115. Cheng AT, Ottemann KM, Yildiz FH: ***Vibrio cholerae* response regulator VxrB controls colonization and regulates the type VI secretion system**. *PLoS Pathog* 2015, **11**:e1004933.