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Review

Insights into the Virulence of *Campylobacter jejuni* Associated with Two-Component Signal Transduction Systems and Single Regulators

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Abstract: *Campylobacter jejuni* is one of the major aetiologies of diarrhoea. Understanding the processes and virulence factors contributing to *C. jejuni* fitness is a cornerstone for developing mitigation strategies. Two-component signal transduction systems, known as two-component systems (TCSs), along with single regulators with no obvious cognate histidine kinase, help pathogens in interacting with their environments, but the available literature on *C. jejuni* is limited. A typical TCS possesses histidine kinase and response regulator proteins. The objective of this review was to provide insights into the virulence of *C. jejuni* associated with TCSs and single regulators. Despite limited research, TCSs are important contributors to the pathogenicity of *C. jejuni* by influencing motility (FlgSR), colonisation (DccRS), nutrient acquisition (PhosSR and BumSR), and stress response (RacRS). Of the single regulators, CbrR and CosR are involved in bile resistance and oxidative stress response, respectively. Cross-talks among TCSs complicate the full elucidation of their molecular mechanisms. Although progress has been made in characterising *C. jejuni* TCSs, shortfalls such as triggering signals, inability to induce mutations in some genes, or developing suitable in vivo models are still being encountered. Further research is expected to shed light on the unexplored sides of the *C. jejuni* TCSs, which may allow new drug discoveries and better control strategies.

Keywords: *Campylobacter jejuni*; virulence; pathogenesis; two-component system; colonisation; fitness

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1. Introduction

The two-component system (TCS) is one of several mechanisms used by bacteria to adapt to their environment [1,2]. Archaea and some eukaryotes other than animals also possess TCSs [3]. Bacteria are known to possess dozens or hundreds of TCSs, which limits a complete understanding of such important systems [4]. A prototype TCS is composed of a histidine kinase (HK) and a response regulator (RR) involved in signal detection and transduction pathways [1,5] but variations exist, including phosphorelay and hybrid TCSs, depending on the number and/or structures of involved domains (Figure 1) [6]. The signal

for a TCS may be a change in nutrient level, osmotic pressure, pH, redox state, antibiotics, or membrane stress [1]. The cytoplasmic domain of sensory HK autophosphorylates when activated by a signal and the phosphoryl group on the histidine of the activated kinase is transferred to an aspartate residue on the RR, which in turn controls cellular behaviours by direct transcriptional repression or activation of target genes depending on the received signal [3,7].

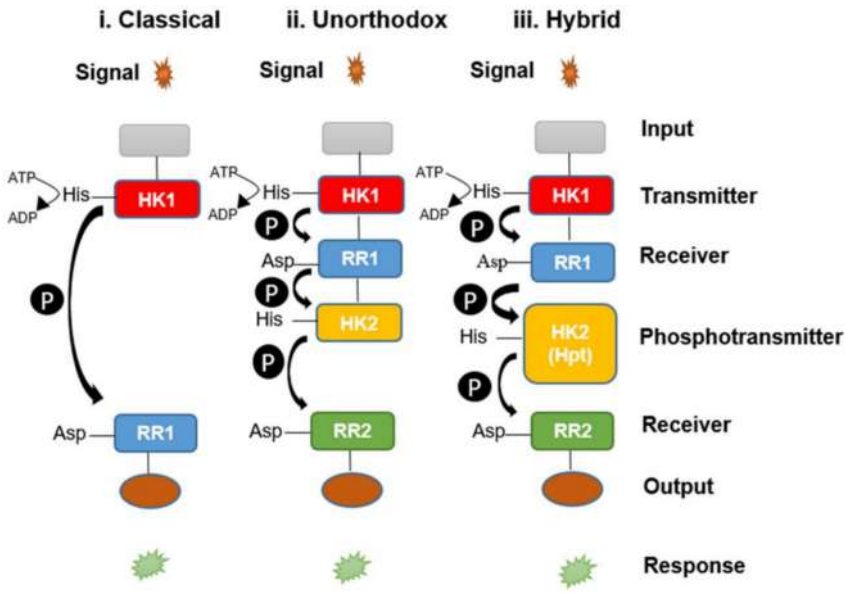

Figure 1. Classical-/Unorthodox-/hybrid signal transduction system modulation patterns: (**i**) the classical version comprises an N-terminal input domain, followed by a transmitter (HK1) with a conserved histidine that can be autophosphorylated. The phosphoryl group (P) is transferred to a conserved aspartic residue of RR1. The classical version is a two-step phosphorelay mechanism; (**ii**) in the unorthodox version, the HK1 is followed by an additional conserved aspartic residue (RR1) and an HK2. The phosphoryl group (P) is transferred to a conserved aspartic residue in the receiver (RR2) domain. The unorthodox version is a four-step phosphorelay mechanism; (**iii**) the hybrid version is similar to the unorthodox version, but HK2 (Hpt) is an external phosphotransfer module that acts as an individual protein. This figure was adapted from Liu et al. [6].

Cross-talk is defined as the communication between pathways that, if eliminated, would leave intact two distinct pathways [4]. Conserved sequences and structural similarity of proteins composing TCSs lead to the conclusion of possible cross-talks and signal integration among various TCSs [4]. However, cross-talks among TCSs are reduced to a minimum and seem to be exceptions, as linear signal transduction is the rule [8,9]. It was previously shown that introducing cross-talk in TCS decreased system performance and led to disastrous consequences for bacteria [8]. Additionally, comparative studies have shown that non-cognate RRs have reduced affinityand slower phosphotransfer kinetics which interfere with cross-talks [5]. The analysis of bacterial genomes found that 15–25% of the TCS proteins could participate in out-of-operon cross-talk which complicates a full elucidation of cell signalling [10]. Occasionally, the HK may function as a phosphatase to control its cognate RR in the absence of a stimulus [8,11]. Therefore, an in-depth understanding of cross-talks and involved mechanisms is of paramount importance in deciphering complex bacterial signalling [10].

Although TCS was first characterised in a non-pathogenic *Escherichia coli*, it is known that many bacteria use several TCSs [1] to regulate their basic processes (metabolism and motility) and other functions such as virulence, drug resistance, or tolerance [1,11]. In short, TCSs are viewed as prerequisites in bacterial virulence, as they help microbes to adapt to various environments, both inside and outside of their hosts [12]. TCSs are also involved in biofilm formation through the integration of input stimuli [6]. Despite the existence of

many TCSs, limited studies have been conducted to identify signals which activate specific TCSs and how this is linked to bacterial pathogenicity [13].

Shedding light on TCSs would be pivotal in antimicrobial chemotherapy and could also support the use of TCSs in agriculture or environmental remediation [1,11]. An attempt has been made to characterise some inhibitors (antibacterial drugs) which target specific TCSs in pathogenic bacteria [13]. TCSs are ideal candidates for drug discovery because (1) halting their functions affects both the upstream and downstream of the targeted step, and (2) selective TCS inhibitors may show slightly negative impacts on mammalian cells based on the phosphorylated histidine for bacteria instead of serine/threonine or tyrosine of higher eukaryotes [13]. Various inhibitors have been shown to target different steps in the TCS functioning including HK, RR, and even the sequestration or inhibition of the triggering signal [1]. A review article established a link between various TCSs and virulence, but the literature about TCSs of *Campylobacter* is still limited [12].

Campylobacter species, mainly *C. jejuni* and *C. coli*, are among the major causes of human gastroenteritis worldwide, and the exposure is often linked to poultry [14,15]. Despite its fragility and lack of secretory systems characterising other pathogenic bacteria, *C. jejuni* is able to withstand harsh environments by adapting to constantly changing conditions [16,17]. However, *C. jejuni* possesses a group of genes and pathways, allowing it to colonise various reservoirs and cause human gastroenteritis [18]. Other features contributing to the survival and virulence of *C. jejuni* include its ability to form biofilm [19] and the ability to enter a state of viable but non-culturable (VBNC) [20].

Similar to other bacteria, *C. jejuni* uses TCSs to control changes in its surrounding environment and responds by the expression of specific genes depending on the incoming signal [21]. *Campylobacter jejuni* genome was predicted to have several TCSs including nine RRs, six HKs, and one hybrid sensor RR protein [16,22]. Research has shown that *C. jejuni* mutants lacking TCSs genes exhibited defects in colonisation or pathogenicity [23–25]. For instance, phenotype analysis and electron microscopy showed that *C. jejuni* strains with defective *Flg*SR genes were not motile and lacked flagella, respectively [23]. Additionally, DccRS *C. jejuni* mutants exhibited a sharp reduction of colonisation and inflammatory potential in the gut of mice with limited flora [24]. The details of each TCS will be given in appropriate sections of this review. *C. jejuni* possesses different well-characterised cognate TCSs—namely, FlgSR regulating the *fla* regulon [23,26], DccRS involved in chicken colonisation [24,27], RacRS controlling various aspects of physiology and metabolism [28,29], PhosSR regulating phosphate acquisition [30], and CprRS involved in biofilm formation and chicken colonisation [20]. Recently, another TCS, called BumSR, has been identified as an indirect sensor of butyrate and could be important for the colonisation of various hosts [31].

Apart from the complete TCSs, the *C. jejuni* genome encodes some single regulators involved in various processes. Some of these regulators include the CbrR involved in bile resistance [32] and CosR which controls oxidative stress response and expulsion of toxic substances [33,34]. The regulation of these single regulators and their interaction with already known TCSs is not fully understood.

Available information on TCSs of *Campylobacter* is fragmented, as the conducted studies cover a single or limited number of TCSs. A better understanding of TCSs would elucidate patterns associated with the survival and pathogenesis of *C. jejuni*. Although not common, cross-talks among members of different TCSs need particular emphasis. The current review provides a comprehensive picture through the synthesis and pooling of the existing literature. This would provide substantial inputs in designing appropriate strategies to control campylobacteriosis and the development of antimicrobial resistance inhibitors. With this background, this review aims to provide insights into the roles played by various TCSs and single regulators of *C. jejuni* with a specific emphasis on their virulence and how they contribute to *C. jejuni* fitness.

2. Detailed Description of TCSs and Single Regulators of *Campylobacter jejuni*

2.1. Two-Component Systems of Campylobacter jejuni

2.1.1. FlgS/FlgR TCS and *Campylobacter jejuni* Motility

Campylobacter jejuni possesses a single flagellum found on either one or both poles, which contributes to its motility [35]. The flagellum is composed of a basal body, hook, and filament [36]. The filament is made of two homologous proteins, FlaA and FlaB, considered major and minor, respectively, but their corresponding genes are under the control of two different promoters [36,37]. The flagellum is an important bacterial virulence factor involved in various processes such as chemotaxis, behaviour, invasion, survival, and colonisation of various hosts [23,35,38,39]. The flagellar assembly of *C. jejuni* is a complex, ATP-requiring, well-organised, and highly regulated process involving more than 50 genes distributed over 32 loci [23,35,40,41]. Despite homology in the structure of flagellar apparatus among bacteria, the flagella of ε-proteobacteria proved to diverge from the rest, but the involved molecular mechanisms are yet to be understood [42]. Additionally, *C. jejuni* lacks global regulatory factors such as FlhDC in *E. coli*, which hampers the complete understanding of flagella synthesis and regulatory mechanisms [43,44].

Campylobacter jejuni flagella genes are classified into three groups-namely, group I, group II, and group III genes [45,46]-regulated by three sigma factors (RpoD, RpoN, and FliA) [23,35,47]. Group I genes encode RpoD (σ^{70})-dependent genes including those coding for the components of the flagellar export apparatus (FEA) (FlhA, FlhB, FliF, FliO, FliP, FliQ, and FliR), the FlgSR TCS, σ^{54} (RpoN), and σ^{28} (FliA) [23,45,48]. RpoN (σ^{54}) controls the transcription of various genes encoding basal body, hook, and FlaB, while FliA (σ^{28}) is associated with the transcription of *fla*A and other minor flagellar proteins [23,35,47].

The flagellar type III secretion system (fT3SS) has been suggested to make a signal sensed by FlgS leading to its autophosphorylation [45]. FlgS monitors fT3SS formation by detecting when FliF and FliG have multimerised into the MS ring and rotor structures [47]. The phosphorylated FlgS activates FlgR by transferring the phosphate group, allowing the interaction with, and stimulation of, σ^{54}, triggering the transcription of group II genes and ending with the formation of the basal body–hook complex [23,26,35,49]. Finally, the transcription of group III genes (*fla*A, *fla*G, *fli*D, etc.) occurs when the FlgM preventing the expression of the σ^{28} factor is detached and secreted out of the cell. Normally, FlgM binds to σ^{28} in the cytoplasm, preventing the expression of group III genes. However, once the basal body and hook are formed, FlgM is secreted out of the cell, allowing the expression of group III genes (Figure 2) [44,48]. FlgR/FlgS system participates in the early stages of *C. jejuni* colonisation and not in the persistence in the chicken caeca [23].

It was shown that FlhF acts before FEA (early stage of flagella assembly) and could be a master protein in the transcription and synthesis of flagellar components [48,50]. However, Balaban et al. [49] mentioned that FlhF was not required for transcription or production of the fT3SS proteins and suggested a lack of fT3SS formation in the flhF mutant. Recently, an in-depth analysis suggested FlhF as a transcriptional regulator directly binding to the promoters of important flagellar genes (σ^{70}, σ^{28}, *flg*S) and indirectly contributing to the transcription of group II genes [44]. FlhF GTPase and FlhG ATPase are hypothesised to influence fT3SS formation and location, which contributes to controlling both the location and number of flagella in polar flagellates [50,51]. If FlhG controls both the active and inactive forms of FlhF [18,46], it may be considered an important contributor to the FlgSR activation (Figure 2). Apart from being suggested to be at the top of the fT3SS, FlhF has been proposed to exert GTPase independent activities such as the activation of σ^{54} factor in RNA polymerase and the genes depending on RpoN, and the stability of σ^{54}-dependent mRNA transcripts [50,51]. Therefore, the complexity of the flagellar regulon, together with various genes involved in motility, make the full elucidation of FlgSR a continuous process.

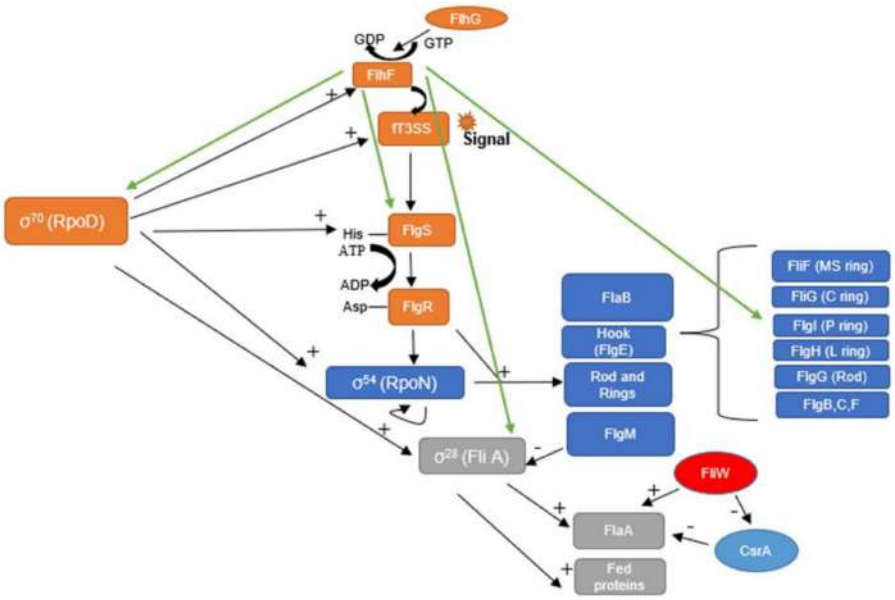

Figure 2. Schematic diagram of *C. jejuni* flagellar regulation. σ^{70} influences several genes (group I) which are part of the *fla* regulon (shown in orange). FlhF is activated by FlhG and becomes phosphorylated. A signal generated in fT3SS, due to formation of FliF and FliM into the MS ring and rotor, is detected by FlgS which in turn autophosphorylates. This phosphate is transferred to the response regulator FlgR. The latter stimulates the transcription of σ^{54} and activates (together with σ^{54}) the transcription of the genes needed for the assembly of the hook-basal body rod complex (Group II). FlgM binds to σ^{28} in the cytoplasm preventing the expression of group III genes. However, once the basal body and hook are formed, FlgM is secreted out of the cell allowing the expression of group III genes. FlhF has a positive regulation on σ^{70}, FlgS, σ^{28}, and the FlgI by binding to their promoters as shown by the green arrows. FliW binds to FlaA as part of the CsrA sensor. Once secreted, the FliW is released. FliW also binds to CsrA and contributes to the translation of FlaA mRNAs. This figure was adapted from Li et al. [44].

2.1.2. *Campylobacter jejuni* DccR/DccS (Diminished Capacity to Colonise) Is Involved in Chicken Colonisation

The *cj1223c-cj1222c* TCS of *C. jejuni*, now named DccRS [24], accelerates *C. jejuni* chicken colonisation and upregulates seven genes coding for proteins exported to the periplasm or cell membrane [24,27]. The DccRS system has also been reported in *C. coli* for the regulation of heat stress at 46 °C [51]. Additionally, the DccRS system and its target genes are believed to contribute to the colonisation of various bacteria because its homologs and regulated genes were found in other *Campylobacter* and *Helicobacter* species [24,52]. DccRS is not necessary for colonisation, but it promotes chick colonisation, and its activation could result from a metabolic product at the end of the stationary phase [27]. The *dcc*R gene was found to be dispensable in vitro, as the growth and colonisation of both mutant and wild-type strains in the presence of various stresses were not distinguishable [24]. In a host, *dcc*RS genes are suggested to be involved in the initial adaptation of *C. jejuni* rather than being required for survival during persistent or acute infection [27]. This is supported by a study which showed that five genes (*cj0200c*, *cj1356c*, and *cj1626*, *cj0606*, and *cj1723c*) of DccRS regulon were upregulated within a few hours after infection, using a novel rabbit soft-tissue chamber model [52].

DccS, as a sensor kinase, selectively phosphorylates the cognate response regulator DccR [27]. MacKichan et al. [24] studied immunocompetent limited flora (I-LF) C3H mice inoculated with 81–176 wild-type strains and isogenic ΔdccS and ΔdccR mutants. The analysis of stool and cecum of the mice after three and seven days showed that the 81–176 dccR mutants were found to be at 10^2-fold lower levels than the dccS mutant, suggesting that the lack of DccS could lead to limited phosphorylation and activation of

DccR, partially raising the colonisation potential [24]. A study using severe combined immunodeficient (SCID) mice inoculated with the DccR mutant reported a greatly reduced inflammatory reaction and a normal appearance of mutant strains [24]. DccRS may also induce the severe combined immunodeficiency (SCID) of limited flora mice [53]. The signal triggering DccRS TCS is yet to be identified, but it is suggested to result from an extended growth of *C. jejuni* and could be a product of metabolism accumulating in the stationary phase [27]. The in vitro studies of DccRS TCS did not reveal any difference between wild-type and mutant strains [24]. The understanding of DccRS TCS would be pivotal in deciphering processes related to *C. jejuni* survival and infection [24].

2.1.3. *Campylobacter jejuni* PhosSR TCS and Phosphate Acquisition

Bacteria use the *pho* regulon under the control of a TCS to cope with the scarcity of inorganic phosphate (P_i) used in various cell functions including signal transduction systems and ATP production [54]. Apart from its role in P_i homeostasis, Pho regulon contributes to bacterial pathogenesis, but the mechanisms involved are yet to be elucidated [54]. *Campylobacter jejuni* PhosS–PhosR TCS detects a drop in phosphate concentration and responds by altering the transcription of genes (the *pho* regulon) involved in phosphate acquisition and other metabolic processes [30]. In general, bacteria obtain P_i from external phosphate-rich sources using alkaline phosphatase (phoA) [54]. Alkaline phosphatases remove P_i from phospho-organic compounds and contribute significantly to the formation of polyP by the polyphosphate kinase 1 (ppk1) [55]. PolyP regulates bacterial response to stresses and virulence [56]. *Campylobacter jejuni* is reported to possess all the genes necessary for polyP metabolism [57]. In a P_i-poor environment, the phosphatase coded by *cj*0145 is upregulated under the control of PhosSR TCS [30,58]. Kumar et al. [56] suggested a model explaining the functioning of PhosSR TCS where the limited concentration of P_i activates PhoS which then interacts with PhosR, allowing the uptake of external organophosphates with the help of PhoA (Figure 3). It is important to mention that there is a positive correlation between lower concentrations of P_i and the biosynthesis of various secondary metabolites including antibiotics such as macrolides and tetracyclines, as reported in the *Streptomyces* genus [58]. Closely related TCSs (PhosBR in *E. coli* and PhoP–PhoR in *B. subtilis*) activate over 30 genes involved in ion transport, degradation of organophosphate, phosphatase, and regulatory proteins [58]. *Campylobacter jejuni* phoA uses phosphomonoesters as a substrate and is transported via unusual twin-arginine translocation (Tat) secretion system [57].

It has been shown that PhosS–PhosR is a special TCS that (1) activates *pstSCAB* and eight other genes in response to the reduction in P_i, and (2) lacks sequence homology and other characteristics common to phosphate sensitive systems in bacteria [30]. Molecular studies revealed that 12 genes located in 3 different operons—*cj0145*, *pstSCAB* (*cj0613-cj0616*), and *cj0727-cj0733*—form a regulon which is regulated by the PhosS–PhosR TCS [30]. The analysis of RNA from wild-type and PhosR mutant using microarray showed only a threefold difference in the *pstS* gene but no differences for the other PhosS–PhosR-regulated genes [30]. The alkaline phosphatase deletion mutant showed (1) significantly decreased polyP accumulation, (2) increased resistance to certain antimicrobials (tetracycline and nalidixic acid, and ciprofloxacin), (3) reduced invasion and survival in human intestinal epithelial human cells and chicken colonisation, and (4) increased biofilm [55]. All these suggest that the increased resistance to antimicrobials could be associated with increased biofilm formation of the mutant strain [55].

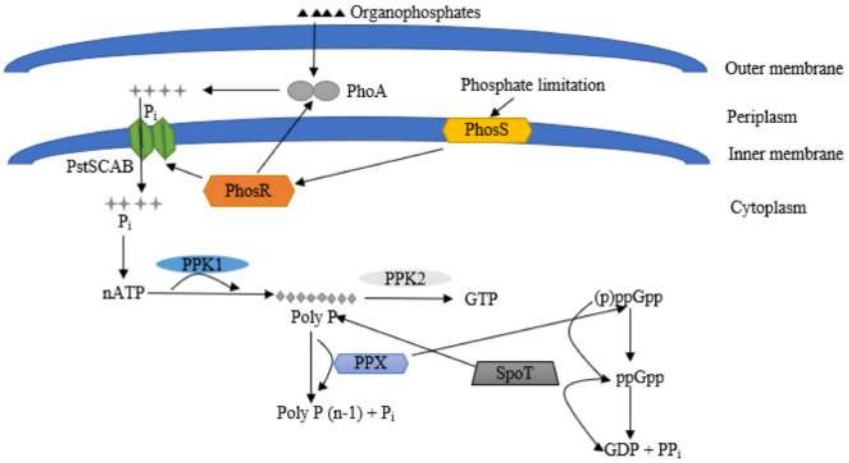

Figure 3. PhosS-PhosR two-component system. Organophosphates are hydrolysed to inorganic phosphate (P_i) by alkaline phosphatase (PhoA) in the periplasm. Phosphate uptake proteins and PhoA are directly regulated by the PhosS-PhosR TCS which is activated by phosphate limitation. P_i is transported across the inner membrane via the high-affinity phosphate transport system PstSCAB. ATP generated from Pi is utilised for polyP synthesis by PPK1. PPK2 utilises polyP to generate GTP, while PPX hydrolyses poly P back to Pi. PPX also affects the conversion of (p)ppGpp to ppGpp. SpoT is a bifunctional enzyme involved in both ppGpp synthesis and its hydrolysis. SpoT is also linked to poly P metabolism. This figure was adapted from Kumar et al. [56].

2.1.4. *Campylobacter jejuni* CprRS (Planktonic Growth Regulation) TCS Regulation of Biofilm Formation and Chicken Colonisation

Biofilms are bacterial communities protected from various environmental conditions and formed in response to particular stimuli [59]. The *Campylobacter* planktonic growth regulation (CprRS), one of the *Campylobacter* TCSs, is involved in biofilm formation, colonisation, and osmotic stress tolerance [20]. It was first described as TCS encoded by *Cj1226c* (HK) and *Cj1227c* (RR) genes conserved in the genus *Campylobacter* and involved in *C. jejuni* pathogenesis [20,60]. The CprRS genes are located near *htr*A and *peb* genes which may suggest their role in the regulation of the *Campylobacter* cell envelope [17]. The molecular analysis proposed that CprRS TCS may be activated by a promoter upstream of CprR and requires CprS only when expression levels beyond minimal ones are required [17]. Proteomics and in vitro studies showed that CprS absence enhances motility (FlaA expression), protein secretion, and biofilm formation [20]. While CprS is dispensable, CprR is essential and contributes to homeostasis by regulating cell envelope-related genes (*htr*A and *peb*4) [17]. Despite being dispensable, CprS mutant resulted in decreased expression of *sod*B, *rrc*, and *lux*S genes [61]. An experiment comparing both a wild-type and *cpr*S mutant concluded that a variety of dysregulated proteins in the mutant strain reflects numerous roles played by CprRS on *C. jejuni* biology [20]. CprRS is suggested to have pleiotropic effects; thus, further studies are needed for a deeper understanding of all the roles it plays [20]. Taken together, CprRS is crucial for the expression of factors necessary for biofilm formation, colonisation, and stress tolerance.

2.1.5. *Campylobacter jejuni* RacRS (Reduced Ability to Colonise) TCS Controls Physiology and Metabolism

RacRS assists *C. jejuni* in mounting a response to stresses associated with heat shock [62] by influencing cell elongation, sustaining motility, and colonising chicken [25]. RacRS, one of the first TCSs discovered in *C. jejuni* [25,62], allows the use of fumarate for respiration in its inactive form [28] and activates glutamate synthesis via upregulation of gamma-glutamyltransferase (*ggt*) [29]. RacRS binds to a consensus sequence upstream *ggt* promoter which affects the transcription levels of *ggt* [29]. The GGT protein contributes to the persistent colonisation of the chicken gut [63] through glutathione and glutamine metabolism, but its regulation is poorly explored [29]. RacRS TCS also regulates vari-

ous aspects of the physiology and pathogenesis of *C. jejuni* [25]. Following the inability to use various sugars [64], *C. jejuni* containing GGT utilised glutamine and glutathione as the sole carbon source in its metabolic pathways [65] with the involvement of RacRS under an oxygen-limited environment [29]. RacRS has also been reported to influence both cellular glutamate production (cytosolic and periplasmic) and the use of extracellular glutamine [29].

The RacRS is a TCS that controls heat shock proteins and is also necessary for a differential expression of proteins at 37 °C and 42 °C [62]. Apart from promoting *C. jejuni* colonisation in chicken, RacRS is known to regulate the in vivo temperature-dependent signalling pathway, facilitating growth beyond 42 °C and contributing to cell elongation and septation [25,62]. Mutants in RacRS exhibited defects in viability at 42 °C, suggesting its influence on the adaptation of *C. jejuni* to higher temperatures [66]. However, a mutation in the *rac*R gene led to the expression of specific proteins in both temperature-dependent and independent manners [67,68]. The activation of RacRS is favoured by limited aerobic conditions and the availability of alternative electron acceptors such as nitrate [28]. Once activated, RacR represses various genes including *asp*A but activates *glt*BD genes [29], ending with glutamate production in the cytoplasm [69]. It is reported that RacR represses the *dna*J gene, which is a heat shock protein, and RacR mutant was unable to grow in Mueller–Hinton Broth (MHB) supplemented with 0.8% sodium chloride [25]. However, details of the mechanism of RacRC TCS functions and involved genes have not been reported.

2.1.6. *Campylobacter jejuni* BumSR (Butyrate Modulated) TCS Regulates Transcription and Colonisation

Campylobacter jejuni is capable of distinguishing various intestinal regions and uses BumSR TCS to regulate transcription and colonisation by sensing butyrate indirectly using an unknown mechanism [31]. By contrast, other pathogens regulate their virulence by using TCSs to sense different short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate released by intestinal microbiota [70]. BumSR was first characterised as Cjj81176_1484 (HK) and Cjj81176_1483 (RR) and was found to repress various genes involved in metabolism, iron/heme acquisition, and respiration [21]. BumS lacks the usual sensor kinase activity in vitro which is a characteristic of other TCSs [31] but acts like phosphatase and dephosphorylates BumR in the absence of butyrate [61]. As such, BumR (RR) was found to work in both phosphorylated and unphosphorylated forms [21]. Among the genes upregulated by butyrate presence are those coding for nutrient acquisition, energy generation, and colonisation factors [31]. The BumR response to butyrate favours commensal chicken colonisation and contributes to human infection [31,71]. It is suggested that BumSR TCS may diminish bacterial colonisation factors outside of the host but contributes to gastrointestinal tract infection in humans [21].

A summary of the known TCSs of *C. jejuni* is provided in Table 1.

Table 1. TCS of *Campylobacter jejuni*.

TCS Name	Genes	Alias	Identity	Role	Stimulus	References
FlgSR	*flgS* *flgR*	*Cj0793* *Cj1024*	HK RR	Regulation of *fla* regulon	FlhF/Flagella export apparatus (FEA)	[23,26,35,49]
DccRS	*dcc*S *dcc*R	*Cj1222c* *Cj1223c*	HK RR	Chicken colonisation	Unknown (product of metabolism)	[24,27]
PhosSR	*phos*S *phos*R	*Cj0889* *Cj0890*	HK RR	Regulation of *pho* regulon	Phosphate limitation	[30]
CprRS	*cpr*S *cpr*R	*Cj1226c* *Cj1227c*	HK RR	Regulation of envelope-related genes	Environmental (unknown)	[17,20]
RacRS	*rac*S *rac*R	*Cj1262* *Cj1261*	HK RR	Regulation of temperature-dependent growth and colonisation	Unknown	[25,28,58,63]
BumRS	*bum*S *bum*R	*Cjj1484* *Cjj1483*	HK RR	Regulation of transcription and colonisation	Exogenous butyrate	[21,31]

2.2. Single Regulators

Apart from the six described TCSs, *C. jejuni* is equipped with single regulators, CbrR and CosR, involved in bile resistance and oxidative stress response, respectively [32,34].

2.2.1. *Campylobacter jejuni* CbrR (Bile Resistance Regulator) Modulates Sodium Deoxycholate Resistance and Chicken Colonisation

The CbrR, considered an orphan two-component RR due to the lack of the HK encoded adjacent as for most TCSs, is encoded by the cj0643 gene and modulates bile salts resistance in *C. jejuni* [32]. Bile salts help in the digestion of lipids but also act as antimicrobial agents against pathogens by disrupting cell membranes [72]. However, *C. jejuni* is equipped with a cmeABC efflux pump, allowing it to withstand bile salts in the chicken gut [32,73]. The mechanism involved in the control of bile resistance by CbrR is unknown, and the interaction between cmeR and CbrR needs to be determined [32].

2.2.2. *Campylobacter jejuni* CosR (Oxidative Stress Regulator) controls Oxidative Stress Response and Expulsion of Toxic Substances

CosR is an orphan regulator encoded by the cj0355c gene [34] which showed 60% amino acid identity with another orphan transcriptional regulator hsrA (hp1043) of H. pylori essential for cell viability [33,74]. In *C. jejuni*, CosR is involved in the adaptation to oxidative stress [33] which is necessary for the viability as confirmed by a knockdown mutation study [34]. A transcriptomic analysis also identified 93 genes regulated by CosR, and 18.3% of these genes are considered essential [75].

Although CosR is an orphan RR, its autoregulation is suggested to be influenced by another oxidative stress response factor called CsrA [76]. A reduced level of CosR protein is suggested to derepress *sod*B coding for superoxide dismutase causing *C. jejuni* resistance to superoxide stress [77]. Molecular studies have shown that CosR binds to the *cme*ABC complex promoter and affects the transcription of *kat*A, coding for efflux pump and catalase, respectively [75]. In addition to CosR, other regulators such as PerR and Fur are also known to be involved in the regulation of oxidative stress [77]. Taken together, CosR collaborates with other genes to monitor an oxidative stress response and the extrusion of toxic compounds in *C. jejuni*.

TCSs in *Campylobacter* are complex based on the number of involved genes. Understanding the functions of TCS has shown progress considering that some genes were previously considered as coding for orphan response regulator (cj1024) or histidine kinase (cj0793), but later, their partner genes were discovered and now are being considered as complete TCS [78]. In 2015, *C. jejuni* was considered to have five TCS [21], but a new TCS (BumSR) has been recently described [31]. Therefore, new TCS may be discovered in the future as a result of progressing research. Another complication is the inability to form mutants for some of the genes which makes the study of particular functions or characteristics impossible [78]. It is worth mentioning that TCSs are subject to the control system that coordinates various signal transduction systems [6]. Further research is needed to understand the triggering signals, possible cross-talk among TCSs or with other regulatory systems, and the deciphering of all the genes involved in TCS pathways.

3. Conclusions

TCSs are important contributors to various aspects of *C. jejuni* survival and successful colonisation through the integration of input stimuli from either internal or external sources. The characterised TCSs of *C. jejuni* are involved in motility (FlgSR), colonisation (DccRS), nutrient acquisition (PhosS-PhosR and BumSR), and stress response (RacRS). However, the mechanisms involved in their regulatory activities are not fully understood. Apart from the TCSs, *C. jejuni* possesses single regulators called CosR and CbrR which mediate bile resistance and oxidative stress, respectively. Cross-talk among TCS and the triggers involved are yet to be fully elucidated. Additionally, the complexity of the regulatory processes needs special attention due to several genes involved. A better understanding of

TCSs would help in designing control strategies, especially in the poultry industry, which is considered the primary source of human campylobacteriosis. Targeted antibacterial drug discovery against TCSs is in its infancy but full of opportunities based on the special features of bacterial TCSs. There is a need for suitable in vivo models which would better mimic the human and avian intestines, thus allowing the study of cross-regulation and cross-talk among TCSs.

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