Virulence factors of Campylobacter

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Abstract

Campylobacter is the most commonly reported bacterial cause of food-borne infection in the United States. Guillian-Barré syndrome and reactive arthritis are the associated complications with Campylobacter infection. Besides, an increasing proportion of human infections caused by C. jejuni are resistant to antimicrobial therapy. Cross contamination in raw poultry and consumption of undercooked poultry are the major risk factors for human campylobacteriosis. Various virulence related mechanisms viz., motility, oxidative stress defense, toxin production, invasive properties, iron acquisition and viable but non-culturable stage shell them in the human body. Since they are actively involved in the food chain, efforts to prevent Campylobacter human illness are needed.

INTRODUCTION

Campylobacter are Gram negative, slim, motile, spirally curved rods. The width and length of the cells varies from 0.2-0.9 µm and 0.5-5µm respectively. The motility of C.jejuni is mediated by single polar flagellum. They are microaerophilic, do not metabolize sugars and use intermediates of the TCA cycle as source of energy. It needs 5-7% oxygen and 10% carbondioxide with optimal growth temperature 37-42°C. [1] C.jejuni is recognized world wide as an important food borne pathogen. Human gastroenteritis caused by the organism is often associated with consumption of red meat and poultry. [2] The natural habitat for Campylobacter spp. is the intestine of birds and warm-blooded animals including sea gulls and other wild animals. Campylobacter may enter the environment through the feces of animals, birds or infected humans. [3]

REVIEW OF THE VIRULENCE FACTORS IN

Animals hosts of Campylobacter spp. have been indicated as a source of contamination and are associated with survival of the organism in nature, since fecal material is shed directly into aqueous environment. [4] This microorganism causes gastroenteritis, further leading to serious neurological disease like Guillain-Barre Syndrome and eventually death. [5] Various virulence factors in campylobacters contribute to survival and establishment of disease in the host.

MOTILITY AND CHEMOTAXIS

Motility and chemotaxis have been shown to play an important role in bacterial colonization of several environments. [6] Chemotaxis towards urine has been demonstrated in Escherichia coli, a common urinary tract pathogen, suggesting that bacterial chemotaxis is involved in the pathogenesis of urinary tract infections. [7]

Flagellum has been suggested to play a vital role in adherence to epithelial cells. Campylobacter jejuni binds to the epithelial cells with the help of flagellin as an adhesin and colonizes the intestinal tracts of rabbits, suckling mice, hamsters etc. The genes coding for flagellin have been isolated from C.jejuni strains. [8] Fla A gene typing studies are done universally to identify the strains of C.jejuni and C.coli isolated from the environment. [9]

Bacterial chemotaxis is a complex signal transduction system by which bacteria are able to sense environmental stimuli and respond to them by flagellar rotation. In 1986, Paster and Gibbons studied the role of chemotaxis in oral colonization in selected Campylobacter and Wolinella species isolated from subgingival plaque, by quantitative method. Studies revealed that cells of C.concisus were attracted towards formate and to no other compounds tested (lactate, amino acids and sugars). Positive chemotaxis towards formate may enable Campylobacter to locate the oral cavity and further colonize plaque laden periodontal pockets. [6] Chemotaxis of Campylobacter was studied towards chemicals like, bile and mucin, which are secreted by gall bladder and the intestinal tract (primary sites of

Campylobacter infection in hosts). Other chemicals experimented with were carbohydrates, inorganic ions, organic acids and amino acids. These chemicals were qualitatively tested by hard agar plug method. Dense zones of bacterial accumulation around the agar plugs containing the attractant were observed. Mucin was the only strong chemoattractant in case of C.jejuni and was verified by flagellar rotation studies. Organic acid intermediates of the tricarboxylic acid cycle were chemoattractive to Campylobacter, Salmonella typhimurium and Pseudomonas aeruginosa all of which possess a tricarboxylic acid cycle uptake system. Chemoattraction of Campylobacter towards bile, mucin and L-fucose proves its ability to colonize the intestine and gall bladder of animals. [7]

INVASION AND ADHESION

One of the most important aspects of virulences in Campylobacter is its nature of interaction with intestinal cell lines. [10] Penetration of the intestinal mucosa play a vital role in pathogenesis of Campylobacter mediated gastroenteritis. Campylobacter adheres to the human intestinal cell lining and then becomes internalized within the cells. The translocation of C.jejuni across epithelial cell barrier reflects the virulent mechanism by which the organism gets access to sub mucosal tissue causing tissue damage, inflammation and thereby gastroenteritis. The virulence factors by which Campylobacter adheres to epithelial cells are proteins, flagella and lipopolysaccharide. [8]

The association of C.jejuni and C.coli with Hela cells was evaluated. Studies revealed that diarrhea and fever occurred more frequently in patients infected with invasive Campylobacter strains than in those infected with non invasive strains. Viable counts and transmission electron microscopy done after killing of extracellular bacteria by gentamycin strongly supports the fact that associated Campylobacter are adherent to cell membrane and are internalized into cytoplasmic vacuoles. [10] Role of flagella in adherence of Campylobacter to human intestinal epithelial cells was studied. C.jejuni flagellar mutants were constructed and examined for the ability to invade cultured human epithelial cells and also to cross the polarized cell monolayer of human epithelial origin. Findings indicated that either motility or flaA gene product or both are essential for translocation of Campylobacter across the polarized monolayer since mutants were not able to cross the cell barrier. In case of Campylobacter, flagellin and adhesion both are required for irreversible binding of bacteria to cells. [8] Graham investigated the ability of clinical isolates and one reference strain of C. fetus to adhere to and invade the human intestinal epithelial cell line, INT 407. During an initial 4-h infection period, all C. fetus strains were detected intracellularly which indicated that C. fetus was capable of adhering, entering, and surviving within the nonphagocytic epithelial cell line, INT 407. [11]

CELL WALL

The cross section of the outer cell membrane of Campylobacter is typical like that of gram-negative cells. Cell wall is three layered, with an outer lipoprotein layer, a middle lipopolysaccharide layer and an inner mucopeptide layer. On enzymatic treatment it was revealed the murein was made up of muramic acid, glucosamine, alanine, glutamic acid and diaminopimelic acid. The disaccharide tetrapeptide were prescribed as Glc NAc-MurNAc-L-ALa-D-Glu-meso-Dpm-D-Ala. Eighty percent of glucose was found in cell wall polysaccharide. [12] Lipopolysaccharides (LPS) are an abundant surface component of the outer membrane of gram-negative bacteria. The LPS consisted of three distinct regions, lipid A moiety which was anchored in the outer membrane and is the endotoxic part of the LPS molecule. Other is the core, which is attached to the lipid, and at last is the O antigen attached to the outer core. The LPS molecules of Campylobacter are involved in adherence and play a role in antigenic variations, as Campylobacter has the ability to shift the LPS antigenic composition. Surprisingly N-acetyl neuraminic acid (sialic acid) is present in the core oligosaccharide, not frequently found in prokaryotes. These sialic acid residues appeared like gangliosides in structure, when attached to –D galactosidase. This molecular mimicry is involved in the neuropathological autoimmune diseases like Guillains Barre' Syndrome and Miller-Fisher Syndrome which eventually leads to the death of the patient. [13]

PROTEINS AND ENZYMES

In Campylobacter, the important virulent factors include cytolethal distending toxin and hemolysin. Cells' major defense mechanisms include superoxide dismutase which breaks down superoxide molecules to hydrogen peroxide and dioxygen thereby protecting several cell components including cytoplasmic enzymes, DNA and membrane factors. [14] Campylobacter resides within epithelial cells lining the gut lumen as well as the granulocytes and parenchyma cells within the lamina propria. The intracellular existence provides the asaccharolytic, slow growing organism a suitable niche when the microbial competition is

either less or absent. Hydrogen peroxide which is generated during aerobic metabolism reacts with myeloperoxidase, reduced iron or products of nitric oxide synthase leading to formation of more toxic intermediates such as hypochlorous anion, hydroxyl radicals, hydroxide anions, nitrogen dioxide and peroxynitrite. Production of catalase that inactivates hydrogen peroxide interrupts the production of these toxic species and aid persistence and survival within host cells and tissues. Catalase is required for Campylobacter hydrogen peroxide resistance as well as persistence in macrophages. KatA is gene coding for catalase in C.jejuni and mutants lacking this gene showed significantly decreased viability than the wild type strain. Catalase plays minor role in intraepithelial cell survival but a significant role in intramacrophage survival by counteracting the effects of nitric oxide synthesis as well as the respiratory burst. [15]

Flagellin proteins are well known to be involved in colonization of Campylobacter in the host. Another virulent protein is Cad F later on termed as Campylobacter adhesion after identification which too is involved in host colonization. Lipoprotein which is a component of a protein binding dependent transport system for the siderophore enterochelin of both C.jejuni and C.coli , is coded by ceu E gene and this too is identified and characterized. Many other cytotoxins in Campylobacter are identified but cytolethal distending toxin (CDT) is the one which is fully characterized. The cdt genes cause cellular distention and eventually death of the cell lines. [16]

ANTIMICROBIAL SUSCEPTIBILITY

Campylobacter resistance to a number of antibiotics, such as tetracycline, erythromycin, ciprofloxacin, kanamycin, nalidixic acid and chloramphenicol has been reported. [17] The increasing rate of human infections caused by antimicrobial resistant strains of C.jejuni makes clinical management of campylobacteriosis cases more difficult. Human and healthy chicken isolates of C.jejuni were investigated for tetracycline resistance. High-level resistance was observed ranging from 32-256 mg/lit. Plasmids were detected in 74% isolates (30-40kb) and all of them carried the tet (O) gene. In only 6 strains resistance was found to be plasmid coded by conjugation experiments. [18] Majority of clinical isolates were found to carry foreign dfr genes coding for resistant variants of dihydrofolate reductase enzymes, the target of trimethorpim. The frequent trimethoprim resistance occurrence in C.jejuni strains was related to the high level exposures of food animals to antimicrobial drugs which would further lead to acquisition of foreign resistance genes in naturally transformable C.jejuni strains. [19] In similar work, commercial broiler chicken flocks were treated with enrofloxacin. Campylobacters were isolated from individual fecal samples before, during and after treatment. Rapid increase in the resistant Campylobacter was observed in the fluoroquinolone treated broilers. [20] During the 10 years (1991-2001), the rate of resistance to ciprofloxacin, ampicillin and tetracycline increased significantly amongst human Campylobacter strains. Different resistance rates to tetracycline amongst chicken isolates suggested the development of resistance during antimicrobial treatment in food animals. [21]

C.jejuni were found to contain undescribed plamids each one approximately 35 Kb size viz., p Tet plasmid carrying tet O gene coding for tetracycline resistance and p Vir coding for virulent proteins. Mutations in these plasmids reduced adherence and invasion to INT407 cell line suggesting that plasmids are involved in virulence of C.jejuni pathogens. [22] A small plasmid (PCJ01) from a poultry isolate of C.jejuni was identified by Luo and Zhang (2001) which was further sequenced and characterized. Results indicated that PCJ01 was a 3212 bp circular molecule with 33.5% G+C content. This plasmid consisted of four open reading frames (ORFs) which share homology with RepA and RepB proteins in C.coli plasmids and which in turn coded for some virulent transmembrane proteins. [23]

River water is often contaminated with detergents due to the daily human activities like personal cleansing, laundry, utensil washing, household cleaning etc. and also with trace amounts of metals via the industrial effluent discharge. [24] Kazmi and colleagues (1985) showed that not only Campylobacter spp. but other pathogens like E.coli, Vibrio, Salmonella and Shigella were also susceptible to cadmium (20-40mg per disk). However, a few isolates of Acinetobacter being resistant to higher levels of silver (1024 mg/lit) and to the metals like aluminium, bismuth, boron and molybdate. [25]

IRON ACQUISITION

The symptoms of campylobacteriosis are variable in humans, ranging from mild to watery stools often accompanied with blood. The ability of pathogenic bacteria to acquire iron in the animal host is important in establishing infection. [26] A major problem for C.jejuni in establishing infection is less availability of free iron in mammalian fluids. Most of the iron in the cells is in the form of heme or ferritin and iron binding glycoproteins, transferrin and

lactoferrin, which are extracellular in plasma or body fluids. [27] Iron sulphur proteins participate in electron transport, anaerobic respiration, amino acid metabolism and energy metabolism. In human immune system, Campylobacter are exposed to the reactive oxygen species. This is important when Campylobacter is internalized by the phagocytes. C.jejuni can use a few siderophores viz.,enterochelin and ferrichrome. Other iron compounds that support the growth of C.jejuni are heme compounds like hemin and hemoglobin, ferric iron and ferrous iron. Campylobacter scavenge siderophores produced by other enteric bacteria. Ferrous iron transport is important for bacterial virulence and can be related to low oxygen tension and variable pH in stomach and intestine. [28] C.jejuni has many systems for siderophore-mediated uptake of iron viz., enterochelin transport system, siderophore systems encoded by cfr A and gene cj0718. Periplasmic binding protein-dependent system is another iron uptake systems present in C.jejuni. This includes a ferrous iron uptake system (Feo B protein) which is important in assimilation of iron under microaerobic conditions. There are 3 sets of exbBD genes, each one linked to ton B gene that codes for Ton B protein, an important energy translocation protein for iron uptake systems and ExbBD which acts as assembly factors for TonB operation. In C.jejuni this iron uptake system is controlled by a global regulator, Fur. [27]

Iron acquisition studies revealed that C.jejuni readily obtains iron from hemin and hemoglobin. Two strains of C.jejuni were tested for their ability to acquire iron from hemin-hemopexin and hemoglobin-haptaglobin. Iron acquisition spot assays of the mutants showed that in addition to being unable to utilize heme, they were all incapable of using hemoglobin, hemo-hemopexin or hemoglobin-haptaglobin as iron sources. [26] Further characterization of these mutants allowed the identification of a 70 KDa outer membrane protein, which was lacking in mutants. This was later identified as Fur A iron repressed protein and the corresponding gene (chuA). On the C.jejuni NCTC11168 genome, the chuA gene is followed by chub, chuC and chuD genes which encode the components of an ABC transporter system. Chu B is a cytoplasmic membrane permease, Chu C an ATPase and Chu D a periplasmic binding protein. [28] Palyada and co-workers used DNA microarrays to identify the C. jejuni genes that were affected by iron availability. The transcript levels of 647 genes were affected after the addition of iron to iron-starving C. jejuni cells. Several classes of genes specific to iron acquisition and metabolism were revealed within 15 min. Directed mutagenesis of these genes identified by the microarray analyses allowed the characterization of the ferric enterobactin receptor, previously named CfrA. Chick colonization assays indicated that mutants defective in enterobactin-mediated iron acquisition were unable to colonize the gastrointestinal tract. This work emphasized the complex response of C. jejuni to iron availability and provided insight regarding the role of iron in C. jejuni colonization in vivo. [29]

OXIDATIVE STRESS DEFENSE

Iron and oxidative stress are interconnected because iron on reacting with oxygen generates reactive oxygen species like superoxide anions (O2-), peroxide (RO2) and hydroxyl radicals (OH). Toxic oxygen species can also be generated by burst of free radical production. These toxic species damage cell lipids, proteins and DNA by oxidation. [27]

Superoxide dismutase removes superoxide by catalyzing the dissimilation of superoxides into hydrogen peroxide and oxygen (2O2- + 2H+ --- H2O2 +O2). Further catalase or peroxidase remove the hydrogen peroxide produced. Superoxide (Sod B) dismutases are classified based on the metal co-factor required. Sod B protein is located in cytoplasm and is fully characterized. [28] Campylobacter converts hydrogen peroxide to oxygen and water with the help of catalase and heme cofactor. Campylobacter adapts to hydrogen peroxide by showing increased response of catalase. C.jejuni and C.coli express a single catalase designated Kat A which is iron repressed and Per R is the regulator. [28]

Alkyl hydroxide reductase (Ahp) is a second peroxidase found in bacteria which converts reactive oxygen hydroperoxides to corresponding alcohols. C.jejuni produces Ahp C homolog in the absence of which it becomes more sensitive to cumene hydroperoxide. [27] Thioredoxin linked thiol peroxidase designated as Tpx belongs to bacterial antioxidant enzymes which protects the enzymes like glutamine synthetase against inactivation by oxidative stress. C.jejuni contains tpx ortholog. In Campylobacter, hydrogen peroxide can also be produced in the periplasm. Catalase is solely cytoplasmic so removal of periplasmic hydrogen peroxide is done via periplasmic cytochromic peroxidases. C.jejuni contains two such peroxidases, Cj0020c and Cj0358. Cft ferritin is an iron binding protein present in C.jejuni and its absence leads to sensitivity to superoxide and peroxide stress inducers. Similarly ferrodoxin Fdx A absence leads to reduced aero tolerance in C.jejuni. Nitric oxide generated by immune system, reacts with superoxide

to give peroxynitrite which can lead to the formation of toxic radicals. Bacteria use flavohemoglobins or single domain hemoglobin to protect themselves from such nitrosative stress. Campylobacter has a single domain called Campylobacter globin (Cgb), mutation in which, leads to hypersensitivity of Campylobacter to nitrosylating agents S-nitroso-glutathione. [28]

VIABLE BUT NON-CULTURABLE FORM

The ability to enter viable but non culturable (VBNC) state has been described for several enteric pathogens, including Salmonella enteritidis, Escherichia coli, Vibrio vulnificus, Vibrio cholereae and Campylobacter jejuni. The VBNC state represents the response to survival stress by not being cultured on laboratory media and also being capable of retaining virulence. Therefore in this form survival can prove to be an important potential public health threat. VBNC state in Campylobacter was 1st reported by Rollins and Colwell, who examined the ability of C.jejuni strain HC, from human campylobacteriosis patient, to survive in sterile stream water microcosm system. Plate counts were compared with direct viable count and acridine orange direct count methods to determine whether non-culturable cells of Campylobacter retain viability. Effects of temperature and aeration were evaluated in terms of transition of the organism to VBNC state. Morphological transition of spiral cells to coccoid cells was monitored by dark phase and electron microscopy as well as density gradient centrifugation. Culturability of Campylobacter was tested by taking plate counts of natural stream water flask microcosms (rotary shaker as well as held stationary) and these were compared with those of broth cultures (stationary biphasic and rotary shaken). The kinetics of decline in culturability of Campylobacter in shaken microcosm and broth were similar. When the microcosm flasks were held stationary the rate of decline was moderate. Transition to the non culturable form was accelerated at higher incubation temperatures so at 37°C the coccoid forms of C.jejuni spp was studied. These forms maintain intact and asymmetric membrane structure while the cell shape and size varies. Production of an extracellular viscous material by Campylobacter is the adaptation made in aqueous environments. The benefits of which are related to organism being able to control oxygen, nutrient and metabolite concentrations. This viscous mat formed at the agar/broth interface remains until it is mechanically disrupted, thereby providing a microenvironment for prolonged survival. This study showed that the VBNC stage is important for understanding the epidemiology of campylobacteriosis. [30] Some authors have reported the possibility of recovering VBNC cells of C.jejuni by animal passage. Cappelier and co-workers chose three human isolates of C.jejuni Bf, 79 and 85 for testing the VBNC state when incubated in filtered, sterilized surface water. After starvation for 30 days, VBNC cells were inoculated in the yolk sacs of embryonated eggs. Culturable cells were further classified as pathogenic, as the maintenance of the adhesion potential indicated that VBNC state of Campylobacter constitutes a public health concern. [31] Solid phase cytometry (SPC) in conjunction with fluorescent viability staining was investigated as a tool to detect viable but non-culturable Campylobacter jejuni in drinking water. SPC distinguished between low numbers of dividing and non-dividing cells of Campylobacter, which had the potential to monitor resuscitation of VBNC cells. [32]

DISCUSSION

The virulence related mechanisms of Campylobacter discussed above throws light upon the insights of the mode of infection in the human body and its survival strategies. Each virulence factor has been studied in detail and based on this, the combinatorial antibiotic therapy can be designed.

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