17 *Yersinia enterocolitica* and *Yersinia pestis*

Introduction

A French bacteriologist, Alexandre Yersin in 1894 first described a bacterium, called *Pasteurella*, which was later renamed *Yersinia pestis*. The genus *Yersinia* belongs to the phylum *Proteobacteria*, class *Gammaproteobacteria*, order *Enterobacteriales*, and family *Enterobacteriaceae*. The genus has 17 species: *Yersinia enterocolitica*, *Y. pseudotuberculosis*, *Y. pestis*, *Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. mollaretii*, *Y. bercovieri*, *Y. aldovae*, *Y. aleksiciae*, *Y. entomophaga*, *Y. massiliensis*, *Y. mollaretti*, *Y. nurmii*, *Y. pekkanenii*, *Y. rhodei*, *Y. similis*, and *Y. ruckeri*. The three most important species that cause infections in humans are *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis*. These are zoonotic pathogens, and among them, *Y. enterocolitica* is responsible for the greatest number of cases of the zoonotic disease. All the three species are facultative intracellular pathogens, harbor a 70 kb virulence plasmid (pYV), and exhibit tropism for lymphoid tissues.

Yersinia enterocolitica is associated with foodborne infections resulting in gastroenteritis, terminal ileitis, mesenteric lymphadenitis, and septicemia. The bacterium emerged as a human pathogen during the 1930s. The Centers for Disease Control and Prevention (CDC) estimates about 97,000 cases of human diseases occur due to *Y. enterocolitica* infection annually in the USA with 533 hospitalizations and 29 deaths. *Yersinia pseudotuberculosis* also causes gastrointestinal disorders, septicemia, and mesenteric adenitis. *Yersinia pestis* causes bubonic or pneumonic plague, and the organism can be transmitted through contact with wild rodents and their fleas. Plague is an old-world disease and often referred to as "Black Death" and occurs in the bubonic or pulmonary forms.

Yersinia species are Gram-negative coccobacilli (short rods) nonspore former and can be differentiated based on their biochemical properties (Table 17.1). *Yersinia* spp. are catalase-positive, oxidase-negative, glucose fermentative organisms, and these may be isolated on MacConkey agar and cefsulodin–irgasan–novobiocin (CIN) agar media. Most *Yersinia* species are noncapsulated except *Y. pestis*, which develops an envelope at 37 °C. In addition, all three species also share *Yersinia* outer membrane proteins (YOPs), V (immunogenic protein), and W (nonprotective lipoprotein) antigens. The fraction 1 envelope antigen (F1) is produced at 37 °C and has two major components: fraction 1A (polysaccharide) and 1B (protein). *Y. pestis* has been identified as a subspecies of *Y. pseudotuberculosis* based on the 16S rDNA sequence. These two species also share 11 common antigens.

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Table 17.1 Classification of *Yersinia* species based on biochemical properties

Characteristics	*Y. pestis*	*Y. pseudotuberculosis*	*Y. enterocolitica*
Motility at 22 °C	–	+	+
Lipase at 22 °C	–	–	v
Ornithine decarboxylase	–	–	v
Urease	–	+	+
Citrate at 25 °C	–	–	–
Voges–Proskauer	–	–	v
Indole	–	–	v
Xylose	+	+	v
Trehalose	+	+	+
Sucrose	–	–	v
Rhamnose	+	+	–
Raffinose	–	–	v

Adapted from Smego et al. (1999). Eur. J. Clin. Microbiol. Infect. Dis. 18, 1–15
+ positive, – negative, *v* variable

Yersinia enterocolitica

Biology

Yersinia enterocolitica is a Gram-negative short rod and facultative anaerobe. *Y. enterocolitica* grows between 0 °C and 44 °C with an optimum growth at 25–28 °C. Growth occurs in milk and raw meat at 1 °C, but at a slower rate. Bacteria can grow in 5% NaCl and at a pH above 4.6 (range pH 4–10). *Yersinia* expresses peritrichous flagella at a lower temperature (25 °C), but it is nonflagellated (nonmotile) at 37 °C. Bacterial swimming and swarming motility are thought to be regulated by bacterial quorum-sensing ability. *Yersinia* is equipped to maintain biphasic lifestyle, one in the aquatic environment/food system and the other in human host. *Yersinia* grows slowly on sheep blood agar, MacConkey agar, and Hektoen enteric agar producing pinpoint colonies after 24 h of incubation. It ferments sucrose but not xylose or lactose. For selective isolation of the bacterium, cefsulodin–irgasan–novobiocin (CIN) and virulent *Yersinia enterocolitica* (VYE) agar can be used.

Classification

Yersinia enterocolitica has been classified into six major biotypes based on their pathogenicity, and ecologic and geographic distributions: 1A, 1B, 2, 3, 4, and 5. 1A is considered the nonpathogenic biotype; however, a minority of biotype 1A strains are found to cause gastroenteritis, while all remaining biotypes are pathogenic, of which biotypes 1B is highly pathogenic and is commonly associated with human infections. The pathogenic biotypes carry virulence plasmid, pYV. *Y. enterocolitica* has about 70 serotypes. Select serogroups for each biogroup include 1A (O:5; O:6, 30; O:7, 8; O:18; O:46), 1B (O:8; O:4; O:13a, 13b; O:18; O:20; O:21), 2 (O:9; O:5, 27), 3 (O:1,2,3; O:5,27), 4 (O:3), and 5 (O:2,3). The predominant serogroups that cause most human infection worldwide are O:3, O:8, O:9, and O:5,27.

Y. enterocolitica is again grouped into two subspecies, *Y. enterocolitica* subspecies *enterocolitica* and *Y. enterocolitica* subspecies *palearctica*. *Y. enterocolitica* subspecies *enterocolitica* contains biotype 1B, and the strains are highly pathogenic and are commonly termed the North American strains. *Y. enterocolitica* subspecies *palearctica* include strains of 1A, 2, 3, 4, and 5 and distributed throughout the world.

Sources

Yersinia enterocolitica is widely distributed in nature, including foods, water, sewage, and animals (cattle, sheep, goats, dogs, cats, rodents); however, the pig is the primary reservoir (bacteria

present as a commensal) of pathogenic strains such as serotype O:3. This serotype has been frequently isolated from pigs and responsible for infections in humans (Fig. 17.1). Thirty-five to 70% of swineherds and 4.5–100% of individual pigs harbor pathogenic *Y. enterocolitica*. Environmental isolates are generally nonpathogenic and belong to the biogroup 1A. The first reported foodborne outbreak of *Y. enterocolitica* occurred in New York state in 1976 affecting 222 children due to consumption of chocolate milk and was caused by serotype O:8. *Y. enterocolitica* outbreaks have also been associated with pasteurized and unpasteurized fluid milk. Chitterlings, a product made of swine intestines, also are implicated in outbreaks in infants in the USA and other countries. Chitterlings are prepared by boiling the intestine of swine and are a traditional winter holiday food for many African American families in the USA. Though the fats and fecal contents are removed before boiling the final product, the chitterlings preparation requires substantial handling, and children in the household may be exposed during its preparation.

Virulence Factors

Yersinia enterocolitica is an invasive intracellular enteric pathogen and interestingly not all strains are virulent. Environmental isolates are generally nonpathogenic, and pathogenic strains are predominant in pigs. The pathogenic strains vary in serological characteristics. In the USA, the most common serovar implicated in yersiniosis is serovar O:8. Pathogenic strains carry several virulence factors encoded on the chromosome, and in a 70 kb virulence plasmid (pYV), which are required for adhesion, invasion, and colonization of intestinal epithelial cells and lymph nodes, growth and survival inside the macrophages, killing of neutrophils and macrophages, and serum resistance (Table 17.2). The nonpathogenic strain (biotype 1A) lacks the virulence plasmid, pYV.

The chromosomal-linked virulence proteins include invasin, attachment invasion locus (Ail), *Yersinia* stable toxin (Yst) (enterotoxin), and siderophore. The environmental isolates are shown to be negative for *inv*, and *ail* genes and are not associated with human disease. The plasmid-linked virulence genes encode *Yersinia* outer membrane proteins (YOPs) that are responsible for bacterial adhesion, a type III secretion system (T3SS) to deliver virulence proteins to the host cell cytosol to allow bacterial growth inside macrophages, serum resistance, and septicemia. YOPs are present on the cell surface as well as secreted into the medium. YOPs are secreted in the presence of very low levels of Ca^{2+}; YOP expression is temperature dependent and occurs

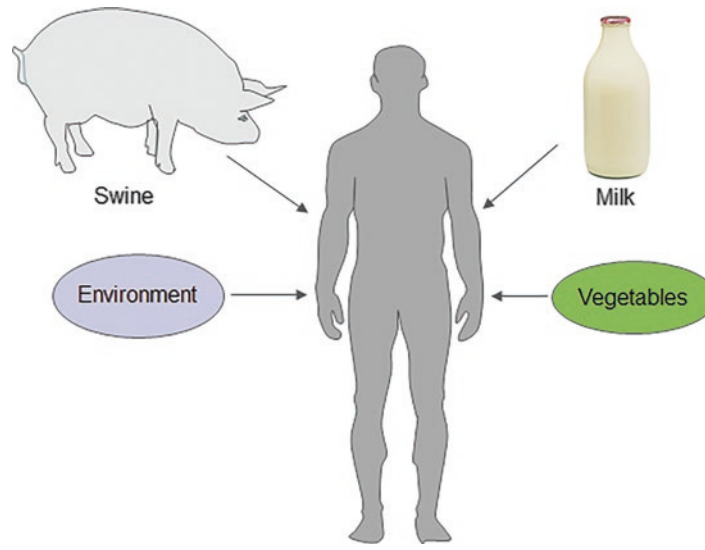

Fig. 17.1 *Yersinia enterocolitica* transmission to humans. Swine is the primary reservoir

Table 17.2 *Yersinia enterocolitica* key virulence factors and their genetic origin

Origin	Protein size	Function
Chromosome		
Invasin (Inv)	92 kDa	Inv binds to β1-integrin and promotes adhesion and invasion
Attachment invasion locus (Ail)	17 kDa	Attachment and invasion; serum resistance
Yersinia stable toxin (Yst)	7.5 kDa	Yst increases cGMP level and fluid secretion
Yersiniabactin (catechol-type)	482 Da	Siderophore, an iron-binding protein
Virulence plasmid (pYV) 70 kb	–	
Ysc (Yop secretion)	–	A type III secretion system (T3SS) made of 28 proteins
YopH	51 kDa	Dephosphorylates host proteins, modulates signaling pathway, and prevents phagocytosis
YopM	41.6 kDa	Kinase activity; inhibits caspase-1
YopD	33.3 kDa	Responsible for translocation of YopE and other effector proteins (YopH, YopM, YopO, etc.) across the membrane
YopE	219-amino acid protein (~22 kDa)	Inactivates Rho family of GTPase, disrupts actin cytoskeleton, and prevents phagocytosis
YopP	33 kDa	Macrophage apoptosis; alters the expression of cytokines
YopT	35.5 kDa	Interferes with actin cytoskeleton formation by inactivating Rho GTPase
YadA (adhesion protein)	160–240 kDa	Adhesion to epithelial cells by interacting with β1-integrin; blocks complement-mediated killing; serum resistance
YopB	41.8 kDa	Inhibits cytokine release from macrophages
LcrV	37.2 kDa	Low calcium response

mostly at 37 °C, which is critical for pathogenesis in the host. Description of important virulence factors is presented below.

Chromosome-Linked Virulence Gene Products

Invasin

Invasin (Inv) is a 103 kDa *Yersinia* outer membrane protein and binds to the β1-integrin receptor located at the apical side of the M cells or the basolateral side of epithelial cells and is an important virulence factor for the early phase of intestinal infection. Invasin is encoded by *inv* gene located on the chromosome, and its expression is high when *Yersinia* is grown in media with a neutral pH at 25 °C but low at 37 °C. Invasin is expressed at high levels during the stationary phase of growth at low temperatures. However, when *Y. enterocolitica* is grown at low pH (equivalent to intestinal pH of 5.6–6) at 37 °C, the invasin expression is enhanced. The *inv* expression is regulated by *rovA* (regulator of virulence A), which is located on the chromosome. RovA is a 143-amino acid protein and is present in all three pathogenic *Yersinia* species. Invasin facilitates bacterial colonization and translocation through M cells located in the follicle-associated epithelium overlying Peyer's patches. Invasin activates multiple signaling cascades including cSrc kinase, Rac1, MAP (mitogen-activated protein kinase) kinases, and transcription factor NF-κB and promotes proinflammatory immune response (IL-8, monocyte-chemoattractant protein-1 (MCP-1), and granulocyte-macrophage colony stimulating factor (GM-CSF)). As a result, chemotactic cytokines are made which recruit neutrophils and macrophages at the site of infection. Macrophages ingest *Yersinia* and disseminate to regional lymph nodes, liver, and spleen.

Attachment and Invasion Locus

Attachment and invasion locus (Ail) is a 17 kDa membrane protein and is involved in adhesion, in invasion, and in the serum resistance. It possibly acts by inactivating complement by-products C3-convertase (C4b2a) thus preventing the formation of C3b and the membrane attack complex (MAC), which are required for lysis of bacterial cells. Ail is the primary serum resistance factor in *Y. pestis* and *Y. pseudotuberculosis* and blocks alternative and lectin pathways thus promoting bacterial growth to high densities during infection.

Iron Acquisition

Iron acquisition is achieved by siderophore such as yersiniabactin (catechol-type), which is encoded by a chromosomal high-pathogenicity island (HPI). Under the iron-starvation condition, *Yersinia* produces large amounts of iron receptors, FoxA and FcuA on the outer membrane, which bind the siderophores to sequester iron. Yersiniabactin also reduces production of reactive oxygen species (ROS) by macrophages and neutrophils thus decreasing bacterial killing during innate immune response.

Yersinia Stable Toxin (Yst)

Y. enterocolitica produces an enterotoxin, Yst, which is a heat-stable (100 °C for 15 min) 7.5 kDa protein and remains active at pH range of 1–11 at 37 °C for 4 h and is methanol-soluble. Yst is structurally and functionally homologous to the heat-stable enterotoxin (ST) of enterotoxigenic *Escherichia coli* (*see* Chap. 14) and is encoded by the chromosomal *yst* gene. Yst is involved in diarrhea. Three subtypes of Yst exist: Yst-a, Yst-b, and Yst-c. Each Yst-a and Yst-b subtype is made of 30 amino acids, while the Yst-c consists of 53 amino acids.

LPS and Flagella

LPS located in the bacterial outer membrane consists of antigenic O specific oligosaccharide, core oligosaccharide, and lipid A. Lipid A of LPS is recognized by host TLR-4 (macrophage) and initiates a signaling cascade resulting in the production of proinflammatory cytokine (TNF-α) and recruitment of macrophages and neutrophils. High levels of LPS in the blood can induce septic shock. LPS produced by bacteria at 21 °C predominantly contains hexa-acylated lipid A, which is recognized by TLR-4 and stimulates monocytes to secrete TNF-α; when bacteria is grown at 37 °C, LPS contains tetra-acylated lipid A, which is a weak TLR-4 agonist and is unable to elicit an immune response by the macrophages. This stealth strategy helps *Y. enterocolitica* to avoid recognition by TLR-4 and consequent immune activation.

Flagella consist of flagellin proteins and are involved in bacterial adhesion. Flagellin proteins bind to TLR-5 on monocytes and induce an innate immune response. Aflagellated strains fail to adhere and stimulate the immune system to produce proinflammatory cytokines (TNF-α and IL-17) and thus unable to recruit macrophages. *Y. enterocolitica* synthesizes flagella only at 22–30 °C and loses when grown at 37 °C. This strategy possibly helps the bacterium to avoid host innate immune defense by avoiding recognition by TLR-5.

Plasmid (pYV)-Linked Virulence Gene Products

pYV (70 kb) encodes for 12 major proteins termed YOPs (*Yersinia* outer membrane proteins) and the two outer membrane proteins called YadA (*Yersinia* autotransporter adhesin) and YlpA (*Yersinia* lipoprotein).

Yersinia Adhesion Protein

Yersinia adhesion protein (YadA) is an important virulence factor and facilitates the bacterial attachment to host cells and protects *Yersinia* from nonspecific immune systems such as phagocytosis and complement-mediated cell lysis. YadA is an outer membrane protein encoded by pYV. It is present in both *Y. enterocolitica* and *Y. pseudotuberculosis* but is nonfunctional in *Y. pestis*. YadA is a 160–240 kDa protein composed of three monomers, each 44–47 kDa, and appears as fibrillar (or lollipop-like) structure covering the entire bacterial cell surface. Each fibrilla is of

50–70 nm in length and 1.5–2.0 Å in diameter. YadA has three parts: N-terminal head, intermediate stalk, and the C-terminal anchor domain. The N-terminal domain contains 25-amino acid-long signal sequence. The stalk binds to host cell receptor and resists host immune system, and the C-terminal domain anchors to the bacterial outer membrane.

YadA serves as a major adhesin and binds to several extracellular matrices (ECM) including cell surface-associated collagen and laminin. YadA also promotes bacterial internalization by interacting with the epithelial β1-integrin proteins by zipper mechanism. Interaction with β1-integrin initiates signaling events that orchestrate actin recruitment to alter the cytoskeletal structure to promote bacterial entry.

YadA is expressed at 37 °C but not at 25 °C. Expression of YadA is regulated by two different gene products: VirF (virulence) and LcrV (low calcium response). VirF senses the optimal temperature, i.e., 37 °C required for protein synthesis, and LcrV regulates the *yadA* expression depending on the availability of extracellular calcium concentration. Furthermore, *yadA* expression is not affected by pH, salt, or sugar concentration.

YadA also disrupts the host cell signaling pathways to block the release of a proinflammatory cytokine such as IL-8, which is a chemoattractant for neutrophils. YadA inhibits oxidative burst in neutrophils and activates YopH, a phosphotyrosine phosphatase that blocks the phagocytic mechanism. YadA also plays a major role in serum resistance. It inhibits the formation of C3b and membrane attack complex (MAC) by activating the proteolytic enzyme, factor H, which degrades the C3b.

YopB and YopD

YopB, a 41 kDa protein, suppresses the secretion of macrophage-derived cytokines, IL-1 and TNF-α, which are important in inflammatory response against infection. YopB also regulates YopD, a 33 kDa protein that inhibits respiratory burst in macrophages. YopD is also responsible for translocation of YopE and other effector proteins (YopH, YopM, YopO) across the membrane.

Type III Secretion System

The type III secretion system (T3SS) apparatus, called Ysc, is responsible for the formation of a supramolecular apparatus called injectisome/injectosome and is essential for secretion of YOPs to extracellular milieu, across the outer membrane, and to the host cell cytosol. It is made of 28 proteins and the genes for which are encoded by the pYV plasmid, and their expression is temperature dependent (37 °C). The T3SS is responsible for delivery of YopH, YopO, YopT, YopP/J, YopE, and YopM effector proteins into the host cell cytosol. These effector proteins affect signaling events and alter actin cytoskeletal structure to induce bacterial entry by zipper mechanism, phagocytosis, apoptosis, and inflammatory response.

YopH is a 51 kDa protein and interferes with the signal transduction pathway to block phagocyte-mediated killing. Phagocytes (macrophages and neutrophils) are important components of the innate immunity and provide the first line of defense against *Yersinia* infection. The process of phagocytosis involves actin rearrangement to form pseudopods for bacterial internalization. YopH dephosphorylates host phosphotyrosine-containing proteins that are involved in actin polymerization to form pseudopods. YopH also interferes with the calcium signaling in neutrophils and downregulates respiratory bursts in the macrophages and neutrophils. In adaptive immune response, YopH suppresses B-cell activation and production of cytokines by the T cells.

YopO/YpkA is an 82 kDa secreted protein and possesses kinase activity and displays cytotoxic action. YpkA induces phosphorylation of VASP (vasodilator-stimulated phosphoprotein), a regulator of actin dynamics, thus disrupts actin polymerization and impairs phagocytosis. YopT possibly has Rho GTPase activity. Rho families of GTPases (Rac1, RhoA, and Cdc42) regulate actin cytoskeleton formation and interference of GTPase activity prevents phagocytosis of bacteria. YopP/J is a 33 kDa protein that blocks inflammation and induces apoptosis in macrophages by inhibiting MAPK signaling pathways and NF-κB

pathway. As a result, TNF-α and IL-8 production are downregulated. YopE has cytotoxic action causing rounding and detachment of host cells from the extracellular matrix, and it regulates the inflammatory response. Controlling activation of serine protease caspase-1 is critical for the progression of the disease. Caspase-1 aids in IL-1β and IL-18 synthesis and induces pyroptosis, a lytic form of cell death. YopM is a 41 kDa protein that inhibits caspase-1 and is required for initiation of pyroptosis.

Pathogenic Mechanism

Foods such as chocolate milk or water that are incriminated in yersiniosis are generally cycled through refrigeration or below 25 °C. Generally, a high dose (10^7–10^9 cells) is required for the disease. Once ingested, bacteria travel through the stomach to the small intestine, and the primary site of infection is the terminal ileum and proximal colon. Survival in the stomach acidity has been proposed to be neutralized by urease produced by the pathogen. It is speculated that, initially, bacteria use chromosomally encoded virulence gene products to colonize the intestine until the temperature shift to 37 °C and then initiate the expression of pYV-encoded gene products. Virulence factors help bacteria to (i) colonize and invade, (ii) prevent activation of cell death pathways, (iii) perturb inflammatory processes, and (iv) evade both innate and adaptive immune response to promote disease.

Bacteria bind to mucus membrane using Invasin, Ail, and YadA and enter through M cells overlying the Peyer's patches (Fig. 17.2). Invasin interacts with the β1-integrin receptor located abundantly on the M cells on the luminal side (apical side). YadA also aids in the invasion by interacting with the β1-integrin as well as the collagen and laminin. Engulfed bacteria are then released from the M cells into the basal layer in the lamina propria, multiply within the lymphoid follicle, and cause necrosis and abscess in Peyer's patches. Bacteria are able to reinvade epithelial

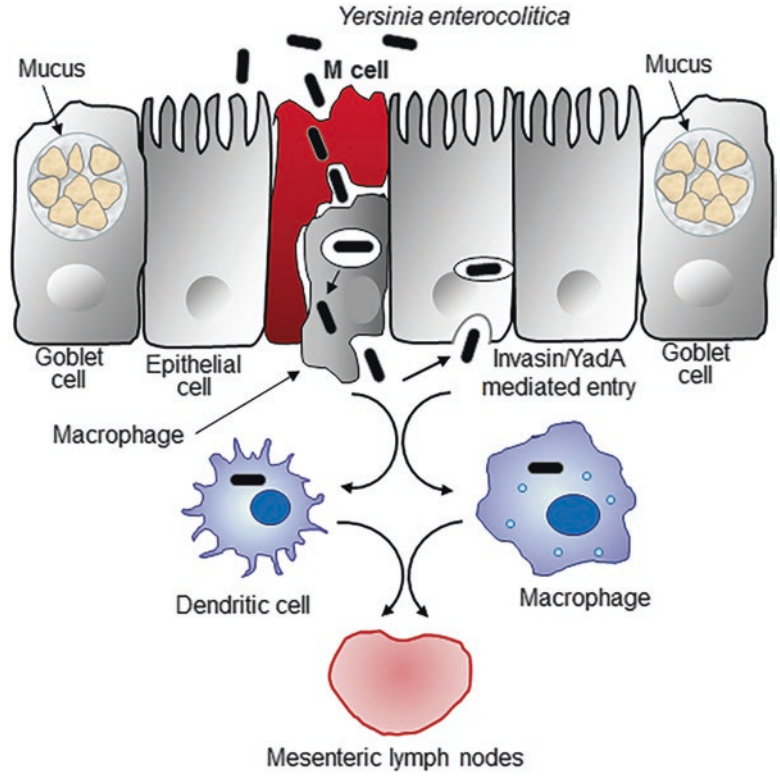

Fig. 17.2 *Yersinia enterocolitica* translocation through intestinal epithelial barrier. After entry into the basal layer via M cells, bacteria invade epithelial cells through interaction with host cell β1-integrin. Macrophage/dendritic cells transport *Yersinia* to mesenteric lymph nodes and to the liver. *Yersinia* prevents phagocytosis, also induces macrophage apoptosis, and prevents cytokine production

cells using the β1-integrin receptor located at the basolateral side. From the Peyer's patches, dendritic cells help bacteria to spread to regional mesenteric lymph nodes to induce characteristic lymphadenitis. Bacteria also disseminate to liver, spleen, and lungs and survive by resisting phagocytosis by macrophages and polymorphonuclear leukocytes. Survival inside macrophage is facilitated by the delivery of YOPs to the macrophage by T3SS that interferes with the cellular signaling events by blocking the phagocytosis process, phagocytic oxidative burst, and apoptosis or pyroptosis (Fig. 17.3). The superoxide dismutase (SOD) enzyme also helps bacterial survival inside the macrophage. Survival inside macrophage is orchestrated by two regulators, OmpR (outer membrane protein R) and GsrA (global stress response A protein, 49.5 kDa).

The lipid A component of *Yersinia* LPS undergoes temperature-dependent modification to avoid detection by TLR-4 and subsequent signaling event to release proinflammatory cytokines (TNF-α, IFN-γ, IL-8) from macrophages and other immune cells thus inhibiting inflammation. IL-8 is a chemoattractant for neutrophils. Bacteria also block complement activation thus C3b- and MAC-mediated inactivation are avoided. Overall, the T3SS system blocks phagocytosis and suppresses immune system thereby ensuring bacterial survival in the lymphoid tissue. In the intestine, bacteria promote the formation of an abscess in the Peyer's patches and induce damage to the epithelial lining.

The enterotoxin, Yst, promotes fluid secretion from cells. All three Yst components Yst-a, Yst-b, and Yst-c stimulate the membrane-bound guanylate cyclase leading to increased accumulation and activation of intracellular cyclic guanosine monophosphate (cGMP), followed by an activation of cGMP-dependent protein kinase, culminating in the final biological event, i.e., inhibition of Na^+ absorption and stimulation of Cl^- secretion. The biological activity of Yst enterotoxin is determined by the suckling mouse bioassay (*see* Chap. 5).

The predominant protective immunity against yersiniosis is the development of $CD8^+$T cell response against YopE antigen in *Y. pseudotuberculosis* and *Y. pestis*.

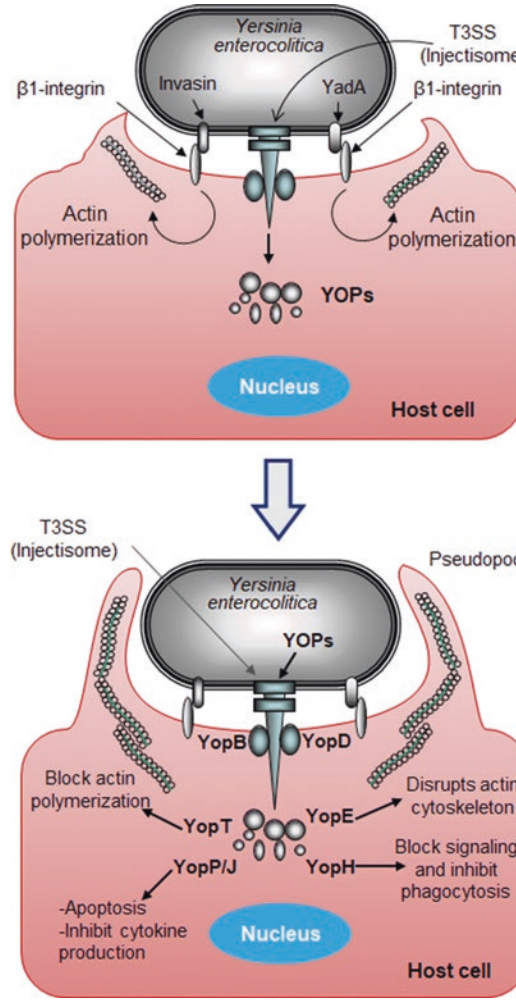

Fig. 17.3 Cellular mechanism of interaction of *Yersinia enterocolitica* with macrophage. After binding to macrophage, bacteria deliver YOPs through type III secretion system (T3SS) or injectisome into the host cell cytosol

Symptoms

Children are more susceptible than adults to foodborne yersiniosis showing acute enteritis. The highest incidence of infection is reported in children under the age of four who typically present with self-limiting diarrhea. Symptoms include severe abdominal pain at the lower quadrant of the abdomen mimicking appendicitis, diarrhea, nausea, vomiting, and fever. It also causes enterocolitis, mesenteric lymphadenitis, and terminal

ileitis. Symptoms generally appear within 24–30 h after consumption of the contaminated food and last for 3–28 days for infants and 1–2 weeks for adults. The disease can be fatal in rare cases. The severity of infection is pronounced in immunocompromised host or individuals with underlying diseases resulting in septicemia, pneumonia, meningitis, and endocarditis and can be fatal. The severity of the disease is also dependent on the serotype of the organism involved. For example, the disease caused by serotype O:8 (biotype 1B) is more severe than that of other serotypes. *Yersinia* is also known to cause nosocomial infection in hospital patients exhibiting symptoms of diarrhea. *Yersinia* infection can result in sequelae in some patients leading to reactive arthritis or Reiter's syndrome.

Prevention, Control, and Treatment

Yersinia enterocolitica is a psychrotroph, and therefore refrigeration is ineffective in controlling growth. Good sanitation at all stages of handling and processing of food and proper heat treatment are important to control the occurrence of foodborne yersiniosis. Chitterling-related outbreaks can be avoided by strict hygienic practices, preventing cross-contamination, and proper cooking. Consumption of raw milk or meat cooked at low temperatures should be avoided. *Yersinia enterocolitica* is susceptible to heat and pasteurization and can be easily destroyed by ionizing radiation, UV radiation, and other food preservation procedures.

Yersinia produces two types of β-lactamases (enzymes that hydrolyze the β-lactam ring of the β-lactam antibiotics) and is thus resistant to the penicillin group of antibiotics; however, the newer β-lactam antibiotics (ceftriaxone, ceftazidime, moxalactam) are found to be effective. *Yersinia enterocolitica* is also sensitive to imipenem and aztreonam antibiotics. Broad-spectrum cephalosporins are also effective against extraintestinal infections.

Detection of *Yersinia enterocolitica*

Culture Methods

Cold and selective enrichment has been used for isolation of *Yersinia*. Cold enrichment (at 4 °C for 3 weeks) is generally used for *Y. enterocolitica* and *Y. pseudotuberculosis*, and the media used are buffered peptone water (BPW), phosphate buffered saline (PBS), PBS containing 1% mannitol and 0.15% bile salts (PMB), and PBS containing 0.25% peptone and 0.25% mannitol (PMP). For selective enrichment, irgasan–ticarcillin–potassium chlorate (ITC) broth at 25–30 °C for 48 h has been used to increase bacterial numbers. *Yersinia* is isolated on several commonly used enteric media: MacConkey agar, Hektoen enteric (HE) agar, *Salmonella–Shigella* with sodium deoxycholate and calcium chloride (SSDC), and xylose–lysine deoxycholate (XLD) agars. Other selective media include cefsulodin–irgasan–novobiocin (CIN) and modified virulent *Yersinia enterocolitica* (mVYE) medium. mVYE agar contains CIN agar, 0.1% esculin and 0.05% ferric citrate. Of all the selective media, CIN agar is the most effective. The mVYE agar can differentiate virulent *Y. enterocolitica* (esculin nonhydrolyzing and produced red colonies) from avirulent environmental *Y. enterocolitica* or other *Yersinia* species that produce dark colonies.

Serodiagnosis

Agglutination-based serodiagnosis of *Yersinia* using host serum has been used. However, it is found to be an inconsistent diagnostic tool because of the cross-reaction of antiserum with several other pathogens. Though ELISA has improved sensitivity, cross-reactions and the false-positive rates are very high. Indirect immunofluorescence assay is used with biopsy specimens. It appears that culture confirmation, in conjunction with serodiagnosis, may be used to correctly diagnose a patient suffering from yersiniosis.

Molecular Detection Method

Standard PCR method that targets virulence genes, namely, *yadA* and *virF* located on pYV, and the 16S rRNA genes is used. Real-time quantitative PCR employing *ail* gene as the target has been used for detection of *Y. enterocolitica* in pig feces and meat.

Molecular Typing Methods

Serotyping based on O (somatic) and H (flagellar) antigens, multilocus enzyme electrophoresis (MLEE), phage typing, and DNA-based schemes have been used to study typing, taxonomy, and epidemiology of *Yersinia enterocolitica*. Among the DNA-based schemes, pulsed-field gel electrophoresis (PFGE), ribotyping, amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD) and multilocus sequence typing (MLST), and whole genome sequencing (WGS) are used that produce reproducible typing information. MLST has also been used to understand the global epidemiology of yersiniosis.

Yersinia pestis

Introduction

Yersinia pestis causes plague, which is either bubonic or pneumonic (pulmonary). The plague was described as early as the 430 BC in Athens, Greece, and is called an old-world disease. Plague is often referred to as "Black Death." *Y. pestis* has been responsible for three pandemics and over 200 million deaths within the last 1500 years. Plague can be transmitted through contact with wild rodents and their fleas, which act as the vector (Fig. 17.4). *Yersinia pestis* has a high affinity for lymphoid tissues and causes acute inflammation, abscess, and swelling of lymph nodes. These inflamed and pus-emitting lymph nodes are called "buboes" (hence bubonic). In recent years, *Y. pestis* has gained significant interest as a possible agent of biological warfare. Pulmonary (pneumonic) plague is of major concern because the infection can be acquired directly by inhalation of infectious aerosols generated by the infected person. Camels and goats are susceptible to *Y. pestis* infection and can transmit to human. *Yersinia pestis* is not a major foodborne concern; however, consumption of camel meat has been implicated in pharyngeal plague and in a rare occasion, gastroenteritis.

Biology

Yersinia pestis is a nonmotile, nonspore-forming, coccobacillary organism measuring 0.5–0.8 μm by 1–3 μm. The optimum growth temperature is 28 °C with a range of 4–40 °C. *Yersinia pestis* is a facultative intracellular organism, and it has three biotypes: antiqua, medievalis, and orientalis. Biotypes can be distinguished by their ability to utilize certain substrates. Antiqua is glycerol-, arabinose-, and nitrate-positive. Medievalis is glycerol- and arabinose-positive but nitrate-negative. Orientalis is glycerol-negative and arabinose- and nitrate-positive. The existence of another biotype called microtus has been proposed. Microtus may have lost genes essential for virulence determinants and host adaptation. Microtus is glycerol-positive and arabinose- and nitrate-negative.

Virulence Factors and Pathogenesis

Yersinia pestis infection starts from fleabite or contact with rats, or through inhalation. Bacteria multiply rapidly inside the midgut (stomach) of the rat flea (*Xenopsylla cheopis*), aided by phospholipase D and form a mass at around 26 °C. During feeding of blood (37 °C) on the host (rat or humans), the fleas regurgitate the bacilli to the wound. Wild animals, particularly rats, are the major reservoir and can be infected asymptomatically. In rural areas, humans coming in contact with the rodents or fleas (vector) carrying *Y. pestis* become infected. Rat-to-human transmission is possible and human-to-human transmission is mostly by the airborne route or by human-adapted flea (*Pulex irritans*) (Fig. 17.4). The bubonic form is most common and starts with the bite of

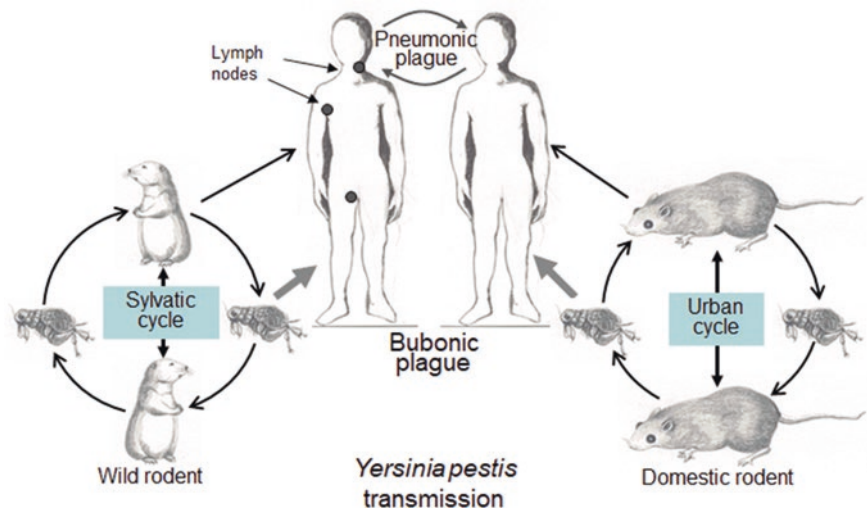

Fig. 17.4 Pathways showing transmission of *Yersinia pestis* to humans. A flea can transmit *Y. pestis* to wild rodents (sylvatic cycle), domestic rats (urban cycle), or humans. Infected flea also helps maintain both sylvatic and urban cycles. Fleabite or direct contact of rodents with a human may result in the development of bubonic plague. Human-to-human transmission via aerosol/droplets may cause pneumonic plague

an infected flea. The incubation period is 2–8 days.

Yersinia pestis virulence genes are located on the chromosome and on three plasmids (pCD1, pMT1, pPCP1). The virulence factors are F1 (fraction 1 antigen) capsule, plasminogen activator (Pla) protein, low calcium response (Lcr) stimulon, pesticin, and a *Yersinia* murine toxin (Ymt). These proteins are used as markers for biotyping of *Y. pestis*. The gene for Lcr is present on pCD1, F1 and the Ymt (phospholipase D) are present on pMT1, and Pla and pesticin are located on pPCP1. Chromosome-encoded invasin and pCD1-encoded YadA are inactive in *Y. pestis* due to mutation. Pla serves as an adhesin, invasin, and proteolytic factor and helps in systemic dissemination of bacteria. F1 blocks phagocytosis. Psa is an adhesion pilus (15 kDa) and forms a capsule-like structure on the surface. It is expressed at high levels at 37 °C and a pH range of 5.8–6.0. It is positively regulated by RovA and negatively by Fur. The virulence plasmid, pCD1, encodes genes for T3SS for secretion of YOPs (YopE, YopH, YopM, YopO/YpkA, YopP/J, and YopT) directly into the host cells to inhibit phagocytosis and blockade of proinflammatory signals.

During the early stage of infection, *Y. pestis* invasion and survival inside macrophages and nonprofessional phagocytes (host epithelial cells) are critical, which are mediated by Ail and Pla, respectively. In nonprofessional phagocytic cells, bacteria enter by zipper mechanism (Fig. 17.3). In the later stage of infection, the bacterium exerts anti-phagocytic activity which is attributed to Psa, F1 antigen (capsule), and YOPs (YopH, YpkA, YopE, and YopT). The key to the successful infection is a switch of bacterial initial invasive status to the anti-phagocytic status at the later stage of infection. It is speculated that initial intracellular lifestyle inside the macrophage may trigger the genes that are responsible for upregulation of surface components to promote bacterial resistance to subsequent phagocytosis during the second stage of the infection process. Temperature shift from 28 °C to 37 °C in vivo also induces F1, Psa, and YOPs expression, which collectively block phagocytosis. Low pH in the phagosome also upregulates Psa expression on the surface of the bacterium. In addition, Psa-mediated binding enhances contact with host cells to facilitate YOP translocation via T3SS to block further phagocytosis.

Symptoms

Clinical manifestation starts with fever, chills, and headache. There are a gradual swelling and enlargement of inguinal (groin) and submaxillary lymph nodes called "buboes." Inflammation or cellulitis may develop around the buboes. Large carbuncles may also develop. Gastroenteritis characterized by abdominal pain, nausea, vomiting, and diarrhea may develop in some cases. Septicemia, disseminated intravascular coagulation, and shock can develop in some patients. Secondary pneumonic plague may result in bronchopneumonia with the production of bloody purulent sputum. Pneumonic plague is highly contagious and can be transmitted easily by the airborne route. In the primary septicemic plague, buboes are absent but bacteremia develops and the mortality rate is 30–50%. In the secondary pneumonic phase, bacteria disseminate to the respiratory tract and develop severe bronchopneumonia, cavitation, and formation of bloody purulent (containing pus) sputum. This form of plague is highly contagious and fatal.

Treatment and Prevention

Early intervention with antibiotics within 1–3 days of exposure may be effective. Streptomycin, gentamicin, chloramphenicol, and tetracycline are effective. Antibiotic-resistant strains have been reported from Madagascar where plague is endemic. These resistant strains are also a serious public health threat. Use of ciprofloxacin as a prophylactic antibiotic has been found to be effective but is not good for treatment. Treatment with other fluoroquinolones has been reported to be promising.

A killed "whole cell" vaccine is available but requires multiple boosters. Efficacy of the vaccine against pneumonic plague is questionable. Other preventive measures include public education about the transmission of the disease by rodents and fleas. Access of rodents to food for human consumption should be prevented. Fleabite should be avoided by wearing protective clothing or by using insect repellant. Insecticides can be used to eliminate fleas from home or pet populations.

Detection of *Yersinia pestis*

Body fluids such as blood, sputum, bubo aspirates, or cerebrospinal fluid can be stained for bipolar staining (Giemsa or Wayson's) to directly visualize the organism under a microscope. The test samples can be streaked onto blood agar plates, brain heart infusion (BHI) agar plates, or MacConkey agar plates and incubated at 28 °C for 48 h (note: at 37 °C, the organism develops an envelope and becomes highly virulent). The colonies are characteristically opaque, smooth, and round-shaped with irregular edges. The bacteria can be extremely slow growing; thus, culture plates should be examined for 1 week before discarding. Biochemical characterization of *Yersinia* is reliable and can be done with diagnostic kits (Table 17.1). Serological tests such as passive hemagglutination (PHA) test could be used for diagnosis. Other immunoassays such as ELISA, direct immunofluorescence assay, and dipstick assays that target F1 antigen or Pla protein are very useful rapid tools. A real-time 5′ nuclease PCR that targets the *pla* gene of *Y. pestis* has been developed for analysis of human sputum or respiratory swabs samples. Whole genome sequencing is a powerful tool in *Y. pestis* identification.

Summary

The genus *Yersinia* consists of 17 species, of which *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* are pathogenic to humans. The former two are enteropathogenic and responsible for gastroenteritis, and the latter one is responsible for the plague. *Y. enterocolitica* and *Y. pseudotuberculosis* are transmitted through food, and pigs serve as the primary reservoir while *Y. pestis* infection is transmitted by fleabite, and rodents act as the intermediate host. All three pathogens carry chromosomal- and plasmid-encoded virulence factors, which are required for adhesion, invasion, and colonization of intestinal epithelial cells and lymph nodes, growth inside macrophages, macrophage apoptosis, and serum resistance. In *Y. enterocolitica*, chromosome-encoded virulence gene products include invasin, attachment invasion locus (Ail), siderophore

(yersiniabactin), and an enterotoxin (Yst), which are important during initial colonization and invasion of intestinal M cells and enterocytes in the intestine. The pYV plasmid-encoded virulence factors include *Yersinia* outer membrane proteins (YOPs) that are responsible for bacterial virulence protein translocation by T3SS to the host cell cytosol and resist macrophage and neutrophil-mediated inhibition and serum resistance. Expression of YOPs is temperature-dependent and occurs mostly at 37 °C, which is critical for bacterial pathogenesis while inside a host. During passage through the digestive tract, bacteria invade M cells overlying Peyer's patches, multiply in the lymphoid follicle, and are engulfed by macrophages. *Y. enterocolitica* is resistant to phagocytic killing by neutrophil, DC, and macrophages, and these cells help disseminate the organism to mesenteric lymph nodes, liver, and spleen.

Y. pestis causes the bubonic and pneumonic form of plague by colonizing the lymphoid tissues of the gastrointestinal and the respiratory tracts. The organisms acquired by either fleabite or aerosol are transported to the lymph nodes by macrophages where the bacteria survive and resist the killing by macrophages and neutrophils by producing several virulence factors: F1 (fraction 1 antigen) capsule, plasminogen activator (Pla), and low calcium response (Lcr) stimulon. In the bubonic form, the submaxillary lymph nodes are enlarged and appear as buboes. In this form, fever, chill, headache, and septicemia develop, and the mortality rate in untreated cases is 30–50%. In the secondary pneumonic phase, the bacteria disseminate to the respiratory tract and develop severe bronchopneumonia with bloody purulent sputum. This form of plague is highly contagious and invariably fatal, if not treated.

Further Readings

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