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Bacterial Ghost Vaccines Against *Yersinia enterocolitica*: A Next-Generation Strategy Linking Zoonotic Transmission, Virulence Genomics, Immune Evasion, Diagnosis, and Antimicrobial Resistance: A Review

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Abstract *Yersinia enterocolitica* remains an underestimated foodborne zoonotic pathogen with a complex biology that links animal reservoirs, contaminated foods, environmental persistence, intestinal invasion, immune evasion, and post-infectious complications. Although most infections are self-limiting, severe disease may occur in children, elderly individuals, immunocompromised patients, and cases associated with septicemia, transfusion-transmitted infection, or invasive extraintestinal spread. The pathogen is distinguished by marked heterogeneity among biotypes, serotypes, virulence plasmid profiles, chromosomal pathogenicity determinants, and antimicrobial susceptibility patterns. Recent genomic surveillance has improved the resolution of outbreak detection, source tracing, and lineage characterization, while molecular diagnostics have reduced the delay associated with culture-based methods. However, underdiagnosis remains a major limitation, especially in regions where yersiniosis is not routinely monitored. The increasing detection of multidrug-resistant isolates from food, animals, and environmental sources has renewed interest in preventive strategies beyond conventional antimicrobial therapy. Bacterial ghosts represent a promising vaccine and delivery platform because they preserve native bacterial surface antigens and pathogen-associated molecular patterns while lacking viable genetic and cytoplasmic contents. This review integrates recent evidence on *Y. enterocolitica* epidemiology, virulence, diagnosis, genomics, resistance, and bacterial ghost technology, and proposes a conceptual framework for developing mucosal bacterial ghost vaccines against enteric yersiniosis.

Keywords: Antimicrobial resistance; bacterial ghosts; foodborne zoonosis; mucosal vaccine; virulence genomics; *Yersinia enterocolitica*

Introduction Yersinia enterocolitica differs from other foodborne pathogens. It has psychrotrophic and facultative anaerobic properties and is a Gram-negative enteropathogen. It can survive cold temperatures, and cycle through the environment and infect humans with illnesses that resemble appendicitis and other bowel and systemic infections. Known for its pathogenic capacity, the Yersinia genus extends to multiple environmental microorganisms and major human pathogens (1-5). The associated human disease for the cold enteropathogen is largely attributed to Y. enterocolitica and Yersinia pseudotuberculosis. Yersinia pestis is associated with the disease of plague. Y. enterocolitica was

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cited as a significant foodborne zoonotic agent associated with pigs, pork products, contaminated water, vegetables, and possible blood transfusion transmission. It is also suggested that bacterial ghost technology serves as a potential vaccine with bacterial protective surface structures that remain after removal of the cytoplasm and genetic materials. This article also aims to integrate to traditionally separated fields, that of the modern pathobiology of Y. enterocolitica, and the bacterial ghost vaccines. The main premise is that, in addition to delivering antigens, a modern vaccine for yersiniosis should take into account the organism's ecology, virulence, and other pathways, including immune evasion, diagnostic problems, genomic variation, and antimicrobial resistance (6–8)

Taxonomy, Biotypes, Serotypes, and Pathogenic Lineages of Yersinia enterocolitica

The genus Yersinia underwent considerable taxonomic changes as Yersinia members were shown to be widely heterogeneous in ecology, range of hosts, potential for virulence, and evolution. Y. enterocolitica is heterogeneous and divided into six biotypes: 1A, 1B, 2, 3, 4 and 5, and multiple O-serotypes. O:3, O:5,27, O:8 and O:9 are the O-serotypes most frequently linked to human illness. This diversity is of direct biological and clinical importance. Biotype 1B is usually considered very pathogenic and is linked to the high-pathogenicity island and yersiniabactin-mediated iron uptake. Biotypes 2-5 are considered low-pathogenic lineages but still have important virulence determinants. Biotype 1A is considered non-pathogenic for the most part as it does not have the classical virulence plasmid and invasion plasmid encoded genes, however, recent genomic and epidemiological data have shown that this view is an oversimplification. Biotype 1A of Y. enterocolitica can be isolated from human illness and can have ystB, inv genes, or even virulence genes of an atypical ail positive profile. This makes the classification of pathogens more complicated from the binary pathogenic and non-pathogenic approach. Typing therefore combines chemical biotyping, O-serotyping, virulence gene analysis, and whole genome analysis. For this reason, a bacterial ghost vaccine from one strain may have a native surface antigen but may only have cross protection if it closely (9-12).

Short of a multivalent vaccine, a single-serotype vaccine will always miss the new lineages that have some of the old ones, and the lineages that have been underrecognized. The multivalent vaccine formulation should be able to cover the majority of the most relevant bioserotype clusters. An original circular taxonomy map of genus Yersinia should be placed in Figure 1 and should have an expanded Y. enterocolitica and biotypes, the dominant O-serotypes, virulence, the major reservoirs, and the expected relevance for vaccine development (1–6) (Figure 1).

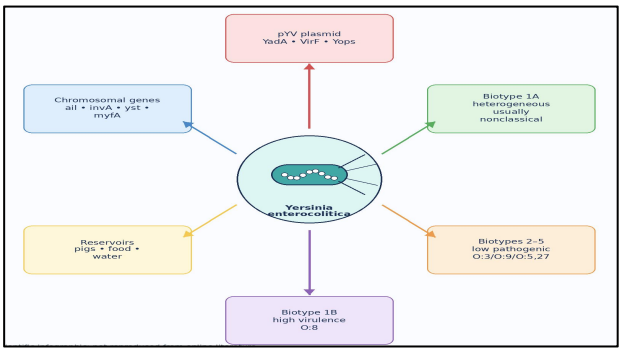

Figure 1: Taxonomy, Biotypes, Serotypes, and Pathogenic Lineages of Yersinia enterocolitica

Global Epidemiology and One Health Transmission at the Human–Animal–Environment Interface

The pathogen of yersiniosis circulates between animals, food, humans, water, and various environments, making it a classic One Health infection. Pigs, especially bioserotype 4/O:3 of Y. enterocolitica, are the major asymptomatic hosts and hence a problem for many European countries. Opportunities to contaminate pork abound at slaughter, the handling of the tonsils, evisceration, and subsequent steps in processing, storage, and meal preparation. The picture of Y. enterocolitica transmission is beyond contaminated pork. Y. enterocolitica has been recently identified in various retail meats, rodents, wildlife, water, ready-to-eat salads, frozen foods, and other environmental matrices. The transmission of Y. enterocolitica perhaps

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varies among countries or regions due to food custom and preferences, climate, surveillance, and the presence or absence of changes in diagnostic capacity. In Europe, yersiniosis is one of the top three most reported yersinia-related infections, and in many developing countries (e.g., the Middle East and Africa), the cases are underreported due to the absence of culture that has been specifically modified for Yersinia or molecular diagnostics. The organism is of particular food safety concern since it can persist or grow in cold environments that other enteric pathogens cannot (13, 14).

Transmission of Yersinia enterocolitica via blood product consumption should not be dismissed because bacterial growth may be fortified by the refrigeration of red blood cell concentrates. These phenomena suggest that the scope for prevention should not be limited to the treatment of patients. It extends to the monitoring of bacterial reservoirs, hygiene of slaughterhouses, control of the entire food chain, and the implementation of molecular trace back, and targeted public health risk communication strategies. A bacterial ghost vaccine would be positioned as the vaccine used for animal reservoirs, high-risk groups of the population, or a vaccine for oral and/or mucosal use in areas that are at high risk for outbreaks. The most appropriate illustration of the One Health concept within the One Health transmission wheel (as shown in Figure 2) is the connection of pigs, porcine blood products, wildlife, rodents, the food chain, water, vegetables, contact surfaces in food production, industrial blood products, humans and the environment, along with arrows of direct, indirect and cold-chain related transmission (7) (Figure 2).

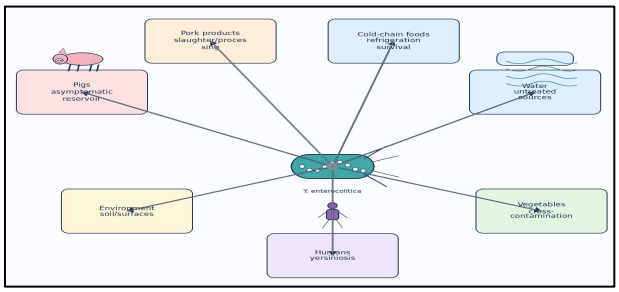

Figure 2: Global Epidemiology and One Health Transmission at the Human–Animal–Environment Interface

Pathogenesis of Yersinia enterocolitica

Yersinia enterocolitica is able to survive the challenges of the stomach, adjust to the temperature of the human body and then to colonize the intestines and interact with epithelial cells, invade M cells, and persist in lymphoid tissue. The bacterium has a strong affinity for colonizing the terminal ileum and the Peyer's patches. Once in the intestinal tract, Y. enterocolitica employs a number of bacterial adhesion factors as well as proteins that are associated with invasion, to adhere to the epithelial cells and traverse the epithelial mucosal barrier, which is facilitated by M cells which are located above the Peyer's patches. The invasion of M cells is facilitated by the activity of the bacterial protein Invasin, which is a product of the invA gene. Additionally, a number of bacterial factors such as Ail, YadA, the Yst enterotoxins, urease, Yersinia's iron uptake systems, and the type III secretion system of plasmid pYV, contribute to the survival and dissemination of the bacterium. Once the bacterium has invaded the lymphoid tissue, it is able to produce microcolonies and evade phagocytosis (15).

This may explain why the patient may have more than just the diarrhea, with symptoms including infection of the terminal ileum, inflammation of mesenteric lymph nodes, inflammation that mimics appendicitis, fever, abdominal discomfort, and in some cases, an inflammatory response that affects the entire body. There is an environmental survival to host-based pathogenicity shift, and these shifts are controlled by temperature, Ca++, nutrient concentration, and the control mechanisms of the plasmid. Hence, when developing a vaccine, it is crucial to determine the antigens that are most likely to be expressed during the infection of the host, which may include antigens that are present during the infection and are not easily found during the passive growth of the bacterium (16). It is likely that bacterial ghosts may be the most promising vaccine candidates, as the ghosts retain their bacterial envelope in a form that is

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more similar to the actual bacteria, including outer membrane proteins, lipopolysaccharides, lipids, and fimbriae, and other PAMPs. To retain the major surface structures that are associated with virulence, ghost preparation must be optimized to maintain the bacterial cells. Fig 3 is expected to explain the complete intestinal infection pathway that occurs following the ingestion of infectious food, including the infection of the ileum, entry through the M cells, invasion of the lymphoid tissue, infection of the mesenteric lymph nodes, infection of the body's immune organs (the liver and spleen), the resultant lymphoid organs, and the various clinical symptoms and immune system barriers (17, 18) (Figure 3).

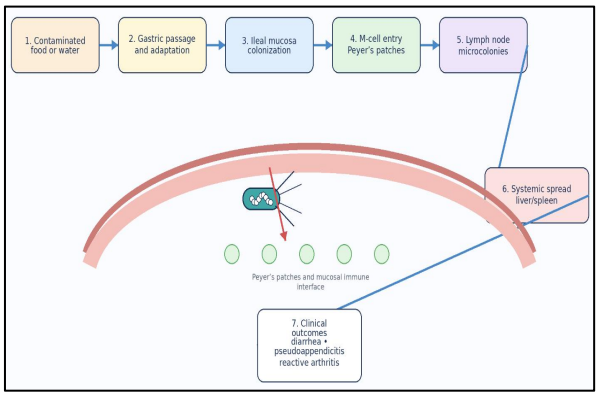

Figure 3: Pathogenesis of Yersinia enterocolitica.

Virulence Architecture: pYV Plasmid, T3SS, YadA, VirF, Yops and Chromosomal Determinants

The virulence system of Y. enterocolitica is characterized by a framework of plasmid- and chromosomally- encoded determinants. The pYV virulence plasmid contains the genes of the Type III Secretory System, and Yersinia outer proteins, YadA and some regulatory proteins, including VirF. At the body temperature of the higher mammals, VirF is also responsible for the synthesis of the proteins that are required for bacteria to infection the host cell and for protection of the bacteria from the immune system. YadA, in addition to mediating the adhesion of the bacteria to the host tissues, also binds the extracellular components of the host body, is resistant to the immune system of the host, and persists in the proximity of host cells to allow the injection of effectors (19).

The type III secretion system operates as a molecular syringe to deliver Yop effectors into host cells to interfere with phagocytosis and cytoskeletal signaling and to inhibit the induction of inflammation and the synthesis of proinflammatory cytokines. Chromosomal genes add additional complexity. ail is involved in adhesion, invasion, and serum resistance. invA is required for intestinal entry. ystA and ystB encode heat-stable enterotoxins, which are involved in the mechanisms of certain types of diarrheal illness. myfA is involved in the formation of fimbrial appendages. ymoA is involved in the control of temperature-dependent expression of virulence. hreP is hypothesized to be involved in a host-adapted proteolytic activity. Additionally, genes related to iron metabolism, such as fepD and fes, play a role in survival in iron-limited conditions in the host. Thus, it is a network, not a single-gene phenomenon, for virulence. Studies of the genomes of pathogens indicate that features such as mobile genetic elements, pathogenicity islands, variability of plasmids, and genes that are characteristic for a certain lineage contribute to the pathogenicity of the organism (20). In the context of bacterial ghost vaccines, this creates two challenges. First, the selection of a strain for the vaccine needs to consider the genomic and antigenic aspects of the pathogen. Second, the ghost vaccine needs to be validated that potentially important surface antigens are still present on the ghost and are not altered due to the lysis or chemical treatment. The virulence of the Y. enterocolitica is shown in a two-ring model with a central Y. enterocolitica cell. The plasmid ring shows genes from the pYV plasmid along with Type III secretion system (T3SS) genes, as well as Yop proteins, and the outer ring shows various elements that are encoded in the Y. enterocolitica chromosome, such as ail, invA, ystA/ystB, myfA, ymoA, hreP, and the iron-related genes fepD and fes. (21, 22) (Figure 4).

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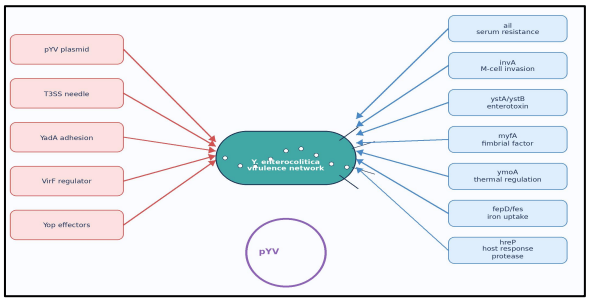

Figure 4: Virulence Architecture: pYV Plasmid, T3SS, YadA, VirF, Yops and Chromosomal Determinants

Host Immune Response and Immune Evasion During Y. enterocolitica Infection

Y. enterocolitica infections witness an aggressive immune response from the host. This is due to a heightened response of the mucosa, and a corresponding, heightened immune evasion response from Y. enterocolitica. At the intestinal mucosa, epithelial cells, M cells, macrophages, dendritic cells, neutrophils, and lymphocytes collectively identify bacterial constituents through a diverse array of pattern-recognition receptors that identify lipopolysaccharide, lipoproteins, peptidoglycan, fimbriae, and other microbial components. This results in the recruitment of innate and adaptive immune responses, including the secretion of proinflammatory cytokines, recruitment of phagocytes, and priming of the adaptive immune response. IFN-γ, TNF-α, IL-1β and IL-6, and other mediators of inflammation play a role in the control of bacterial growth and the coordination of the immune response. However, all Y. enterocolitica pathogenic strains have evolved the ability to thwart immune clearance. The T3SS and Yop proteins interrupt phagocytosis, cytokine, and chemokine signaling and inflammasome activation and leukocyte stimulation. Y. enterocolitica also employs YadA and the Ail proteins to resist the action of complement and to persist in the tissues. It is this immune-evasion ability that is responsible for why Y. enterocolitica can, in some cases, grow extracellularly in lymphoid tissues, and why the infection can produce inflammatory sequelae such as reactive arthritis and erythema nodosum in sensitive persons. This means that a bacterial ghost vaccine must go beyond inducing passive and poor antibody responses and should invoke the production of mucosal IgA and systemic IgG along with the activation of macrophages and dendritic cells (23). This vaccine should also tilt the immune response to the Th1 and Th17 pathways and provide protective immunity in the form of a rapid and effective response towards a microbial invasion. The ghosts, due to the preservation of the PAMPs, should have an intrinsic adjuvant property and would provide a means of sustaining the immune response along with protecting the balance between the safety and the immunogenicity of the ghosts, giving the best rationale for the implementation of the ghost-based vaccines. The protective immunogenicity of the ghosts is the best rationale for ghost-based vaccines. This balance is the best rationale for ghost-based vaccines. Figure 5 shows the Yop blockade along with all the immune evasion tactics next to ghost preservation of PAMPs. The immunogenicity of ghost vaccines (24–27) (Figure 5).

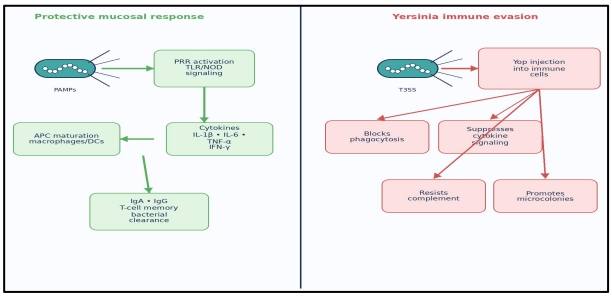

Figure 5: Host Immune Response and Immune Evasion During Y. enterocolitica Infection

Culture-Based and Molecular Diagnosis: From CIN Agar to Real-Time PCR and Genomic Detection

Diagnosis of Y. enterocolitica remains difficult because routine stool culture can easily neglect the organism unless the laboratories request or employ Yersinia-specific methods. Culture can be performed using selective media (e.g. cefsulodin–irgasan–novobiocin agar), enrichment steps, biochemical identification, and serological confirmation. However, the process can be slow and is influenced by competing flora, sample type, the stage of infection, and the incubation atmosphere. Some isolates can be

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recovered from stool, blood, lymph nodes, wounds, bile, throat swabs, and others depending on the clinical presentation and the disease (28). Molecular methods have improved detection by targeting specific markers of the species or particular markers of virulence such as ail, invA, ystA, ystB, yadA, virF, ymoA, and the 16S rRNA. Real-time PCR, multiplex PCR, and syndromic gastrointestinal panels have improved the timeliness and the sensitivity of the assays, and even allowed direct testing from the clinical samples. Nevertheless, molecular methods can yield positive results, without isolating the organism from the clinical sample, making the necessary tests to the antimicrobial sensitivity, comparison of outbreak isolates and selection of isolates for vaccine studies infeasible. Therefore, the ideal diagnostic system can be based on rapid PCR screening, combined with culture recovery and whole-genome sequencing, whenever additional public health investigations are necessary. Recent qPCR methods have been developed for patho-serotyping with the intention of targeting the important clinical O-serotypes and the virtual profiles. In areas with inadequate surveillance, the use of short-amplicon qPCR can be regarded as an advancement in diagnostic methods that targets clinical samples and can aid in decreasing the underreporting of disease (29). Figure 6 is to show the diagnostic pathway for the suspected case of yersiniosis, commencing with stool/blood/food sampling followed by culture on selective media with biochemical confirmation, PCR/qPCR, virulence gene profiling, antimicrobial susceptibility testing, and sequencing for genome mobile genetic elements for outbreak investigations (28–31) (Figure 6).

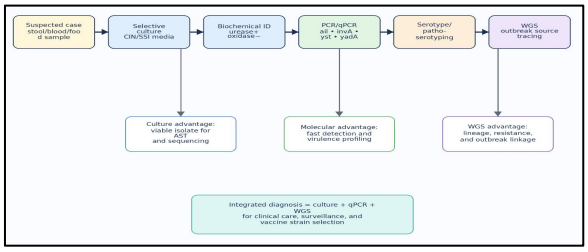

Figure 6: Culture-Based and Molecular Diagnosis: From CIN Agar to Real-Time PCR and Genomic Detection

Whole-Genome Insights, Mobile Genetic Elements, and Evolution of Pathogenicity

Whole genome sequencing (WGS) has shifted the understanding of Y. enterocolitica from a phenotype classification to a population genomic approach. First, the classical biotyping and serotyping approaches are still dependable and helpful, however, WGS has the ability to determine the core genome of a biotype, the types of SNPs and clusters, the virulence gene, plasmid (and resistance) content, and the relationship of a genome to a previous outbreak. Recent studies that apply genomic surveillance to Y. enterocolitica have illustrated the ability for isolates to cluster by phenotype, biotype, and pathogenicity, however, some non-pathogenic or low-pathogenic groups still have a virulence gene or pathogenicity factor. Mobile genetic elements are critically important for the pathogen due to the direct ability for a strain to modulate its pathogenicity to a host or environmental matrix (28). It appears that within the elements of YGI, YGI elements, environmental persistence, and host adapted pathogenicity, also plasmid and iron acquisition are also employed. In the context of outbreaks, WGS has the ability to differentiate and show case sporadic outbreaks (common source) and the connectivity of the environmental/food sources to outbreaks. Regarding the area of biotechnological research and vaccine development, WGS has the capability and the ability to analyze the selection and surface of proteins that are potentials to be vaccine targets especially those that are perceived to be virulence elements are also a factor (29).

Designing a future bacterial ghost vaccine should be done with a genomic-first framework: choose representative strains, confirm coverage of important antigens, ascertain absence of unacceptable genetic hazards, and assess the preservation of ghost preparation structures in bacterial envelopes that are likely to be immunologically relevant. Figure 7 should illustrate a genomic-to-vaccine paradigm in

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which raw isolates are subjected to WGS, cgMLST/SNP, virulence, and AMR analysis are done, antigen preservation and bacterial strain selection are applied, and then bacterial ghost preparation and production are done. Preclinical immune screening should be done afterwards (4-12, 19–22, 32) (Figure 7).

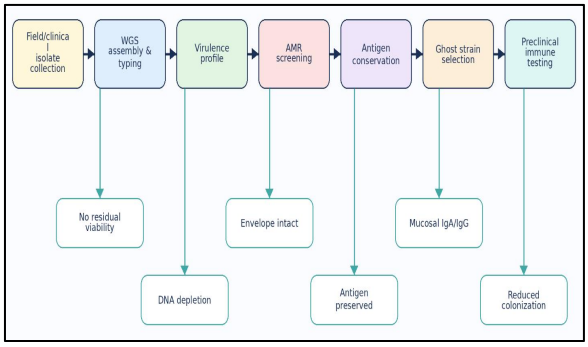

Figure 7: Whole-Genome Insights, Mobile Genetic Elements, and Evolution of Pathogenicity

Antimicrobial Resistance in Y. enterocolitica: Clinical Relevance and Food-Chain Risk.

Supportive care continues to be the primary treatment for uncomplicated yersiniosis. There are important clinical considerations for antimicrobial therapy in severe enterocolitis cases and others with septicemia, systemic invasion, and extraintestinal complications, particularly in the case of high-risk and immunocompromised patients. Yersinia enterocolitica resistance should be considered for these scenarios, and for these reasons, enterocolitis and yersiniosis in general should be classified as a threat. Y. enterocolitica expresses intrinsic resistance to a few of the β-lactams, particularly ampicillin and first-generation cephalosporins, because of its intrinsic β-lactamase activity (33). There is variability in resistance to other β-lactams, and fluoroquinolone, aminoglycoside, trimethoprim-sulfamethoxazole, and carbapenem susceptibility. Recent studies have documented multidrug resistance in wild and domestic animals, humans, food, and the environment, and documented resistance to polymyxins, azithromycin, sulfonamides, cefotaxime, ciprofloxacin, and tetracycline. Variability in these studies and resistance profiling was the result of different research designs, project scales, sources, venues, and country variability. Food-chain resistance is especially worrying, as Y. enterocolitica is able to grow in aerobic refrigerated environments, and enter the home via raw or undercooked pork, contaminated meats, veggies, or even water. Biofilms have been shown to persist in the food environment. Current research suggests that calcium, temperature, glucose, and the status of the pYV plasmid, may influence the biofilm, motility, and extracellular polymeric substances. Biofilms may also create a persistent level of difficulty in management and sanitation, especially on food-contact surfaces (34). These trends support the need for preventative measures and control of Y. enterocolitica in the food environment, as well as improvements in clinical management of Yersinia spp. to control disease in the food supply. The development of bacterial ghost vaccines for the animal reservoir, particularly for pigs, may decrease internal colonization in the food chain. The development of mucosal vaccines to protect the high-risk population may also be a viable option (35).

Figure 8 should show an antimicrobial resistance and persistence network connecting intrinsic β-lactam resistance and acquired resistance, biofilm formation, cold-chain survival, food-processing contamination, clinical treatment challenges, and vaccine-prevention challenges (33–35).

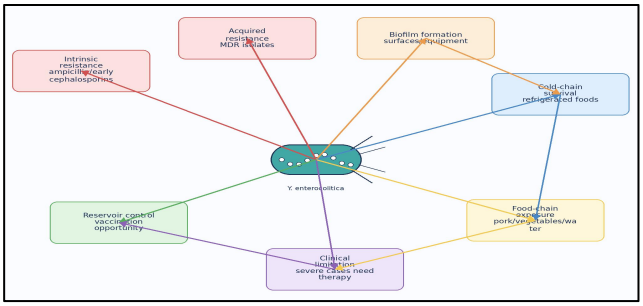

Figure 8: Antimicrobial Resistance in Y. enterocolitica: Clinical Relevance and Food-Chain Risk.

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Bacterial Ghost Technology: Production, Structure, Quality Control, and Immunological Logic

Bacterial ghosts retain the three-dimensional appearance and surface structures of the original bacteria and consist of nothing but an empty cell envelope. This is an exciting concept, especially in the case of Gram-negative pathogens, as the outer cell membrane contains lipopolysaccharide, outer membrane proteins, adhesion, fimbriae, and other PAMPs, which can act as innate as well as adaptive immune stimulators. Ghosts can be produced by genetic lysis systems, especially by the lysis of the PhiX174 gene E bacteriophage, or by some chemical methods, like the sponge-like methods using NaOH, SDS, H_2O_2, Triton X-100, Tween 80, different acids, or some other optimized methods. The right method must be chosen, as harsh inactivation of the membranes can lead to the loss of surface structures and epitopes, while an insufficient method can leave viable bacteria and/or nucleic acids. Quality control must involve sterility and recultivation testing, DNA and protein assessments, electron microscopy, antigen preservation testing, an endotoxin safety evaluation, and stimulation assays of immune cells (36). Some ghost vaccines against both non-enteric and enteric pathogens have shown that ghosts can elicit humoral responses, immune cell responses, serum bactericidal responses, and production of cytokines, along with protection in various animal models. Their intrinsic adjuvant effect is one of the major advantages, as APCs can recognize bacterial PAMPs and processed PAMPs as well as associate antigens. In addition, ghosts can be packed with various antigens, DNA, peptides, drugs, or immune modulators, which makes the design very versatile and broad (37).

For Y. enterocolitica, ghost production may sustain YadA, Ail, and InvA bacterial envelope structures, along with O-antigen and other target immunogens. In this regard, the technology would be more biologically accurate than subunit vaccines with purified components. Figure 9 presents a comparison between gene E-mediated lysis and a sponge-like, ghost-production technology. These will be illustrated in two, parallel workflows, and will conclude with the same quality-control checks: absence of growth, depletion of DNA, preservation of the envelope, and preservation of the antigens along with stimulation of the immune system (36-40) (Figure 9).

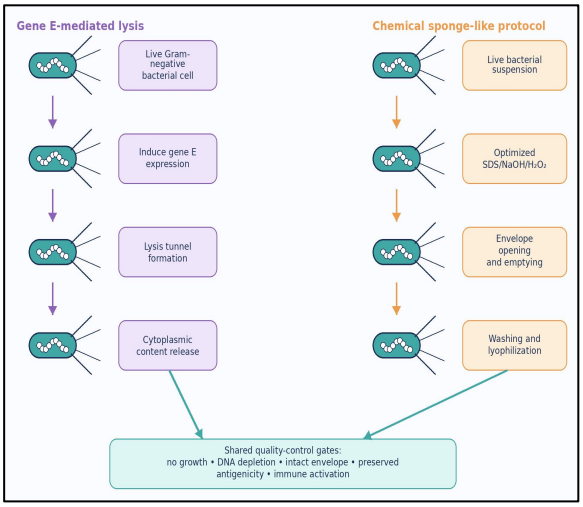

Figure 9: Bacterial Ghost Technology: Production, Structure, Quality Control, and Immunological Logic

Hypothesized Bacterial Ghost Vaccine Model Against Y. enterocolitica

An example of a rational bacterial ghost vaccine for Y. enterocolitica would build on a mucosal, rigorous, digital, and more sophisticated platform for a killed-bacterial preparation. The first step would involve the selection of cultured strains based on WGS, bioserotype and prevalence, air-virulent gene, antigenic profile, and depletion of resistance and/or gene-moving factors. These strains would have to belong to the more ubiquitous, human-related bioserotypes, or be a related variant of the 4/O:3, or of 2/O:9 of the 1B/O:8, or any other related lineage. The second step would be the technology of ghost production for the elimination of cytoplasm and DNA along with the preservation of the outer membrane. The third step would involve the determination of the integrity and the preservation of the antigens (30). This would require the application of Galactic electron microscopy, immunoblot, ELISA, proteomic surface profiling, and receptor-binding studies.

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The final step would involve the testing of the immunology of the intestinal epithelium, in addition to immune cells of the gut, along with the use of models of live mammals. In this model, the most significant endpoints would be the production of mucosal IgA, and IgG, along with the provision of immunity, protection of the gut, of the mesenteric immune system, and of the systemic channels, along with the provision of immunity against the invasion of the gut, and the provision of protection against colonization (32).

Mucosal vaccination via the intranasal, oral, or other routes deserves special attention since naturally occurring Y. enterocolitica infections initially colonize the intestinal mucosa. The most advanced version of this vaccination platform will have a combination of preserved Y. enterocolitica ghost envelopes with either preserved antigens or immunomodulators that could expand the breadth of protection to other bioserotypes. Using this technology on the reservoir animals may reduce the contamination of the pork-chain and would apply to One Health prevention (34). However, safety needs to be established prior to consideration of this technology for the clinic. this would involve an assessment of the residual DNA (Deoxyribonucleic acid), the burden of viable bacteria, the balance of endotoxins, inflammatory toxicity, potential impact on the microbiome and the selection of an appropriate dose. The final graphical abstract should be Fig. 10 showing, WGS guided strain selection, bacterial ghost formation, mucosal delivery, activation of professional antigen presenting cells, the differentiation of B and T lymphocytes, production of immune memory, reduction of intestinal colonization, reduction of food-chain transmission and reduction of antimicrobial pressure (37–40).

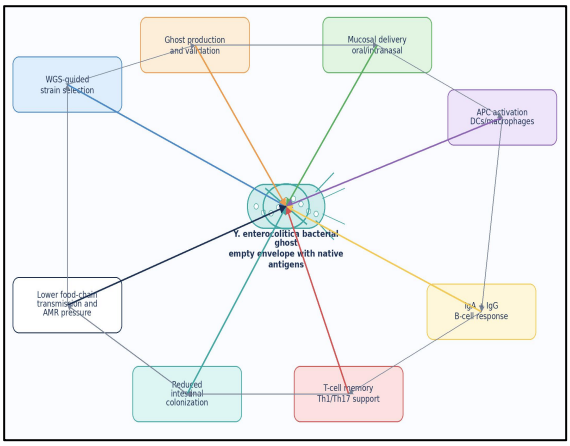

Figure 10: Hypothesized Bacterial Ghost Vaccine Model Against Y. enterocolitica

Conclusion

Yersinia enterocolitica is a biologically intricate and clinically overlooked zoonotic pathogen. The public health significance of Y. enterocolitica stems from its pathogen reservoir animals, its persistence in the food-chain at refrigeration temperatures, the extensive range of symptoms it can cause, the elements of its pathogenicity carried on a plasmid, factors present in its chromosome that make it a pathogen, its diagnosis being routinely overlooked, its genomic variability, its ability to form biofilms and a recent rise in its resistance to treatment with antibiotics. The same complexity that makes this organism hard to control also provides opportunities for the design of next generation vaccines. The ghost technology provides a compelling platform for vaccines since it preserves the integrity of the bacterial surface, gives a preserved bacterial surface with elements that enhance the immune response and can be designed to be a multiple antigen system. A successful Y. enterocolitica bacterial ghost vaccine should be WGS guided, bioserotype aware, delivered via the mucosal route and formulated to meet the immune, microbiological, genomic and food safety endpoints. This technology may take prevention from a treatment focus to an early intervention that breaks the chain of transmission along the continuum between the human–animal–environment nexus.

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Conflict of interest
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