Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.249

REVIEW ARTICLE

# A review on the Use of Nanomaterials for Control and Prevention of *Clostridium perfringens*: An Organism of Zoonotic Importance

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ARTICLE HISTORY (24-515)

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|---|---|
| Received: | August 19, 2024 |
| Revised: | September 13, 2024 |
| Accepted: | September 15, 2024 |
| Published online: | September 27, 2024 |

**Key words:**
Bacteria
Clostridium
Control
Diseases
Nanoparticles
Pathogenesis
Public Health
Zoonotic

**A B S T R A C T**

*Clostridium perfringens* is a Gram-positive species of bacteria known for their zoonotic importance and intestinal diseases in various species of animals and humans. *C. perfringens* produces 7 different toxins and based on these toxins they are categorized into various serotypes, which are commonly called toxinotypes. These bacteria are widely found in the environment and in animals. The major control of these bacteria depends upon vaccination and the use of antibiotics. Vaccines are commonly reported to be less effective because of issues of vaccine failure and lack of proper immune response. On the other hand, daily used antibiotics are also being compromised because of antimicrobial resistance and public health concerns. The severity of these issues demands to search the alternates, and the most suitable alternate is the use of nanoparticles. Various types of nanoparticles, including metallic, metal oxides, and polymeric nanoparticles are being used against *C. perfringens* for vaccine and antimicrobial drug delivery. Multiple nanoparticles can control *C. perfringens*-caused intestinal disease by acting on the various cellular processes. Several other nanoparticles can control *C. perfringens*-caused infections directly by working as delivery agents of various types of vaccines. Research proves that the use of nanoparticles can help to control *C. perfringens*. Nanomaterials, despite their benefits, however, need to be improved a little especially regarding their synthesis and toxicities.

**To Cite This Article:** Almuzaini AM, 2024. A review on the use of nanomaterials for control and prevention of *Clostridium perfringens*: an organism of zoonotic importance. Pak Vet J, 44(3): 563-570. http://dx.doi.org/10.29261/pakvetj/2024.249

## INTRODUCTION

*Clostridium perfringens* is a species of Gram-positive rod-shaped (bacillus) bacteria that are anaerobic, however, have survivability in aerobic environments (Talukdar and Sarker, 2022). *C. perfringens* can persist in multiple environmental, geographical, and climatic environments (Camargo *et al.*, 2022). It is a spore-forming bacillus that has no motility organelles (Banerji *et al.*, 2021). It is a common inhabitant of the gastrointestinal tract of humans, animals, and birds and has the potential to cause serious illnesses in its hosts (Mora *et al.*, 2020). These are toxin-producing bacteria that cause pathogenesis because of the toxins released in the body (Grenda *et al.*, 2023). Based on the classes and natures of toxins produced by them, they are classified into several groups (Table 1). These toxins determine the type and nature of pathologies inside the body (Lee and Lillehoj, 2021).

The serotyping of *C. perfringens* based on toxin production (toxinotyping) depends upon their capacity to carry the specific set of genes that are responsible for the production of these toxins (Hussain *et al.*, 2022) (Table 1). Based on these toxins *C. perfringens* is divided into seven different groups named A to G (Mehdizadeh Gohari *et al.*, 2021). These different serotypes cause various diseases in humans and animals including bovine necrotizing hemorrhagic enteritis, necrotic enteritis, gas gangrene, and common food poisoning (Boulianne *et al.*, 2020). It is among the most prevalent bacteria causing intestinal diseases (Huang *et al.*, 2023). Multiple strains have zoonotic significance i.e., infect multiple species and remain infecting humans (Bendary *et al.*, 2022). Several outbreaks of *C. perfringens* have been reported in the last decade, causing high morbidities and economic losses (Fancher *et al.*, 2020). Clostridial infections remain at the top of the food-borne illnesses worldwide despite prevention and hygienic measures (Kanaan and Tarek, 2020).

*C. perfringens* is a major pathogen to be considered for control and prevention because of the severity and

**Table 1:** Toxins of *Clostridium perfringens,* their site of production, mechanisms of toxicities, and referring strains.

| Sr. | Toxin | Responsible Gene | Site of Production | Mechanisms of Action | Toxic effects | Carrying strains | Reference |
|---|---|---|---|---|---|---|---|
| 1. | Alpha (CPA) | *cpa/0plc* | Chromosome | Destroys plasma membranes by disturbing sphingomyelin and phosphatidylcholine. Inhibits maturation of the neutrophils Causes shortening of vascular lumen and leads to aggregation of neutrophils | Necrosis, hemolysis, vasoconstriction, gas gangrene | A, B, C, D, E, F, G | (Wang *et al.,* 2020) |
| 2. | Beta (CPB) | *cpb* | Plasmid | It perforates in cell structure. Releases "substance P" causing neuropathy. Causes the symptoms of enterocolitis | Bovine hemorrhagic enteritis, pore-forming toxin | B, C | (Benz *et al.,* 2022) |
| 3. | Epsilon (ETX) | *Etx* | -do- | Activated by enteric proteases and increases intestinal permeability. Oedema around the vasculature Gets accumulated in the central nervous system and organs of the renal system because of undetermined reasons | Dermal necrosis, vasculature edema, vasoconstriction | B, D | (Jiang *et al.,* 2020) |
| 4. | Iota (ITX) | *iap/ibp/itx* | -do- | Disturb the cytoskeletal structures by interfering with the depolymerization of actions. | Necrotic effects | E | (Mada *et al.,* 2023) |
| 5. | Enterotoxin (CPE) | *cpe* | Chromosome, Plasmid | Binds to Claudin receptors are present on the surface of cells. Forms a hexamer resulting in calcium influx. | Toxic or intestinal structures | F (possibilities also in C and D) | (Mehdizadeh Gohari *et al.,* 2021) |
| 6. | Necrotic Enteritis B-like toxin (NetB) | *net*B | Plasmid | Causes hemolysis. Pore formation in poultry | Hemolysis, enteritis in poultry | G | (Lee and Lillehoj, 2021) |

diversity of illnesses (Grenda *et al.*, 2023). *C. perfringens* has different serotypes and there is a diversity of toxins and all of them have different mechanisms of action, so control and prevention of *C. perfringens* is crucial and needs to be done on a priority basis (Lhermie *et al.*, 2020; Heidarpanah *et al.*, 2023). Prevention from infection of *C. perfringens* depends on immunization through vaccination (Fancher *et al.*, 2020; Saadh *et al.*, 2022). Two types of vaccines i.e., bacterin and toxoid vaccines are being used in humans and poultry for the control of necrotic enteritis and other diseases produced by *C. perfringens* (Alizadeh *et al.*, 2021; Fu *et al.*, 2022). These vaccines are being used on the human and veterinary side for the prevention and control of infections produced by *C. perfringens* depending upon the serotype and toxin involved (Fathima *et al.*, 2022; Wang *et al.*, 2022). Treatment of these diseases needs high doses of broad-spectrum antibiotics (Kowalczyk *et al.*, 2021). These drugs have been successful in controlling *Clostridium* spp., but several issues are making the use of these drugs limited in the future (Vamsi Krishna *et al.*, 2022).

The emergence of antibiotic resistance is the most threatening issue being seen against multiple pathogens, including *C. perfringens* (Bendary *et al.*, 2022). *C. perfringens* has been reported to adopt multiple ways to counter antibiotics (Khan *et al.*, 2021). They develop antibiotic-resistant genes that destroy the molecules of these drugs and stop their antibacterial activity (García-Vela *et al.*, 2023a). Other pathways include modification of transport channels, hence reducing the intake of antibiotics, shifting the targets of antibiotics, etc. (Naveed *et al.*, 2020; Uruén *et al.*, 2020) Resistance to multiple antibiotics has been reported in various parts of the world (Anju *et al.*, 2021; Yadav *et al.*, 2022; García-Vela *et al.*, 2023b). Along with resistance, there are issues of secondary metabolites and antibiotic toxicity with the antibiotic chemicals (Kongkham *et al.*, 2020). The management of resistance needs to be done by alternative measures.

Multiple compounds are being suggested for the control of *C. perfringens* infections, but the most suitable substances being recommended are nanoparticles which have direct antibacterial activities and may be used for the delivery of antimicrobial agents and vaccines effectively (Mohd Yusof *et al.*, 2021; Gomaa *et al.*, 2023; Ibrahim *et al.*, 2024). Metallic, metal oxides and polymeric nanoparticles are among the most used nanomaterials for these purposes (Begines *et al.*, 2020). Literature proves that nanoparticles are effective against various diseases, including *C. perfringens*-born enteric diseases (Kannan *et al.*, 2020; Fatima *et al.*, 2024). Recently, wide research on their antimicrobial efficacy has been done. This article summarizes the role of metallic nanoparticles against *C. perfringens*-caused infections as antimicrobial, drug, and delivery agents.

**Review Methods:** This review was performed using "Google Scholar (www.scholar.google.com)" as the primary search engine, and the results were refined using "Web of Science", "PubMed", "ScienceDirect" and "ResearchGate". The Keywords used were "*Clostridium perfringens"* AND "Nanoparticles"; "*C. perfringens"* AND "Nanomaterials"; "Necrotic Enteritis" AND "Nano vaccine". Almost 885 articles resulted using these keywords. Only the original papers of the last 5 years were included in the study; review articles and secondary articles were excluded from the study. The Journal articles were included and the thesis, dissertation, Conference proceedings, abstracts, books, and chapters were not included. The papers on the effect of nanomaterials on the detection, the indirect effects of nanoparticles, and *C. perfringens* toxin conjugations for control of any other disease were not included. Only 10 studies were refined for the formation of Table 2 and Table 3. Before the review of these nanoparticles, understanding the predisposing factors and pathogenesis of *C. perfringens* is mandatory (Fig. 1).

**Predisposing Factors and Pathogenesis:** Necrotic enteritis is a disease of zoonotic importance that primarily infects humans and poultry birds (Fathima *et al.*, 2022). *C. perfringens* is the etiological agent of this disease, commonly present in the environment as spores, which are resistant to harsh environmental conditions (Mora *et al.*, 2020). In addition, in the external environment, vegetative forms of *C. perfringens* can be found in various animals, decaying organic materials, and raw vegetables (Balali *et al.*, 2020). Mainly, transmission of this pathogen depends on the fecal-oral route (Khan *et al.*, 2023). It belongs to the anaerobic bacterial group, so its replication requires an environment that is deprived of oxygen (Mehdizadeh Gohari *et al.*, 2021). *C. perfringens* remains in the intestine and is incubated until entry into the intestine (Mora *et al.*, 2020). The main predisposing factors of necrotic enteritis include dietary factors, physical damage to the intestine, immunosuppressive circumstances, and prevailing intestinal diseases (Fathima *et al.*, 2022; Shamshirgaran and Golchin, 2024) (Fig. 2). Because of these factors, the intestine is damaged, creating a hypoxic environment and facilitating the stay of *C. perfringens* (Finnie and Uzal, 2022). This environment enables the incubation of *C. perfringens* in the intestine and the replication of *C. perfringens* starts in the intestine (Hustá *et al.*, 2020; He *et al.*, 2022).

The predilection site of *C. perfringens* in the intestine is the small intestine, especially the duodenum and jejunum (Campos *et al.*, 2024). The main pathologies of *C. perfringens* arise when the organism secretes toxins (Mehdizadeh Gohari *et al.*, 2021). The various toxins of *C. perfringens* produce various types of lesions in the intestine and liver (Rizk *et al.*, 2020; Lee and Lillehoj, 2021). Molecular studies have revealed recently that most of the toxins of *C. perfringens* cause cell death by induction of necrosis (Takehara *et al.*, 2020; Lan-Xin *et al.*, 2024). The individual mechanisms of induction of toxicities of various toxin types and their respective toxins are presented in Table 1. These toxicities lead to the development of gas gangrene in the intestine and necrosis of various tissues (Hussain *et al.*, 2024). The damage may extend to the vascular supply of the intestine and toxemia and bacteremia may develop (Hussain *et al.*, 2024). Bacteremia is rare with C. *perfringens* infection in animals or birds (<4%), but if it occurs, a high risk of mortality occurs (Kalender *et al.*, 2023).

**Nanomaterials against *C. perfringens:*** Nanomaterials are among the most used carriers of the medicinal agents (Yetisgin *et al.*, 2020; Mabrouk *et al.*, 2021). Several nanomaterials are being used, including metallic, natural polymeric, synthetic polymeric, lipids, etc. (Ren *et al.*, 2022). They may have direct antimicrobial activities or be used as carriers of antibacterial agents or vaccines (Rajak *et al.*, 2020; Rosli *et al.*, 2021). The nanomaterials have been used in all the mentioned aspects against *Clostridium perfringens* (Zgheib *et al.*, 2021). The use of nanoparticles and nanoparticles-based therapeutic agents for the control of *C. perfringens* is given in the following sections:

**Antibacterial activity of nanomaterials against *C. perfringens*:** Nanomedicine is an emerging concept in which nanoparticles with direct antimicrobial properties are being prepared to avoid the expense of extra medicine (Eleraky *et al.*, 2020; Thapa *et al.*, 2021). Nanoparticles can easily penetrate bacterial cell walls because of their smaller size (Linklater *et al.*, 2020; Li *et al.*, 2021). They can cross the cell membrane either by endocytosis (invagination of membrane) (Makvandi *et al.*, 2021; Cong *et al.*, 2022), simple diffusion (Jiménez-Jiménez *et al.*, 2020; Liu *et al.*, 2020), or the transport channels present in the plasma membrane (Wang *et al.*, 2021; Yang *et al.*, 2021). These nanoparticles, after entry, show their antibacterial properties by interfering in various cell mechanisms (Salleh *et al.*, 2020; Godoy-Gallardo *et al.*, 2021). These properties include the disruption of cellular enzymes (Guan *et al.*, 2021; Gudkov *et al.*, 2021), the production of reactive e-oxygen species (Zhang *et al.*, 2020; Bochani *et al.*, 2023), and the release of metallic charged particles (Godoy-Gallardo *et al.*, 2021; Maťátková *et al.*, 2022), which induce toxicities directly (Abbasi *et al.*, 2023).

Mostly metallic nanoparticles are used for the control and treatment of *C. perfringens* in *in vitro, in vivo,* and *in silico* experiments (Xu *et al.*, 2023). The studies showed that the nanomaterials effectively controlled the *C. perfringens* in the above-mentioned way directly, controlled the toxins of *C. perfringens,* and eliminated the biofilm formed by the *C. perfringens.* These studies have been summarized in the Table 2. Nanoparticles have also shown great antibacterial efficacy by working as delivery agents for antimicrobial drugs (Vassallo *et al.*, 2020). Research studies show that nano vehicles improve the drug's therapeutic efficacy and targeted delivery. Recent searches are focusing on the observation of the conjugated nanoparticles i.e. medicine or drug-coated nanoparticles so that dual activities can be achieved i.e. the action of drug and combination of antibacterial nanomaterial be used to form antibacterial nanoparticles (Zhang *et al.*, 2024).

**Nanomaterials as Vaccine adjuvants:** Nanoparticles are widely being explored to deliver vaccines against clostridial diseases (Dykman, 2020). For this purpose, mostly polymeric nanoparticles are being conjugated with the vaccine peptides (Rodrigues Dos Santos Junior *et al.*, 2020; Koirala *et al.*, 2023). Chitosan, alginate, and various derivatives are among the most searched materials for the delivery of anti-clostridial vaccines (Niculescu and Grumezescu, 2022). These nanocarriers adsorb the anti-clostridial subunits or peptides and deliver them to the body (Akerele *et al.*, 2020). These materials dissolve in the body slowly and cause a slow release of vaccine (Dmour and Islam, 2022). They have been proven effective in providing various vaccine types including proteins, DNA, and RNA-based proteins (Şenel and Yüksel, 2020). The research states that the addition of these nanoparticles makes them suitable options for the delivery of vaccine (Abdolmohammadi Khiav and Zahmatkesh, 2021; Ibrahim *et al.*, 2021). The use of

**Table 2:** Nanomaterials for the control of *Clostridium perfringens* of veterinary importance.

| Sr. No | NP class | NP name | Conjugated substance | Synthesis of NP | Size (nm) | Type of Experiment | Animal source/ experiemnt | Target strain | Mechanism of action | Result | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Natural polymeric | Alginate | Leaderless antibacterial peptides | Ball milling methods | 172 | *In vitro* | Simulated guts of human and poultry | *C. perfringens* Type A, alpha+, and Clin1 | Peptides have antibacterial activity and alginate protect them from the intestinal environment | Shows activity against all strains | (Thapa *et al.*, 2021; Zgheib *et al.*, 2021) |
| 2. | Metallic | Silver | Hydrogen peroxide, and mint | Purchased stock solution | 45 | *in vitro* | Isolates from chicken, humans, camels, and pigeons | *C. perfringens* Type A | Anti-film formation activity of NP was observed | Biofilm formation was reduced | (Ahmed *et al.*, 2022) |
| 3. | Metal oxides | Zinc oxide | - | Ultrasonic irradiation method | - | *In vivo* | Broiler chicks (COBB) | *C. Perfringens* Type A | Antibacterial activity reported | Birds' health improved, lesions were reduced | (Shakal *et al.*, 2024) |
| 4. | -do- | -do- | Leaves of *Nelumbo nucifera* | Green synthesis | 38 | *In vivo* | Fish (*Oreochromis niloticus*) | Type A | Penetrates the cell membrane of bacteria, and active transport is inhibited. | Improvement in survival percentage and improved health conditions | (Ibrahim *et al.*, 2024) |
| 5. | Organic | delivery vehicles | Specifically targeted antimicrobial peptides | - | 222.1 | *In silico* and *In vivo* | Mice C57BL/6 e | - | Penetration into cell membranes and controlling the growth | The mice show rapid recovery | (Xu *et al.*, 2023) |

**Table 3:** Nanoparticles for vaccine delivery against *Clostridium perfringens.*

| Sr. No | NP -Class | NP | Type of vaccine | Properties of NP | Type of Experiment | Type of animals | Target strain | Result | References |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Natural Polymeric | Chitosan | Native toxoid and extracellular proteins | - | *In vivo* and *in vitro* | Chicken (COBB 500) | Field isolates | Provided sufficient titers of antibiotic immunity | (Zgheib *et al.*, 2021) |
| 2. | Polymeric | Poly lactic-co-glycolic acid | Epsilon protoxin NPs coated with RBC membrane coating | Spherical, smooth 105 nm | *In vitro* | - | - | The addition of polymeric NPs improved the uptake of toxin NPs | (Pudineh Moarref *et al.*, 2023) |
| 3. | Natural polymeric | Chitosan | Extracellular proteins in conjugation with *Salmonella* proteins | | *In vivo* | Broiler Chicks | Field strains | Increase antigen-specific mucosal immunoglobulins A and G | (Akerele *et al.*, 2020) |
| 4. | | Chitosan | Epsilon toxin | 100-1000 nm size after loading of toxin | *In vivo* | BALB/c mice | *C. perfringens* Type D | IgA and IgG titers were improved | (Poorhassan *et al.*, 2021) |
| 5. | Polymeric | Mesoporous silica | Ribosomal RNA (chimeric antigen) based NetB and alpha toxins | Spherical; 90nm diameter | *In vivo* | Broiler chicken | *C. perfringens* CP58 | Humoral immunity response was improved | (Hoseini *et al.*, 2021) |

nanoparticles helps to maintain high titers of humoral immunity, especially IgA and IgG (Csaba *et al.*, 2009). Several studies have been conducted which report the efficacy of nanoparticle conjugation with vaccine candidates of *C. perfringens* (Table 3).

**Interaction with Eukaryotic cells:** The literature presents that the nanomaterials effectively penetrate the prokaryotic cell of *C. perfringens*, (Shanker *et al.*, 2020; Tungare *et al.*, 2024) which is a bacterium of zoonotic importance, and our studies suggest that a single serotype (toxinotype) of *C. perfringens* may be present in several species (Rizk *et al.*, 2023; Wahdan and Elhaig, 2024), so targeting the bacteria needs to be specifying that the nanomaterials should be easily applicable (Bhattacharjee *et al.*, 2023; Brar *et al.*, 2023). Reports are presented that there are varying intestinal and metabolic environments in the various organisms, so a single drug specifically cannot target the *C. perfringens* (Kuo *et al.*, 2024). There is a need to understand the complex nature of eukaryotic cells and the mechanism of action of nanoparticles (Hernández-Abril *et al.*, 2024).

This review states that most of the metallic and metal oxide nanoparticles are effective against *C. perfringens*

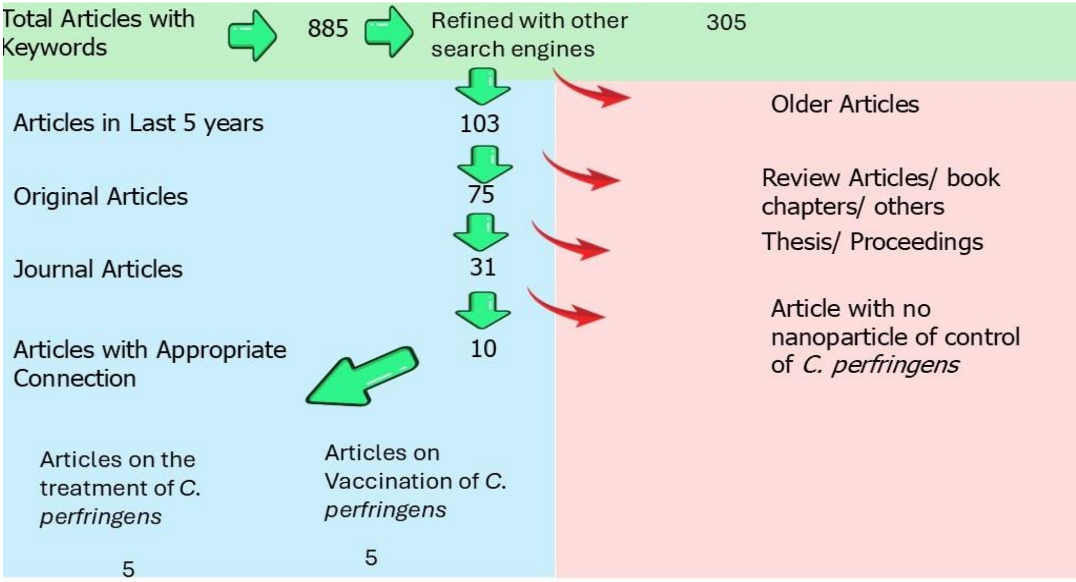


**Fig. 1:** The diagram of exclusion and inclusion criteria.

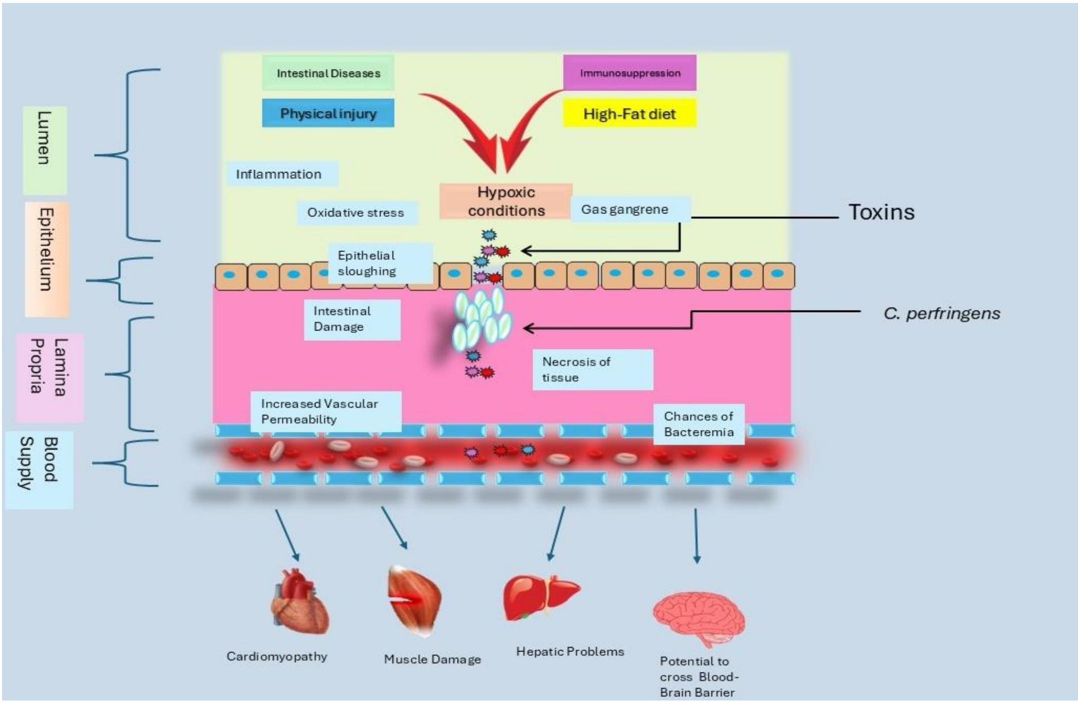


**Fig. 2:** Predisposing factors and pathological events of *Clostridium perfringens* infections in the intestine.

for its control via therapeutic purposes (Packialakshmi *et al.*, 2023). Their entry is facilitated by the similar way they can penetrate the eukaryotic cells. After the entries same mechanisms of cell death can be observed which are observed for the bacterial cells (Balog *et al.*, 2024). If their interaction and mode of entry have not justified them, using the nanoparticles can be dangerous, not only for humans but the environment (Bhat *et al.*, 2023) because the excretion of these nanoparticles can be a source of the killing of several other organisms present in the environment (Bhardwaj *et al.*, 2023). Although the studies have reported that the nanoparticles showed no toxicities when given in *in vivo* experiments (Hu *et al.*, 2023; Lopes *et al.*, 2023) but the mechanisms of protection of eukaryotic cells have not been explained by any of the researchers (Epple *et al.*, 2023; Hernández-Abril *et al.*, 2024). This deficiency may be damaging when any nano preparation is applied on the clinical grounds in any of species (Ingole *et al.*, 2023; Saberi Riseh *et al.*, 2023). The mechanisms of interaction of nanoparticles will play a crucial role for their safety and clinical implications soon.

**Horizons and Perspectives:** This study highlights that the nanoparticles can be beneficial for controlling the zoonotic pathogen *C. perfringens* in several animal and human species. The NPs can be used to treat and prevent necrotic enteritis in various animals and humans. The research states that they are a suitable option for managing *C. perfringens*. Despite the current success, the therapeutic and clinical use of Nanoparticles against this pathogen needs to be improved and verified.

Several aspects of delivery need to be specified especially the size and shape of the nanoparticles should be adjusted accurately to specify and deliver in the body. The safety aspects especially the environmental safety of the nanoparticles must be addressed. A few considerations in the solubility of polymeric articles need to be addressed. The clinical and long-term exposure studies of nanoparticles are especially needed to evaluate the hazards related to their exposure to the body.

**Conclusions:** Nanomaterials are effective for the control of *C. perfringens* as a vaccine and therapeutic drug delivery. They have strong antibacterial and immunological efficacy, however, their safety and effects on the eukaryotic nanoparticles need to be studied extensively. Further work can be done to improve their safety and delivery issues.

**Acknowledgements:** The Researchers would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (QU-APC-2024-9/1)

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