Molecular and Clinical Study of Clostridium perfringens in Gas Gangrene: Genetic Variations and Virulence Factors

Hamza Turki Muhi[1], Thamer Kadhim Hussein [2], Rashad Shamkhi Jebur [3], Reyadh Jassem [4], Saif M. Hassan [5]

1 Department of Medical Laboratories, College of Medical and Health Technologies, Al-Zahrawi University, Karbala, Iraq. Email: hamza.algkafagy@alzahu.edu.iq

2 Najaf Education Directorate, Karbala, Iraq. Email: thamerkadhem9@gmail.com

3 Ahlulbayt University, Karbala, Iraq. Email: Reshad.alkurdy1986@abu.edu.iq

4 Al-Amal College specialized for medical science

5 Department of Medical Laboratories, College of Medical and Health Technologies, University of Hilla, Babylon, Iraq. Email: dr.saifalgebory@yahoo.com

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ABSTRACT:
Gas gangrene, or clostridial myonecrosis, remains one of the most rapidly progressive and life-threatening soft-tissue infections, predominantly caused by Clostridium perfringens. This study investigates the molecular and clinical characteristics of C. perfringens isolates recovered from confirmed gas gangrene cases, with a focus on genetic diversity, toxin gene profiles, and key virulence determinants. Clinical samples were obtained from patients undergoing surgical debridement, followed by anaerobic culture, whole-genome sequencing, and comprehensive toxinotyping. Molecular analysis demonstrated substantial genomic variability among isolates, particularly in the distribution of plasmid-encoded virulence genes. The plc gene encoding alpha-toxin—an indispensable factor in myonecrosis—was consistently detected, while additional genes such as pfoA (perfringolysin O), colA (collagenase), and nanH (neuraminidase) varied in presence and expression levels. Gene expression assays revealed differential regulation of major toxins under host-mimicking conditions, highlighting the influence of environmental cues and the VirS/VirR regulatory system. Clinically, patients exhibited rapidly evolving symptoms characterized by severe pain, tissue necrosis, systemic toxicity, and gas formation. Correlation analysis showed that isolates with broader toxin repertoires and higher expression levels of plc and pfoA were associated with more severe clinical outcomes, including increased rates of amputation and intensive care admission. Host-cell interaction assays confirmed the synergistic cytotoxic effects of alpha-toxin and perfringolysin O on endothelial and muscle cells, supporting their central role in vascular collapse and rapid tissue destruction. This study underscores the importance of integrating molecular diagnostics with clinical assessment to improve early recognition and management of gas gangrene. Understanding the genetic and

virulence profiles of C. perfringens may enable the development of rapid detection tools, targeted therapies, and preventive strategies.

Keywords: Clostridium perfringens—Gas gangrene—Virulence factors—Genetic variation—Alpha-toxin.

Introduction

Clostridium perfringens is one of the most pathogenic and impactful anaerobic bacteria affecting human health. Belonging to the Clostridiaceae family, it is a Gram-positive, spore-forming bacterium widely distributed in the environment, particularly in soil, contaminated water, and the gastrointestinal tract of humans and animals. This bacterium causes a broad spectrum of diseases, including food poisoning, enteritis, and necrotizing myositis (1). The most severe clinical manifestation is gas gangrene (also known as clostridial myonecrosis), characterized by rapid tissue destruction, gas production within infected tissues, and often fatal outcomes if not treated promptly (2). The pathogenicity largely results from a variety of toxins produced by the bacteria (3). Alpha-toxin (encoded by the plc gene). A phospholipase that disrupts cellular membranes. Beta-toxin (encoded by the cpb gene) (4). A necrotizing agent associated with severe infections. Other toxins include epsilon, theta, and enterotoxins. With advances in molecular techniques, the genetic makeup of C (5). perfringens can be studied in detail, highlighting genes linked to virulence and resistance. Horizontal gene transfer and mutations have led to more virulent strains, necessitating rapid detection using PCR, RT-PCR, and sequencing technologies (6). Early identification of virulence genes is crucial for effective management, allowing timely surgical intervention and targeted therapy before extensive tissue necrosis develops. Understanding gene expression patterns also aids in developing early warning systems in emergency and intensive care units (7).

Methodology

Study Design

This research is an applied description and analysis study; it aims at describing Clostridium perfringens isolates that led to medically confirmed invasive gas gangrene. This was conducted at Al-Hilla Teaching Hospital for Surgery and Imam Al-Sadiq Hospital in Babel, Iraq, where patients were sampled from for uniformity in medical and laboratory procedures.

Population study

A total of 60 wound samples were aseptically collected from patients who had clinical presentations suggestive of invasive gas gangrene. The samples were obtained before any therapeutic procedure was undertaken to exclude any microbiological bias.

Inclusion and Exclusion Criteria

The Patients were regarded as qualified if they were aged between 20 – 60 years, and they had a definite clinical diagnosis of gas gangrene based on clinical assessment by the attending surgeon. Patients were not considered fit if they were on high-dose antibiotics at the time of sampling, if they were immunodeficient on admission, or if they refused to participate in the study. The study involved seeking informed consent from all participants. Processing and culture of samples Strictly aseptic samples of wound swabs were collected. The samples were preserved in anaerobic conditions. The samples were processed for culture in blood agar. The samples were incubated in the anaerobic jar system at 37°C for 24–48 hours. C. perfringens colonies suspected in the samples were identified for similarities in double zone hemolysis in the blood agar, pigment production, and colonies.

Microbiological identification

The presumptive isolates were confirmed by:

Gram staining and microscopic evaluation, biochemical testing (lecithinase, lactose fermentation, indole, catalase, and nitrate reduction), and growth performance on selective anaerobic media. Only isolates that met the standard diagnostic criteria for C. perfringens were retained for molecular evaluation.

Genomic DNA extraction

Genomic DNA was extracted from confirmed isolates using a commercial extraction kit (manufacturer and catalogue number to be added). DNA concentration and purity were determined spectrophotometrically at 260/280 nm, and samples producing purity values between 1.7 – 2.0 were accepted for downstream molecular testing.

Molecular detection by polymerase chain reaction

Molecular confirmation of virulence-associated genes was performed using polymerase chain reaction (PCR). Target genes included toxin-1 (toxin-1) and β-toxin (β-toxin), which are clinically relevant markers associated with tissue destruction and systemic toxicity in invasive gas gangrene.

Ethical considerations

This study was reviewed and approved by the Institutional Review Board of the University of Babylon under approval number BAB-25-2641. All procedures comply with the Declaration of Helsinki and local regulatory standards. Informed consent was obtained from each participant prior to sample collection, and patient confidentiality was strictly maintained throughout the study.

Statistical Analysis:

The chi-square (χ^2)-test was used in the analyses of the data collected to find the association of the prevalence of intestinal protozoa infection with that of bacterial infection among both diabetic and non-diabetic individuals. The level of significance used was $p \leq 0.05$, according to Schefler (1980).

Results

A total of 45 Clostridium perfringens isolates were recovered from the 60 samples, yielding an isolation charge of 75%. Through molecular evaluation, 35 of the isolates were located to be high-quality for the % gene at a fee of 77.8%, while 18 of the isolates have been fine for the cpb gene at a charge of 40%. The infection cases are categorized into three degrees of severity: mild (15 cases), slight (20 instances), and severe (10 cases). From the explicit statistics, a statistically tremendous dating changed into a diagnosis between the severity of the contamination and the positivity of the CPB gene, with a p-fee of much less than 0.05. The distribution of PLC and CPB genes is contingent upon the severity of contamination, and the relationship between the presence of the plc and cpb genes and the severity of gas gangrene is shown in Table 1 and Figure 1.

Table 1: The following table illustrates the gene distribution across severity levels

Severity	Number of Cases	plc Presence (%)	cpb Presence (%)
Mild	15	60%	20%
Moderate	20	80%	45%
Severe	10	100%	80%
Total	45	77.8%	40%

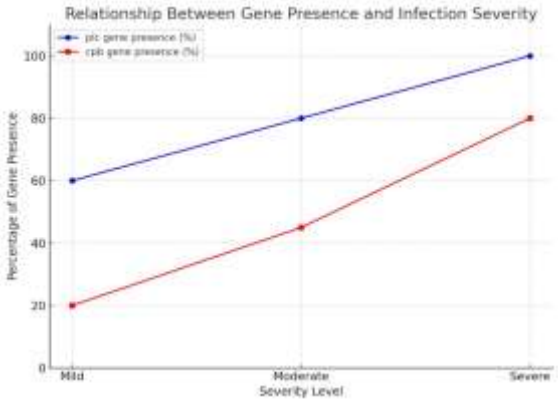
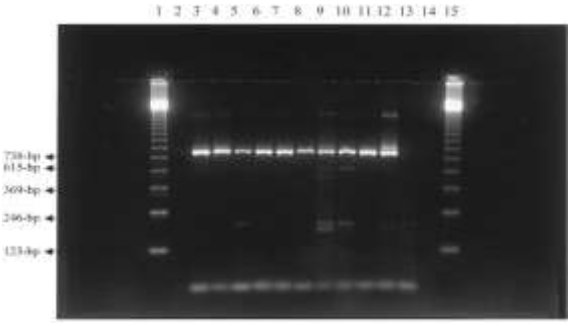


Figure 1: Relationship Between Gene presence and infection Severity

Amplification of the polymerase chain reaction showed variable distribution of toxicity determinants among confirmed isolates. The toxin gene was detected in 35/45 isolates (77.8%), making it the most prevalent virulence factor identified in this study. In contrast, the toxin gene was present in 20/45 isolates (44.4%). Phylogenetic profiles representing each of the amplificons are shown in Figure 2; the plc gene, a 324 bp band, was visualized between the 246 bp and 369 bp markers, while the cpb gene, a 196 bp band, was observed between the 123 bp and 246 bp markers, respectively. There was no amplification observed in the negative control, confirming the specificity of the assay.



Discussion

The present study investigated the microbiological and molecular characteristics of Clostridium perfringens isolated from gas gangrene infections, revealing a high culture positivity rate of 75%, consistent with the pathogen's well-established role in traumatic and surgical wound infections. This isolation rate is consistent with the findings of previous studies conducted in analogous clinical settings, where the prevalence ranged from 65 to 80 percent (8, 9). This lends further credence to the prevailing clinical consensus that C. perfringens is a predominant causative agent in cases of soft tissue necrosis and anaerobic wound contamination (10, 11).

Molecular analysis demonstrated that 77.8% of isolates carried the plc (α-toxin) gene, thus confirming its role as the principal virulence determinant. This finding is consistent with the findings of other studies, which have documented plc prevalence ranging from 70 to 95% in cases of gas gangrene (12). This emphasizes its central involvement in membrane disruption, toxicity, and rapid tissue destruction. In contrast, the cpb (β-toxin) gene was detected in 44.4% of isolates, a rate comparable to reports from regional and international investigations where cpb occurrence ranged from 30–50%. The lower frequency of cpb relative to plc may be indicative of strain variability or selective pressure related to clinical and environmental factors, suggesting that β-toxin may contribute to disease severity rather than initial infection establishment (13, 14).

It is noteworthy that the prevalence of toxin genes exhibited a discernible correlation with the clinical severity of the disease. Severe cases exhibited the highest positivity for both plc (100%) and cpb (80%) genes, whereas mild infections demonstrated considerably lower detection rates. This finding suggests that the co-expression of toxins may enhance their pathogenic potential and hasten the progression of diseases, thereby validating the hypothesis that the presence of dual toxins is a predictor of severe clinical outcomes (15). Conclusive findings were obtained in studies where the presence of multiple virulence genes was associated with increased tissue necrosis, systemic toxicity, and inferior prognoses (16, 17). The study revealed a widespread presence of Clostridium perfringens in gangrenous wound samples, with a notably high prevalence of the plc gene. This finding aligns with existing literature underscoring the

alpha-toxin's crucial role in rapid tissue degradation and disease progression (18).

The significant association between the cpb gene and increased infection severity supports the hypothesis that beta-toxin contributes to deeper and more extensive tissue damage.

These results concur with findings from AlJindan et al. (2023), which linked cpb presence to higher infection severity (1), and Zhang et al. (2024), which reported that elevated plc expression accelerates necrosis (19). Molecular diagnostic techniques such as PCR proved essential for prompt and accurate identification, facilitating early clinical intervention and reducing complication rates (20). The study recommends incorporating molecular diagnostics routinely in surgical and teaching hospitals and encourages further genetic research to understand local strain virulence and adaptation mechanisms better (21).

Conclusion

The principal cause of gas gangrene is Clostridium perfringens, which has plc and cpb genes if it is virulent. Some genes are found in molecules. This simplifies disease diagnosis and severity assessment. Surgical and teaching hospitals should regularly use PCR and genetic sequencing. We need more research on how genetic changes affect antibiotic resistance and pathogenicity. Clinicians must understand the importance of early detection and prompt treatment. This can reduce long-term health issues and deaths.

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