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# Genetic Relatedness of Multidrug Resistance and Biofilm Production Genes in *C. perfringens* Isolates from Broilers

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**Abstract**

*Clostridium perfringens* remains a leading etiological agent of necrotic enteritis in broiler chickens, as control efforts are increasingly challenged by multidrug resistance (MDR) and biofilm formation. We characterized the genetic determinants of antibiotic resistance and biofilm production in 36 pathogenic *C. perfringens* type A isolates (24% of 150 samples) recovered from broilers exhibited diarrhea and necrotic enteritis. All isolates harbored the alpha-toxin (*cpa*) and enterotoxin (*cpe*) genes; 75% carried the pore-forming *netB* toxin gene. Phenotypic assays demonstrated that 69.4% of isolates produced biofilm, of which 84% were MDR. Antimicrobial susceptibility profiling revealed highest resistance rates against chloramphenicol (80.5%), penicillin (80.5%), and oxytetracycline (77.7%), with universal sensitivity to vancomycin and ofloxacin. PCR screening detected tetracycline resistance gene *tetK* in 100% of isolates, β-lactamase gene *bla* in 62.5%, and macrolide resistance gene *ermB* in 37.5%. Biofilm-associated loci *appB* and *proA* were ubiquitous (100%), whereas *iolD* and *ripH* occurred in 62.5% and 50% of isolates, respectively. Statistical correlation analysis revealed a statistically significant association between MDR and biofilm gene carriage ($r = 0.994$; $p < 0.05$), suggesting co-selection and potential linkage on mobile genetic elements. These findings clarify the genetic interactions that contribute to antibiotic resistance in biofilms formed by *C. perfringens* . They highlight the urgent need for responsible antimicrobial use and the development of alternative prevention strategies, such as targeted vaccines and probiotic treatments, to reduce the incidence of necrotic enteritis in high-density poultry farming.

**Keywords:** *C. perfringens*, necrotic enteritis, broiler chickens, multidrug resistance, biofilm genes, antibiotic resistance genes.

## Introduction

*Clostridium perfringens (C. perfringens)* is a spore-forming anaerobic bacterium that typically exists harmlessly in the intestines of healthy poultry and other animals. Nevertheless, certain environmental or physiological factors can trigger its shift to a pathogenic state, leading to serious disease outbreaks[1]. This bacterium is a key pathogen in poultry, primarily responsible for necrotic enteritis (NE)—a disease that ranks among the most economically damaging conditions in the poultry sector, especially in broiler chicken production.[2]. Necrotic enteritis often emerges when certain predisposing factors disturb the balance of the gut environment. These include dietary modifications, weakened immune function, and concurrent infections—particularly with *Eimeria* species, the causative agents of coccidiosis. Such disturbances promote the excessive proliferation of *C. perfringens* in the intestines, enabling it to secrete a range of toxins that are crucial to the development and progression of the disease [3].

A major virulence factor implicated in *C. perfringens*-induced necrotic enteritis is the alpha toxin. This toxin functions as a phospholipase C enzyme, disrupting host cell membranes by degrading phospholipids. The resulting cellular lysis leads to tissue damage and the formation of necrotic lesions within the intestines. The destructive action of alpha toxin is especially critical during the early phase of the disease, playing a key role in the onset of the intestinal damage that defines necrotic enteritis [4]. In addition to alpha toxin, other virulence factors like *NetB*—a pore-forming toxin—have been

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identified as significant contributors to the development of necrotic enteritis. Research indicates that *C. perfringens* strains capable of producing *NetB* exhibit greater virulence and are more frequently associated with the formation of necrotic intestinal lesions compared to strains that produce only alpha toxin[5].

The combined action of multiple toxins—most notably alpha toxin and *NetB*—is believed to be essential for the full manifestation of necrotic enteritis in broiler chickens. In parallel, the extensive use of antibiotics such as tetracyclines, macrolides, and β-lactams in broiler farming—for both prophylaxis and growth promotion—has significantly contributed to the rise of antibiotic resistance in pathogens like *C. perfringens*. This heavy antibiotic usage has imposed strong selective pressure, fostering the emergence and spread of multidrug-resistant (MDR) strains[6].

The capacity of *C. perfringens* to develop MDR and form biofilms presents significant challenges in the treatment and control of infections within poultry flocks [7]. MDR strains often harbor multiple antibiotic resistance genes, which can be horizontally transferred between bacterial populations, complicating treatment efforts. Biofilm formation adds another layer of complexity, as it encases bacterial cells within a protective matrix, contributing to persistent infections and enhanced resistance to antimicrobial agents. This makes biofilms a critical survival mechanism for *C. perfringens* in broiler production environments [8]

The genetic basis of both MDR and biofilm formation is complex and may be interlinked. Resistance genes are frequently located on mobile genetic elements, such as plasmids, which facilitate their spread among diverse bacterial strains [9]. Concurrently, genes associated with biofilm production help *C. perfringens* endure harsh environmental stresses, including antibiotic exposure [10]. The convergence of these two mechanisms—antibiotic resistance and biofilm formation—creates a formidable barrier to infection control, as biofilms not only shield the bacteria from antimicrobial treatment but also serve as hotspots for gene exchange, accelerating the spread of MDR within microbial communities [11]. Although recent research has explored the genetic markers of MDR and biofilm formation in various bacterial pathogens, investigations focusing specifically on *C. perfringens* isolates from broilers remain limited [7]. Gaining insight into the genetic relationship between resistance and biofilm-related genes could provide valuable information for developing targeted control strategies. Since these genetic traits enhance bacterial persistence and treatment evasion, understanding their interaction may support the design of more effective therapeutic and preventive approaches. This study seeks to investigate the genetic correlation between multidrug resistance and biofilm-forming genes in *C. perfringens* isolates from broiler chickens, with the goal of uncovering mechanisms that drive co-selection and long-term survival of this pathogen in poultry farming systems.

## Material and Methods

*Sample Collection*

A total of 150 samples, 50 cloacal swabs samples obtained from broilers suffering from diarrhea, 50 liver and 50 intestines of recently deceased broilers that previously showed symptoms of severe diarrhea, specifically those suspected to have succumbed to necrotic enteritis.

*Isolation and Identification of Clostridium perfringens from examined samples*: according to [12,13]

*Antibiogram activity*

According to the Clinical and Laboratory Standards Institute (CLSI) [14], antimicrobial susceptibility testing should follow standardized protocols to accurately determine resistance profiles. These guidelines help classify bacterial isolates, such as *C. perfringens*, as susceptible, intermediate, or resistant, ensuring reliable and consistent results that support effective treatment decisions and resistance monitoring.

Strains exhibiting resistance to three or more classes of antibiotics are classified as MDR, as defined by the guidelines of the CLSI [15].

*Detection of Biofilm Production*[16]:

To assess the biofilm-producing ability of the isolates, The Congo Red binding assay (CRA) method was carried out. The Congo Red Agar (CRA) method is a commonly used approach for detecting biofilm production in *C. perfringens* and other bacteria. This method relies on the ability of biofilm-producing bacteria to bind Congo red dye, which leads to distinct colony morphologies indicating biofilm formation.

*Molecular identification* (Table 1)

Confirmed isolates will be subjected to further classification and typing using Multiplex PCR Genotyping to determine the genetic characteristics of the isolates[17] this include:

**-Multiplex PCR Genotyping** of the isolates for detection of the presence of specific virulence genes (Alfa- Beta- Epsilon and Iota toxin genes) (*cpa, cpb, etx* and *iap*)

**-Multidrug Resistance genes** such as *tetA, ermB, and Bla* was performed.

**-Biofilm Associated genes** including *appB, iolD, ripH* and *proA*, was conducted [18].

*Study of the Relationship Between Resistance Genes and Biofilm Genes*:

The genetic relatedness between multidrug resistance genes and biofilm production genes were analyzed using correlation analysis. The presence of both gene types in the same isolate statistically analyzed to determine the strength of association using Correlation test. The results considered significant if the P-value is less than 0.05.

## Results

*Incidence of C. perfringens from the examined Samples* (Table 2)

*Phenotypic Biofilm Production Testing:* The Congo Red Agar (CRA) test revealed that 69.4% (25/36) of the isolates were capable of biofilm production. Colonies with strong black pigmentation on CRA were considered biofilm producers. Notably, the majority of biofilm-positive isolates 84% (21/25) were multidrug-resistant, while only 16% (4/25) were sensitive to antibiotics. These findings suggest a potential relationship between biofilm formation and antibiotic resistance.

*Antimicrobial susceptibility of the C. perfringens Isolates* (Table 3).

*Phenotypic Biofilm production in the C. perfringens isolates***. (**Table 4)

*Multiplex PCR Genotyping*

A total of eight strains, including two from swabs, two from liver samples, and four from intestinal samples, were analyzed for toxin type identification using multiplex PCR. The screening targeted common toxin genes: *cpa, cpb, etx,* and *iap* (which correspond to the Alpha, Beta, Epsilon, and Iota toxins). All eight strains were confirmed to produce only the alpha toxin, classifying them as *C. perfringens* type A, as depicted in (Fig. 2).

Additionally, conventional PCR was performed using specific primers targeting other virulent genes, such as *Cpe* and *netB*. The results showed that 100% (8/8) of the strains carried the enterotoxin *Cpe* gene (Fig. 1), while 75% (6/8) tested positive for the *netB* gene (Fig. 3).

*Genetic Detection of Multidrug Resistance***. (**Fig.4-6)

*Genotypic Detection of Biofilm Associated Genes.* (Fig. 7-10)

PCR analysis for resistance and biofilm genes confirmed the presence of multiple resistance and biofilm-related genes among the eight isolates:

Multidrug resistance genes: The *tetA* gene (tetracycline resistance) was detected in all examined isolates, *ermB* (macrolide resistance) in 37.5%, and *Bla* (beta-lactam resistance) in 62.5%. (Fig. 3,5 and 4 respectivelly)

Biofilm production genes: The *appB* and *proA* gene was the most frequently detected biofilm gene, found in all examined isolates and the *iolD* gene in 62.5% of the isolates, while the *ripH* gene was detected in 50%. (Fig. 6,10,8 and 9 respectively)

*Relationship Between Antibiotic Resistance Genes and Biofilm Genes*. **(**Tables 5, 6 and Fig. 11)

Statistical analysis revealed a significant association between the presence of multidrug resistance genes and biofilm production genes ($p < 0.05$). Among the 25 multidrug-resistant isolates, 84% (21/25) were also biofilm producers, whereas only 16% (4/25) of the antibiotic-sensitive isolates were capable of biofilm formation. This indicates a strong genetic correlation between multidrug resistance and biofilm production in *C. perfringens* strains isolated from broilers.

The relationship between the *tetK* gene and the *appB* and *proA* genes was particularly significant, with 100% of *tetK*-positive isolates also carrying the *appB* gene and *proA* gene. Similarly, the *Bla* and *iolD* genes were frequently co-detected, suggesting a potential co-selection mechanism driving the persistence of these traits in the poultry environment.

## Discussion

The findings demonstrated a high prevalence of MDR strains with strong biofilm-forming capabilities and identified significant correlations between specific antibiotic resistance genes and biofilm-associated genes.

Among 150 collected samples, 36 (24%) were confirmed pathogenic *C. perfringens*, predominantly from intestinal samples (68%). All tested isolates were confirmed as type A (*cpa*-positive) and carried *cpe* (100%) and *netB* (75%) virulence genes (Figures 1–3). The high isolation rate from intestines supports previous findings that *C. perfringens* is an enteric pathogen frequently associated with necrotic enteritis in poultry [1, 19–21]. The dominance of type A strains confirms earlier studies identifying it as the most prevalent type causing enteric disease in broilers [22, 23]. The presence of the *netB* gene in 75% of isolates agrees with reports linking *netB* to increased virulence and lesion formation in necrotic enteritis [24–26].

Antimicrobial susceptibility testing (Table 3) showed high resistance to chloramphenicol (80.5%), penicillin (80.5%), and oxytetracycline (77.7%). MDR was detected in 25 out of 36 isolates (69.4%). These results are consistent with previous work reported high resistance to commonly used antibiotics in poultry farms [27–29]. The complete sensitivity to vancomycin and ofloxacin matches findings from other studies, although their use in poultry is limited due to regulatory restrictions [30]. The high MDR prevalence aligns with a study

emphasized the role of antibiotic overuse in driving resistance in *C. perfringens* [31].

Phenotypic analysis (Tables 4) revealed biofilm production in 69.4% of isolates, with the majority being MDR strains (84%). Genotypically, biofilm genes *appB* and *proA* were detected in 100% of the tested isolates, followed by *iolD* (62.5%) and *ripH* (50%) (Figures 7–10). These results confirm the strong biofilm-producing ability of *C. perfringens* as previously described [7, 32]. The consistent detection of *appB* and *proA* in all tested isolates declared that these genes are essential for the biofilm matrix. However, this finding is not aligning with previous studies suggested that biofilm formation appears to be more strongly influenced by type IV pili-associated secretion systems and extracellular polymeric substances like *BsaA* proteins [33]. Of note, biofilm presence enhances survival in adverse environments and contributes to persistent infections, complicating control and treatment strategies [34].

Molecular detection revealed universal presence of *tetK* (100%) among tested isolates, with *bla* (62.5%) and *ermB* (37.5%) also detected (Figures 4–6). The dominance of *tetK* confirms its widespread distribution due to frequent tetracycline use, in line with previous observations [35, 36]. The detection of *bla* and *ermB* also agrees with previous studies highlighting the increasing prevalence of macrolide and β-lactam resistance in *C. perfringens*[37–39].

Statistical analysis (Table 6, Figure 11) showed a strong correlation ($r = 0.994$, $p < 0.05$) between antibiotic resistance genes and biofilm genes. Notably, *tetK* was co-detected with *appB* and *proA* in all cases, and *bla* was commonly associated with *iolD*. This correlation supports the hypothesis that biofilm formation and antibiotic resistance are genetically linked and may be co-selected under antibiotic pressure [40, 41]. The co-presence of these genes may be due to plasmid-mediated gene clustering or stress-induced gene expression. Such associations enhance bacterial persistence and resistance transmission in poultry environments.

*C. perfringens*type A, particularly its α- toxin, is the primary causative agent of necrotic enteritis in broiler flocks. Most *C. perfringens* isolates in this study exhibited multidrug resistance (MDR) and biofilm-producing capabilities, which contribute to the pathogen's virulence and complicate treatment efforts. A strong correlation between MDR and biofilm formation in the isolates was observed, highlighting significant challenges for effective disease control.

To mitigate the spread of *C. perfringens* and reduce the impact of necrotic enteritis, it is essential to implement stringent hygiene measures in poultry farms, including regular application of potent disinfectants. The misuse of antibiotics, especially for random treatment or as growth promoters, should be strictly avoided. Furthermore, responsible antimicrobial usage is crucial, and the development of alternative interventions such as probiotics and vaccines is recommended to address *C. perfringens*-associated diseases.

Additional research is needed to better understand the mechanisms of biofilm formation, the spread of resistance genes, and to evaluate the effectiveness of new therapeutic strategies aimed at controlling *C. perfringens* infections in broiler production systems.

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There's no funding source.

*Conflict of Interest*

The authors declare that there is no conflict of interest.

**TABLE 1. Primers sequences, target genes, amplicon sizes and cycling conditions**

| Aspect | Target gene | Primers sequences(5'-3') | Amplified segment (bp) | Amplification (35 cycles) | | | | Final extension | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Primary denaturation | Second denaturation | Annealing | Extension | | |
| Virulence Genes | Alpha (*cpa*) | F:GTTGATAGCGCAGGAC ATGTTAAG R:CATGTAGTCATCTGTT CCAGCATC | 402 | 94˚C 5 min. | 94˚C 45 sec. | 50˚C 45 sec. | 72˚C 45 sec. | 72˚C 10 min. | [42] |
| | Beta (*cpb*) | F:ACTATACAGACAGATC ATTCAACC R:TTAGGAGCAGTTAGA ACTACAGAC | 236 | | | | | | |
| | Epsilon (*etx*) | F:ACTGCAACTACTACTC ATACTGTG R:CTGGTGCCTTAATAGA AAGACTCC | 541 | | | | | | |
| | Iota (*iap*) | F:GCGATGAAAAGCC-TACACCACTAC R:GGTATATCCTCCAC-GCATATAGTC | 317 | | | | | | |
| | Entero-toxin (*cpe*) | F:ACATCTGCAGA-TAGCTTAGGAAAT R:CCAGTAGCTGTAATT-GTTAAGTGT | 247 | 94˚C 5 min. | 94˚C 30 sec. | 55˚C 30 sec. | 72˚C 30 sec. | 72˚C 7 min. | [43] |
| | *netB* | F:CGCTTCACATAAAGGT TGGAAGGC R:TCCAGCACCAGCAG-TTTTTCCT | 316 | 94˚C 5 min. | 94˚C 30 sec. | 55˚C 30 sec. | 72˚C 30 sec. | 72˚C 7 min. | [44] |
| Antibiotic Resistance Genes | *tetK* | F:TTATGGTGGTTGTAGC TAGAAA R:AAAGGGTTAGAAACT CTTGAAA | 382 | 94˚C 5 min. | 94˚C 30 sec. | 50˚C 40 sec. | 72˚C 40 sec. | 72˚C 10 min. | [45] |
| | *ermB* | F:GAA AAG GTA CTC AAC CAA ATA R: AGT AAC GGT ACT TAA ATT GTT TAC | 638 | 94˚C 5 min. | 94˚C 30 sec. | 57˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | [46] |
| | *Bla* | F:ATGAAAGAAGTTCAA AAATATTTAGAG R:TTAGTGCCAATTGTTC ATGATGG | 780 | 94˚C 5 min. | 94˚C 30 sec. | 50˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | [47] |
| Biofilm productio nGenes | *appB* | F: AGTGATGCCGCTCCTAAAAT R:GCGTTGTAACAGAAG GGTCT | 300 | 94˚C 5 min. | 94˚C 30 sec. | 50˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | [18] |
| | *iolD* | F:TTTGTGGTGGTGGAGT TAGA R:CTTACCTGCTTGTGTT TCGC | 400 | 94˚C 5 min. | 94˚C 30 sec. | 50˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min. | |
| | *ripH* | F:GACATGGAGTAGAGG AAGAGA R:CTCCGGCATATTTTCC TGATTT | 105 bp | 94˚C 5 min. | 94˚C 30 sec. | 56˚C 30 sec. | 72˚C 30 sec. | 72˚C 7 min. | |
| | *proA* | F:AAGAGGAGGAAGTGA GGCTAT R:TTGAACTGATCCCTCT GGAA | 97 bp | 94˚C 5 min. | 94˚C 30 sec. | 56˚C 30 sec. | 72˚C 30 sec. | 72˚C 7 min. | |

**TABLE 2. Incidence of *C. perfringens* from Examined Samples (n = 50)**

| Broilers State | Sample Type | Number of Positive *C. perfringens* Isolates | Isolation Rate (%) | Number of Pathogenic *C. perfringens* Isolates | Isolation Rate (%) |
|---|---|---|---|---|---|
| **Diseased (Diarrheic)** | Swabs | 13 | 26% | 7 | 14% |
| **Deceased (Necrotic Enteritis)** | Liver | 6 | 12% | 2 | 4% |
| | Intestine | 34 | 68% | 27 | 54% |
| **Total** | 150 | 53 | 35% | 36 | 24% |

**TABLE 3. Antimicrobial susceptibility of the *C. perfringens* Isolates (n = 36)**

| Antimicrobial Agent | Disc Concentration | Number of Resistant Isolates | Resistance (%) |
|---|---|---|---|
| **Azithromycin** | 15 µg | 20 | 55.5 |
| **Chloramphenicol** | 30 µg | 29 | 80.5 |
| **Clindamycin** | 2 µg | 26 | 72.2 |
| **Erythromycin** | 15 µg | 20 | 55 |
| **Gentamicin** | 10 µg | 3 | 8.3 |
| **Kanamycin** | 30 µg | 25 | 69.4 |
| **Nalidixic acid** | 30 µg | 26 | 72.2 |
| **Ofloxacin** | 5 µg | 0 | 0 |
| **Oxytetracycline** | 30 µg | 28 | 77.7 |
| **Penicillin** | 10 UI | 29 | 80.5 |
| **Rifampicin** | 5 µg | 3 | 8.3 |
| **Vancomycin** | 10 µg | 0 | 0 |

**TABLE 4. Phenotypic Biofilm Production in *C. perfringens* Isolates (n = 36)**

| Isolate Category | Number of Biofilm-Positive Isolates | Percentage (%) |
|---|---|---|
| **MDR Isolates** | 21 | 58.3% |
| **Antibiotic-Sensitive Isolates** | 4 | 11.1% |
| **Total** | 25 | 69.4% |

**TABLE 5. Comparative results among antibiotics resistance genes and biofilm genes in different confirmed PCR samples**

| Types | Gene | No. | Total |
|---|---|---|---|
| **Antibiotics resistance gene** | *tetK* | 8 | 16 |
| | *Bla* | 5 | |
| | *ermB* | 3 | |
| **Biofilm genes** | *IolD* | 5 | 25 |
| | *AppB* | 8 | |
| | *proA* | 8 | |
| | *ripH* | 4 | |

**TABLE 6. Correlation among antibiotics resistance genes and biofilm genes in different confirmed PCR samples**

| Samples | No. Of Antibiotics Resistance Gene | No. Of Biofilm Genes | Correlation | |
|---|---|---|---|---|
| | | | r | P |
| **Swabs** | 5 | 5 | 0.994192 | <0.05 |
| **Liver** | 4 | 8 | | |
| **Intestinal** | 7 | 12 | | |

Significant = P<0.05

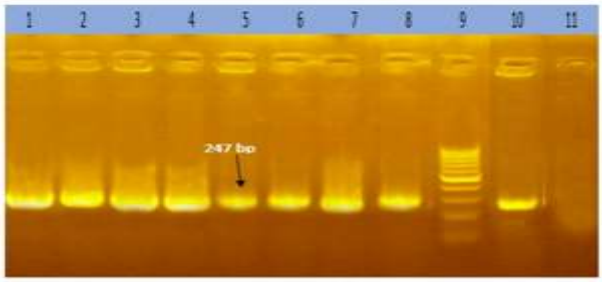


**Fig. 1. Gel electrophoresis of the *cpe* gene for *C. perfringens*.** Lanes 1 to 8 show positive amplification of the *cpe* gene at 247 bp for *C. perfringens*. Lane 9 contains the 1000 bp DNA ladder. Lane 10 represents the positive control, and lane 11 represents the negative control.

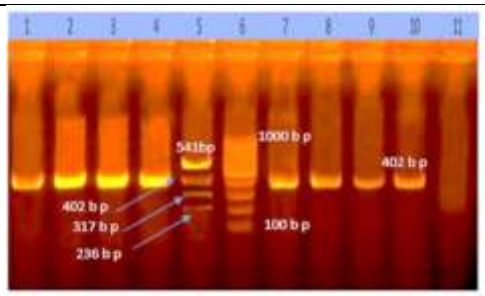


**Fig. 2. Toxin typing pattern (Alpha, Beta, Epsilon, and Iota) of *C. perfringens* isolates by multiplex PCR for the detection of toxin genes (*cpa*, *cpb*, *etx*, and *iap*).** Lanes 1 to 4 show the examined *C. perfringens* strains, which exhibited the Alpha toxin at 402 bp. Lane 5 contains the toxin-positive control. Lane 6 shows the 1000 bp DNA ladder. Lanes 7 to 10 represent the remaining strains, and lane 11 is the negative control.

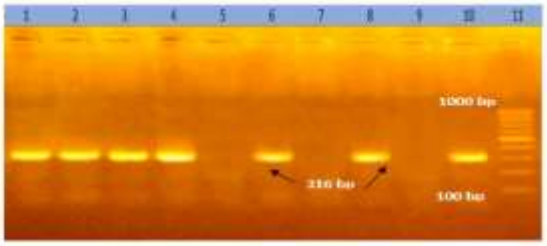


**Fig. 3. Gel electrophoresis of the *netB* gene for *C. perfringens*.** Lanes 1 to 8 display the results for the *netB* gene, with positive amplification observedat 316 bp for *C. perfringens*. Lane 9 represents the negative control, and lane 10 represents the positive control. Lane 11 contains the 1000 bp DNA ladder.

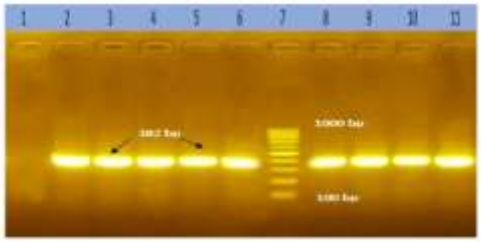

**Fig. 4. Gel electrophoresis of the *tetK* gene for *C. perfringens*.** Lanes 3 to 6 and lanes 8 to 11 show positive amplification for the *tetK* gene at 382 bp for *C. perfringens*. Lane 7 contains the 1000 bp DNA ladder. Lane 2 represents the positive control, and lane 1 represents the negative control.

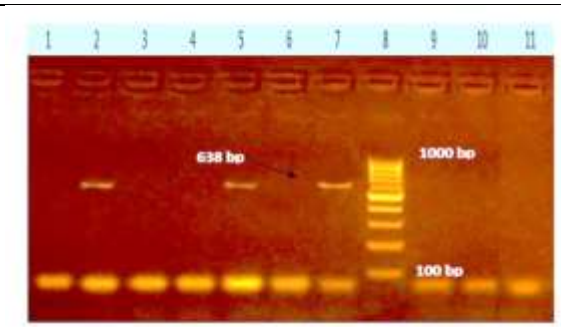


**Fig. 5. Gel electrophoresis of the *ermB* gene for *C. perfringens*.** Lanes 3 to 7 and lanes 9 to 11 show positive amplification for the *ermB* gene at 638 bp for *C. perfringens*. Lane 8 contains the 1000 bp DNA ladder. Lane 2 represents the positive control, and lane 1 represents the negative control.

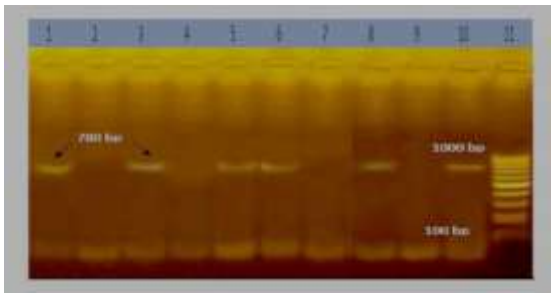

**Fig. 6. Gel electrophoresis of the *bla* gene for *C. perfringens*.** Lanes 1 to 8 show positive amplification for the *bla* gene at 780 bp for *C. perfringens*. Lane 10 represents the positive control, lane 9 represents the negative control, and lane 11 contains the 1000 bp DNA ladder.

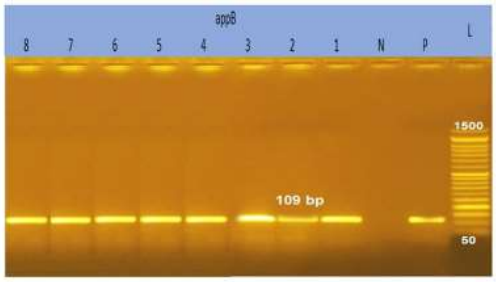


**Fig. 7. Gel electrophoresis of the *appB* gene for *C. perfringens*.** Lanes 1 to 8 show positive amplification for the *appB* gene at 109 bp for *C. perfringens*. Lane P represents the positive control, lane N represents the negative control, and lane L contains the 1500 bp DNA ladder.

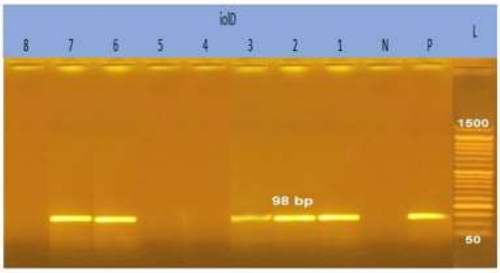


**Fig. 8. Gel electrophoresis of the *iolD* gene for *C. perfringens*.** Lanes 1 to 8 show positive amplification for the *iolD* gene at 98 bp for *C. perfringens*. Lane P represents the positive control, lane N represents the negative control, and lane L contains the 1500 bp DNA ladder.

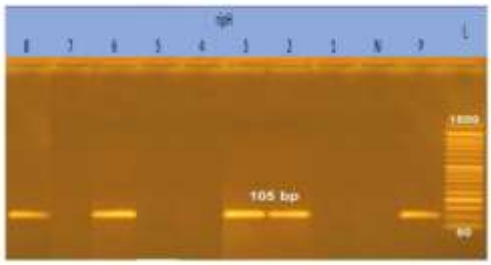


**Fig. 9. Gel electrophoresis of the *ripH* gene for *C. perfringens*.** Lanes 1 to 8 show positive amplification for the *ripH* gene at 106 bp for *C. perfringens*. Lane P represents the positive control, lane N represents the negative control, and lane L contains the 1500 bp DNA ladder.

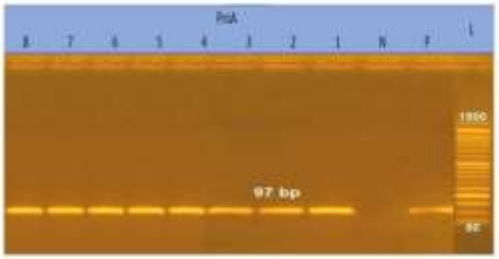


**Fig. 10. Gel electrophoresis of the *proA* gene for *C. perfringens*.** Lanes 1 to 8 show positive amplification for the *proA* gene at 97 bp for *C. perfringens*. Lane P represents the positive control, lane N represents the negative control, and lane L contains the 1500 bp DNA ladder.

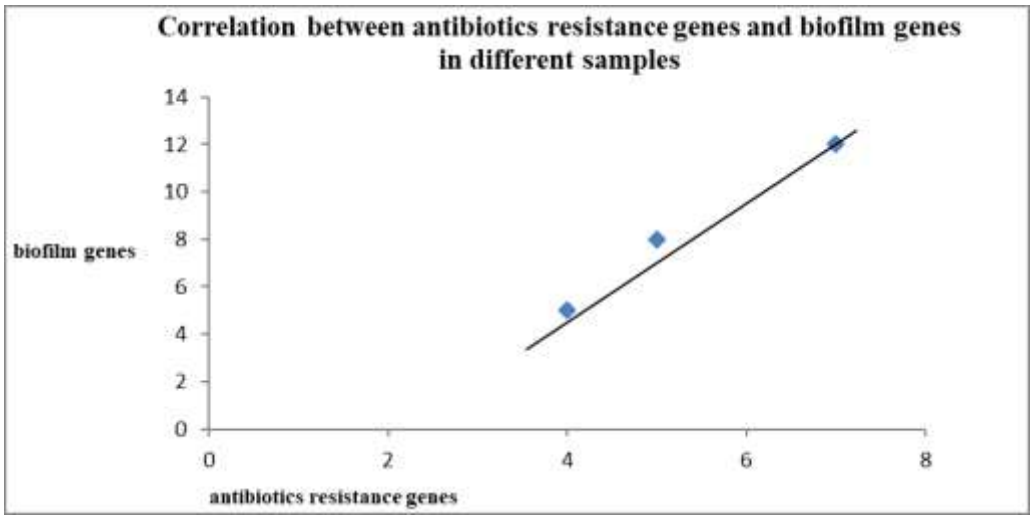


**Fig. 11. association between the presence of multidrug resistance genes and biofilm production genes**

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# الارتباط الجيني للجينات المقاومة المتعددة للادويه وجينات تكوين البيوفيلم لعترات الكلوستريديمبرفرنجينزالمعزولة من دجاج التسمين

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**الملخص**

تُعد بكتيريا المطثية الحاطمة (*Clostridium perfringens*) من أبرز المسببات لالتهاب الأمعاء التنخري (Necrotic Enteritis) في دجاج التسمين، حيث تعيق مقاومتها المتعددة للمضادات الحيوية (Multidrug Resistance - MDR) وقدرتها على تكوين الأغشية الحيوية (Biofilm Formation) جهود المكافحة والوقاية. تهدف هذه الدراسة إلى توصيف العوامل الوراثية المرتبطة بمقاومة المضادات الحيوية وتكوين الأغشية الحيوية في ست وثلاثين عزلة مرضية من النوع (A) ، تمثل 24% من أصل 150 عينة جُمعت من دجاج مصاب بالإسهال أو التهاب الأمعاء التنخري.

أظهرت النتائج أن جميع العزلات تحمل الجينات المسؤولة عن إنتاج السم ألفا (cpa) والسم المعوي(cpe) ، بينما وُجد جين السم المثقِّب (netB) في 75% من العزلات. أظهرت الاختبارات الفينوتيبية أن 69.4% من العزلات قادرة على تكوين الأغشية الحيوية، وكان 84% منها مقاومًا لعدة أنواع من المضادات الحيوية.

أظهرت اختبارات الحساسية للمضادات الحيوية أن أعلى نسب المقاومة كانت تجاه الكلورامفينيكول (80.5%)، البنسلين (80.5%)، والأوكسي تتراسيكلين (77.7%)، في حين أظهرت جميع العزلات حساسية تامة للفانكومايسين (Vancomycin) والأوفلوكساسين(Ofloxacin) .

كشفت تحاليل تفاعل البوليميراز المتسلسل (PCR) عن وجود جين مقاومة التتراسيكلين (tetK) في جميع العزلات، وجين بيتا-لاكتاماز (bla) في 62.5%، وجين مقاومة الماكروليدات (ermB) في 37.5%. كما وُجدت الجينات المرتبطة بتكوين الأغشية الحيوية مثل (appB) و (proA) في جميع العزلات، بينما ظهرت الجينات (iolD) و (ripH) في 62.5% و50% على التوالي.

أظهرت التحاليل الإحصائية وجود ارتباط وثيق بين المقاومة المتعددة للمضادات الحيوية ووجود جينات الأغشية الحيوية (معامل الارتباط $r = 0.994$ ؛ قيمة $p < 0.05$ ، مما يشير إلى احتمال وجود انتقال مشترك لهذه الصفات من خلال عناصر وراثية متنقلة (Mobile Genetic Elements)

توضح هذه النتائج التفاعل الجيني الذي يُعزز مقاومة المضادات الحيوية المرتبطة بتكوين الأغشية الحيوية لدى هذه البكتيريا، وتؤكد على ضرورة الاستخدام الرشيد للمضادات الحيوية، إلى جانب أهمية تطوير استراتيجيات وقائية بديلة مثل اللقاحات الموجهة والمكملات الحيوية (Probiotics) للحد من انتشار المرض في نظم إنتاج الدواجن المكثفة.

**الكلمات الدالة:** المطثية الحاطمة، التهاب الأمعاء التنخري، دجاج التسمين، مقاومة متعددة للمضادات، الأغشية الحيوية، جينات المقاومة.