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Mastitis in Cattle: Role of *C. perfringens*

Rania S. Mohammed and Asmaa. E. GamalEldeen

Bacteriology Department, Animal Health Research Institute, Agriculture Research Center, Giza, Egypt.

Abstract

BOVINE mastitis, a leading cause of economic loss in dairy industry, has traditionally been associated with aerobic pathogens; however, emerging evidence implicates anaerobic bacteria such as *Clostridium perfringens* in intramammary infections. The present study aimed to isolate and identify *C. perfringens* from milk samples of cows with clinical (n=30) and subclinical (n=30) mastitis, determine toxin profiles using ELISA and PCR genotyping, assess antimicrobial susceptibility, and detect key resistance genes via multiplex PCR. All 30 subclinical samples tested positive by the California Mastitis Test (CMT), confirming 100% mastitis prevalence among screened cows. Overall, *C. perfringens* was recovered from 53% of clinical and 30% of subclinical samples (41.6% overall), with pathogenic strains present in 30% of clinical and 13.3% of subclinical cases. ELISA revealed that 100% of the 13 isolates produced alpha toxin, while none expressed beta or epsilon toxins, indicating a dominance of toxinotype A in both clinical and subclinical manifestations. Antimicrobial susceptibility testing demonstrated high resistance rates to penicillin (69.2% clinical, 23% subclinical), oxytetracycline (61.5% clinical, 23% subclinical), and chloramphenicol (53.8% clinical, 15.3% subclinical), whereas all isolates remained susceptible to ofloxacin and vancomycin. Multidrug resistance (MDR) was identified in 38.5% of isolates (3 of 6 clinical; 2 of 3 subclinical). Multiplex PCR confirmed that all isolates harboredcpa (alpha toxin). The cpe (enterotoxin) gene was detected in 2 of 5 tested isolates; tetK (tetracycline resistance) was present in 4 of 5; bla (β-lactamase) in 4 of 5; and ermB (macrolide-lincosamide-streptogramin B resistance) in 4 of 5—results that match the corresponding phenotypic resistance profiles. These findings underscore the emerging role of *C. perfringens* as a significant mastitis pathogen with notable virulence and resistance determinants, highlighting the need for vigilant anaerobic diagnostics, judicious antibiotic stewardship, and alternative control measures in dairy herds.

Keywords: Clostridium perfringens; bovine mastitis; toxinotype A; alpha toxin; antimicrobial resistance; multidrug resistance; enterotoxin; resistance genes.

Introduction

Mastitis in dairy cows remains a leading health issue worldwide, imposing heavy financial burdens through decreased milk output, compromised milk quality, treatment expenses, and the need to cull infected animals[1, 2]. This ailment can present as clinical mastitis, with obvious udder inflammation, or as subclinical mastitis, where there are no visible indicators yet milk yield and quality decline[3]. While *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus* spp. are traditionally recognized as predominant mastitis pathogens, emerging evidence highlights the involvement of anaerobic bacteria, including *Clostridium perfringens*, in the pathogenesis of bovine mastitis [4].

Clostridium perfringens is a Gram-positive, spore-forming, anaerobic bacillus commonly found in soil, feces, and the gastrointestinal tracts of animals and humans [5]. It is widely known for its role in enteric and histotoxic diseases, yet its contribution to intramammary infections in cattle is increasingly acknowledged [6]. The organism produces an array of potent exotoxins, with the alpha (α) toxin-encoded by the cpa gene—being the most universally expressed virulence factor among all types (A–E) [7]. Strains producing other toxins such as beta (β), epsilon (ε), iota (ι), and enterotoxin (CPE) are also known to cause systemic or localized

*Corresponding authors: Rania Saleh Mohammed, E-mail: raania.saleh@yahoo.com, Tel.: +20/100 1962479
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infections in livestock and may pose zoonotic risks [8].

Despite its known pathogenicity, *C. perfringens* is often underreported in mastitis cases, possibly due to limitations in routine aerobic culturing methods that fail to detect anaerobes [9]. The application of selective culturing, toxinotyping, and molecular tools has allowed more sensitive detection and characterization of *C. perfringens* in mastitic milk, providing a clearer understanding of its epidemiological and clinical relevance [9].

Furthermore, the widespread and often indiscriminate use of antibiotics in veterinary medicine has contributed to the emergence of antimicrobial-resistant *C. perfringens* strains, complicating treatment outcomes [10]. Resistance genes such as tetK (tetracyclines), bla (β-lactams), and ermB (macrolides-lincosamides-streptogramin B) have been increasingly identified in isolates from livestock, reflecting significant public health concerns due to potential transmission via the food chain [11].

In light of these issues, this investigation set out to recover and characterize *Clostridium perfringens* from milk specimens of cows suffering from both clinical and subclinical mastitis. The study objectives included defining the toxin repertoire of each isolate through ELISA and PCR genotyping, evaluating their antimicrobial susceptibility profiles, and screening for major antibiotic resistance determinants using multiplex PCR. By combining traditional bacteriological techniques with molecular assays, this work aims to clarify the involvement of *C. perfringens* in bovine mastitis and deliver current information on its virulence and resistance patterns, ultimately supporting improved control measures and more prudent antibiotic application in dairy herds.

Materials and methods

Collection of Milk Samples.

Milk specimens were obtained from dairy cows presenting both clinical and subclinical mastitis. Subclinical infections were identified using the California Mastitis Test (CMT), a quick and straightforward screening method commonly employed for mastitis detection[12].Sixty milk samples were obtained in total, with thirty from cows exhibiting clinical mastitis and thirty from cows identified with subclinical mastitis.

Isolation and Identification of Clostridium perfringens from Examined Milk Samples

Milk specimens were first centrifuged at 3,000 rpm for 20 minutes. The pellet obtained from each sample was then transferred into cooked meat broth (CMB). These inoculated broths were incubated under anaerobic conditions according to standard protocols[13].A loopful from each enriched broth was inoculated onto sheep blood agar plates supplemented with 150 µg/mL neomycin sulfate and then incubated anaerobically at 37 °C for 24 hours. Anaerobic conditions were maintained using AnaeroGen sachets in sealed jars (OXOID Ltd, England)[14].

Presumptive *C. perfringens* colonies were characterized by their distinctive double zone of hemolysis on blood agar, confirmed by a positive Nagler reaction for lecithinase activity, and verified as Gram-positive rods through Gram staining[15]. *Clostridium perfringens* is differentiated from other *Clostridium* species by a series of biochemical assays, including oxidase and catalase reactions, motility, nitrate reduction, hemolysis on blood agar, indole formation, urea hydrolysis, lecithinase activity, and various sugar fermentation profiles[16].

Antibiotic Sensitivity Testing of the Isolates

Antibiotic susceptibility was evaluated on Mueller–Hinton agar (Oxoid) plates using the disc diffusion technique in accordance with Clinical and Laboratory Standards Institute guidelines[15][17].

Typing of C. perfringens isolates using ELISA

The assay was carried out following the manufacturer's instructions. A sandwich ELISA kit (Bio K 270/2 ENT21H10, Bio-x Diagnostics, Belgium) was employed to detect *Clostridium perfringens* alpha, beta, and epsilon toxins in culture supernatants and biological fluids across various species.

Molecular identification

In Table 1,typed isolates by ELISA will be subjected to further confirmation and typing using Multiplex PCR Genotyping to determine the genetic characteristics of the isolates [18]using :

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*)

Detection of Resistance genes by Multiplex PCR such as *tetA, ermB, and Bla* was performed.

Results

To investigate the role of *Clostridium perfringens* in bovine mastitis and its associated virulence and antimicrobial resistance profiles, a multifaceted diagnostic approach was employed, combining clinical screening, bacteriological culture, toxin detection, antimicrobial susceptibility testing, and

molecular characterization. Initial screening with the California Mastitis Test (CMT) provided a broad overview of mastitis prevalence in the studied herd, followed by targeted isolation of *C. perfringens* from clinical and subclinical cases to assess pathogen distribution. Toxinotyping of recovered isolates using ELISA helped elucidate the dominant virulence factors involved, while antimicrobial susceptibility testing offered insight into emerging resistance patterns affecting treatment options. Finally, PCR-based detection of toxin and resistance genes further validated phenotypic observations and clarified the genetic basis for toxigenicity and multidrug resistance among the isolates. The integrated findings presented in Tables 2–6 and Figures 1–5 offer a detailed overview of *C. perfringens* epidemiology, pathogenicity, and resistance dynamics in mastitis-affected cattle.

The outcomes of the California Mastitis Test conducted on 30 milk samples. All 30 samples tested positive by CMT, yielding a 100 % isolation rate. These results indicate a complete prevalence of somatic cell increases consistent with mastitis across the examined cohort, underscoring the widespread occurrence of mammary gland inflammation in the studied herd (Table 2).

Additionally, table 3 details the isolation rates of *Clostridium perfringens* from milk samples associated with clinical and subclinical mastitis. Of 16 clinical-mastitis samples, 53 % yielded *C. perfringens*, and 30 % harbored pathogenic strains. In contrast, subclinical-mastitis samples showed lower recovery rates, with 30 % positive for *C. perfringens* and 13.3 % identified as pathogenic. Overall, 41.6 % of all samples contained *C. perfringens*, with 21.7 % bearing pathogenic isolates, highlighting a notable but variable bacterial presence in different mastitis states.

We also reported the toxin profiles of the 13 *C. perfringens* isolates (9 from clinical mastitis, 4 from subclinical) as determined by ELISA. Every isolate (100 %) produced alpha toxin, while none exhibited beta or epsilon toxins. The uniform alpha-toxin positivity across both clinical and subclinical cases suggests that this toxin is the predominant virulence factor of *C. perfringens* in bovine mastitis within the sample set (Table 4).

The resistance profiles to ten antimicrobial agents for isolates from clinical and subclinical mastitis represented in table 5. Penicillin showed the highest resistance in clinical isolates (69.2 %) and moderate resistance in subclinical (23 %). Oxytetracycline and chloramphenicol also demonstrated substantial resistance rates, particularly in clinical cases (61.5 % and 53.8 %, respectively). Notably, all isolates remained susceptible to ofloxacin and vancomycin, indicating these agents' potential utility in managing *C. perfringens* mastitis infections.

The results also categorize isolates exhibiting resistance to multiple antimicrobial classes. Three of six resistant clinical-mastitis isolates qualified as multidrug-resistant (MDR), while two of three resistant subclinical isolates were MDR. In total, 5 of the 13 isolates (38.5 %) met MDR criteria, emphasizing the challenge of treating *C. perfringens* mastitis due to emerging resistance patterns (Table 6).

Molecular Characterization of Clostridium perfringens Isolates Using PCR-Based Detection of Toxin and Resistance Genes.

To determine the toxigenic and antimicrobial resistance profiles of *Clostridium perfringens* field isolates, a series of targeted PCR assays were performed to amplify specific toxin and antibiotic resistance genes. The results from agarose gel electrophoresis are presented in Figures 1–5.

Detection of Toxin Genes by Multiplex PCR

Figure 1 shows a multiplex PCR assay targeting the major toxin genes (*cpa*, *cpb*, *etx*, and *iap*). Lanes 1–5 correspond to five different field isolates. All five isolates produced a single band at 402 bp, indicating the presence of the *cpa* (alpha-toxin) gene. No amplification products were observed for *cpb* (beta), *etx* (epsilon), or *iap* (iota), confirming that these isolates are all *C. perfringens* type A. The positive control (P) amplified as expected, demonstrating primer specificity and PCR efficiency; the negative control (N) showed no bands, ruling out contamination or non-specific amplification. Lane L (1 kb DNA ladder) served as the molecular weight reference.

Presence of Enterotoxin Gene (cpe)

Figure 2 presents PCR results for the *cpe* (enterotoxin) gene in the same five *C. perfringens* isolates. Only lanes 3 and 4 produced bands at the expected size (~233 bp, depending on primer set), indicating that 2 of the 5 isolates are enterotoxigenic. Lanes 1, 2, and 5 showed no amplification for *cpe*. The positive control (P) confirmed correct assay function, and the negative control (N) remained blank. Lane L (1 kb ladder) was used to validate product size. These data indicate that two isolates carry *cpe* rather than all five.

Detection of Tetracycline Resistance Gene (tetK)

Figure 3 shows PCR amplification for the *tetK* gene (tetracycline resistance). Bands of the correct size (~705 bp) appear in lanes 1, 2, 3, and 5. Lane 4 lacks a band, indicating that 4 of the 5 isolates harbor*tetK*. The positive control (P) worked as expected, and the negative control (N) showed no amplification. The 1 kb ladder (L) provided a size reference. The presence of *tetK* in four isolates correlates with their phenotypic tetracycline resistance in Table 5.

Detection of β-Lactamase Gene (bla)

Figure 4 depicts PCR results for the *bla* gene (β-lactamase). Amplification bands (~856 bp, depending on primer set) are visible in lanes 1–4, indicating that these four isolates carry *bla*. Lane 5 shows no band, so only 4 of 5 isolates possess *bla*. The positive control (P) confirmed assay validity, while the negative control (N) remained blank. Lane L (1 kb ladder) served as the molecular weight marker. Detection of *bla* in these four isolates explains their β-lactam resistance observed phenotypically in Table 5.

Detection of Macrolide-Lincosamide-Streptogramin B Resistance Gene (ermB)

Figure 5 shows PCR amplification for the *ermB* gene. Lanes 1, 2, 3, and 5 display bands of the expected size (~412 bp), indicating that four of the five isolates carry *ermB*. Lane 4 has no band, so one isolate is negative for *ermB*. The positive control (P) amplified correctly, and the negative control (N) showed no product. The 1 kb ladder (L) confirms band sizes. The presence of *ermB* in these four isolates corresponds with their erythromycin and clindamycin resistance in Table 5.

Discussion

This research comprehensively examined how often *Clostridium perfringens* appears in milk from cows with clinical and subclinical mastitis, characterizing its toxin genes, antibiotic susceptibility, and underlying genetic markers. The results shed light on the pathogen's involvement in bovine mastitis and highlight worrying trends in its developing resistance to antimicrobial agents.

The California Mastitis Test (CMT) yielded a 100% positivity rate across all 30 subclinical milk samples (Table 2), underscoring the high burden of subclinical mastitis within the sampled dairy population. This aligns with findings emphasized the silent and widespread nature of subclinical mastitis in dairy herds, contributing significantly to economic losses due to decreased milk quality and yield [2, 19].

Additionally, *C. perfringens* was isolated from 53% of clinical and 30% of subclinical mastitis samples, resulting in an overall prevalence of 41.6%. These rates indicate a substantial involvement of *C. perfringens* in bovine mastitis cases, consistent with reports documented *C. perfringens* as an emerging mastitis pathogen, particularly under anaerobic or immunosuppressive conditions [9, 20, 21]. The higher isolation frequency in clinical cases supports the hypothesis that *C. perfringens* plays a more active role in overt mastitis episodes compared to its opportunistic role in subclinical infections [22].

Moreover, the identification of pathogenic strains in 30% of clinical and 13.3% of subclinical cases reinforces the potential virulence variability within *C. perfringens* populations, suggesting a stronger pathogenic profile in cows with clinical signs.

All 13 *C. perfringens* isolates tested positive for alpha toxin using ELISA (Table 4), while none produced beta or epsilon toxins. This uniform detection of alpha toxin confirms the predominance of type A strains in bovine mastitis, which agree with previous findings [23, 24]. Type A strains, characterized by the cpa gene encoding alpha-toxin (a phospholipase C), have been frequently implicated in a range of ruminant infections, including gas gangrene and mastitis [25].

The study reveals concerning resistance rates among the isolates, with penicillin resistance reaching 69.2% in clinical mastitis and 23% in subclinical cases. Resistance to oxytetracycline (61.5%) and chloramphenicol (53.8%) was also prominent. These data reflect growing antimicrobial resistance trends among anaerobic pathogens and corroborate earlier studies highlighted penicillin and tetracycline resistance as emerging threats in *Clostridium* species [10]. On the other hand, several studies is not agree with penicillin but only tetracycline [26–29].

On the contrary, all isolates remained fully susceptible to ofloxacin and vancomycin, offering potential treatment avenues. The preserved sensitivity to vancomycin is particularly noteworthy, as glycopeptides have retained efficacy against resistant Gram-positive bacteria, although they are not routinely used in veterinary settings due to cost and regulatory concerns. However, these findings is not in align with previous reports confirming the resistance of glycopeptides toward Gram-positive bacteria [30, 31].

Multidrug resistance (MDR) patterns among isolates: 3 of 6 resistant clinical strains and 2 of 3 resistant subclinical strains exhibited MDR phenotypes. In total, 38.5% of isolates met the MDR criteria. This aligns with the global pattern described MDR rates among *C. perfringens* isolates from livestock have steadily increased, raising concerns for both veterinary and public health [32, 33].

The results illustrate multiplex PCR results showing amplification of only the alpha toxin gene (cpa), with a 402 bp band present in all tested isolates. This molecular confirmation corroborates ELISA findings and reaffirms that the isolates belong to toxinotype A. No cpb, etx, or iap genes were detected, which agrees with the toxin profile in Table 4. This finding is consistent with previous genotyping studies that have reported a predominance of cpa-positive, cpb/etx/iap-negative strains in bovine mastitis [34, 35].

The detection of the *cpe* gene (enterotoxin) in two of the five isolates (lanes 3 and 4, Fig. 2) aligns with

previous reports linking *cpe* to enterotoxigenic potential in *C. perfringens*[36, 37]. Its presence in mastitis-associated strains may reflect horizontal gene transfer events and underscores the need for ongoing genomic surveillance of livestock isolates.

Regarding antimicrobial resistance, four of the five isolates (lanes 1, 2, 3, and 5) harbored the *tetK* gene, which encodes a tetracycline efflux pump (Fig. 3). These same four isolates exhibited high-level tetracycline resistance in disk-diffusion assays (Table 5), consistent with previous findings documenting widespread tetracycline resistance in veterinary *C. perfringens*[38, 39]. Similarly, four of the five isolates (lanes 1–4, Fig. 4) carried the *bla* gene, encoding β-lactamase; these isolates were phenotypically resistant to penicillin, confirming the genetic basis for β-lactam resistance observed in Table 5 and mirroring trends reported in other anaerobic pathogens [40]. Finally, four of the five isolates (lanes 1, 2, 3, and 5; Fig. 5) tested positive for the *ermB* gene, which mediates resistance to macrolides, lincosamides, and streptogramin B antibiotics. Those four isolates displayed resistance to erythromycin and clindamycin in Table 5, in agreement with prior studies of livestock- and foodborne *C. perfringens*[29, 41].

These results collectively suggest that the resistance phenotypes in *C. perfringens* are largely driven by acquired genes, possibly through plasmid-mediated transfer, and call for urgent interventions to reduce antibiotic misuse in animal agriculture.

Conclusion

This study demonstrates that *Clostridium perfringens* emerges as a notable pathogen in both clinical and subclinical bovine mastitis, with all isolates identified as toxinotype A producing alpha toxin and a minority carrying the enterotoxin gene, hinting at food-safety concerns. The high levels of resistance to common antibiotics—particularly penicillin, tetracycline and chloramphenicol—and the frequent occurrence of multidrug-resistant strains contrast with preserved susceptibility to ofloxacin and vancomycin. Molecular detection of tetK, bla and ermB genes confirms plasmid-mediated resistance mechanisms. These findings highlight the urgent need for prudent antibiotic stewardship, regular genomic monitoring of mastitis pathogens and the pursuit of alternative treatment strategies in dairy herds.

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Declaration of interest

The authors have no conflicts of interest in this study.

Ethical approval

The samples were collected according to the protocol approved by the scientific committee of the Institute.

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

Aspect	Target gene	Primers sequences(5'-3')	Amplified segment (bp)	Amplification (35 cycles)				Reference
				Primary denaturation	Second denaturation	Annealing	Extension	Final extension	
Virulence Genes	Alpha (*cpa*)	F:GTTGATAGCGCAGGACATGTTAAG R:CATGTAGTCATCTGTTCCAGCATC	402	94˚C 5 min.	94˚C 45 sec.	50˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	[8]
	Beta (*cpb*)	F:ACTATACAGACAGATCATTCAACC R:TTAGGAGCAGTTAGAACTACAGAC	236						
	Epsilon (*etx*)	F:ACTGCAACTACTACTCATACTGTG R:CTGGTGCCTTAATAGAAAGACTCC	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.	[9]
Antibiotic Resistance Genes	*tetK*	F:TTATGGTGGTTGTAGCTAGAAA R:AAAGGGTTAGAAACTCTTGAAA	382	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	[10]
	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.	[11]
	Bla	F:ATGAAAGAAGTTCAAAAATATTTAGAG R:TTAGTGCCAATTGTTCATGATGG	780	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	[12]

TABLE 2. Results of California Mastitis Test (CMT)

Number of examined samples	Number of Positive CMT	Isolation Rate (%)
30	30	100%

TABLE 3. Incidence of *C. perfringens* from Examined Milk Samples (n = 30).

Cattle State	Number of Positive *C. perfringens* Isolates	Isolation Rate (%)	Number of Pathogenic *C. perfringens* Isolates	Isolation Rate (%)
Clinical mastitis	16	53%	9	30%
Subclinical mastitis	9	30%	4	13.3%
Total	25	41.6%	13	21.7%

TABLE 4. Typing of *C. perfringens* Isolates using ELISA.

Cattle State	Positive *C. perfringens*	*C. perfringens* toxin		Alpha toxin		Beta toxin		Epsilon toxin	
		No.	%	No.	%	No.	%	No.	%
Clinical mastitis	9	9	100%	9	100%	0	0%	0	0%
Subclinical mastitis	4	4	100%	4	100%	0	0%	0	0%
Total	13	13	100%	13	100%	0	0%	0	0%

TABLE 5. Antimicrobial susceptibility of the *C. perfringens* Isolates (n = 13).

Antimicrobial Agent	Disc Concentration	Clinical mastitis		Subclinical mastitis	
		Number of Resistant Isolates	Resistance (%)	Number of Resistant Isolates	Resistance (%)
Azithromycin	15 µg	6	46.1	1	7.6
Chloramphenicolo	30 µg	7	53.8	2	15.3
Clindamycin	2 µg	6	46.1	2	15.3
Erythromycin	15 µg	2	15.3	0	0
Gentamicin	10 µg	1	7.6	0	0
Kanamycin	30 µg	5	38.4	1	7.6
Nalidixic acid	30 µg	5	38.4	2	15.3
Ofloxacin	5 µg	0	0	0	0
Oxytetracycline	30 µg	8	61.5	3	23
Penicillin	10 IU	9	69.2	3	23
Rifampicin	5 µg	1	7.6	0	0
Vancomycin	10 µg	0	0	0	0

TABLE 6. Multidrug Resistant Isolates of the *C. perfringens* (n = 13).

Cattle State	Resistant Isolates	MDR Isolates
Clinical mastitis	6	3
Subclinical mastitis	3	2
Total	9	5

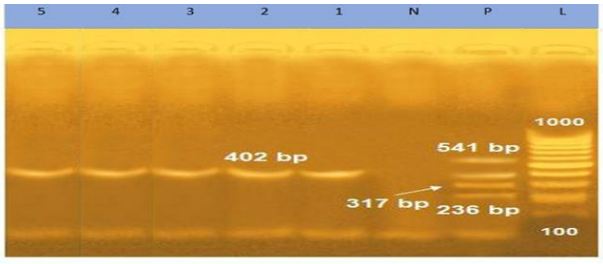

Fig.1. In this multiplex PCR designed to detect key toxin genes of *C. perfringens*, primers targeted cpa (alpha toxin, 402 bp), cpb (beta), etx (epsilon), and iap (iota). Lanes 1–5, corresponding to field isolates, each produced a single 402 bp amplicon, confirming the presence of cpa and classifying them as type A. No PCR products were observed for cpb, etx, or iap, indicating those toxin genes were absent. Lane P is the positive control, Lane N the negative control, and Lane L the 1 kb DNA ladder.

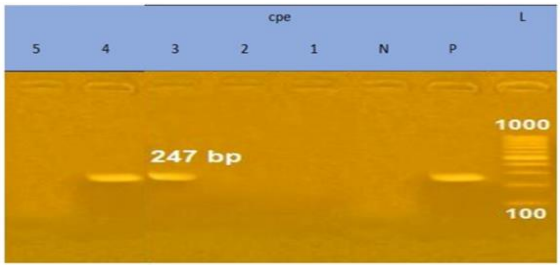

Fig.2. Gel electrophoresis of PCR amplification products for the *cpe* (enterotoxin) gene in *C. perfringens* isolates. Lanes 3 and 4 represent amplification of the *cpe* gene at the expected size, indicating that tested strains are enterotoxigenic. Lane P: positive control; Lane N: negative control; Lane L: 1 kb DNA ladder.

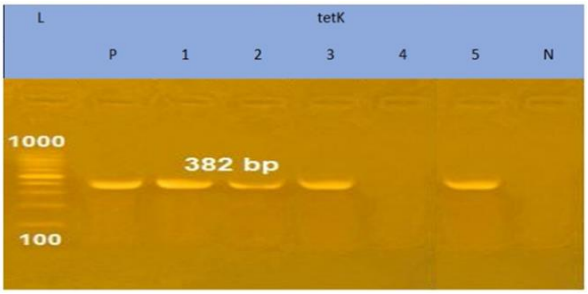

Fig. 3. Gel electrophoresis of PCR amplification products for the *tetK* gene in *Clostridium perfringens* isolates. Lanes 1,2,3 and 5 represent exhibiting bands at the expected size for *tetK*, indicating the presence of tetracycline resistance genes. Lane P: positive control; Lane N: negative control; Lane L: 1 kb DNA ladder.

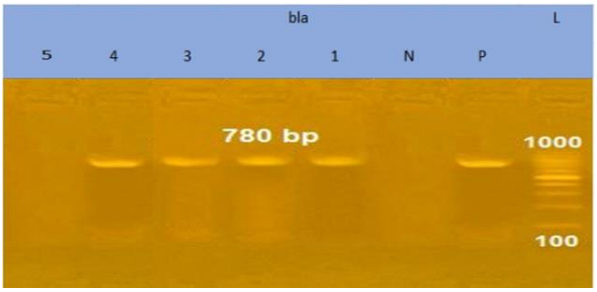

Fig.4. Gel electrophoresis of PCR amplification products for the *bla* gene in *Clostridium perfringens* isolates. Lanes 1–4 showing amplification bands corresponding to the *bla* gene, indicating the presence of β-lactamase-encoding resistance. Lane P: positive control; Lane N: negative control; Lane L: 1 kb DNA ladder.

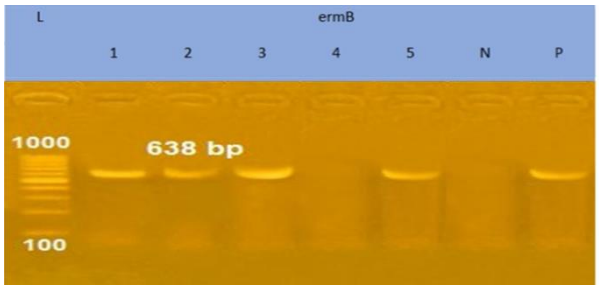

Fig. 5. Gel electrophoresis of PCR amplification products for the *ermB* gene in *Clostridium perfringens* isolates. Lanes 1,2 ,3 &5 showing positive amplification of the *ermB* gene, associated with resistance to macrolide, lincosamide, and streptogramin B antibiotics. Lane P: positive control; Lane N: negative control; Lane L: 1 kb DNA ladder.

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إلتهاب الضرع في الابقار: دور كلستريديم برفرنجينز

رانية صالح محمود محمد و أسماء السيد جمال الدين

قسم البكتريولوجى، معهد بحوث الصحة الحيوانية، مركز البحوث الزراعية، الجيزة، مصر.

الملخص

يُعَدُّ التهاب الضرع في الأبقار من الأسباب الرئيسية للخسائر الاقتصادية في صناعة الألبان، وكان يرتبط تقليديًا بالعوامل الممرضة الهوائية، غير أن الأدلة الحديثة تشير إلى تورط البكتيريا اللاهوائية مثل كلوستريديوم بيرفرينجنز في حالات العدوى داخل الغدة اللبنية.

هدفت هذه الدراسة إلى عزل وتحديد كلوستريديوم بيرفرينجنز من عينات حليب لأبقار تعاني من التهاب ضرع سريري (n=30) وتحت السريري (n=30)، وتحديد أنماط السموم باستخدام اختبار ELISA وتقنية تحديد النمط الجيني PCR، وتقييم الحساسية تجاه المضادات الحيوية، والكشف عن الجينات المقاومة الرئيسية باستخدام PCR متعدد الأهداف (Multiplex PCR).أظهرت نتائج اختبار كاليفورنيا لالتهاب الضرع (CMT) إيجابية جميع العينات تحت السريرية (٣٠ من ٣٠)، مما يؤكد انتشار التهاب الضرع بنسبة ١٠٠% في الأبقار التي تم فحصها. تم عزل كلوستريديوم بيرفرينجنز من ٥٣% من العينات السريرية 30% من العينات تحت السريرية (بنسبة إجمالية ٤١.٦%) .وكانت السلالات الممرضة موجودة في ٣٠% من حالات التهاب الضرع السريري و١٣.٣% من الحالات تحت السريرية.

أظهر اختبار ELISA أن جميع العزلات الـ١٣ أنتجت سم الألفا (alpha toxin) في حين لم تُظهر أي عزلة إنتاج سموم بيتا أو إبسيلون، مما يدل على سيطرة النمط السمي toxinotype A في كل من الحالات السريرية وتحت السريرية.

أظهرت اختبارات الحساسية للمضادات الحيوية مقاومة عالية تجاه: البنسلين: ٦٩.٢% (سريري)، ٢٣% (تحت سريري) أوكسي تتراسايكلين: ٦١.٥% (سريري)، ٢٣% (تحت سريري) كلورامفينيكول: ٥٣.٨% (سريري)، ١٥.٣% (تحت سريري) في حين أن جميع العزلات كانت حساسة للأوفلوكساسين والفانكوميسين. تم تحديد وجود مقاومة متعددة للمضادات (MDR) في ٣٨.٥% من المعزولات (٣ من ٦ سريرية؛ ٢ من ٣ تحت سريرية)وأكد PCR المتعدد الأهداف أن جميع المعزولات احتوت على جين cpa (سم الألفا) كما تم الكشف عن جين cpe (السم المعوي) في ٢ من ٥ معزولات جين tetK (مقاومة التتراسايكلين) في ٤ من ٥ وجين bla (بيتا لاكتاماز) في ٤ من ٥ وجين ermB (مقاومة ماكروليد-لينكوساميد-ستربتوغرامين B) في ٤ من ٥.وهي نتائج تتطابق مع أنماط المقاومة الظاهرية. تُبرز هذه النتائج الدور المتزايد لكلوستريديوم بيرفرينجنز كمُمْرِض مهم في حالات التهاب الضرع، مع امتلاكه لعوامل ضراوة ومقاومة واضحة، مما يستدعي تشخيصًا دقيقًا للبكتيريا اللاهوائية، وإدارة حذرة للمضادات الحيوية، والبحث عن بدائل للسيطرة عليها في قطعان الألبان.

الكلمات الدالة: كلوستريديوم بيرفرينجنز؛ التهاب الضرع البقري؛ النمط السمي A؛ سم الألفا؛ مقاومة المضادات الحيوية؛ المقاومة المتعددة؛ السم المعوي؛ جينات المقاومة.