

ANTIMICROBIAL SUSCEPTIBILITY OF *Clostridium perfringens* ISOLATED FROM BROILER CHICKENS WITH NECROTIC ENTERITIS IN TIARET, WESTERN ALGERIA

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ABSTRACT

The present study was carried out to evaluate *in vitro* susceptibility of *Clostridium perfringens* (*C. perfringens*) to antimicrobial agents. A total of 22 *C. perfringens* type A strains, previously isolated from intestinal contents of broiler chickens displaying gross lesions suspected to be necrotic enteritis (NE) reared at different locations in Tiaret province, were tested on a panel of 7 antimicrobial agents using the disk diffusion method. The following antibiotics were used: Amoxicillin-Clavulanic acid (Co-amoxiclav); Penicillin G; Bacitracin; Tetracycline; Clindamycin; Trimethoprim-Sulfamethoxazole (Co-trimoxazole); Erythromycin. Our results showed a widespread resistance to multiple antibiotics including Tetracycline (86.36%), Erythromycin (77.27%), Co-trimoxazole (68.18%) and Co-amoxiclav (50%). However, *C. perfringens* type A isolates tested demonstrated a low resistance to Clindamycin (36.36%), Bacitracin (36.36%) and Penicillin G (31.81%). We concluded that the highest rate of resistance was showed for commonly used antibiotics in comparison with antibiotics used in specific cases.

Keywords: *Clostridium perfringens*; necrotic enteritis; antimicrobial susceptibility; broiler chickens; Tiaret.

INTRODUCTION

For many years, the antimicrobial growth promoters were widely used in poultry feed to prevent and control intestinal pathogens including *C. perfringens* [1], which is the major cause of NE disease, responsible for great financial losses to the poultry production industry [2].

C. perfringens infection causes clinical and subclinical NE. The acute clinical disease is mostly found in 2-5 week-old-chickens, characterized by severe intestinal necrosis, leading to a sudden 50% increase in flock mortality rates [3], while the subclinical infection can show clinical signs including decreased appetite and diarrhea

leading to poor feed conversion and impairment of body weight gain [4]. Gross lesions are diverse, usually affecting the small intestine and liver; jejunum and ileum are the most affected portions of the gut [5].

Due to the important economic losses to poultry production caused by NE disease [6], the use of antibiotics to prevent great costs has become more widespread, leading to the emergence of *C. perfringens* strains with resistance mechanisms against these antibiotics [7]. Furthermore, previous studies reported that *C. perfringens* strains isolated from poultry have shown a marked difference in resistance patterns for different geographic regions [8,9,10,11,12]. However, there is a lack of data about this

microorganism susceptibility to antimicrobial drugs in Tiaret province (Western Algeria).

In This study, we aimed to evaluate *in vitro* susceptibility of *C. perfringens* Type A strains, isolated from broiler chickens with NE in Tiaret province, to antimicrobial agents, which are relevant to poultry production, used in therapy and prophylaxis.

MATERIALS AND METHODS

Source of Isolates

A total of 22 *C. perfringens* strains, previously isolated from intestinal contents of broiler chickens displaying gross lesions suspected to be NE reared at different locations in Tiaret province, were analyzed. All the isolates were genotyped by multiplex polymerase chain reaction as *C. perfringens* type A and confirmed as NetB negative strains by uniplex PCR [3]. The study was conducted during the period of February to April 2018 in Hygiene and Animal Pathology Laboratory, University of Tiaret, Algeria.

Antimicrobial Susceptibility Testing

The isolates were subjected to antimicrobial susceptibility by using disc diffusion test. The following antibiotics manufactured for analytical purpose were tested: Co-amoxiclav (AMC: 30 µg) (CYPRESS DIAGNOSTICS, Belgium), Penicillin G (P: 10 IU) (Liofilchem, Italy), Bacitracin (BA: 10 IU) (Liofilchem, Italy), Tetracycline (TE: 30 µg) (CYPRESS DIAGNOSTICS, Belgium), Clindamycin (CD: 10 µg) (Liofilchem, Italy), Erythromycin (E: 15 µg) (Liofilchem, Italy), (Co-trimoxazole) (SXT: 25 µg) (Liofilchem, Italy).

Each *C. perfringens* isolate was plated on Clostridium-selective medium

(perfringens agar base containing 400 µg/ml of Cycloserine) (Oxoid, UK), and incubated under anaerobic condition provided by AnaeroGen atmosphere generation system for 24 h at 37°C. Typical black colonies of *C. perfringens* were taken out, re-streaked onto plate of 5% defibrinated sheep blood agar (Biokar, France) and incubated anaerobically for 24 h at 37°C [13,14]. 3-4 colonies were then suspended into 10 ml of 0.9% NaCl. The suspension was adjusted to achieve an optical density equivalent to that of McFarland 0.5 standard, and diluted 10-fold. Approximately 1×10^7 colony forming units were streaked on Muller Hinton agar plates using a sterile cotton swab and the antibiotic discs were applied at the doses shown previously. The plates were incubated under anaerobic condition at 37°C for 24 h. The inhibition zone was measured according to standard interpretive criteria charts [15]. The *C. perfringens* isolates with intermediate susceptibility classification were considered susceptible to that drug and the Multidrug resistance was defined as resistance to more than two drugs [16].

RESULTS

Antibiotic Resistance of *C. perfringens* Type A Isolates

The resistance rates to different antibiotics tested against *C. perfringens* type A strains are shown in Table 1. According to the results, *C. perfringens* type A isolates were highly resistant to Tetracycline with the rate of 86.36% (19/22), followed by Erythromycin, Co- trimoxazole and Co-amoxiclav at the rates of 77.27% (17/22), 68.18 (15/22) and 50% (11/22), respectively. Clindamycin and Bacitracin demonstrated a low rate of resistance with 36.36% (8/22), while the lowest rate of resistance was recorded for Penicillin G with 31.81% (7/22).

Table 1. Antimicrobial susceptibility test results of 22 *C. perfringens* type A isolated from NE in broiler chickens

Antimicrobial agent	Disc concentration	Number of resistant	Number of sensitive	Percent of resistance (%)
Co-amoxiclav (AMC)	30 µg	11	11	50
Penicillin G (P)	10 IU	7	15	31.81
Tetracycline (TE)	30 µg	19	3	86.36
Erythromycin (E)	15 µg	17	5	77.27
Bacitracin (BA)	10 IU	8	14	36.36
Clindamycin (CD)	10 µg	8	14	36.36
Co-trimoxazole (SXT)	25 µg	15	7	68.18

Co-amoxiclav: amoxicillin-acid clavulanic; Co-trimoxazole: Trimethoprim-sulfamethoxazole

Multidrug Resistance Patterns for *C. perfringens* Isolates

As shown in Tables 2 and 3, the multidrug resistance results revealed that 19 out of the total 22 *C. perfringens* type A isolates demonstrated multiple resistance (resistance to 3 and more antibiotics) at the rate of 86.36%. 31.81% (7/22) and 27.27 (6/22) were resistant to 3 and 4 antibiotics, respectively. Furthermore, 9.09% (2/22) and 18.18% (4/22) were resistant to 5 and 6 antibiotics, respectively. However, the multidrug resistance results revealed that none of the 22 isolates demonstrated resistance to all 7 antibiotics tested.

DISCUSSION

The resistance of *C. perfringens* isolates to different antibiotics, used for the treatment or as growth promoters in-feed for the prevention of NE outbreak in poultry, was reported in different geographic regions by several studies [17,8,18,19]. In order to study the susceptibility of *C. perfringens* isolated from broiler chickens with NE disease in Tiaret province, 22 *C. perfringens* type A isolates were tested on a panel of 7 antimicrobial agents using the disk diffusion method.

According to our results, Tetracycline demonstrated the highest rate of resistance with 86.36%, which is in accordance with the results of Shojadoost et al. [20] who reported a high level of resistance to Tetracycline at the rate of 80%, while Park et al. [10] indicated that tested *C. perfringens* stains exhibited a partial resistance to Tetracycline. The high level of resistance to this antimicrobial drug recorded in our study may be explained by the fact that Tetracycline is one of the most antibiotics used in the treatment of respiratory and digestive infections in Tiaret poultry farms. In addition, other investigations have shown that the resistance to this antibiotic has been attributed to the presence in clostridium strains of Tetracycline resistance genes, such as *tetA* and *tetB* [21,17,22,23].

As shown in Table 1, Erythromycin demonstrated a high level of resistance with 77.27%. Our results are partially in agreement with these reported by Osman and Elhariri [24] who identified 125 isolates of *C. perfringens* from broiler chickens displaying gross lesions suspected to be NE and found that all the isolates were resistant to Erythromycin. Additionally, Mwangi et al. [11] indicated that most *C. perfringens* isolates (67%) were resistant to

Erythromycin, and explained this resistant by its widespread use to treat *Mycoplasma gallisepticum* infection in poultry, which may contribute to increase the resistance of *C. perfringens* to this drug. Other studies reported that the resistance to macrolide group has been attributed to the presence of *ermQ* and *ermB* genes which codified enzymes responsible for the 23 SrRNA demethylation [25,26].

Table 2. Multidrug resistance results of 22 *C. perfringens* type A isolated from NE in broiler chickens

Number of antibiotics	Number of <i>C. perfringens</i> type A isolates	Percent of multidrug resistance (%)
3	7/22	31.81
4	6/22	27.27
5	2/22	9.09
6	4/22	18.18
Total	19/22	86.36

The Multidrug resistance was defined as resistance to more than two drugs [16]

Table 3. Profiles of antibiotic resistance to 22 *C. perfringens* type A isolated from NE in broiler chickens

	Co-amoxiclav G	Penicillin	Tetracycline	Erythromycin	Bacitracin	Clindamycin	Co-trimoxazole
1	R	S	R	R	S	S	R
2	S	S	R	R	S	S	R
3	S	S	R	R	S	S	R
4	S	S	R	R	S	S	R
5	S	S	S	R	S	R	S
6	S	S	R	R	S	R	S
7	S	S	R	R	S	S	S
8	R	S	S	R	R	R	R
9	R	S	R	R	R	S	S
10	R	R	R	S	S	S	S
11	R	S	R	S	S	S	S
12	S	R	R	R	R	R	R
13	S	S	R	R	R	S	R
14	S	R	R	R	R	R	R
15	R	S	R	S	S	S	R
16	R	R	R	R	S	R	R
17	R	S	R	S	R	S	R
18	R	R	R	R	S	R	R
19	S	S	R	R	R	S	R
20	R	R	R	R	S	S	R
21	R	S	S	R	S	R	R
22	S	R	R	S	R	S	S

Co-amoxiclav: amoxicillin-acid clavulanic; Co-trimoxazole: Trimethoprim-sulfamethoxazole;
R: resistant; S: sensible

In the current study, 68.18% and 50% of resistance were recorded for Co-trimoxazole and Co-amoxiclav, respectively. Park et al. [10] reported that isolated *C. perfringens* strains demonstrated a partial resistance to Co-trimoxazole. However, Llamco et al. [25] indicated that all tested strains were resistant to Sulphaquinoxaline and explained that Sulfamide is drug commonly used in the treatment of respiratory diseases in poultry. In contrast to our study, Co-amoxiclav showed an excellent activity against the evaluated *C. perfringens* strains [25], similar results were reported by several studies performed in Jordan, Norway and Sweden who suggested that Amoxicillin is the most effective antimicrobial against *C. perfringens* infection in poultry [17,18]. The partial resistance to Co-amoxiclav demonstrated in our study may be explained by the widespread use of this drug for prevention and treatment of omphalitis in the first days of broiler chickens in Tiaret poultry farms, which may contribute to the outbreak of resistant *C. perfringens* strains to this drug.

In regard to Bacitracin and Clindamycin, both of them presented a low rate of resistance (36.36%). Llamco et al. [25] found that 36% of the *C. perfringens* strains were resistant to Clindamycin, while Johansson et al. [17] recorded a low rate of resistance to Bacitracin in Swedish and Danish isolates, countries which this antimicrobial was no longer in use. Furthermore, Penicillin demonstrated a lowest rate of resistance in our study with 31.81%. Silva et al. [9] found that 100% of their studied *C. perfringens* isolates were susceptible to penicillin; likewise, Nhung et al. [27] concluded that penicillin and Bacitracin are the most effective drugs in the treatment and prevention of NE in poultry. We suggest that the low resistance rate recorded in the current study may be due to

the absence of using these drugs in prevention or treatment of infections in Algerian poultry farms, which may explain why *C. perfringens* had a low resistance to these drugs.

The results of the present study demonstrated that most *C. perfringens* type A isolates (86.36%) had a multidrug resistance profile that include Tetracycline, Erythromycin, Co-trimoxazole and Co-amoxiclav. Previous studies conducted by several researchers in different geographic regions concluded that *C. perfringens* strains, isolated from NE cases in poultry, had a multidrug resistance profile but this resistance was demonstrated to different antimicrobial drugs used in each region according to local or national policy for the use of antibiotics [24,10,11,12].

CONCLUSIONS

In summary, this study was carried out to evaluate *in vitro* susceptibility of *C. perfringens* type A strains, isolated from broiler chickens with NE in Tiaret province, to antimicrobial agents. Our results showed a widespread resistance to multiple antibiotics including Tetracycline, Erythromycin, Co-trimoxazole and Co-amoxiclav. However, *C. perfringens* type A isolates tested demonstrated a low resistance to Clindamycin, Bacitracin and Penicillin. We concluded that the highest rate of resistance was showed for commonly used antibiotics in comparison with antibiotics used in specific cases. More attention should be taken by veterinarians in choosing the most specific antimicrobial drug to combat NE in the field, and this by performing antimicrobial susceptibility to avoid the outbreak of multi-resistant *C. perfringens* strains. Moreover, the use of antibiotic alternatives such as competitive exclusion, organic acids, probiotics,

prebiotics and vaccines in conjunction with good management practices throughout the whole rearing cycle should be considered to improve the gut health, which may control NE outbreak and reduce the selection of antibiotic resistant *C. perfringens* strains.

This is the first report of antibiotics susceptibility of *C. perfringens* Type A isolated from broiler chickens with NE disease in Tiaret province (Western Algeria).

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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