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ISOLATION OF INTESTINAL *CLOSTRIDIUM SPIROFORME* FROM BROILER CHICKEN AND THEIR SUSCEPTIBILITY TO EIGHT ANTIBACTERIAL AGENTS *IN VITRO*

A.M. Abd El-Gwad and A.El-R-Thabet

Animal Health Research Institute, Assiut Regional Laboratory

ABSTRACT :

A total of 240 intestinal samples of broiler chickens (2–6) weeks, 90 from freshly dead broiler chickens showing enteritis and positive for coccidiosis in 66.7% of samples, 150 from slaughtered broiler with grossly normal intestine and negative for coccidiosis. All samples were collected from private farms at Assiut Governorate. According to morphological characters and biochemical reactions 2 isolates of *Clostridium spiroforme* were isolated with an incidence of 0.83%. *In vitro* sensitivity tests to eight different antibiotics showed that the examined isolates were highly sensitive to amoxycillin, ciprofloxacin, ampicillin and enrofloxacin.

INTRODUCTION :

Bladen *et al.*, (1964) reported that helically coiled gram-positive anaerobic organism (*Cl.spiroforme*) isolated from animal faeces and caecum contents. Kaneuchi *et al.*, (1979) recovered coiled spore-forming organism (*Cl.spiroforme*) from faeces of healthy chickens. The organism was proteolytic and non gelatinolytic, fermented glucose and produced terminal to subterminal spores. Cato *et al.*, (1986) stated that cells of *Cl.spiroforme* are non motile and gram positive and grows vigorously at a temperature between 20-50°C with an optimum of 45°C for most strains. Borriello *et al.*, (1986) studied the cellular morphology of the helically-colied bacterium (*Cl.spiroforme*) and they found that it consists of an ardered aggregation of numerous individual semicircular cells joined end to end. Peeters *et al.*, (1986) detected *Cl.spiroforme* by gram stain in 52.4% of 149 caecal samples of rabbits with enteritis complex and the iota-like toxin was present in 7.4%. Carman and Wilkins (1991) found that lincomycin and erythromycin were inactive against *Cl.spiroforme* in vitro conversely penicillin G was active. Ellis *et al.*,(1991) were found that an enterotoxaemia caused by *Cl.spiroforme* responsible for significant losses in commercial rabbits. Abd-ElGwad (1993) recovered one isolate of *Cl.spiroforme* from rabbits with an incidence of 0.61% in Assiut Governorate. Ebtehal (2000) isolated 2 strains of *Cl.spiroforme* out of 470 intestinal samples from broiler chickens in Assiut and El-Minia

Governorates with an incidence of 0.43% and she tested it against different antibiotics *in vitro.*

The present work was designed to cover the following items:

- Isolation and identification of *Cl.spiroforme* from broiler chickens at Assiut Governorate.
- *In vitro* sensitivity test of the isolated organisms against eight different antibiotics.

MATERIAL AND METHODS :

MATERIAL:

1- Samples:

A total of 240 intestinal samples were collected from freshly dead and freshly slaughtered broiler chickens (2-6 weeks old) were obtained from private farms at Assiut Governorate. A direct microscopic examination of intestinal scraping were carried out.

2- Culture media:

a- Cooked meat medium "Mast DM 120".

b- Neomycin blood agar medium. (neomycin sulphate solution was added to the media just before the addition of blood to make the final concentration of 150 μg/ml).

3- Media used for biochemical tests :

Sugar fermentation (glucose, lactose, maltose, sucrose and manitol), gelatin medium, glucose phosphate broth medium, peptone water, triple sugar iron agar (T.S.I.), urea agar base, semi-solid agar media.

4- Reagents, chemicals and stains used

were Kovac's reagent, urea, methyle red, andrade's indicator, Gram's stain, glucose 1%.

5-Gas-pak anaerobic jar "BBL-814-21":

It was used for production of anaerobiosis by using disposable hydrogen-carbon dioxide bags with socket.

6-Antimicrobial sensitivity discs:

were produced by Oxoid Laboratories including ampicillin (10 μg), ciprofloxacin (5 μg), enrofloxacin (5 μg), amoxycillin (25 μg), erythromycin (15 μg), gentamycin (10 μg), streptomycin (10 μg), lincomycin (2 μg).

METHODS:

1-Isolation and identification of Cl. spiroforme.

The intestinal tract of freshly dead and slaughtered broiler chickens were collected, and the intestinal, caecal contents were subjected to direct microscopic examination for coccidial infestation. Small pieces of the intestines with their contents from each sample were inoculated into cooked meat media tubes. The inoculated media were incubated anaerobically at 37°C for 48 hrs. subcultures from each of 48 hrs culture tubes were made on duplicated neomycin blood agar plates. One of the inoculated solid media was incubated anaerobically and the other aerobically at 37°C for 24 hr. Only strict anaerobic isolates were examined and identified for microscopical appearance, culture characters, motility then transfered to cooked meat medium for other biochemical tests as described by Konemann *et al.,* (1983), Kaneuchi *et al.,* (1979).

2- Sensitivity test:

The isolated *Clostridium spiroforme* were tested for sensitivity to different chemotherapeutic agents. One ml of 48 hr broth cultures was spread on the surface of blood agar. Antibiotic sensitivity discs were placed on the surface of seeded agar. Plates were incubated anaerobically at 37°C for 24 hr. The sensitivity was judged according to the diameter of clearance zone arround the disc (Perelman *et al.,* 1991).

RESULTS :

1-Isolation and identification of *Cl. spiroforme:*

The examined intestine of freshly dead broiler chickens showed enteritis varied from catarrhal to haemorrhagic and necrotic enteritis and ballooning of the intestine. Direct microscopic examination revealed that 60 (66.7%) samples were positive to intestinal and caecal coccidiosis of different degrees, while birds with grossly normal intestine were free from coccidia. All isolates were non haemolytic on neomycin blood agar. Gram stained smears of suspected colonies revealed that a semicircular gram positive rods which tended to join end-to-end to produce long spiral coiled forms. According to morphological and biochemical studies of suspected *Cl.spiroforme* organisms (sugar fermentation, Indole production, gelatin liquefaction, H_2S production, urease test, methyl red), 2 isolates were identified to be *Cl.spiroforme* (one isolate was noticed in birds positive for coccidiosis and the other isolate was recovered from salughtered birds with grossly normal intestine. Frequency and percentage of infection are summarized as presented in Table (1).

2-The effect of different antibiotics

on the isolated *Cl.spiroforme* isolates are illustrated in Table (3).

DISCUSSION :

In the present work we tried to throw some light on the role of the recently isolated enteric microorganism (*Cl.spiroforme*) from broiler chickens aged 2-6 weeks in Egypt.

Our results revealed recovery of *Cl.spiroforme* (0.83%) from the intestinal samples of freshly dead and freshly slaughtered broiler chickens and this is in agreement with Kaneuchi *et al.,* (1979) who found *Cl.spiroforme* in the faeces of healthy chickens and Ebtehal (2000) who recovered *Cl.spiroforme* from broiler chickens aged 2-7 weeks in Egypt but with low incidence (0.43%).

The morphological and biochemical characters of the newly discovered microorganisms (*Cl.spiroforme*) are summarized in Table (2) and it was similar to the data observed by Kaneuchi *et al.,*(1979); Cato *et al.,* (1986); Borriello *et al.,*(1986); Peeters *et al.,* (1986) and Abd ElGwad (1993).

***In vitro* sensitivity testing of the isolates to eight antimicrobial agents revealed that the isolates examined were highly sensitive to amoxycillin, ciprofloxacin, ampicillin and enerofloxacin, moderately sensitive to lincomycin, erythromycin while gentamycin, streptomycin had no effect at all. In this respect our results disagreed but to some extent to those reported by Carman and Wilkinson (1991) who found that erythromycin, lincomycin were inactive against *Cl.spiroforme* in vitro, conversly penicillin G was active. On the other hand, our results agreed with those reported by Ebtehal (2000) who found that *Cl.spiroforme* isolates were highly sensitive to ampicillin, ciprofloxacin, enerofloxacin but gentamycin, streptomycin had no effect.**

Finally, it may be concluded from the present investigation that *Cl.spiroforme* found in the intestine of broiler chickens but with small incidence so further studies are needed around the recently discovered *Cl.spiroforme* organism in broiler chickens especially for prevention and control.

Table (1): Frequency and percentage of isolated *Cl.spiroforme.*

Examined specimens	No. of samples	*Cl.spiroforme*	
		No.	%
Intestine of freshly dead and freshly slaughtered broiler chickens	240	2	0.83

Table (2): Morphological characters and biochemical identification of the suspected *Cl.spiroforme* isolates.

Gram stain	B.haemolysis	motility	spore location	Biochemical reactions											No. of reacted isolates
				Indole	M.R.	V.P	H_2S	Urease	gelatin hydrolysis	Acid production from					
										glucose	lactose	maltose	sucrose	mannitol	
+ve coiled spiral shaped bacteria	-	-	T/St	-	-	+	-	V	-	+	+	-	+	-	2

+ = positive - = negative V = variable T/St = terminal to subterminal

Table (3): Results of sensitivity of *Cl.spiroforme isolates*

Antimicrobial agents	Sensitivity of Cl.spiroforme isolates
amoxycillin	+++
ciprofloxacin	+++
ampicillin	+++
enrofloxacin	+++
lincomycin	++
erythromycin	++
gentamycin	R
streptomycin	R

+++ = highly sensitive ++ = moderate sensitive R = resistant

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عزل ميكروب الكلوستريديم سبيروفورمى من أمعاء بدارى التسمين ودراسة تأثير ثمانية أنواع من المضادات الحيوية عليه فى المعمل

عبد التواب محمد عبد الجواد وعبد الراضى ثابت

فى هذا البحث تم فحص عدد ٢٤٠ عينة امعاء لبدارى التسمين ٢-٦ أسابيع منها ٩٠ عينة أخذت من كتاكيت تسمين نافقة حديثاً وكان بها التهابات معوية مختلفة مع وجود إصابة بالكوكسيديا فى ٦٠ عينة بنسبة ٦٦,٧% ، ١٥٠ عينة لبدارى تسمين مذبوحة ، وكانت الأمعاء سليمة ظاهرياً ولا تحتوى على الكوكسيديا عند إجراء الفحص المجهرى ، وهذه العينات كلها كانت لمزارع خاصة فى محافظة أسيوط . أوضحت الخواص المورفولوجيية والتفاعلات البيوكيميائية عزل ٢ عترة من الكلوستريديم سبيروفورمى بنسبة ٠,٨٣%. وبإجراء اختبار الحساسية فى المعمل للعترات المعزولة لثمانية أنواع من المضادات الحيوية وجد أنها عالية الحساسية لكل من الاموكسيسلين ، السبروفلوكساسين ، الامبيسلين ، الانروفلوكساسين.