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STUDIES ON *E.COLI ISOLAT ES* FROM RESPIRATORY AFFECTED BROILERS AND PROTECTION EVALUATION OF DIFFERENT PREPARED BACTERINES

(With 3 Tables)

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دراسات على معزولات الميكروب القولونى من الجهاز التنفسى المصاب لبدارى التسمين وتقييم الحماية للقاح الميت والمحضر بطرق مختلفة

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فى هذه الدراسة تم تصنيف ١٩٠ عزلة إيشريشيا كولاى من الجهاز التنفسى المصاب لبدارى تسمين (عمر ٢-٦ أسبوع) من منطقة قناة السويس كما تم التصنيف السيرولوجى وتحديد شدة الضراوة وكذلك إجراء إختبار الحساسية (باستخدام ١٥ نوع من مضادات البكتريا). تم تحديد ١٢ نوع من الايشريشيا كولاى وهى كالتالى : ($O_{114}K$-, $O_{158}K$- .$O_{125}K_{70}$, $O_{119}K_{69}$, $O_{126}K_{71}$, $O_{86}K_{61}$, $O_{55}K_{60}$, $O_{28}K_{67}$, $O_{111}K_{58}$, $O_{26}K_{60}$, $O_{78}K$- and $O_{127}K_{63}$) لتحديد شدة الضراوة لعدد ٤٦ عزلة فى كتاكيت عمر أسبوعين وجد أن ١٤ عزلة شديدة الضراوة ، ٢١ عزلة متوسطة الضراوة و ١١ عزلات ضعيفة الضراوة . كما وجد أن معظم العزلات حساسة لمضادات البكتريا الآتية : الجنتاميسين ، الانروفلوكساسين ، الدانوفلوكساسين ، النورفلوكساسين. تم تقييم ثلاثة لقاحات ميتة من الايشريشيا كولاى بثلاثة طرق مختلفة ، الأولى باستخدام الفورمالدهيد ، الثانية باستخدام الاشعاع أما الثالثة فكانت باستخدام جهاز الموجات فوق الصوتية وجد أن اللقاحات الثلاثة حمت الكتاكيت عمر أسبوعين عند إجراء إختبار تحدى المناعة بنسب متفاوتة كما وجد أن اللقاح الذى تم تحضيره باستخدام جهاز الموجات فوق الصوتية أعطى أفضل نتائج بالمقارنة باللقاحين الآخرين .

SUMMARY

One hundred and ninety E.coli isolates were recovered from broiler chickens 2-6 weeks of age, with respiratory manifistation at Suez Canal area.

Serotyping of 46 isolates demonstrated a predominance of serotypes $O_{114}K$-, $O_{78}K$-, $O_{158}K$- and $O_{125}K_{70}$. Other eight serotypes were identified ($O_{119}K_{69}$, $O_{126}K_{71}$, $O_{86}K_{61}$, $O_{55}K_{60}$, $O_{28}K_{67}$, $O_{111}K_{58}$, $O_{26}K_{60}$ and $O_{127}K_{63}$). The virulence of the 46 identified isolates was confirmed in 2 weeks old chicks, 14 serotypes were highly pathogenic, 21 isolates were intermediate while 11 serotypes were low pathogenic. The in vitro sensitivity testing of 190 isolates using 15 antimicrobials revealed that the majority of the isolates were sensitive to Gentamycine. Enrofloxacin, Danoflaxacin and Norofloxacin. Three types of bacterine were prepared from the most virulent serotype ($O_{125}K_{70}$) by using formaldhyde, irradiation and sonication. Evaluation of protection were studied in three groups of 2 week old chicks. The preliminary results revealed that prepared bacterins could protect chickens vaccinated at two weeks of age and the degree of protection provided varried with the method of inactivation. Bacterin based on ultrasonic inactivation provided the best protection when compaired with other methods used for inactivation.

Key words: *Broilers - Respiratory affections E-coli - Bacterines*

INTRODUCION

Escherchia coli involved in many health problems in poultry, they may be a direct cause of disease i.e collibacillosis or coliseptcaemia and respiratory tract infection (Sojka and Cornaghan, 1961) or associated with other agents as IBV, NDV including vaccine strains, Mycoplasma, pasteurella sharing in what is known as Respiratory disease complex (Gross, 1991). Recently E.coli has been implicated in swollen head syndrome, dermatitis and cellulitis (Morley and Thomson, 1984; Randall *et al.*, 1984). In Egypt E.coli infection of broiler chickens is one of the major causes of increased mortality and condamenation (Awaad, 1972; Hassanin, 1977; Abdel El-Ghfar, 1979; Farid *et al.*, 1983; Mashhoor *et al.*, 1987; Ali, 1989; Khaled, 1990 and Zouelfakar, 1993). Control measures for E.coli associated diseases have depended mostly on the preventive hygienic, therapeutic use of antimicrobial agents and vaccination. However, the widespread transferrable resistance to antibiotic have been reported (Heller and Smith, 1973; Linton, 1979; Nazer, 1980; Rosenberger and Cloud, 1981; Allan *et al.*, 1993 and Ginns *et al.*, 1996). The prophylactic use of antimicrobial agents in animal

production may result in development of resistant bacteria to single or multiple antibiotics which can be transferred via plasmids within species, between species or between genera (Nivas *et al.*, 1976). The increasing resistance leads to a considerable attension to the development of E.coli vaccines especially in breeder flocks which could be of major importance to protect chicks during the first week post hatch (Heller, 1976; Rosenberger *et al.*, 1985). The potenial success of vaccination depend on the antigen used (Dep and Harry, 1976 and 1987; Melamed *et al.*, 1991). The objective of this study is to obtain a collection of pathogenic E.coli isolates from different pathological condition at Suez Canal area serotyping antibiogram, pathogenicity and to evaluate the immunogenicity of different prepared bacterins.

MATERIAL and METHODS

A) Material:

1-Experimental birds:

Day old chicks were obtained from commercial hatcheries, raised in an isolation, for use in laboratory trials, commercial feed without antimicrobial and drinking water were provided adlibitum.

2- Samples for bacteriological examination:

Lung, heart blood and liver samples were taken from moribund broiler chickens in Suez Canal area which suffered from respiratory problems.

B) Methods:

1- Bacteriological examination:

Tissues were streaked directly into MacConkey's agar plates and incubated at 37°C for 16-24 hr. A single lactose fermenting colony, furthest from the primary streak was selected and subcultured into nutrient agar then incubated at 37°C, 16-18 hr. for biochemical identification. Confirmation of E.coli isolates was done on the basis of cultural morphological, Gram staining and biochemical characterstic according to Cruickshank (1975).

2- Serotyping:

Fourty six isolates out of a total 190 identified isolates of E.coli were serotyped by using O & K antisera (Behringwerke, Marburg, Germany).

3- Antimicrobial sensitivity testing:

The sensitivity of 190 E.coli isolates was assessed using 15 different antibacterial agents produced by Difco, oxoid and upjhon. Disc diffusion

method was carried out by using Muller hinton agar media (Bauer *et al.*, 1966).

4- E.coli pathogenicity:

The relative pathogenicity of 46 serotyped E.coli was evaluated in two week old chicks. Five birds per serotyps were inoculated intra airsac with 0.2 ml. of 24 hr. nutrient broth culture containing 10^8 colony forming unit (CFU) of E.coli. Birds were observed daily for mortality and lesions. Live birds one week post inoculation were secraificed, examined for growth lesions and reisolation. E.coli serotypes were grouped according to there digree of pathogenicity (Rosenberger *et al.*, 1985).

5- Bacterin preparation:

Strain O_{125} K_{70} of E.coli suspended at a concentration of 10^8 colony forming unit (CFU)/ml, were inactivated by three different methods.

1- Inactivation by formaldhyde: A 37% of formaldehyde solution was added to bacterial suspension to a final concentration of 0.4% and incubated at 4°C for 48 hours with periodical shaking to prepare formaline inactivated E.coli bacterin (FIEB).

2- Inactivation by irradiation: The bacterial suspension was exposed to Gamma radiation at a dose of 25K rad at National Center for Radiation and Research Technology, Nasr City, Cairo to prepare irradiated killed E.coli bacterin (IKEB).

3- rasonicatin: E.coli suspension was ultrasonicated by using of an ultrasonic disintegration untill a transparent solution was obtained to prepare sonicated E.coli bacterin (SEB).

Each preparation was streaked on MacConkey agar for sterility verification. Inactivated suspensions were aliquated and stored at -20°C until used.

Experimental vaccination:

The immunogenicity of the prepared bacterin was studied using one hundred and fifty day old chicks which were reared under hygenic condition, for 2 weeks. Then devided into 5 equal groups. Groups from (1-3) were injected intramuscularly with 0.2 ml (FIEB, IREB or SEB) prepared bacterine respectively. Group (4) and (5) were left as non injected control, 10 days post vaccination chicks of groups 1-4 were challenged with 10^8 live bacteria per/birds. Group 5 was left as non challenged negative control. Chicks were observed daily for one week and mortalities were recorded. Surviving chicks were killed and inspected for lesions.

RESULTS

One hundred and ninty E.coli organisms were isolated from 2-6 week old broiler with respiratory manifistation from different flocks at Suez Canal area. Fourty six isolates were chosen for serotyping and pathogenicity study. In table (1) the predominant serogroups isolated were identified as $O_{114}K$-, (17.4%), 13% for $O_{158}K$-, $O_{78}K$- and $O_{125}K_{70}$, while $O_{119}K_{69}$ and $O_{126}K_{71}$ represent 8.7% and $O_{55}K_{60}$, $O_{111}K_{58}$ were at frequency of 6.5%. $O_{28}K_{67}$, $O_{26}K_{60}$ and $O_{127}K_{63}$ represent 4.3%. The lowest frequency (2.2%) was $O_{86}K_{61}$.

Pathogenicity:

Results of pathogenicity according to serogroup are presented in Table (1). Fourteen serotypes were highly pathogenic and 21 serotypes were intermediate while the low pathogenic were 11 serotypes. The most high pathogenic serotype was $O_{125}K_{70}$.

Antimicrobial characterization:

Table (2) shows the in vitro antimicrobial sensitivity results of 190 isolates for 15 types of antimicrobials. Organisms were arranged in a descending order according to frequency of sensitivity. More than 60% of the isolates were sensitive to Gentamycin, Enrofloxacin, Danofloxacin an Norofloxacin. While 20-50% of isolates were sensitive to Doxycycline, Neomycin, flumequine, Ampicilline and Chlorumphenicol, less than 17% of the isolates were sensitive to the remaining antimicrobials tested.

Protection evaluation of prepared bacterines:

Table (3) shows dead and live chicks that have lesions following challenge of three groups injected with different bacteriens and the control group.

Challenge of vaccinated groups showed protection which differed according to the type of used bacterine these as 76.7%, 73.3% and 80% for FIEB, IKEB and SEB while control groupe showed 6.7% protection.

DISCUSSION

In this study the pathogenicity of 46 isolated E.coli serotypes was evaluated using standard procedure described by (Rosenberger et al., 1985). The different serotypes could be evaluated as highly pathogenic (14 serotyped isolates), intermediate (21 isolates) while 11 isolates were less

pathogenic. There was a relationship between the pathogenicity and the frequency of isolated serotype.

In vitro antimicrobial sensitivity:

Our results indicat that most isolates (60-90%) were sensitive to Gentamycin, Enrofloxacin, Danofloxacin and Norofloxacin. To some extent our findings are in agrement with those of Ashgan *et al.*, 1996 and El-Gohary et al., 1996. Many of the other antibacterials, such as the Doxycycline, Flumequine, Streptomycin, Neomycin, ampicillin, Colistin and Chloramphenicol are effective for 20-50% of the isolates. More than 80% of the tested isolates were resistant to Amaxicilline, Oxytetracycline Oxalinic acid and Nalidixic acid. The resistance of the majority of isolates to common antibacterials reflect the extensive use or abuse of these agent. Certainly other methods for controlling E.coli should be evaluated, so that enaergence of resistant strains will be limited and the cost involved in prophylactic and therapeutic treatment programs will be reduced.

Our rêsults presented in Table (3) show that the inoculation of inactivated E.coli bacterines may confer protection to inoculated chicks and the degree of protection provided varried with the method of inactivation. Bacterine prepared by ultrasonic inactivation provided the best protection to inoculated chicks from challenge when compared with other methods used for inactivation. The efficiency of ultrasonic inactivation of bacterine in protection of chicks from challenge are in agreement with that obtained by Heller et al. (1990) and Melamed et al. (1991). This efficiency might be due to the ultrasonic disruption of the bacteria leads to expression of some important immunogenic determinants and the antibodies to these determinants may provide effective protection to inoculated chicks. These determinants might be remain obscured in other method of inactivation and therefore they were less protection.

Conclusion:

Prilimenary results revealed that prepared bacterins could protect chickens vaccinated at two weeks of age and the degree of protection provided varried with the method of inactivation. Bacterin based on ultrsonic inactivatioin provided the best protection when compaired with other methods used for inactivation with those bactrine.

The ultrasonic bacterine might be of use for vaccination of breeder flocks in order to produce chicks that are more resistant against colibacillosis during their first weeks of life.

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Table (1): Serotyping and pathogenicity of 46 E.coli isolates from respiratory tract of broiler chickens.

E.coli serogroup	Isolates		Pathogenicity per serogroup					
			High		Intermediate		Low	
	No	%	No	%	No	%	No	%
$O_{158}K$-	6	13	4	66.6	1	16.7	1	16.7
$O_{114}K$-	8	17.4	1	12.5	6	75.0	1	12.5
$O_{78}K$-	6	13	4	66.6	1	16.7	1	16.7
$O_{119}K_{69}$	4	8.7	0	0	1	25	3	75
$O_{126}K_{71}$	4	8.7	0	0	3	75	1	25
$O_{125}K_{70}$	6	13	5	83.3	1	16.7	0	0
$O_{86}K_{61}$	1	2.2	0	0	1	100	0	0
$O_{55}K_{60}$	2	43	0	0	2	100	0	0
$O_{28}K_{67}$	2	4.3	0	0	2	100	0	0
$O_{111}K_{58}$	3	6.5	0	0	3	100	0	0
$O_{26}K_{60}$	1	2.2	0	0	0	0	1	100
$O_{127}K_{63}$	2	4.3	0	0	0	0	2	100

Table (2): In vitro antibiotic characterization of E.coli isolates.

Antimicrobials		No. of tested isolates	Sensitive		Resistant	
			No	%	No	%
Gentamycin	10 µg	190	182	95.8	8	4.2
Enrofloxocin	10 µg	190	148	77.9	42	22.1
Danofloxacin	5 µg	190	140	73.9	50	21.1
Noroflaxacin	10 µg	190	117	61.6	73	38.4
Doxycyclin	30 µg	190	63	38.2	127	61.8
Flumequine	30 µg	190	63	38.2	127	61.8
Streptomycin	10 µg	190	51	26.8	139	73.2
Neomycin	30 µg	190	48	25	172	75
Ampicillin	10 µg	190	47	24.7	143	75.3
Colistin	25 µg	190	47	24.7	143	75.3
Choloramphenicol	30 µg	100	22	22	78	78
Naledixic acid	30 µg	180	8	4.4	172	95.6
Amoxicillin	25 µg	190	32	16.8	158	83.2
Oxytetracycline	30 µg	100	7	7	93	93
Oxalinic acid	2 µg	130	9	6.9	121	93.1

Table (3): Results of challenge of chicks injected with different bacterins.

Group	Bacterin	No. of birds per group	Challenge	Birds with lesions				Protection %
				dead	live	total	%	
1	Inactivated by formaldhyde	30	+	2	5	7	23.3	76.7
2	Inactivated by irradiation	30	+	3	5	8	26.7	73.3
3	inactivated by ultrasonication	30	+	0	6	6	20	80
4	-	30	+	7	18	25	83.3	6.7
5	-	30	non	0	0	0	0	0