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STUDIES ON PYOCIN TYPING, DERMONECROSIS AND PATHOGENICITY OF E.COLI RECOVERED FROM SEPTICAEMIC BROILER CHICKENS

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SUMMARY

E..coli was recovered from 59.00% of septicaemic broiler chickens suffering enteritis, perihepatitis and airsacculitis. Pyocin typing of chosen E. coli isolates revealed two types. The first type was able to grow in the presence of pyocin (PREC). The second type was unable to grow in the presence of pyocin (PSEC). There is no correlation between pyocin typing and dermonecrotic test or pyocin typing and pathogenicity for day-old chickens. But, there is a correlation between dermonecrotic test and pathogenicity for day-old chicks. Thus, virulent types of E.coli can be detected through the dermonecrosis of their whole cell lysates (WCL) in the skin of guinea-pigs.

INTRODUCTION

E. coli is the bacterial species recovered commonly in clinical laboratories. It has been incriminated in infectious diseases involving virtually every tissue and organ system. Stress resulting from food and water deprivation for 36-48 hours or from exposure to 42°C for one hour seemed to cause penetration of the pathogenic E.coli into the blood stream of chickens causing severe disease and mortality (Koneman et al., 1988; and Leitner and Heller, 1982).

It may be a primary or a secondary agent. As a secondary agent, Nakamura et al., (1992) suggested that infectious brounchitis virus may facilitate E.coli invasion into the lower respiratory tract of chickens. Generally, E. coli causes a variety of pathological conditions in chickens including colibacillosis (colisepticaemia), coligranuloma, egg peritonitis, salpingitis, omphalitis, synovitis, panophthalmitis, enteritis, tracheitis and localized abscesses as recorded by Jordan (1990) and Calenk et al., (1991).

From the economic point of view, the food intake of infected chickens with E.coli was reduced. It was accompanied by altering the growth of whole body, eviscerated carcass, skeletal muscles, heart and liver. Also, protein accumulation in muscles was severely inhibited. The infected chicks did not reach the same body weight as controls by day 30 post-infection (Tian and Baracos, 1989).

Accordingly, this investigation was done to determine the incidence of E.coli from septicaemic broiler chickens. Then , to study the pyocin typing, the dermonecrotic test in guinea-pigs and the pathogenicity for day-old chicks to some recovered E.coli isolates

MATERIAL AND METHODS

One hundred and fifty septicaemic broiler chickens of 25 days old were collected from El-Gharbia

Governorate. Intestine, liver, lung and air sacs of examined birds were cultivated onto the surface of MacConkey's agar and eosin methylene blue agar (EMB). The inoculated plates were incubated aerobically at 37°C for 24-72 hours. Suspected colonies of E.coli were subcultured onto the same media to get a pure culture and preserved on sem-solid agar for furthr studies. Morphological, cultural and biochemical identificaion of the isolates was made with the help of Koneman et al, (1988).

One hundred and twenty isolates of identified E.coli were pyocin typed by scrape and streak method following Gillies and Govan (1966) using a standard strain of Pseudomonas aeruginosa. Pyocin-types E.coli isolates were grown on brain heart ifusian broth, incubated aerobically at 37°C for 18-24 hours and centrifuged. Whole cell lysates (WCL) of these isolates were prepared from the sedimented bacterial cells by sonication as described by Kohler (1971). Then, the lysates were inoculated intradermally in guinea-pigs in a dose of 0.2ml.

Ten isolates of E.coli, 5 from each of PREC and PSEC, their WCL produced dermonecrosis in guinea-pigs, were administered orally in day old chicks in 1 ml dose containing 1.9 X 10^9 CFU. Five chicks were used for each isolate. Death of inoculated chicks was recorded durig one week post-infection. Reisolation of E.coli was done from the internal organs of dead chicks.

RESULTS AND DISCUSSION

E.coli predominates among the commensal bacteria in the healthy gut. It causes opportunistic infections in other parts of the body where there is some abnormality or impairment of defences . Colibacillosis (colisepticaemia) is one of these infections characterized by diarrhoea, enteritis, septicaemia and/or bacteremia. Viruses and coccidia may be involved (Collee et al., 1989; and Carter and Chengappa, 1991).

Table (1) demonstrates the recovery of E.(from 59.00% of the examined tissues septicaemic broiler chickens. Highest recov rate was from intestine (21.50%) followed by er (15.67%), lungs (12.17%) and air sacs (9.67 Recovery of E.coli from ill-chickens was recor by Ike et al., (1990), Vidotto et al., (1990), Pa grahy and Ling (1990), Joya et al., (1990); a Char and Rao (1991).

Pseudomonas aeruginosa produces four disti types of pyocins (R,F, trypsin-sensitive and tr sin-resistant S.pyocins). Individual strains of t organism may produce more than one type pyocins. Sensitive bacterial cells are killed follo ing attachment of pyocin to specific receptors the cell surface (Collee et al., 1989).

As shown in Table (2 & 4), two types of E.co were recognized on the basis of pyocin typin The first one, PREC was abled to grow in th presence of pyocin. The second type, PSEC wa unable to grow in the presence of pyocin. PSE isolates represented the highest rate (65.00%) i comparison to PREC isolates (35.00%).

There is no correlation between the pathogenicit of E.coli isolates and their pyocin typing as th mortalities among day-old chicks infected wit PREC or PSEC isolates were almost the same (80.00% and 88.00% respectively).

These results open the way to make further studies on the use of pyocin for the treatment of E.coli infection and for the epidemiological typing of E.coli isolates in poultry.

Cytotoxic necrotizing factor (CNF) was produced by E.coli as reviewed by Pohl et al., (1992) who detected CNF in 61 out of 115 E.coli strains, Also, Emery et al., (1992) found that 6 out of 80 (7.5%) E.coli isolates from fowl produced a heat-

Table (1): Incidence of *isolation of* *E.coli* from the examined tissues of septicaemic broiler chickens.

Examined tissues	Recovered *E. coli* isolates	
	Number *	Percentage **
Intestine	129	21.50%
Liver	94	15.67%
Lung	73	12.17%
Air sacs	58	9.67 %
Total	354	59.00%

* : Number of examined samples from each tissue was 150.

** : Percentages were calculated according to the total number of examined samples (600).

Table (2): Pyocin typing of studied *E. coli* isolates.

Examined tissues *	*E. coli*			
	PREC		PSEC	
	No.	% **	No.	% **
Intestine	8	6.67	22	18.33
Liver	11	9.17	19	15.83
Lung	14	11.67	16	13.33
Air sacs	9	7.50	21	17.50
Total	42	35.00	78	65.00

PREC : Pyocin resistant *E. coli*

PSEC : Pyocin sensitive *E. coli*

* : Number of pyocin typed *E. coli* isolates from each tissue was 30.

** : Percentages were calculated according to the total number of pyocin typed E. coli isolates (120).

Table (3): Results of dermonecrosis of whole cell lysates (WCL) of pyocin-typed *E. coli* isolates in guinea-pigs.

Examined tissues	The positive *E. coli* isolates for dermonecrosis					
	PREC (42)		PSEC (78)		Total (120)	
	No.	* %	No.	** %	No.	*** %
Intestine	3	7.14	6	7.69	9	7.50
Liver	4	9.52	4	5.13	8	6.67
Lung	4	9.52	5	6.41	9	7.50
Air sacs	2	4.76	8	10.26	10	8.33
Overall total	13	30.95	23	29.49	36	30.00

* : Percentages were calculated according to the number of PREC isolates (42).

** : Percentages were calculated according to the number of PSEC isolates (78).

*** : Percentages were calculated according to the total number of pyocin-typed E. coli isolates (120).

Table (4): Results of death of 1-day old chicks infected orally with five isolates from each of PREC and PSEC (their WCL produced dermonecrosis in guinea-pigs).

Examined tissues	Death of 1-day old chicks					
	PREC		PSEC		Total	
	No.	* %	No.	* %	No.	*** %
Isolate No. 1	5	100.00	4	80.00	9	90.00
Isolate No. 2	5	100.00	5	100.00	10	100.00
Isolate No. 3	4	80.00	4	80.00	8	80.00
Isolate No. 4	3	60.00	5	100.00	8	80.00
Isolate No. 5	3	60.00	4	80.00	7	70.00
Overall total ***	20	80.00	22	80.00	42	84.00

* : Percentages were calculated according to the number of infected chicks for each isolate (5).

** : Total Percentages were calculated according to the total number of infected chicks for each isolate from PREC and PSEC (10).

*** : Overall total percentages were calculated according to the overall total number of infected chicks (50).

abile toxin (LT) that was cytotoxic for both Vero and Y-1 cells and 18 (22.50%) E.coli isolates produced a distinct LT that was cytotoxic only for Vero cells.

In Tables (3 & 4), WCL of 36 out of 120 (30.00%) studied E.coli isolates produced dermonecrotic reactions in the skin of guinea-pigs. Moreover, the studied isolates of dermonecrotic E.coli produced high mortalities in day-old chicks (84.00%). So there is a correlation between the pathogenicity of E.coli and dermonecrotic test.

On the other hand, there is no correlation between pyocin typing and dermonecrotic test as PREC and PSEC isolates produced nearly the same results of dermo-necrotic reactions (30.95% and 29.49% respectively).

Correlation between the pathogenicity of E.coli and dermonecrosis was recorded by Rycke et al., (1990)., who identified two toxic categories of CNF in cell sonic extracts from 22 animal and human E.coli isolates. They caused both necrosis in rabbit skin and multinucleation in tissue cultures. The original toxic properties of these recognized categories of E.coli strains together with their association with enteritis and septicaemia suggest that these strains may play a role in pathogenicity.

On the other hand, Katiyar et al., (1992) examined the permeability response after intradermal injection of E.coli endotoxin. It included a chemotactic effect of basophils . Also, hyperaemia, oedema, necrosis and formation of perivascular lymphoid aggregates were recognized.

In conclusion, E.coli isolated from colisepticaemia in chickens can be typed by pyocin. Sensitivity of many strains of E.coli to pyocin requires further studies on the use of pyocin for the treatment of E.coli infection and for the epidemiological typing of E.coli isolates in poultry. Dermonecrotic test to E.coli whole cells lysate in the skin of guinea-pigs is a significant test for. detection of virulent E.coli isolates.

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