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IN VITRO AND *IN VIVO* CHARACTERIZATION OF SOME PATHOGENIC MICROORGANISMS ISOLATED FROM BROILERS IN BEHERA PROVINCE WITH SPECIAL REFERENCE TO THE EFFECACY OF *ALLIUM SATIVUM* "GARLIC"

(With 4 Tables)

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الخصائص المعملية والحياتية لبعض الميكروبات المعزولة من بدارى التسمين بمحافظة البحيرة مع إشارة خاصة لتأثير الثوم

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أجريت الدراسة على ٧٣ عينة من دجاج التسمين بمحافظة البحيرة ,تم عزل الميكـــروب القولونى والميكروب العنقودى الذهبى والميكروب الســبحى وميكــروب الســودوموناس وميكروب الهيموفيلس وميكروب البروتيس وميكـــروب الكلوســتريديا بنســبة ٦٥,٧%, ١٧,٨% ,٦,٨% ,٦,٨% ,٤,١% ,١,٣% و١,٣% وذلك حسب الترتيب. تـم تصنيـف عترات الميكروب القولونىوعددها ٤٨ عترةإلى ثلاث عترات أساسية وأختير كـــل مـــن الميكروب القولونى والميكروب العنقودى الذهبى لدراسة الخصاص المعملية والحياتية لها إضافة الى تأثير الثوم عليها. ودلت نتائج العدوى التجريبية بتلك الميكروبـــات أن إعطـــاء عصير الثوم المائى للطيور المصابة متزامنا مع العلاج بالمضادات الحيوية حسب نتـــائج اختبار الحساسية كان تأثيره جيدا.

SUMMARY

The study was carried out on 73 samples of broilers in Behera Province, The isolated Microorganisms were E.Coli, Staph.aureus, Streptococcus avium, Pseudomonas spp., Haemophilus paragalinarum, Proteus vulgaris, Clostridia spp. with an incidence of 65.7, 17.8, 6.8, 6.8, 4.1, 1.3, 1.3, percent respectively. The *In vitro* and *In vivo* characterization of some pathogenic microorganisms (E.Coli-Staph.aureus) were studied with special reference to the effecacy of

Allium sativum "Garlic", the best results were seen when both Garlic and the effective antibiotic were used.

Key Words: *Broilers, Allium Sativum, Garlic*

INTRODUCTION

One of the important factor affecting poultry industry is disease particularly on intensive production. Coliform infection (E.coli) plays a part include colisepticaemia, colibacillosis, chronic respiratory disease, air saculitis, yolk sac and navel infection resulting in significant losses. While Staphylococcosis refers to the various diseases of poultry and other avian species caused primarily by the bacterium Staphylococcus aureus causing lameness, swollen joints, spondolitis, subdermal staphylococcal abscesses, gangrenous dermatitis and staphylococcal septicaemia (Prukner, 1986; and Jensen and Skeeles 1989).

Among baby chicks, broilers and laying hens different E.coli serotypes 026,055,086,0111,0114,0119,0125,0126,0127,0128,0142, and 0158 were isolated by many investigators, (Sahar 1994; Torky et al., 1995).

Immunosuppression may contribute to the cause of complex diseases and immunization failures. Immunosuppression may increase a bird's susceptibilty to infection and to development of disease, and it may allow infectious agents to persist (Winterfield, 1989).

Control measures of E.Coli associated diseases were mostly relying on prophylactic measures and use of the therapeutic agents, However, increase of antibiotic resistance has been encountered on many sites where outbreaks have been occuring repeatidly (Rosenberger and Cloud, 1985). Antibiotic multiresistant which can resist more than one type of antibacterial agent is considered now as an acutual hazard both in human and/or Veterinary medical fields (Werner, 1986)

The misuse of antibacterials, and other antimicrobial agents have many side effects. However the herbal therapy if found to be effective serves at both synergist and corrective factors, the antibacterial properties of Allium sativum (Garlic) were studied by many investigators (Elnima et al., 1983; Black, 1985; Ray et al., 1992; Ahlam and Omayma 1996).

Investigations of antibacterial activity in plants particularly those largely consumed by humans is highly indicated because it may be considered in the future an effective, available and inexpensive source of antibacterial agent especially under the new international economic system (GAT) so, The aim of the present study was to discuss the character of most common microorganisms causing problems in broiler industry in Behaira province and the antibacterial effecacy of Allium sativum on these microorganisms.

MATERIALS and METHODS

Material:

A total of 73 samples from broiler private farms were collected, the birds were sent directly to the laboratory, sacrified and examined bacteriologicaly for isolation and identification of pathogenic microorganisms.

Culture media:-

Trials for isolation of bacteria were carried out by culturing on, blood agar,chocolate blood agar, broth nutrient agar, Manitol salt phenol-red agar, Baird parker's medium, Christen's urea agar, MacConkey's agar, peptone water, MacConkey broth, Glucose phosphate broth, nutrient broth, malonate broth, simmon citrate agar, Triple sugar iorn agar.

Reagents:

Gram's stain, Kovac's reagent, urea, Methyle red in absolute ethanol, rabbit plasma, H_2O_2 3%.

Antisera:-

The serological identification of E.coli was done using monovalent E.coli antisera produced by "*Behring*" against K and O antigens.

Sensitivety discs:

A-Antibiotic discs (ABD): commercial antibiotic discs produced by *(Oxoid)* includes: (Amoxycillin "Aml" 20mcg-Enrofloxacin "En" 5mcg-spictinomycin "Sp" 100mcg-gentamycin "Gm" 10mcg-streptomycin "S"10mcg-flumequene "Ub"30mcg-neomycin "N"30mcg-oxytetracyclin "Ot" 30mcg) and (lincospectin "Lin"100mcg which produced by *Upjhon*)- were used in this investigation.

B-Garlic discs (g): Sterile discs of Whatman filter paper were impregnated in aquaous garlic juice.

C-Antibiotic garlic discs (ABGD): was prepared after impregnation of the commercial antibiotic discs *(Oxoid)&(Upjhon)* in aquous garlic juice to take the symboles (Amlg-Enrg-Spg-Gmg-Sg-Ubg-Ling-Ng-Otg)

Methods:

Isolation,purification and identification of the isolates:

After surface sterilization of Liver, spleen and heart of freshly sloughtered birds,it were minced,using a sterile loop, a streaking over plates of blood agar, MacConkey's agar and Nutrient agar was occured, the inoculated plates were incubated at 37 °C for 18-24 hours .The colonies were examined according to their growth on various media, Films were prepared from the suspected pure isolates and stained with Gram's stain then examined microscopically ,the identification were carried out using the criteria adopted by Merchant and Packer (1969), Edwards and Ewing (1972), Cruckshank et al.,(1975) , Finegold and Martin (1982).

The in vitro and invivo sensitivity test:

Sixty one serovares of the bacterial isolates (48 were related to E.coli and 13 isolates of Staph.aureus strain) were used in this test using (ABD-GD-and ABGD).

Experimental procedures:

Sixty, one day old broiler chicks were kept under hygienic condition, they divided to 6 groups each of 10 chicks in a separate floor pen (A-B-C-D-E-F). The groups (A-C-E) at 6 day old were infected via the oral route with 1ml of the isolated Staphylococcus aureus strain containing a total count of $1.2x10^6$, the other groups (B-D-F) were infected also via the oral route with 1ml of the isolated E.coli strain 0114K-(C) containing a total count of $1.2x10^6$, all the groups were treated as illustrated in table (1):

Table 1. Experimental procedure:

Group	Treatment at			
	Day 3-5 old	Day 6 old	day 7-9 old	day10-12 old
A	Garlic juice in drinking water	oral infection with Staph.aureus	garlic juice in drinking water	No medication
B	Garlic juice in drinking water	oral infection with E.coli "0114k-(C)	garlic juice in drinking water	No medication
C	no medication	oral infection with Staph.aureus	no medication	Garlic juice in drinking wa +systemic enrofloxa "Cidotril-CID Co.-Egy according to Sensitivity tes
D	no medication	oral infection with E.coli "0114k-(C)	no medication	Garlic juice in drinki water+systemic enrofloxa "Cidotril-CID Co.-Egy according to Sensitivity test
E	no medication	oral infection with Staph.aureus	no medication	Systemic enrofloxa "Cidotril-CID Co.-Egy according to Sensitivity test
F	no medication	oral infection with "0114k-(C)	no medication	Systemic enrofloxa "Cidotril-CID Co.-Egy according to Sensitivity test

The chicks were kept under observation, sacrified birds were taken for reisolation and typing the isolated serovar after the treatment course according to Finegold and Martin(1982).

RESULTS and DISCUSSION

As shown in table (2) it was found that the pathogenic microorganisms isolated were as follows: Echerichia coli (48/73) in an incidence of 65.7%, Staphylococcus aureus (13/73) with an incidence of 17.8%, Haemophilus paragalinarum (5/73) with an incidence of 6.8%, Streptococcus avium (5/73) with an incidence of 6.8%, Pseudomonas spp (3/73) in an incidence of 4.1%, Clostredial spp. (1/73) in an incidence of 1.3%, Proteus vulgaris (1/73) in an incidence of 1.3% which means the most common pathogenic microorganisms were E.coli and Staphylococcus aureus.

The serotyping of the E.coli strains (No=48) revealed three serotypes which represented as [055K59(A) (No=24/48)- 0114K-(C) No.=(13/48), and 086K61(B) No.=(11/48)].

The *In vitro* sensitivity test (table 2) for the 48 different strains of E.coli [055K59(A)- 0114K-(C), 086K61(B)] revealed that a 37.5%, 20.8%, 20.8% and 16.6% of these isolates were sensitive to

enrofloxacin, spectinomycin, lincospectin, and flumequine respectively, moreover the sensetivity to Garlic (GD) revealed an 83.3%, while the sensitivity to (ABGD) ranged between 91.6-100%. Our results coincids with many authors (Hassan and Shawkat, 1982; Abonorage et al., 1992; and Ahlam and Omayma 1996) who reported a powerful bactericidal action of Allium sativum extract on E.coli.

On respect to Staphylococcus aureus it revealed a sensitivity of 100%, 83.3%, 33.3% to amoxycillin, enrofloxacin and lincospectin respectively.While the Garlic (GD) showed intermediate effectivness , this results disagree with Abonorage ,et al.,(1992) who reported high effecacy of garlic extract on Staph.aureus.

In contrast the (ABGD) showed a synergistic action represented in an 100%, 100%, 66.6%,66.6% and 50% inhibition with (amoxycillin, enrofloxacin, spectinomycin, lincospectin and gentamycin).

The experimental infection to the chicks with serovars 0114K-(C), and Staph.aureus with oral route showed a morbidity rate of 100% in all groups after 72 hours of the infection except group Aand B which showed (4/10) 40% morbidity in each, which means that garlic reduced the morbidity rate when offered to this groups (A-B) three days before and after infection through its antibacterial action and stimulation of the host's defense mechanism. Petkov, (1985) reported that Garlic was found to stimulate the reticuloendothelial system of treated animals.

The sacrified birds from group (B,D, and F) showed sever congested lungs airsaculitis, perihepatitis,enlarged heart muscles, enlarged gall bladder. These results agree with these of Butura et al., (1973). while the subcutaneous haemorrhages, peticheal haemorrhage on thigh, brest the provent, and coloaca, with nicrotic focci in liver were the most prodominant in the sacrified birds from groups (A,C,and E) the *In vivo* results (table 3) revealed a highly synirgistec action of garlic juice when offered in drinking water with the systemic injection of enrofloxacin (Cidotril) which used according the *In vitro* sensitivity test represented as failure of reisolation of the infected strain (0114K-(C) (Group D), from the sacrified birds while a few scattered colonies (4 colonies) of Staph aureus could be isolated from the sacrified bird (groupC).That means the garlic juice when used in parallel to the effective antibiotic have a powerful synergistic action.These findings were in complete agreement with the results of

Ahlam and Omayma (1996) who reported that the watery extract of Allium sativum juice evoked high antibacterial effects against most of bacterial strains.

The results in group (B-F) a very small number (12, to 14 colonies) of E.Coli strain 0114K-(C) could be isolated, In case of group (A-E) the number of colonies which reisolated were much more (47and 44 Staph.aureus colonies) respectively.

In conclusion the using of Garlic juice alone (group A-B) was not completely satisfied to overcome the infected pathogens (E.coli-Staph.aureus), the same results for the antibiotic group (E-F) but the best results were seen when both Garlic and the sensitive antibiotic were used so it is so important to put garlic on its right position through further investigations.

The misuse of antibacterial agents especially in the small private broiler industry have many side effects, since the key pathogen for the flock disease is the E.coli and from the obtained results we can advice in prophylactic measures the periodic using of garlic juice in broiler flocks can be an alternative path way to many antibiotcs and in combination with the effective antibiotics in cases of problems sustain its antibacterial action, on the other hand it may help to some extent in minimizing the risk of bacterial resistance to the antibacterial in both human and veterinary fields.

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Table 2. Incedence of the pathogenic microorganisms isolated from Broilers In Behera Province

Microorganism	No.	Serotyping	Percent
E.coli	48	055K59(A) -NO=24 0114K(C)--No=13 086K61(B)_No=11	65.7
Staphylococus aureus	13		17.8
Streptococcus avium.	5		6.8
Pseudomonas spp.	5		6.8
Haemophilus paragalinarum	3		4.1
Proteus vulgaris	1		1.3
Clostridia spp.	1		1.3
Total	73		100

Table 3. The in vitro sensitivity test to the different serovars of E.Coli & Staph.aureus.

	055K59(A) (No.=24)			0114K(C)(No.=13)			086K61(B) (No.=11)			Total No=48			Total No. of Staph.aureus (13)		
	s	i	r	s	r	s	I	R	s	s	i	r	S	i	r
Aml	4	8	12	7	7	2	3	6	9		14	25	4	3	6
Amlg	24			13		11			48						
Enr	12	12		6	2	3	6	2	21		23		5	5	3
Enrg	24			13		11			48						
Sp	4	4	16	2	7	1	5	5	7		16	28	1	2	10
Spg	24			13		11			48						
Gm	4	16	4	3	4	1	6	5	8	28	12		2	5	6
Gmg	24			13		11			48						
S		4	20		13			11	48						
Sg	24			13		11			48						
Ub	4	4	16	5	6	1	4	6	10	10	24		2	3	8
Ub	24			13		11			48						
Lin	4	12	8	5	3	2	5	4	9	17	25		2	3	8
Ling	24			13		11			48						
N		8	16		4		4	7		21	27		1	1	11
Ng	24			13		11			48						
Ot			24		13		1	10		1	47			1	12
Otg	24			13		11			48						
G	24			13		11			48						

S=sensitive
I=intermediate
r=resist

Table (4) The clinical improvement and bacterial isolates after treatment for the different groups

Groups	Morbidity	mortality	Clinical improvement	Number of isolated Bacterial colonies fro sacrified birds
A	3	-	6	47 colonies of Staph.aureus
B	3	-	6	12 colonies of E.coli
C	1	-	8	4 colonies Staph.aureus
D	1	1	7	2 colonies of E.coli
E	3	-	6	44 colonies of Staph.aureus.
F	4	-	5	14 colonies of E.coli