ANTIBACTERIAL AND IMMUNOLOGICAL EVALUATION OF GENTAMICIN AND SPECTINOMYCIN IN CHICKENS

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ABSTRACT

Two hundreds and ten one day old hubbard chicks were used in this study. They were divided into 6 groups. The first group was kept as control. The second group was experimentally infected with 0.2 ml of broth containing 10^{-8} C.F.U. of S. pullorum given orally and left as positive control. The third group was experimentally infected orally with S. pullorum and intramuscularly injected with gentamicin; 5 mg/kg b.wt. for 5 successive days.

The fourth group was experimentally infected with S. pullorum and injected I/M with spectinomycin 20 mg/kg b.wt. twice with one day apart. The fifth group was injected I/M with gentamicin in the same dose regimen, while sixth group was I/M treated with spectinomycin 20 mg/kg b.wt. twice with one day apart. Non infected and gentamicin treated birds demonstrated significant decrease in total proteins. Infected non treated birds showed a significant increase in the total leucocytic count and serum total proteins. While treatment of infected birds with spectiomycin elicited a significant decrease in total leucocytic count. Non infected and spectinomycin treated birds divulged non significant changes in the total leucocytic counts. Both gentamicin and spectinomycin exerted more inhibitory effect on S. pullorum compared with other aminoglycosides, colistin and tetracyclines but less potent compared with chloramphenicol and enrofloxacin . It was concluded that both gentamictn & spectinomycin devoid any significant effects on chicken immune system turned by S. pulorum infection and high efficacy of both drugs either In vivo or In vitro.

INTRODUCTION

The consensus amongst the veterinary clinicians is that aminoglycosides are highly effective in treatment of diseases caused mainly by gram negative bacteria **(Sande and Mandel, 1985)**. Gentamicin has been proved as a broad spectrum aminoglycoside, being superior than other

commonly used antibiotics **(Kirby & Saniford, 1969)**.

In a like manner, high efficacy of spectinomycin, with a number of properties common with aminoglycosides, was evinced against Colibacillosis & Salmonellosis in chickens **(Hardi, 1973)**. Some chemotherapeutics have immunosuppressive effect either by their ability to interfere with protein synthesis by promoting rapid elemination of antigens, or by interferance with phagocytosis **(Kruger, 1965)**.

The current study was carried out to evaluate the efficacy of both gentamicin & spectinomycin against chicken Salmonellosis and highlighting their possible effect on the immune system.

MATERIAL AND METHODS

Drugs :

Gentamicin : Gentamicin 5% injection (Gentamicin)_ Egyptian Co. for Chemicals and Pharmaceuticals (ADWIA), Egypt.

Spectinomycin : Spectinomycin injection (Spectam.)_ CEVA Abbot Co., France.

Experimental chicks :- Two hundreds and ten healthy, one day old hubbard broiler chicks were obtained from "Star Farms Co., Tanta, Egypt". The chicks were reared under hygienic conditions and were fed on a balanced commercial starter poultry ration. At the age of 8 days they were divided into 6 groups. The forty five chicks in the first group received neither infection nor medications (control). The Sixty chicks in the second group were experimentally infected orally with 0.2 ml of broth containing 10^8 C.F.U. of S. pullorum **(Heller & Drabkin, 1977)**. The twenty five chicks in third group were experimentally infected and after 2 days they were intramuscularly injected with gentamicin; 5mg/kg b.wt. for 5 successive days **(Greg & Linda, 1986)**. The twenty five chicks in the fourth group were experimentally infected and intramuscularly injected with spectinomycin; 20mg/kg.b.wt. twice with one day interval **(Nicholas & Leslie, 1986)**.

The thirty five chicks in the fifth group were intramuscularly treated with gentamicin; 5 mg/kg b.wt. for 5 successive days. The twenty chicks in the sixth group were intramuscularly treated with spectinomycin, 20mg/kg b.wt. twice with one day interval .

Blood sampling :- Five blood samples from each group were collected after slaughtering on the 1^{st}, 7^{th}, 14^{th} and 21^{st} days post treatment. Each sample was divided into two parts. The first part : 5 ml was taken in clean, dry vials containing EDTA to be used for the total leucocytic count **(Natt & Herick, 1952)** and differential counts **(Schalm et al., 1975)**. The second part :- (5ml) was taken without anticoagulant in sterile Wasserman tube for separation of serum which is kept at - 20°C until used for estimation of total serum proteins **(Weichselbaum, 1946)** and its

fractions **(Laemmli, 1970).**

Antibacterial activity :-

1) In vitro : Sensitivity of pathogenic microorganism (S.pullorum) to gentamicin and spectinomycin in comparison with other antimicrobials was studied using the disc diffusion method **(Bauer, 1963).**

2) In vivo :- Clinical signs, P.M. findings, mortality rate were recorded during the experiment.

- Statistical analysis : Data obtained were statistically analysed using Student "t" test **(Snedecor & Cochran, 1967).**

RESULTS

Chicks treated with gentamicin and non infected showed a significant decrease in serum total proteins on the 2nd and 3rd week post treatment and a significant decrease in serum albumin, a, b and s globulins in the 2nd & 3rd week post treatment (Table 1). Chicks infected with S. pullorum and treated with spectinomycin showed a significant decrease in total leucocytic count on the 14th and 21st day post treatment (Table 2). They portrayed also a significant increase in the serum albumin level on the 2nd and 3rd week post treatment (Table 3), along with a significant decrease in the serum a globulin level on the 2nd week. Whereas serum s globulins level showed a highly significant decrease on the 2nd and 3rd week post treatment compared with infected non treated (Table 6).

The experimental infection of chicks with S. pullorum evoked a highly significant increase in the total leucocytic count and serum total protein on the 1st, 7th, 14th and 21st day post infection compared with control group (Table 4 & 5). As well as a significant decrease in the serum albumin (Table 5). Serum a and s globulin level demonstrated a highly significant increase. Serum b globulins level were non significantly affected (Table 1) .

Birds experimentally infected with S. pullorum and treated with gentamicin demonstrated non significant decrease in the total leucocytic count on the 1st day post treatment and they displayed a highly significant decrease on the 7th, 14th and 21st day post treatment compared with infected, non treated group (Table 4). They showed also a decreased total serum protein on the 7th & 14th day post treatment (Table, 5). They illustrated also a highly significant decrease in a globulins, on the 7th and 14th day post treatment (Table 1). Gamma globulins level depicted highly significant decrease on the 7th, 14th and 21st day post treatment compared with the infected non treated group (Table 1). Non infected, spectinomycin treated chicks showed non significant changes in total serum proteins and its fractions (Tables 3, 6) .

Antibacterial activity :

1) **In vitro** : Both gentamicin & spectinomycin exerted a more potent inhibitory effect on S. pullorum compared with other aminoglycosides, colistin and tetracyclines and less potent than chloramphenicol and enrofloxacin. In the same line, gentamicin has been found to possess a more potent inhibitory effect on S. pullorum compared with spectinomycin (Table 7).

2) **In vivo** : Chicks experimentally infected with S. pullorum and non treated displayed characteristic clinical symptoms represented by depression, loss of appetite, poor growth, dropped wings, ruffled feathers and whitish faeces covered vents .

- Chicks infected and treated either by gentamicin or spectinomycin showed milder degree of clinical symptoms compared with infected, non treated.

Mortality rate was 15% in S. pullorum infected, non treated. On the other hand deaths were not recorded in other groups. Gross pathological lesions in non treated birds, experimentally infected were enlarged & congested liver, enteritis, pericarditis, enlarged kidneys, spleen and haemorrhagic pneumonia .

DISCUSSION

In the present study, there has been bountiful evidence that healthy birds administered gentamicin in a therapeutic regimen displayed non significant effects on total leucocytic count throughout the experimental period. Our findings fit in with the results previously obtained **(Elchenworld, 1966)**, Who recorded that aminoglycosides failed to elicit significant effect on the leukogram in infants.

It has been shown that healthy birds treated with therapeutic regimen of gentamicin divulged a significant decrease in the serum total proteins, albumin, a, b and s globulins levels on the 14th and 21st day post treatment. No better evidence of the significant reduction in the serum total proteins, albumin and globulins levels can be cited than an alleged suppressive effect of gentamicin on the humoral immune response. The observed inhibition of mice immune response proved by different doses of gentamicin, have suggested that a suppression of the immune system is far and away the likeliest candidate **(Artsimovich, 1991)**. Gentamicin may cause a decrease in synthesis of plasma proteins in liver and lead to appearance of protein in large amounts in the urine **(El-Deeb, 1995)**.

In the face of absence of an antigen, that could be represented by Salmonella microorganism in our study, a conceivable immuno suppressive effect of gentamicin could be easily detected.

On the same basis **(Exon et al., 1989)**, stated that a possible immuno-suppressive effect of gentamicin based on the concept that the drug, in high doses, produces modest increase in the granulocytes adherence and decrease their migration. The significant reduction in the total serum proteins and its fractions is due to the effect of gentamicin on both hepatic and renal cells function. Our results coordinated with that previously reported **(Pashove et al., 1989 and Koesk et al., 1974)**. They reported degenerative changes in hepatic & renal tissues.

It has been evidenced that, multiple oral administration of spectinomycin evoked leucopenia in dogs **(Brown et al., 1990)**. In fact, this apparent controversy could be best resolved in the view of the notion that leukopenia could be set down to the long duration of drug administration which may depress the granulopaeisis in the bone narrow **(Eisa, 1998)**.

Administration of spectinomycin in therapeutic regimen, evoked no significant effect on the level of serum total proteins or any of its fractions throughout the experimental period. Nearly similar results has been previously reported **(Fathy, 1995)**. The author found no alteration in the picture of the serum total proteins, albumin and globulin fractions post treatment by spectinomycin (20 mg/kg b.wt.) twice with one day interval in chickens.

Non treated chicks, experimentally infected with S. pullorum disclosed significant increase in the serum total proteins on the 1^{st}, 7^{th} & 14^{th} day post treatment compared with control. He assigned the increase in the serum total proteins to the increase of globulins level more than the decrease in the albumin level in response to immunogen. Chicks experimentally infected with S. pullorum and administered gentamicin, in therapeutic regimen showed significant increase in the total leucocytic count. Our results coordinated with **(Wenstein, 1975)** who mentioned that there were a significant increase in total leucocytic count and heterophils in chicks experimentally infected with S. pullorum & administered gentamicin in therapeutic regimen in the 1^{st} week post-treatment.

Chicks experimentally infected with S. pullorum and treated with the therapeutic regimen of spectinomycin elicited a significant decrease in the serum total proteins on the 14^{th} day post treatment with significant increase in the albumin level on the 14^{th} & 21^{st} day post treatment **(Eisa, 1998)**, credited this findings to the regenerative power of hepatic cells that were recovered from the lethal effect of S. pullorum infection under the spectinomycin therapy.

Both gentamicin & spectinomycin exert a potent inhibitory effect on S. pullorum either in vitro or in vivo. Our result were compatable with **Olga et al. (1973)**, they stated that gentamicin given via drinking water or injection was effective in controlling Salmonella in chickens. Our outcome is highly commendable by **Smith & Tucker (1975)**. The author recorded that, spectinomycin in a dose level of 50 mg/kg b.wt. controls efficiently S. typhimurium infection in chickens.

Table 1 : Effect of gentamicin in therapeutic regimen; 5 mg/kg.b.wt. administered intramuscularly once a day for 5 successive days on the serum a, b and s-globulins level (gm/dL) in both healthy and experimentally S.pullorum infected chicks. Mean ± S.E. n = 5.

Group	Fraction	Time (post treatment)			
		1st day	7th day	14th day	21st day
Control (non-infected, non treated)	α	0.61 ± 0.02	0.64 ± 0.01	0.66 ± 0.03	0.69 ± 0.01
	β	0.73 ± 0.01	0.75 ± 0.02	0.80 ± 0.01	0.79 ± 0.02
	σ	0.43 ± 0.01	0.45 ± 0.03	0.41 ± 0.02	0.40 ± 0.01
Non infected + gentamicin	α	0.59 ± 0.02	0.62 ± 0.01	0.55 ± 0.02*	0.56 ± 0.05*
	β	0.72 ± 0.01	0.72 ± 0.02	0.71 ± 0.07*	0.70 ± 0.03*
	σ	0.41 ± 0.03	0.44 ± 0.01	0.35 ± 0.01*	0.33 ± 0.02*
Infected, non treated	α	0.93 ± 0.03***	0.97 ± 0.03***	0.97 ± 0.02***	0.98 ± 0.03***
	β	0.74 ± 0.02	0.74 ± 0.03	0.71 ± 0 06	0.70 ± 0.06
	σ	0.86 ± 0.03***	0.98 ± 0.05***	0.88 ± 0.05***	0.72 ± 0.02***
Infected + gentamicin	α	0.91 ± 0.03	0.71 ± 0.05 ++	0.76 ± 0.04 ++	0.69 ±0.04
	β	0.70 ± 0.03	0.76 ± 0.02	0.82 ± 0.01	0.74 ± 0.02
	σ	0.85 ± 0.03	0.72 ± 0.03 ++	0.68 ± 0.02 ++	0.50 ± 0 04 ++

* P < 0.05 ** P < 0.01 *** P < 0.001

(* Compared with the control group) (+ Compared with the infected non treated group)

Table 2 : Effect of spectinomycin in therapeutic regimen; 20 mg/kg.b.wt. administered intramuscularly twice with one day interval on the total leucocytic counts (X 103 cells /mm3) in both healthy and experimentally S. pullorum infected chicks. Mean ± S.E. n = 5

Group	Time (post treatment)			
	1st day	7th day	14th day	21st day
control (non-infected, non treated)	30.30±0.30	30.60±0.99	31.80±0.70	32.10±0.94
non infected + spectinomycin	30.50±0.69	30.30±0.78	31.00±0.35	31.70±0.41
infected, non treated	52.90±1.03***	55.20±1.08***	56.10±0.60***	46.60±0.80***
infected + specti-no-mycin	52.10±0.70	54.30±1.06	49.80±0.75+++	40.80±1.12++

* P < 0.05 ** P < 0.01 *** P < 0.001

(* Compared with the control group) (+ Compared with the infected non treated group)

Table 3 : Effect of spectinomycin in therapeutic regimen; 20 mg/kg.b.wt. administered intramuscularly twice with one day interval on the serum total proteins level and the Serum albumin level (gm/dL) in both healthy and experimentally S.pullorum infected chicks. Mean ± S.E. n = 5.

Group	Serum	Time (post treatment)			
		1st day	7th day	14th day	21st day
Control (non-infected, non treated)	Total proteins	4.10 ± 0.05	4.20 ± 0.05	4.50 ± 0.09	4.30 ± 0.06
	Albumin	2.28 ± 0.09	2.30 ± 0.10	2.46 ± 0.16	2.34 ± 0.07
non infected + spectinomycin	Total proteins	3.94 ± 0.18	4.30 ± 0.09	4.40 ± 0.09	4.20 ± 0.11
	Albumin	2.13 ± 0.08	2.40 ± 0.11	2.44 ± 0.19	2.35 ± 0.07
Infected , non treated	Total proteins	4.46 ± 0.08**	4.49 ± 0.06**	4.80 ± 0.05*	4.60 ± 0.04**
	Albumin	1.96 ± 0.08*	2.00 ± 0.06*	2.00 ± 0.07*	2.05 ± 0.06*
Infected + spectinomycin	Total proteins	4.36 ± 0.09	4.42 ± 0.10	4.60 ± 0.07 +	4.37 ± 0.07
	Albumin	1.90 ± 0.09	1.92 ± 0.05	2.20 ± 0.02 +	2.25 ±0.04 +

* P < 0.05 ** P < 0.01 (* Compared with the control group)

(+ Compared with the infected non treated group)

Table 4 : Effect of gentamicin in therapeutic regimen; 5 mg/kg.b.wt. administered intramuscularly once a day for 5 successive days on the total leucocytic counts (X 103 cells /mm3) in both healthy and experimentally S. pullorum infected chicks. Mean + S.E. n = 5.

Group	Time (post treatment)			
	1st day	7th day	14th day	21st day
control (non-infected, non treated)	30.70 ± 0.34	31.20 ± 0.46	31.40 ± 1.13	30.20 ± 1.08
non infected + gentamicin	30.90 ± 0.29	31.10 ± 0.60	30.20 ± 1.01	29.00 ± 0.82
Infected, non treated	55.30 ± 1.10***	55.50 ± 0.55***	57.30 ± 0.51***	47.20 ±0.72***
Infected + gentamicin	54.50 ± 0.63	50.80 ± 0.34+++	46.90 ± 0.64+++	37.80±0.82+++

*** P < 0.001 (* Compared with the control group)

(+ Compared with the infected non treated group)

Table 5 : Effect of Gentamicin in therapeutic regimen; 5 mg/kg.b.wt. administered intramuscularly once a day for 5 successive days on the Serum total proteins level and serum albumin level (gm/dL) in both healthy and experimentally S. pullorum infected chicks. Mean + S.E. n = 5.

Group	Serum	Time (post treatment)			
		1st day	7th day	14th day	21st day
Control (non-infected, non treated)	Total proteins	3.90 ± 0.17	4.00 ± 0.09	4.20 ± 0.13	4.10 ± 0.07
	Albumin	2.13 ± 0.04	2.16 ± 0.06	2.33 ± 0.10	2.22 ± 0.08
Non infected + gentamicin	Total proteins	3.80 ± 0.17	3.90 ± 0.09	3.65 ± 0.17*	3.53 ± 0.18*
	Albumin	2.07 ± 0.04	2.11 ± 0.03	2.01 ± 0.08*	1.93 ± 0.09*
Infected , non treated	Total proteins	4.48 ± 0.09*	4.65 ± 0.19*	4.55 ± 0.07*	4.35 ± 0.15
	Albumin	1.95 ± 0.05*	1.91 ± 0.07*	2.00 ± 0.05*	2.11 ± 0.04
Infected + gentamicin	Total proteins	4.40 ± 0.23	4.12 ± 0.07+	4.30 ± 0.08 +	4.09 ± 0.09
	Albumin	1.94 ± 0.07	1.90 ± 0.06	1.98 ± 0.04	2.10 ± 0.07

* $P < 0.05$ (* Compared with the control group)

(+ Compared with the infected non treated group)

Table 6 : Effect of spectinomycin in therapeutic regimen; 20 mg/kg.b.wt. administered intramuscularly twice with one day interval on the serum a, b and s-globulins level (gm/dL) in both healthy and experimentally S.pullorum infected chicks. Mean + S.E. n = 5.

Group	Fraction	Time (post treatment)			
		1st day	7th day	14th day	21st day
Control (non-infected, non treated)	α	0.63 ± 0.02	0.69 ± 0.02	0.71 ± 0.01	0.69 ± 0.04
	β	0.77 ± 0.02	0.74 ± 0.02	0.89 ± 0.03	0.88 ± 0.03
	σ	0.42 ± 0.02	0.47 ± 0.02	0.44 ± 0.05	0.39 ± 0.01
non infected + spectinomycin	α	0.64 ± 0.03	0.70 ± 0.02	0.70 ± 0.03	0.65 ± 0.04
	β	0.74 ± 0.02	0.75 ± 0.01	0.85 ± 0.04	0.84 ± 0.05
	σ	0.43 ± 0.02	0.45 ± 0.02	0.41 ± 0.04	0.36 ± 0.03
Infected , non treated	α	0.94 ± 0.01***	0.91 ± 0.02***	0.90 ± 0.02***	0.80 ± 0.04
	β	0.78 ± 0.03	0.77 ± 0.01	0.95 ± 0.02	0.90 ± 0.04
	σ	0.80 ± 0.03***	0.82 ± 0.04***	0.87±0.02***	0.70±0.02***
Infected + spectinomycin	α	0.91 ± 0.04	0.88 ± 0.01	0.79 ± 0.01++	0.73 ± 0.03
	β	0.76 ± 0.01	0.80 ± 0.03	0.90 ± 0.05	0.89 ± 0.02
	σ	0.79 ± 0.01	0.78 ± 0.01	0.72 ± 0.04 ++	0.50±0.03++

* P < 0.05 ** P < 0.01 *** P < 0.001

(* Compared with the control group) (+ Compared with the infected non treated group)

Table 7 : Sensitivity of S. pullorum to gentamicin and spectinomycin in comparison with some antimicrobial agents. Mean ± SE (n = 5).

Antimicroblal agents discs	Mark	Potency of disc (ug)	Inhibition zone diameter in (mm)
Chloramphenicol	C	30	22.4 ± 0.2
Colistin	CL	25	12 ± 0.12
Enrofloxacin	Enr.	5	28.7 ± 0.31
Gentamicin	GN	10	18.55 ± 0.11
Neomycin	N	10	10.91 ± 0.15
Spectinomycin	SH	25	16.01 ± 0.5
Streptomycin	S	10	13 ± 0.22
Tetracyclines	TE	30	14.1 ± 0.25
Toblamycin	Tob.	10	14.31 ± 0.31

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الملخص العربى

التقييم المناعى والمضاد للبكتريا للجنتاميسين والاسبكتينومايسين فى الدجاج

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أجريت هذه الدراسة لتقييم التأثير المضاد للبكتريا للجنتاميسين والاسبكتينومايسين وكذا الاستجابة المناعية للدواجن المعداه بميكروب السالمونيلا بللورم وذلك باستخدام الجنتاميسين بجرعة ٥مجم/كجم من وزن الطائر حقناً فى العضل لمدة ٥ أيام متتالية والاسبكتينوميسين بجرعة ٢٠مجم / كجم من وزن الطائر حقناً فى العضل مرتين يوم بعد يوم. ولقد أجريت هذه الدراسة على ٢١٠ كتكوت هابارد عمر يوم، قسمت إلى ٦ مجموعات وهم : المجموعة الأولى : مجموعة ضابطة) غير معداه وغير معالجة والمجموعة الثانية : غير معالجة ومعداه بميكروب السالمونيلا بللورم، والمجموعة الثالثة : معداة بالسالمونيلا بللورم ومعالجة بالجنتاميسين، والمجموعة الرابعة : معدا بالسالمونيلا بللورم ومعالجة بالاسبكتينومايسين.

ولقد حدثت زيادة معنوية فى البروتين الكلى للمصل والعدد الكلى لخلايا الدم البيضاء فى الطيور الغير معالجة والمعداه بالسالمونيلا مقارنة بالمجموعة الضابطة بينما أظهرت الطيور المعداه والمعالجة بالجنتاميسين نقصاً معنوياً فى العدد الكلى لخلايا الدم البيضاء فى الإسبوع الأول والثانى والثالث بعد العلاج بينما لم يحدث أى تغيير فى الطيور الغير معداة والمعالجة بالجنتاميسين فى العدد الكلى لخلايا الدم البيضاء وقد حدث نقص معنوى فى البروتين الكلى للمصل بينما أظهرت الطيور المعداه والغير معالجة زيادة معنوية فى العدد الكلى لخلايا الدم البيضاء طول فترة التجربة وكذا فى البروتين الكلى للمصل بينما أظهرت الطيور المعداه ومعالجة بالاسبكتينوميسين نقصاً معنوياً فى العدد الكلى لخلايا الدم البيضاء فى الإسبوع الثانى والثالث بعد العلاج مقارنة مع الطيور المعداة والغير معالجة ولقد أثبتت التجارب نقصاً معنوياً فى البروتين الكلى للمصل أما الطيور المعداه والمعالجة بالاسبكتينوميسين لم تظهر أى تغييرات معنوية فى العدد الكلى لتلك الخلايا.

كما أظهرت النتائج أن لعقارى الجنتاميسين والاسبكتينومايسين تأثيراً مميزاً وفعالاً ضد ميكروب السالمونيلا بللورم معملياً.

نستخلص مما سبق أن كل من العقارين بالجرعات المستخدمة ليس له تأثير على الإستجابة المناعية فى الدجاج المعدى.