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Studies on The Antibiotic Resistance of Salmonellae Isolated from Imported Frozen Poultry and Their Virulence to Chickens

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Studies of the antibiotic sensitivity pattern declared that all 101 salmonellae isolated from imported frozen poultry meat were resistant to the following polymyxin B, penicillin G. cloxacillin, erythromycin pusicbic acid, Lincouycin, colistin Sulphate, novobiocin and vancomycin

All these salmonella isolants showed variable resistance pattern against the following nitrofurantion, kanamycin, tobramycin, cepholothin, gentamicin, carbe *nicillin*, *Nalidixic* acid, chlorete-tracycline, oxtetracline, chloramphenicol, Mandelamine, ampicillin cotrimoxazole and streptomycin.

These data is of great importance for the correct use of antibiotic in poultry industry. The results of the pathogenicity of the isolated salmonella serotypes to one day old chicks varied from 20-45% which is of great economical importance for poultry industry.

salmonellosis is today the most important food borne disease in nearly all parts of the world. (Beside medical expenses, the effects on trader has been of great importance due to salmonella contamination. Several countries have closed their frontiers regarding the import of food of animal origin). Hummel (1979) reported that the common practice of feeding fowls continuously on diets containing nutritional concentration of terracycline or penicillin led us to determine the resistance patterns for salmonella cultures isolated from fowls.

Accordingly Ryder *et al.* (1980) observed an increase in the frequency of antibiotic resistance among salmonella isolates. The causes of this increase probably include not only use of antibiotic in humans but also use of antibiotics in animals for therapy and for "growth promotion".

Henderson *et al.* (1960) administered 7 different salmonella serotypes orally in graduated doses to inbred White leg-horn day-old chicks. All 7

serotypes produce rates of mortality varying from about 2% (*S. anatum*) to 80% (S. *typhi-murium*). Hofstad *et al.* (1972) reported that mortality from paratyphoid infections in poultry was encountered most frequently during the first 2 weeks after hatching with the highest losses occurring between the 6th and 10th days.

The aim of this investigation was to determine the significance and the role of some newly introduced salmonella serotypes the to our country through the imported frozen poultry in :

1. Their antibiotics sensitivity to detect the role of continuous use of antibiotics.

2. Determination of the pathogenicity of 10 Salmonella serotypes to one-day old. chicks.

Material and Methods

Ten serosypes, which was isolated from imported frozen chicken meat by Safwat *et al.* (1984) in the Animal Health Research Insititute, were tested for their sensitivity to antibiotics and pathogenicity to one-day-old chicks. The serotypes were as follows ; *S. typhi-murium, S. heidelberg, S. chester, S. breedenburg, S. agona, S. colindal, S. virchow, S. taksony, S. farcham and S. carrau.*

The antibiogram of the 10 salmonella serotypes was recovered from frozen meat tested against 22 antibiotics (multidiscs Oxoid) and nitrofurans by the disc agar diffusion method (Sojka *et al.*, 1972). The interpretation of the results were carried out according to Oxoid (1970).

For detection of pathogenicity, 220 one day-old salmonella free chicks obtained from Generai Poultry Organization were used. They were grouped into 10 groups (20 chicks / group) and one control group. They were fed on a commercial ration suitable for their age without any antibiotics additives. They dosed orally 5×10^9 bacteria/ml suspension of one of the following 10 serotypes, S. *typhi-murium, S. agona S. chester, S. breedenburg* (group B), *S. virchow, S. colindale* (C_1) *S. farcham S. taksony* (E_4) and *S. Carrau* (H). All chicks were observed daily for 6 weeks and subjected to clinical examination. At the end of the experiment the survived chicks were sacrificed. Dead as well as the sacrificed birds were subjected to post-mortem and bacteriologically for salmonella isolation recommended by (Edwards and Ewing, 1972).

Results

Results of sensitivity test of 101 salmonella isolates belong to 10 salmonella serotypes to 22 antibiotics and nitrofurans agents revealed that the

sensitivity patterns was varied from each serotypes to another (Tables 1 and2). In the meantime,all isolated were resistant to the following 9 agents : colistin sulphate, cloxacillin, erythromycin, novobiocin, lincomycin, fusidic acid, polymyxin B, penicillin G and vancomycin.

The results of pathogenicity of the 10 serotypes isolated from imported frozen meat are tabulated in Table 3 concerning the mortalities and survival after the period of experiment. The incubation period in orally experimentally infceted chicks varied from 1 to 7 days which depends mainly on the virulence of serotypes examined.

The noticed clinical signs were generally the same and constituted of loss of appetite, increase thirst, dullness, depression off food, diarrhoea, where death occurred with in 24 hr from onset of symptom. The post-mortem findings, in chicks dying within the first week after infection typical picture of septicemia, congestion of internal organs and petechical haemorrhages on heart and occasionally in liver and spleen, while those dying after the lst week and later showed necrotic foci in the liver and heart, with enlargement of gall-bladder.

Discussion

In the present work, generally the antibiogram of 10 serotypes from the imported frozen meat showed the classification of the 23 chemotherapeutic agents into 2 groups. The first ineffective groups of antibiotics, where the 10 serotypes were completely resistance to their action, were the following 5 broad spectrum antibiotics, erythromycin, lincomycin, novobiocin, polymyxin B and vancomycin in addition to 4 agents affected only on Gram positive bacteria as colistin sulphate, cloxacillin, fusidic acid and pencillin. This agreed with Smith (1977) who stated that all Salmonella were resistant to almost members of pencillin group except ampicillin.

The action of second groups of antibiotics varied considerably according to the type of strains, where most of them were sensitive (Table 2). The only uneffective 4 agents were streptomycin (rate of resistant 77.2%), co-trimoxazole 29 %), oxtetracycline (20.7 %) and chloretetracycline (21.71). Such variation in the resistance can be concluded to its uses as additive in diets. Although Smith (1977) reported that tetracycline group are among the widest antibacterial spectrum against Gram-negative and Gram-positive bacteria, yet its wide spread use and unscientific application induced a large number of resistant strain (Hummel, 1979 and Ryder *et al.*, 1980), the case which was noticed in strepytomcin. The results of the present work showed that 77.7 % of the strain were resistant to streptomycin, which supported by Panhotra *et al.* (1980), Viaene *et al.* (1970) and Safwat *et al.* (1984) who stated that some salmonella strains were resistant to dihydrostreptomycin and tetracycline. This leads to suggestion that the broad application of the

TABLE 1. Type and frequency of antibiotic resistance among salmonella strains examined from imported frozen chicken meat.

Drugs.	Conc. per disc	Salmonella serotypes																					
		S. typhimurium (20)		*S. heidelberg* (12)		*S. chester* (10)		*S. bredenbury* (9)		*S. agona* (8)		*S. colindale* (8)		*S. Virchow* (9)		*S. taksony-* (8)		*S. jurchan* (7)		*S. Carrau* (10)	
		NR	%	NR	%	NR	%	NR	%	NR	%	NR	%	NR	%	NR	%	NR	%	NR	%
Ampicillin	10ug	4	20	—	—	—	—	—	—	—	—	1	12.5	3	33.3	—	—	7	100	1	10
Carbenicillin	100	—	—	—	—	—	—	3	33.3	—	—	4	50	3	33.3	—	—	—	—	—	—
Cephalothin	30	—	—	4	33.3	—	—	—	—	2	25	3	37.5	2	22.2	—	—	3.4	2.8	4	40
Chloramphenicol	30	3	15	—	—	2	20	2	22.2	—	—	—	—	—	—	—	—	—	—	—	—
Chlortetracycline	30	7	35	2	16.6	—	—	4	44.4	—	—	—	—	4	44.4	—	—	2	28.5	3	30
Co-Trimoxazole	25ug	2	10	4	33.3	1	10	1	10	—	—	8	100	5	55.5	—	—	7	100	1	10
Gentamicin	10	—	—	3	2.5	—	—	—	—	1	12.5	—	—	—	—	—	—	1	14.2	—	—
Kanamycin	30	—	—	4	33.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mandelamine	3mg	12	60	—	—	—	—	—	—	—	—	—	—	4	44.4	—	—	—	—	—	—
Nalidixic acid	30	4	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Nitrofurantion	300ug	6	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Oxtetracycline	30	8	40	2	16.6	—	—	—	—	2	25	3	37.5	4	44.4	—	—	—	—	2	20
Tobramycin	30	—	—	—	—	—	—	—	—	3	37.5	—	—	—	—	—	—	—	—	—	—
Streptomycin	10ug	20	100	12	100	10	100	5	55.5	—	—	6	75	—	—	8	100	7	100	10	100

( ) Number of tested NR Number of resistant

TABLE 2. Resistance Patterns of 101 salmonella Strains isolated form imported forzen poultry meats to antimicrobial agents in vitro.

Antibiotic and Chemo-thera peutic agent	Disc Potency /mg	Number of Resistant Isolates	%
Tobramycin.	30	3	2.9
Nalidixic acid.	30	4	3.9
Kanamycin.	300	4	3.9
Gentamicin.	10	5	4.9
Nitrofurans.	300	6	5.9
Chloramphenicol.	30	7	6.9
Carbenicillin.	100	10	9.9
Mandelamine.	3mg	16	15.8
Ampicillin.	10	16	15.8
Cephalothin.	30	18	17.8
Oxtetracycline.	30	21	20.7
Chloretetracycline.	30	22	21.7
Co-trimoxazole.	25	93	29.0
Streptomycin.	10	78	77.2

TABLE 3. Results of experimental infection of Salmonella serotypes to chicken chicks.

Inoculated Solmonella Serotypes	Salmonella Groups	No. of Survival Cases.	Total No. of deaths.	Mortality rate due to salmonella infection %
S. typhi-murium		12	8	40 %
S. heidelberg.		14	6	30 %
S. chester	B	14	6	30 %
S. breedenburg		14	6	30 %
S. agona		16	4	20 %
S. Colindale	C_1	11	9	45 %
S. Virchow		16	4	20 %
S. taksony	E_4	11	9	45 %
S. farcham		12	8	40 %
S. Carrau	H	13	7	35 %

antibiotic as prophylactic, growth promotion factor or therapeutic dose resulting in the induction of resistant strains to the used antibiotic as streptomycin or tetracycline resistant salmonella strains. This conclusion was supported by Ryder *et al.* (1980) who stated the cause of the increase of the resistant of the strains was probably due to the use of antibiotics in animal and poultry for growth promotion.

This can be proved in comparing the results of this work in the resistant *S. typhi-murium* of the imported strains and that of local strains recorded by Safwat *et al.* (1984) where the imported strains was resistant to nitrofurantion, chloramphenical, ampicillin, oxtetracycline, and streptomycin in a rate of 6·9 %, 6·9 %, 15·5 %, 20·7 %, and 77·2 %, . While the local strains was resistant in a rate of 21·5 %, 16·1 %, 40·8 %, 45·1 % and 100 % respectively

Such wide variation between the resistant pattern of the newly introduced strains and that of local strains may be due to the unscientific application in Egypt of the antibiotics in the poultry farms.

Therefore, hence for controlling Salmonella infection in Egypt, it is important to select the effective antibiotic according to the antibiogram of the isolates. Also it must put in consideration on the public health significance in case of infection of human with salmonella resistant strains on the danger of food poisoning.

The results of pathogenicity of isolated salmonellae to one day old chicks varied from 20-45 %. This agreed with the reported by Schaffer *et al.* (1957) who found marked variation in the response of one day old chicks to either oral or parentral inoculation with various type of salmonellae. Sieburth and Johnson (1957) reported that orally administred *S. typhi-murium* organism are very infective for the day-old chick.

Also Vestal and Stephens (1966) suggested that difference in pathogenicity of salmonella serotypes for chicks may be due to the fact that cells of some types are rapidly lysed following injection with the release of a large quantity of endotoxin and early mortality.

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دراسات عن مقاومة السالمونيلا المعزولة من الدواجن المجمدة المستوردة للمضادات الحيوية وكذلك ضراوتها فى الكتاكيت

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لقد أظهرت دراسات نموذج الحساسية للمضادات الحيوية ١٠١ عترة سالمونيلا معزولة من لحوم الدواجن المجمدة المستوردة أن كل السلالات المعزولة كانت مقاومة للآتى :

بولى مكسين ـ بنسيلين ج ـ كلوكساسلين ـ أرثيروميسين ـ حامض الفيوزيك ـ الليتلكوميسين ـ كولستين سلفات ـ النوفوبيسوسين والفانكوميسين .

بينما كانت تختلف درجة مقاومتها ل نيتروفيورنتوين ـ كاناميسين ـ توبراميسين ـ سيفالوثين ـ جنتاميسين ـ كاربنسيللين ـ حامض ناديلك ماندلامين ـ امبسلين ـ كوترای میکسازول والستربتوميسين .

ولقد تراوحت النتائج الباثولوجية للعترات السالمونيلا المعزولة مع كتاكيت عمر يوم من ٢٠ ـ ٤٥٪ من حيث الضراوة وهذه لها أهمية كبرى فى صناعة الدواجن .