

Prevalence and antimicrobial susceptibility of *salmonella* species isolate from slaughtered cows in Iraq

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Summary

This study was planned to determine the prevalence and antimicrobial susceptibility of *Salmonella* spp. One hundred bovine with different age and both sexes at slaughterhouse were examined. Four hundred samples were collected from different internal organ (bile, Liver, spleen and mesenteric lymph nodes). Diagnostic study depended upon the morphological and cultural properties of the isolates on some selective media like Brilliant green, XLD, SS agar which used in addition to different biochemical tests, API-20E (Enterobacteriaceae Identification Kit.). Isolates were serotyped at the Central Public Health Laboratories (National Center of *Salmonellae* in Baghdad, Iraq). Eleven isolates consisting of *Salmonella anatum* (9.09%), *S.newport* (27.27%), *S.enteritidis* (45.45 %) and *S.ohio* (18.18%). The percentage of infection were 3% in the mesenteric lymph node and liver, (4%) in bile, and (1%) in spleen. The antibiotic susceptibility pattern of *Salmonella* against 15 antimicrobial drugs revealed that all isolates were sensitive (100%) to ciprofloxacin and ampicillin. And most of isolates were resistance to cloxacillin, cefixime, amoxicillin. and gave intermediate results to neomycin, gentamycin, tetracycline and streptomycin.

Keywords: *Salmonella*, Prevalence, slaughtered cows, antimicrobial, susceptibility.

Introduction

Salmonellosis is a global problem for animals and human and the association of the disease between man and food animals has been investigated by many workers (1). *Salmonella* species are among the most important foodborne pathogens, leading to millions of cases of diarrheal illness, salmonellosis is associated with medium to severe morbidity and even mortality in farm animals representing major economic productivity loss in food and animal industries. *Salmonella* has been widely reported in cows (2, 3 and 4). Infection in animals is of importance because of the direct economic effect. Of even greater importance is that animals constitute a vast reservoir of these organisms for human infection (5). In humans, in addition to concern about foodborne zoonoses caused by *Salmonella* organisms, concern has also been raised about the impact of acquired antimicrobial resistance transferred among these organisms (6) which limits therapeutic options both in veterinary and public health practice.

This study aimed to determine the distribution of *Salmonella* spp in different

internal organs of cows in slaughterhouse and determining the antimicrobial susceptibility of isolated *Salmonella*.

Materials and Methods

The study was performed on (100) cows with different ages and both sexes. Four hundred samples from internal organs were collected aseptically in sterile container (100) samples of each bile, mesenteric lymph nodes, spleen and liver. Samples were transferred to AL-Khairat Veterinary Laboratory immediately on cool box.

The isolation and biochemical identification of *Salmonella* was made according to (7). Each sample was cultured immediately on Tetrathionate broth and Brilliant green broth (HiMedia Laboratories Pvt.Ltd, India) and incubated at 37°C for 24h. A loopful of inoculum from each of enrichment cultures sample were inoculated on the surface of different type of medium containing Brilliant Green agar, Xylose-Lysine Deoxycholate agar (Oxoid, Hampshire, England), *Salmonella-Shigella* agar then incubated aerobically at 37°C for 24 h. for confirmation, five presumptive

Salmonella colonies both from XLD agar, were selected and streaked onto the surface of pre-dried nutrient agar (Oxoid, Hampshire, England) plates and incubated at 37°C for 24 h. Pure cultures from the nutrient agar were used for biochemical confirmation (motility, triple sugar iron agar, indol, methyl red and citrate utilization tests, urea agar) were inoculated and incubated at 37°C for 24h.

Biochemical confirmation of *Salmonella* organisms were made according to (7). API-20E (Enterobacteriaceae Identification Kit), this kit is a standardized colorimetric identification system utilizing conventional biochemical tests and carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. (8). Serotyping; All isolates were cultured on Kligler Iron media (HiMedia Laboratories Pvt.Ltd , India) for serotyping at the Central Public Health Laboratories (National Center of *Salmonellae* in Baghdad).

Each isolate was tested for susceptibility to fifteen commonly antimicrobials using the disk diffusion method according to the Kirby Bauer method (9). Well-isolated colonies were selected from brain heart infusion agar just the top of colonies touched and the growth transferred to tube containing 4-5ml of nutrient broth was inoculated and incubated at 37°C for 4 h. Culture of each isolate was compared with McFarland tube NO 2. Muller Hinton agar plates (HiMedia, India) were inoculated by swabs immersed in each of the culture and held at room temperature for 30 minutes to allow drying. The disc was placed on to the agar surface using sterile forceps. Each disc was gently pressed with the point of sterile forceps to ensure complete contact with the agar surface cultures of Muller Hinton agar and incubated at 37°C for 20 h. The diameters of the zones of inhibition were recorded to the nearest millimeter and classified as resistant, intermediate, or susceptible. Characterization of strains as sensitive or resistant was based on the size of inhibition zone around the disc compared with the interpretation standards provided by the manufacturers from Bionalyse, Turkey Company.

The categories susceptible or resistant were assigned on the basis of the critical points recommended by the Turkey Committee on Guidelines for susceptibility testing. Their concentration in the discs and their zone of inhibition in deciding susceptibility are given in (Table,1).

Results and Discussion

The prevalence of *Salmonella* in animal and sample level was observed. At animal level, overall prevalence of 7% (7 of 100) was bacteriologically positive for *Salmonella*. At sample level, *Salmonella* was observed with prevalence of 2.75% (Table,2). Detection of *Salmonella* from liver, mesenteric lymph nodes, bile, and spleen (each n=100) with percentage rate of 3%, 3%, 4%, and 1%, respectively. Four different species of *Salmonella* were confirmed by national center of *Salmonella* in Baghdad (Table,3). *Salmonella enteritidis* and *Salmonella newport* were isolated at the highest frequency of 45.45% (5 of 11) and 27.27 (3 of 11%) respectively, *Salmonella ohio* and *Salmonella anatum* were isolated at frequency of 18.18% and 9.09% respectively of the total isolates.

According to age of cows at slaughter house the results shows that *Salmonella* was highest (7.70%) in 1.5 – 3 year age group and lower percentage (6.25%) in animals. More than 3 years, insignificantly difference between age group of cows (Table,4). Table, 5 revealed distribution of *Salmonella* species according to sex, six isolates from (56) samples recorded in male and (5) isolates from (44) recorded in female. insignificant difference between male and female.

The results of the present study showed that *Salmonella* were isolated at a percentage of (7%) in cow at slaughter house and this is in agreement with the study of (2) who isolate *Salmonella* from 2% fecal, 2% rumen and 7.6% carcasses of cows at a commercial abattoir in Irish, while (10) recorded low percentage (2.95%) of *Salmonella* from cow in Bangladesh authors (11) isolated *Salmonella* (1.1%) from ground cattle beef samples. Other studies have also shown different results, (4) found a high percentage

(38.89%) of *Salmonella* in cow and a percentage of (11.11%) from one hundred and eight samples (20 buffaloes and 88 cows) were collected from different Cairo slaughter house and abattoirs. Also (12) recorded isolation rate of salmonellae of 19% from carcass samples of cattle.

In the present study, four different species were confirmed which constituted about (2.75%), this result similar to many researcher (13) who recorded different species in cows (*S.typhimurium*, 8; *S.agona*, 2; *S.infantis*, 1) and a study of other workers (2) who recorded three species in cows (*S.typhimurium*, *S.agona*, and *S.dublin*). Also in Egypt (4) found two species in cows (*S.entritidis* and *S. typhimurium*). While (14) isolated a new serovar from cattle in the Khartoum State in Sudan the new serovar was named *Salmonella umbadaha* and assigned as *Salmonella enterica* subspecies *enterica* on the basis of its biochemical characteristics. *Salmonella Umbadaha* is now listed in the 9th edition of the Antigenic formulae of the *Salmonella* Serovars.

In Iraq (15) isolate *S.typhimurium* from bovine 320 fecal samples which collected from Cows of different ages and sexes present in farms and slaughter house in Diwaniya and Najaf provinces. Also (16) isolate *S.typhimurium* in a percentage of 4.5% from 150 sample isolate from calf and cows in some of Baghdad village and dairy cattle station.

The difference in the percentage of *Salmonella* in our study with others may be due to the difference in geographical region and the difference in temperature, humidity and ways of animal living and its management with the handling in abattoir.

In this study, higher percentage of *Salmonella* isolates from gall bladder from summation of *Salmonella* isolates in followed mesenteric lymph node, liver and spleen. A similar result has been recorded by (17) who found that the *Salmonella* organisms

concentrated in gall bladder, liver, mesenteric lymph node and spleen in carrier animals. The results of present study according to sex show that the incidence of *Salmonella* in male is higher than in female, and this is agreement with, (18) who reported a percentage of *Salmonella* (5%) of in female and higher in male (8%) of the animals sampled at Uganda meat industries. While many studies indicate that the percentage of infection in female is higher than in male as in study by (15) who reported higher percentage of *Salmonella* (13.5%) in female and lower in male (7.7%).

The Antibiotic susceptibility pattern of *Salmonella* against the antimicrobial disc revealed that all isolates were resistant (100%) to Cloxacillin, (91%) of isolates are resistant to Cefixime and (81.8%) of isolates are resistant to Amoxicillin. And all isolates sensitive (100%) to Ciprofloxacin and Amicacin (Table,6). The results of present study was in agreement with those of (18) who found that *Salmonella* was sensitive to Ciprofloxacin and Amicacin, but disagreed about resistant to Gentamycin and Streptomycin. All isolates were susceptible to ciprofloxacin probably because the drug was relatively new on the market, fairly expensive and had not been used extensively in treatment of diseases in cattle. Resistance to ciprofloxacin also agreement with study of (19) who recorded that all isolates of cows showed (100%) sensitive to ciprofloxacin. Ciprofloxacin showed a good antimicrobial activity against both human and cow isolates. Though no data has indicated this, the effectiveness of such drugs like ciprofloxacin may be because they are not widely used in countries like Iraq.

Most of the isolate in this study were resistant to erythromycin. Erythromycin is an old antibiotic belong to class macrolides that has been used to prevent infection caused by enteric pathogens, where almost 100% resistance have been reported from different parts of world (20).

Table, 1: Antimicrobial discs used with their remarks

No.	Antimicrobial agent	Code	Conc.	Diameter of zone (mm)		
				R	I	S
1	Chloramphenicol	C	30	12	13-17	18
2	Neomycin	N	30	12	13-16	17
3	Suphamethoxazole	SMZ	100	10	11-15	16
4	Gentamicin	CN	10	12	13-14	15
5	Cefixime	CFM	5	15	16-18	19
6	Erythromycin	E	15	13	14-22	23
7	Ciprofloxacin	CIP	5	15	16-20	21
8	Kanamycin	K	30	13	14-17	18
9	Amoxicillin	AX	25	13	14-17	18
10	Streptomycin	S	10	11	12-14	15
11	Tetracycline	TE	30	14	15-18	19
12	Amicacin	AK	30	14	15-16	17
13	Trimethoprim	TMP	5	10	11-15	16
14	Cloxacillin	CX	1	14	15-18	19
15	Nitrofurantoin	F	300	14	15-16	17

Table, 2: Percentage of infections with *Salmonella* in cow

Location	No. of cow	No. of affected animal	%	No. of sample	No. of sample positive to <i>Salmonella</i>	%
Slaughterhouse	100	7	7%	400	11	2.75%

Table, 3: Number and distribution of *Salmonella* species in different organs

No. of animal	Status of <i>Salmonella</i>				No. of isolates
	Liver	Spleen	Bile	MLN	
1	<i>S.anatum</i>	–	–	–	1
2	<i>S.newport</i>	–	–	<i>S.newport</i>	2
3	–	<i>S.newport</i>	–	–	1
4	–	–	<i>S.enteritidis</i>	<i>S.enteritidis</i>	2
5	–	–	<i>S.ohio</i>	<i>S.ohio</i>	2
6	<i>S.enteritidis</i>	–	<i>S.enteritidis</i>	–	2
7	–	–	<i>S.entritidis</i>	–	1
Total	3	1	4	3	11

Table, 4: Distribution of *Salmonella* isolate according to age of animals

Cow age	No. of animal examined	No. of effected animal	%	X ² value Cal X ² =0.05 P =0.972 df= 2
6month 1.5years	45	3	6.66%	
1.5years-3 years	39	3	7.70%	
More than 3 years	16	1	6.25%	
Total	100		7%	

Chi =1.2 non-significant difference between age group of cows.

Table, 5: Distribution of *Salmonella* species according to sex of animal

Cow sex	No.of examine animal	No. of affected animal	%	X ² value
Male	56	4	7.14%	Cal X ² = 0.004 P=0.95 df= 1
Female	44	3	6.81%	
Total	100	7	7%	

Chi = 0.7 non-significant difference between male and female.

Table, 6: Antibiotic susceptibility pattern of *Salmonella*

No.	Antibiotics used	Total No. of isolate	R	%	I	%	S	%
1	Chloramphenicol	11	6	54.6%	0	0%	5	45.4%
2	Neomycin	11	3	27.2%	4	36.4%	4	36.4%
3	Suphamethoxazol	11	8	72.7%	0	0%	3	27.3%
4	Gentamicin	11	6	54.6%	4	36.4%	1	9%
5	Cefixime	11	10	91%	0	0%	1	9%
6	Erythromycin	11	8	72.7%	0	0%	3	27.3%
7	Ciprofloxacin	11	0	0%	0	0%	11	100%
8	Kanamycin	11	5	45.4%	2	18.2%	4	36.4%
9	Amoxicillin	11	9	81.8%	0	0%	2	18.2%
10	Streptomycin	11	0	0%	11	100%	0	0%
11	Tetracycline	11	5	45.4%	4	36.4%	2	18.2%
12	Amicacin	11	0	0%	0	0%	11	100%
13	Trimethoprim	11	0	0%	1	9%	10	91%
14	Cloxacillin	11	11	100%	0	0%	0	0%
15	Nitrofurantoin	11	3	27.3%	0	0%	8	72.7%

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نسبة انتشار انواع السالمونيلا المعزولة من الابقار المذبوحة و حساسيتها للمضادات الحيوية في العراق

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الخلاصة

صممت الدراسة لمعرفة نسبة انتشار انواع السالمونيلا و انماط مقاومتها للمضادات الحيوية. خضعت لهذه الدراسة 100 بقرة بمختلف الاعمار و من كلا الجنسين في المجزرة اذ جمعت 400 نموذج من الاعضاء المختلفة (المرارة، الكبد، الطحال و العقد اللمفاوية المساريقية). اعتمدت الدراسة على الصفات الشكلية و الزراعية على مختلف الاوساط الزرعية مثل وسط الاخضر اللماع او الالكس ال دي و السالمونيلا-شيكولا اجار بالاضافة الى الاختبارات البايوكيميائية، واختبار الابي (العدة الخاصة بعائلة المعويات). صنفت العزلات سيروولوجيا في مختبر الصحة المركزي (المركز الوطني للسالمونيلا-بغداد-العراق). تم عزل (11) عزلة سالمونيلا شكلت *Salmonella anatum* نسبة (9.09%) وكانت *Salmonella newport* بنسبة (27.27%) وكانت نسبة *Salmonella enteritidis* (45.45%) بينما كانت نسبة *Salmonella ohio* (18.18%)، شكلت نسبة الاصابة في حيوانات المجزرة نسبة مختلفة بين الاعضاء المفحوصة ففي الغدد اللمفية المساريقية و الكبد كانت نسبة الاصابة (3%) و (4%) في الصفراء و (1%) في الطحال. درست حساسية العتر المعزولة لخمسة عشر نوع من المضادات البكتيرية و كانت جميع العزلات حساسة بنسبة (100%) لـ Ciprofloxacin and Amicacin وكانت اغلب العزلات مقاومة لـ Gloxacillin, Neomycin, Gentamycin, Tetracyclin and Cefotaxim and amoxicillin وكذلك كانت متوسطة المقاومة لكل من Streptomycin.

الكلمات المفتاحية: السالمونيلا، الانتشار، الابقار المذبوحة، حساسية، المضادات الحيوية.