Prevalence of Salmonella enterica in Slaughterhouses in Kalubia Province

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Abstract

100 samples were taken from the surface of the skin of slaughtered carcasses of cattle in slaughterhouses at Kalubia Province, 25 samples were positive to *Salmonella enterica serovar enteritidis* with a percentage of (25%). and 100 samples were taken from human stools of workers in contact with cattle in slaughterhouses at Kalubia Province, suffering from diarrhea and/or fever. *Salmonella enterica serovar enteritidis* was represented in 13 samples with a percentage of (13%). were examined for isolation and identification of *Salmonella enterica serovar enteritidis* by different methods of isolation and testing.

Human phage typing sets of isolated strains from animals and cattle workers demonstrated three strains having the possibility of cross infection between cattle workers and cattle carcasses. The effect of two different disinfectants on to strains of *Salmonella enterica serovar enteritidis* strains revealed the most effective disinfectant in different dilutions was Betidin then formalin. Antimicrobial sensitivity test proved that those *Salmonella enterica* isolated strains were sensitive to three antibiotics, gentamicin, nalidixic acid, and ciprofloxacin, but multiply resistant to four commonly available antibiotics, including ampicillin, chloramphenicol, cotrimoxazole and tetracycline

The public health significance of the isolated strains and the implications of this experiment for understanding the path of Salmonellae from domestic animals to man is discussed.

Introduction

Most of the attention to date regarding raw meat diets has been on evaluating diets for enteropathogen contamination and disease in animals from ingestion of *Salmonella*-contaminated meat. Little attention has been paid to indirect infection via handling of contaminated meat or items that have been in contact with contaminated food. Food bowls may be a potential source of infection for animals or humans, if contaminated meat is fed and if the food bowl is not adequately disinfected. The objectives of this study were to evaluate persistence of *Salmonella* spp **(1).**

The sourc and mode of transmission in the African context have remained unknown, although it is thought that human-to-human transmission may play an important role, and we have been unable to demonstrate the presence in humans of multidrug-resistant that are phenotypically and genotypically similar to which found in food animals in Kenya **(7).**

multidrug-resistant *Salmonella enterica serovar enteritidis* is the predominant cause of bacteraemic illness in children, while this serotype is the predominant isolate in children with salmonellae bacteraemia. The source and mode of transmission have remained unknown, although it is thought that human-to-human transmission may play an important role, and we have been unable to demonstrate the presence in humans of multidrug-resistant that are phenotypically and genotypically similar to which found in food animals **(6)** and **(7).**

The current study was carried out to throw light on the following:

1-Prevalence of *Salmonella enterica serovar enteritidis* from the surface of the skin of slaughtered cattle carcasses in slaughterhouses of Kalubia Province.

2- Prevalence of *Salmonella enterica serovar* from human stools of workers in contact with cattle in slaughterhouses at Kalubia Province.

3-Detection of the possibility of cross infection between cattle workers and cattle carcasses

4- Results of uses of antimicrobial sensitivity test to *Salmonella enterica serovar enteritidis*.

5- Effect of some disinfectants on *Salmonella enterica serovar enteritidis* strains of the isolated Salmonella strains.

Materials and Methods

Case material:

In the present study 100 swabs were randomly collected taken from the surface of the skin of Cattle carcasses samples at the slaughterhouses, as well as100 samples were obtained from human stools of the workers in contact with Cattle carcasses in Cattle slaughterhouses at Kalubia Province according to the methods recommended by **(2).** Again human samples were mainly taken from persons suffering from diarrhea and/or fever.

Sampling For Bacteriological Examination:

Collection of cattle swab:

Cattle carcasses were swabbed with large swabs made by wrapping several layers of cotton on small artery forceps. The premoisted swabs were rubbed to-and-fro six times on the selected area the cotton wool pushed off into a cotton capped sterile test tube containing 10 ml Selenite-F broth (BBL). Collected swabs were carried to the laboratory with a minimum of delay in ice bag to be immediately examined. The interval between the collection of the swabs and the inoculation of primary plates was usually between 1.1/2 and 4 hr., and did not exceed 5hr **(10).**

Collection of human swab:

Cotton tipped swabs may be used to collect specimens. If the swabs prepared locally, it is important to ensure that the cotton fits tightly on the stick. The swab should be moistened with sterile non bacteriostatic fluid or transport medium (not lubricating gel). The swab should be placed in sterile empty tube with a cotton plug or screw cap. If immediate inoculation is not possible, the specimens the specimens should bee stored at 4°C. Suspend the feces swab in tube containing 1 ml. of sterile saline per swab, washing the swab thoroughly in the saline by swirling the tube and rotate the swab against the side of the tube to express the fluids portions of formed stools should be suspended in saline to make a turbid suspension **(2).**

Bacteriological Examination of the Collected Samples:

The following bacteriological media were used: brilliant green agar (BBL), MacConkey agar (BBL) for direct plating of specimens, Selenite-F broth (BBL).

Biochemical identification of isolates: was made on the basis of the following tests according to (11):

glucose metabolism positive; production of indole negative, Methyl red reaction positive (MR) and Voges Proskaur test (VP) negative and positive utilization of Citrate and H2S production and hydrolysis of urea negative.

Phage typing:

Phage typing was performed in accordance with the methods of Dutch Phage typing system described by **(4)** and **(14)**. Briefly, 4 ml of double-strength nutrient broth (Difco) was inoculated with a single colony of *S. enterica serotype enteritidis* strains and incubated at 37°C for 1 h 15 min. By means of a sterile Pasteur pipette 2 ml of the broth culture was then used to flood a dried double-strength nutrient agar plate (30-ml volume of agar, dried for 1 h 30 min), and the excess broth was removed. After surface drying for 15 min, a series of typing phages were applied to the plate surface according to a defined template using a multipoint inoculator. Each plate was incubated overnight at 37°C, and the pattern of lyses produced by the phages was recorded and interpreted by comparison to standard charts.

Serological identification:

The isolated proved biochemically to be Salmonella microorganism were subjected to serological identification according to Kauffman white scheme. Isolates were subcultured on nutrient slope for 24 hours at 37°C. For application of slide agglutination technique, two homogenous suspensions were made on a slide by suspending a piece of suspected colony in a drop of sterile physiological saline .A drop of each separate O and H Salmonellae factors were added separately to each of the suspensions with standard loop and thoroughly mixed to bring the microorganism in close contact with the antisera. Positive agglutination occurred within a minute and could be easily seen with the naked eye. A delayed or partial agglutination was considered as negative or false result **(8)**.

Effect of some disinfectants on *Salmonella enterica serovar enteritidis* strains:

Strains used:

Salmonella enterica serovar enteritidis strains.

2-Reagents used:

1-Formalin37%.

2-Betidin10% Iodophore compound.

I- stock inoculum:

The tested strains were growing on nutrient agar in petri dishes at 37°C. for 24 Hrs, the stock inoculums were prepared by harvesting the culture in sterile saline solution.

2- Disinfectants:

Sterilized test tubes containing 5 ml. of the diluted disinfectant under test form 1: 10, 1:20, 1:40 1:60.dilutions was used, 0.5ml. Of the Cell suspension were added by using sterilized pipetes to the test tubes containing the disinfectant and shacked after 2.30, 5,10, and 15 minutes. Loopfulls were transferred to sterile test tube containing nutrient broth in order to eliminate the risk of faulty results due to the residue action of disinfectants, then Transfer a loopfulls to petri dish containing nutrient agar from each dilution at 2.30, 5, 10 and 15 minutes then incubated at 37°C for 24 Hrs *Salmonella enterica serovar enteritidis* strains, growth were observed.

Results were shown in Table (6).

Antimicrobial susceptibility testing: Antimicrobial sensitivity test was done to seven antibiotics, gentamicin, nalidixic acid, ciprofloxacin, ampicillin, chloramphenicol, cotrimoxazole and tetracycline were determined by both controlled disc diffusion and measuring MICs using E-test strips (AB BIODISK) according to the manufacturer's instructions. Disc diffusion susceptibility tests and MICs were interpreted according to the guidelines provided by **(12)**. The results were recorded in tables (1, 2,3,4,5, 6&7).

Results

Table (1): The percentage of *Salmonella enterica serovar enteritidis* isolated from human stools of the workers:

Total No. of examined samp	No. of individuals positive to *Salmone* of individual case	%
100	13	13%

Table (2): The percentage of *Salmonella enterica serovar enteritidis* isolated from Cattle carcasses:

Total No. of examined	No. Cattle carcasses samples positive to *enterica*	%
100	25	25%

Table (3): The numbers and percentage of phage typing isolated from Human stools in the same locality of cattle:

Human phage type	No. of isolates	%
Phage type No.4	3	23.07%
Phage type No.6	1	7.69%
Phage type No.21	5	38.46%
Untypable strains	4	30.77%
Total	13	100%

Table (4): The numbers and percentage of phage typing isolated from Cattle carcasses:

Cattle phage type	No. of isolates	%
Phage type No.4	3	12%
Phage type No.6	5	20%
Phage type No.21	7	28%
Untypable strains	10	40%
Total	25	100%

Table (5): phage typing of isolated strains from Cattle meat and human stools of workers:

Source of samples	Isolates	Typeable strains	Phage produced Typeable strains	Untypeable strains	Possibility of cross infection between man and Cattle Carcasses
Man	13	9	phage type No. 4,, 6, and 21	4	Phage type No. 4, 6 and 21
Cattle carcasses	25	15	Phage type No. 4,, 6 and 21	10	

Table (6): Effect of some disinfectants on human strains of *Salmonella enterica serovar enteritidis*:

		Betidin				**Formaline**			
	Time	**Dilution**				**Dilution**			
		1/10	1/20	1/40	1/60	1/10	1/20	1/40	1/60
Salmonella enterica serovar enteritidis	2.30	N	N	N	G	N	G	G	G
	5	N	N	N	G	N	G	G	G
	10	N	N	N	N	N	N	G	G
	15	N	N	N	N	N	N	G	G

N.B (G) GROWTH (N) NO GROWTH

Table (7): Summarized results of antimicrobial sensitivity test of the isolates:

Antimicrobic agent	**Disc potency**	**Inhibited zone**	**Results**
Gentamycin	(10 µg)	12 or less	S
Nalidixic acid	(300 µg)	14 or less	S
Ciprofloxacin	(10 µg)	14 or less	S
Tetracycline	(30 µg)	14 or less	R
Chloramphenicol	(30 µg)	14 or less	R
Cotrimoxazole	(20 µg)	19 or less	R
Ampicillin	(10 µg)	20 or less	R

S= Sensitive **R= Resistant**

Discussion

100 Samples from the surface of Cattle carcasses and 100 stool samples from the workers in contact with the Cattle in slaughterhouses at Kalubia Province were examined for isolation and identification of *Salmonella enterica* serovar enteritidis.

The data recorded in Table (1) showed that out of 100 fecal samples obtained from human stools in the same locality of the Cattle carcasses, only 13 (13%) contained *Salmonella enterica* serovar enteritidis. The incidence recorded was agreed with those obtained by **(5).**

The result displayed in Table (2) revealed that, out of 100 swabs collected from the surface of Cattle carcasses, *Salmonella enterica serovar enteritidis* was Isolated from 25 (25%). The incidence recorded was agreed with those reported by **(9).**

From Table (3) it is evident that, out of 13 *Salmonella enterica serovar enteritidis* isolated from human stools, 9 (69.23%) were typed by human set phage, while 4 (30.77%) were untyped. The typable strains were phage type No. 4, 6, and 21with the incidence of 3, 1, and 5 with a percentage of 23.07%, 7.69%, and 38.46% respectively.

From Table (4) it is evident that, out of 25 *Salmonella enterica serovar enteritidis* isolated from Cattle carcasses, 15 were typed by human set phage, while10 were untyped. The typable strains were phage type No. 4, 6, and 21 with the incidence of 3, 5 and 7 with a percentage of 12%, 20%, and 28% respectively.

The presence of untypeable strains my be due to the use of the common ordinary human phage set only and not all human sets. The incidences recorded in table 3 and 4 was agreed with those obtained by **(14).**

From Table (5) it is clear that out of Cattle carcasses, and human stool isolates the possibility of cross infection between Cattle and human in the same locality of Cattle, were demonstrated by 3strains (4-6-21).. Phage-typing result nearly substantiates what had been observed by **(3).** From the results achieved, it can be concluded that cross contamination between Cattle and human in the same localities of Cattle may occur by some strains *of Salmonella enterica* serovar enteritidis.

Table (6) showed the effect of two different disinfectants in the same serial dilution 1:10, 1:20, 1:40, 1:60, on to strains *of Salmonella enterica* serovar enteritidis mentioned before. The most effective disinfectant in

different dilutions was Betidin then formalin which had moderate effect similar results obtained by **(13).** That effect of some disinfectants on strains *of Salmonella enterica* serovar enteritidis were the most common causes of public health investigations of outbreaks and individual cases have led to a long list of confirmed food sources that includes undercooked beef, camel, sheep and goat and the role of that strains in cases of diarrhea in both infants and different animals species and the zoonotic problem of strains *of Salmonella enterica* serovar enteritidis in both man and animals.

From Table (7) the *Salmonella enterica* serovar enteritidis multiply resistant to four commonly available antibiotics, including ampicillin, chloramphenicol, cotrimoxazole and tetracycline. **(15).** It is noteworthy that the availability of these antibiotics over the counter and without prescription mainly for self-treatment of suspected infection in humans may have played a major role in the high prevalence of the multidrug-resistance phenotype.

In conclusion, most ecological evidence warns that better control of antibiotics on an international scale is the key factor needed to reduce the emergence of antibiotic-resistant *Salmonella enterica* serovar enteritidis, including their maintenance in carriers. It may be necessary to avoid such practices as prophylactic and broad-spectrum therapy, therapy without sensitivity testing, and dissemination of residual antibiotics into the environment of man and animals.

To avoid contamination of Cattle carcasses with such pathogens, food handlers must be free from diseases that may be transmitted by foods, should have medical certificate and subjected to periodical medical examination. Proper examination of the Cattle at the farm & at the slaughter houses in both antemortem and postmortem examinations. Personal hygiene, good sanitation and application of good hygienic conditions at the slaughterhouses are also recommended. The most ecological evidence warns that better control of antibiotics on an international scale is the key factor needed to reduce the emergence of antibiotic-resistant organisms, including their maintenance in carriers. It may be necessary to avoid such practices as prophylactic and broad-spectrum therapy without sensitivity testing, and dissemination of residual antibiotics into the environment of man and animals

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مدى أنتشار ميكروب السالمونيلا انتريكا في مجازر محافظة القليوبيةِ

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الملخّص

أخِذتْ ١٠٠عينة مِنْ سطحِ الماشيةِ المذبوحةِ في مجازر محافظةِ القليوبية، 25 عينة كانتْ إيجابية إلى السالمونيلا انتريكا سيروفار انتراتيدس بنسبة مئوية (٢٥ %). وكذالك أخِذتْ ١٠٠ عينة مِنْ المخالطين للماشيةِ في مجازر محافظةِ القليوبية يعانون من الاسهال و/ أو حُمَّى، مُثّلتْ السالمونيلا انتريكا سيروفار انتراتيدس في ١٣ عينةِ بنسبة مئوية (١٣ %). وقد تم عزل سالمونيلا انتريكا سيروفار انتراتيدس بالطرق المختلفةِ وباستخدام لاقمات البكتريا للعترات المَعْزُول مِنْ الماشية والمخالطين، وجد أن ثلاثة عترات لهم إمكانيةُ العدوى التبادلية بين المخالطين للماشيةِ و الماشيةِ. وقد تم اختبار تأثير مطهرين مختلفين على عترات السالمونيلا انتريكا سيروفار انتراتيدس فكشفت أن المطهرَ الأكثر فاعلية في التخفيفاتِ المختلفةِ هو البيتادين ثمّ الفورمالين.
أثبتَ اختبار حسّاسية مقاومة الميكروب للمضادات الحيوية بأنَّ عترات السالمونيلا انتريكا كانتْ حسّاسة إلى ثلاثة مضادات حيوية الجينتاميسين و حامض النالديكسك و السيفالوكسين، و مقاوماً إلى أربعة مضادات حيوية هي الامبسلين والكلورامفنيكول و كتريمكسازول و تتراسيكلين.
و قد نوقشت الأهمية الصحة العامة للعترات المَعْزُولة ونتائج التجارب لعترات السالمونيلا انتريكا سيروفار انتراتيدس لمعرفة للعلاقة بين الماشية و الإنسان.