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SALMONELLA ENTERICA SEROVAR ENTERITIDIS IN POULTRY MEAT AND THEIR EPIDEMIOLOGY

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SUMMARY

Out of 100 samples taken from the surface of the skin of poultry carcasses in poultry slaughterhouses, 54 samples were positive to *Salmonella enterica* serovar enteritidis with a percentage of (54%). 100 fecal samples were taken from human stools of workers in contact with poultry in poultry slaughterhouses at Kalyobia Governorat, suffering from diarrhaea and/or fever. *Salmonella enterica* serovar enteritidis represented in 42 samples with percentage of (42%). Phage typing of isolated strains from poultry and poultry attendant demonstrated three strains 6, 21 and 28 having the possibility of cross infection between poultry men and poultry carcasses. Antimicrobial sensitivity test proved those *Salmonella enterica* isolated strains were sensitive to Ampicillin (10 µg), Amoxicillin (20 µg), Gentamycin (10 µg), Kanamycin (30 µg), Nitrofurantoin (300 µg), and Cephalothin (30 µg) and medium resistant to Streptomycin (10 µg), and Tetracycline (30 µg). The public health significance of the isolated strains was discussed.

INTRODUCTION

Food of animal origin can be the vehicle for transmission of salmonellae to man, meat and meat products which may be contaminated by human excreta at any step in the chain of processing, meat handling from raw material to the preparation of meat and meat products (Fathi et al., 1994).

Salmonella enterica is the cause of the foodborne salmonellosis pandemic in humans, in part because it has the unique ability to contaminate

poultry meat (Jean Guard-Petter 2001). The incidence of Salmonella food poisoning in the United States in 1988 was estimated to be between 840,000 and 4 million (Tauxe, 1991).

Salmonella enterica can be divided into two broad groups on the basis of pathogenesis and infection biology. One group consists of a large number of serovars, including Salmonella enterica serovar typhimurium and Salmonella enterica serovar enteritidis that can colonize the alimentary tract of food animals or cause gastrointestinal disease in a range of hosts including humans. The other group comprises a small number of serovars that cause systemic typhoid-like disease in a restricted range of host species, such as Salmonella serovar typhi in humans, Salmonella enterica serovar dublin in cattle, and Salmonella enterica serovars pullorum and gallinarum in poultry.Salmonella enterica serovar enteritidis localizes in the reproductive tract of chickens and as a consequence may be transmitted vertically to chicks by transovarian transmission of the bacteria into developing hatching eggs. The diseased poultry is an acute systemic disease that results in a high mortality rate in young chicks but rarely causes such severe clinical disease in adult birds, though it can result in loss of weight, decreased laying, diarrhea, and lesions and abnormalities of the reproductive tract (Snoyenbos , 1991). Therefore the present investigation was planned out to throw some light on Salmonella enterica serovar enteritidis contamination of poultry carcasses , the workers and also antimicrobial sensitivity test of the isolated Salmonella strains.

MATERIALS AND METHODS

Case material: The isolates of *Salmonella enterica* serovar enteritidis used in this study originated from swabs obtained from the surface of the skin of poultry carcasses samples at the slaughterhouses. Fecal samples were obtained from human stools of the workers in contact with poultry carcasses in poultry slaughterhouses at Kalyobia Governorat according to the methods recommended by (Sheila Polakoff et al., 1967); (Varnam & Evans 1991) and (Collins et al. (1995).

The collected samples were labeled and transferred to laboratory without delay in ice bag.

Bacterial culturing: The following bacterioligcal media were used: brilliant green agar (BBL). MacConkey agar (BBL) fro direct plating of specimens, Selenite-F broth (BBL). Swabs were taken from the surface of the skin of the chicken carcasses.

Biochemical identification of isolates: was made on the basis of the following tests according to (McFadden, 1980): glucose metabolism negative; production of indole, Methyl red reaction positive (MR) and Voges proskaur test (VP), and do not 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 (pomeroy and Nagaraja. 1991) and (Wierup M. et al., 1995). Briefly, 4ml 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 lysis produced by the phages was recorded and interpreted by comparison to standard charts.

Serological identificaiton: The isoalted proved biochemically to be Salmonella microorganism were subjected to serological identification according to Kauffman white scheme (Kauffman, 1974). 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 phage thping for serovar enteritidis strains were phage typed using the Dutch. Phage typing system described by (Wierup et al., 1995) at Faculty of Veterinary Medicine Zagazig University Benha Branch.

Antimicrobial susceptibility testing: The disk diffusion method recommended by Bauer et al., 1966 was used for susceptibility testing.

Eight drugs was routinely used to test Gram-negative enteric bacteria: Ampicillin (10µg), Amoxicilin (20µg), Gentamycin (10µg), Kanamycin (30µg), Nitrofurantoin (300µg), Streptomycin (10µg), Tetracyclin (30µg), and Cephalothin (30µg),

The results were recorded in tables (1,2,3,4,5, & 6).

RESULTS

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

Total No. of examined samples	No. of individuals positive to *Salmonella enterica* of individual case	%
100	42	42%

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

Total No. of examined samples	No. of individuals positive to *Salmonella enterica* of individual case	%
100	54	54%

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

Human phage type	No. of isolates	%
Phage type No.1	12	28.5%
Phage type No.4	6	14.2%
Phage type No.6	7	16.6%
Phage type No.2	9	921.4%
Phage type No.28	1	2.3%
Untypable strains	7	16.6%
Total	42	100%

Table (4): The numbers and percentage of phage typable isolated from poultry meat:

Human phage type	No. of isolates	%
Phage type No.1	17	31.4%
Phage type No.4	10	18.5%
Phage type No.6	5	9.2%
Phage type No.2	11	20.3%
Phage type No.28	2	3.7%
Untypable strains	9	16.6%
Total	54	100%

Table (5): phage typing isolated strains from poultry carcasses and human stools of workers:

Source of samples	Isolates	Typeable strains	Phage produced Typeable strains	Untypeable strains	Possibility of cross infection between man and poultry Carcasses
Man	42	35	phage type No.1, 4, 6, 21and 28	7	phage type No. 6, 21and 28
Pooultry carcasses	54	45	phage type No.1, 4, 6, 21and 28	9	

Table (6): Summarized results of antimicrobial sensitivity test of isolates:

Antimicrobic agent	Disc potency	Inhibited zone	Results
Ampicillin	(10 µg)	20 or less	S
Amoxicillin	(20 µg)	19 or less	S
Gentamycin	(10 µg)	12 or less	S
Kanamycin	(30 µg)	13 or less	S
Nitrofurantoin	(300 µg)	14 or less	S
Cephalothin	(10 µg)	14 or less	S
Tetracycline	(30 µg)	14 or less	R
Streptomycin	(30 µg)	14 or less	R

S= Sensitive R= Resistant

DISCUSSION

100 Samples from poultry carcasses at the slaughter house and 100 fecal samples from human stools of the workers in contact with the poultry were examined for isolation and identification of Salmonella enterica serovar enteritidis by using, glucose metabolism negative; production of indole, Methyl red reaction positive (MR) and Voges proskaur test (VP), and do not utilization of Citrate.

The result displayed in Table (1) revealed that out of 100 swabs collected from poultry carcasses, Salmonella enterica serovar enteritidis was Isolated from 54 (54%). The incidences recorded were agreed with (Humphrey 1999). Salmonella enteritidis was found in both poultry

meat and eggs (Hinton et al. 1990). Capita et al. (2003) could detect Salmonella enteritidis in 34.4 % of poultry carcasses in Spain.

The data recorded in Table (2) showed that out of 100 fecal samples obtained from human stools in the same locality of the poultry, only 42 (42%) contained Salmonella enterica serovar enteritidis. The incidences recorded were agreed with obtained by (Barrow and Duchet-Suchaux, 1997). In Denmark from 1991-1999, the number of patients suffered from salmonellosis was 26947 patients, while the number of deaths occurred between them was 838in percentage of 3.1 % (Helms et al., 2003). Salmonella species could be detected in 5.6% of 606faeces specimen taken from Patients with diarrhea aged 0-60and living in the area of Fanon(Italy) (Baffone et al., 2001). In the Greek island of Crete during a 5 years period (1995-1999), Salmonella species could be detected in 6% stool samples obtained from human patients (Maraki et al., 2003).

From Table (3) it is evident that out of 42 Salmonella enterica serovar enteritidis isolated from human stools, 35 (83.3%) were typed by human set phage, while 7 (16.6%) untyped. The typable strains were phage type No. 1, 4, 6, 21 and 28 with the incidence of 12,6,7,9, and 1, with percentage of 28.5%, 14.2%, 16.6%, 21.4%and 2.3% respectively

From Table (4) it is evident that out of 54 Salmonella enterica serovar enteritidis isolated from poultry carcasses, 45 (83.3%) were typed by human set phage, while 9 (16.6%) untyped. The typable strains were phage type No. 1, 4, 6, 21 and 28 with the incidence of 17, 10,5,11. And2, with percentage of 31.4%, 18.5%, 9.2%, 29.6%and 3.7% respectively .The incidences recorded in table 3,4 were agreed with obtained by the (Saeed et al., 1999), and also the same results were recorded by(Pomeroy and Nagaraja. 1991).

From Table (5): out of 54 poultry carcasses isolates 45 were typed, 9 untyped The possibility of cross infection between poultry and human in the same locality of poultry were demonstrated by 3 strains (6-21-28). It's worthy to mention that the number of the untypeable strains may be due to the use of the common ordinary human phage set only and not all human sets.

Phage-typing result nearly substantiates what had been observed by (Barrow and Lovell. 1991). From the results achieved, it can be concluded that cross contamination between poultry and the human in the same localities of poultry may occur by some strains of Salmonella enterica serovar enteritidis.

Table (6): Mention that antibiotics can influence carrier-states significantly. Antibiotic susceptible resident flora can be replaced by Salmonella enterica serovar enteritidis with multiple antibiotic resistances and in hospital environments, by epi-

demiologically virulent strains. In veterinary hospitals antibiotics excreted in urine and feces may dry as droplet nuclei and be carried into the atmosphere by movement of patients or personnel. Also antibiotic resistant strains are met with in previously treated patients than untreated ones.

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.

Antimicrobial sensitivity test proved those Salmonella enterica isolated strains were sensitive to Ampicillin (10 µg), Amoxicillin (20 µg), Gentamycin (10 µg), Kanamycin (30 µg), Nitrofurantoin (300 µg), and Cephalothin (30 µg) and medium resistant to Streptomycin (10 µg), and Tetracycline (30 µg) (Smith and Tucker 1975).

To avoid contamination of poultry carcasses with such pathogens,

Food handlers must be free from diseases which can be transmitted by foods, should have medical certificate and subjected to regular medical examination. Personal hygiene and good sanitation. Proper examination of the poultry at the farm& at the slaughter house in both antimortem and postmortem examinations. Application of good hygienic conditions at the slaughter house.

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