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RESEARCH ARTICLE

Identification of Human and Animal Salmonella spp. isolates in Najran region and control of it.

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Abstract

Animals including the poultry and its by product are major sources of human salmonellosis aswell as animals raised for food play an important role in transmission of antimicrobial resistant Salmonella strains to humans. This study was under-token to determine the prevalence of Salmonella spp. In human, animals, food items, and their antimicrobial resistance patterns in Najran region, Saudi Arabia. Samples for analysis were collected from human (n. = 532) and animals ((sheep n. = 330, camels n. = 44 and cattle n. = 59) faecal samples, Poultry by product (meat) (n. = 120) including egg (n. = 506), wild pigeons (n. = 43), Fish (n. = 85) and fresh she-camels milk (n. = 13). Salmonella isolation, identification and antibiotic sensitivity were performed according to standard previously described methods. The prevalence of Salmonella isolates in human, sheep, camel and cattle was (2.4%, 6.4%, 2.3% and 8.5) However, the prevalence of Salmonella isolates in fish, poultry meat, table eggs, fresh camel milk and wild pigeon was (0 %, 4.1%, 2.1%, 0%, 11.6%, and 2.7%).The prevalence of Typhoid and non-typhoid salmonellosis were 46.1% and 53.8 % respectively. Isolated Salmonella spp. were Salmonella subsp.1, S. arizonae subsp.3A, Salmonella subsp.5, Salmonella typhimurium, Salmonella typhi, Salmonella paratyphi A and Salmonella pullorum. (37.7%, 3.3%, 4.9%, 24.6%, 8.2%, 1.6% and 19.7%) Respectively. All isolated salmonella sp. were significantly higher sensitive ($P<0.05$) to Ciprofloxacin (5µg) (97.1 %).In conclusion, the serious implications associated with drug-resistant Salmonella species, a more deliberate use of antibiotics in both human medicine and animal industry is warranted. Furthermore, attentions must be taken to wild pigeons that played important role as source of Salmonella typhimurium infection to human, animals and domestic birds in Najran region, Saudi Arabia. Non-Saudi Residentworkers should be have health certificate and periodically renewed specially Restaurant workers because they were a source of new isolates, serotypes and clones of Salmonella species in Saudi Arabia.

INTRODUCTION

Salmonella are the major pathogenic bacteria in humans as well as in animals. Salmonella is ubiquitous in the environment originating from the gastrointestinal tracts of domesticated and wild animals and can be present without causing apparent illness. Most infections result from the ingestion of foods of animal origin contaminated with Salmonella species such as beef, chicken, turkey, pork, eggs, and milk (Olsen et al., 1997).

The genus Salmonella currently has two species, Salmonella enterica and S. bongori. S. entericais divided into the following subspecies: S. enterica subsp. enterica (subspecies I), S. enterica subsp. salamae (subspecies II), S. enterica subsp. arizonae (subspecies IIIa), S. enterica subsp. diarizonae (subspecies IIIb), S. enterica subsp. houtenae (subspecies IV), and S. enterica subsp. indica (subspecies VI). S. enterica subsp. I strains are usually isolated from humans and warm-blooded animals. Most of the Salmonella strains isolated in clinical laboratories belong to S. enterica subsp. I. The majority of the Salmonella serotypes belong to S. enterica subsp. I (Cuff, et al., 2000 and Dice, 1945). Human stool acts as an important reservoir of Salmonella serovars that are the grouping of microorganisms based on their cell surface antigen. Species isolated from human stool are Salmonella typhi, S. paratyphi A, S. typhimurium, S. wrothington and S. enteritidis (Kumar et al., 2009). Salmonella paratyphoid in poultry (PT) play a very important role in food – borne salmonellosis in humans (Gast, 1997). certain serotypes of Salmonella such as S. Enteritidis, which can penetrate poultry reproductive organs resulting in the contamination of egg contents has been a prominent cause of human illness for several decades (Gantois et al. 2009). In many countries, Salmonella typhimurium is one of the most common and harmful pathogens found in livestock, poultry, and vegetables, and is a causative agent of salmonellosis, food poisoning, gastroenteritis, abdominal pain, and typhoid fever (Liu et al., 2001 and Nandakumar, 2008). Salmonellosis is the most common pigeon disease, caused by S. Typhimurium and S. Enteritidis. In the chicken, Salmonella entericaserovarPullorum may persist for a number of months in the spleen, leading to infection of the reproductive tract and, consequently, to vertical transmission of the infection to eggs or to progeny (Shivaprasad, 2000).

Majority of Salmonella resistant to nalidixic acid show decreased susceptibility to ciprofloxacin as well (Poutanen and Low, 2003). In Saudi Arabia, serovartyphimurium and enteritidis were the most frequently isolated serovars from humans and animals (Boyen et al., 2008; Abdullahi, 2010). Ampicillin and cotrimoxazole resistance occurred in 29% and 24% of the human Salmonella spp., Isolates in Saudi Arabia, respectively, but only 0.6% were resistant to ceftriaxone and 1.8% had intermediate resistance to ciprofloxacin (Elbashier et al, 2003). Somily et al., 2012 concluded that the majority of Salmonella isolates in Riyadh, Saudi Arabia were non-typhi serotypes. Significantly, higher proportions of Salmonellae were resistant to nalidixic acid and ciprofloxacin and a vast majority of nalidixic acid resistant organisms exhibited decreased susceptibility to ciprofloxacin.

In view of these considerations, the present study was undertaken with the following objectives of (i) to isolate and identify Salmonella serovars from human stool, (ii) to isolate and identify Salmonella serovars from animal samples, (iii) to characterize the isolated Salmonella serovars using cultural, biochemical and (iv) to study the antibacterial sensitivity of the isolated Salmonella serovars.

Material and Methods

Study population: The study subjects were selected by proportional random stool sampling from the Najran hospitals and clinical laboratories and were obtained from relevant authorities. 532 human stool samples were collected (33 diarrhoeic and 499 non-diarrhoeic).

The animal samples: 433 animal faecal samples were collected (sheep = 330, camels = 44 and cattle = 59) between October 2013 and july 2014. Fecal animal samples were collected from Najran farms and slaughterhouses. 330 sheep faecalsamples (30 diarrhoeic and 300 non-diarrhoeic), 44 camel faecal samples (7diarrhoeic and 37 non-diarrhoeic) and 59 cattle faecal samples (12 diarrhoeic and 47 non-diarrhoeic). Poultry by product (meat) (n. = 120) including egg (n. = 506) were collected from Najran farms. wild pigeons (n. = 43) were examined for Salmonella spp. Fish (n. = 85) and fresh she-camels milk (n. = 13) samples were included in sample study.

Ethical consideration: The university ethical board gave permission to conduct the study within the institutional research mandate as stipulated by the National Ethical Board.

Study design and area: A cross sectional study was conducted in the Department of Applied Medical Sciences, Community College, Najran University. Najran, south of Saudi Arabia lies between 17° 30' 20" North, 44° 11' 3" East (1260 kms away from Riyadh). Average temperature in Najran ranges from 14.6 to 30.9 °C and the average annual rainfall is 83 mm.

Sample collection and transport: Human and animal specimens were collected in a clean cup by medical laboratory technicians and transported into the Microbiology laboratory in the Department of Applied Medical Sciences, Community College, Najran University within an hour of collection.

Culture and identification: The collected 25 g samples were put into an Erlenmeyer flask. 225 ml buffered peptone water (Scharlau, India) was added to obtain 1 part sample + 9 part buffered peptone water then well mixed and Incubated at 37oC overnight (16-20 hours) to recover sub-lethally injured cells due to heat, cold, acid, or osmotic shock (Sandel et al. 2003; Gracias and McKillip 2004). 1 ml of the pre-enrichment buffered peptone water sample was transferred with a pipette to 10 ml Selenite-F broth (HiMedia Laboratories) and incubated at 37°C for 18 hours

for maximum recovery of the isolates (Leifson, 1936). The inoculates were obtained from Selenite-F medium using a sterile cotton swab onto MacConkey agar media (Oxoid, UK) and was incubated for 18 hours at 37°C. These were then subcultured into Deoxychocolate Citrate Agar (XLD) (HiMedia Laboratories). These culture methods were according to the current ISO horizontal method (ISO 6579 Standard, 2002) (updated in 2007) for the detection Salmonella, including Salmonella Typhi and Salmonella Paratyphi applicable to products intended for human consumption and the feeding of animals, and to environmental samples in the area of food production and food handling. The isolates were then subcultured in Triple Sugar Iron agar (TSI) (Scharlau, India) and was incubated for 18-24 hours at 35 ± 2°C (ISO 10272 Standard, 1995). The suspected Salmonella spp. colonies according to TSI and selective media were followed by biochemical identification, the Microbact™ Gram-negative system (Oxoid, UK) which used for the identification of aerobic and facultatively anaerobic Gram-negative bacteria (Enterobacteriaceae and miscellaneous Gram- negative bacteria) (Mugg and Hill, 1981). The Salmonella positive specimens were then subcultured in nutrient broth and stored in the refrigerator at 8ºC for Antimicrobial susceptibility testing.

Antimicrobial susceptibility testing: Antimicrobial susceptibility tests were performed on Mueller-Hinton agar (Oxoid, Hampshire, UK) by disc diffusion method (Bauver et al., 1966). The antimicrobial agents tested were: Ciprofloxacin (5 µg), Gentamicin (10 µg), Ceftriaxone (30 µg), Cotrimoxazole (25 µg), Amoxicillin &Clavulinic acid (30 µg), Norfloxacillin (10 µg), Amikacin (30 µg), cephradine (30 µg), Ampicillin (10 µg), Tetracycline (10 µg), Nalidixic acid (30 µg) and Choloramphenicol (10 µg) (Oxoid, UK). The resistance and sensitivity were interpreted according to the National Committee for Clinical Laboratory Standards (National Committee, 1993).

Statistical analysis: The data were analyzed with Chi, ×2. Probabilities of $P<0.05$ were considered significant and analysis of variance (ANOVA) according to Fisher's PLSD test. Differences were considered significant when $P\leq0.05$.

Result and Discussion

The overall Salmonella infection prevalence in Najran area, Saudi Arabia was 3.5% table (1). The prevalence rate salmonella isolates was significantly higher ($P<0.05$) in cattle, sheep than in human and camel. Salmonella isolates were recorded in diarrheic and non-diarrhoeic samples. Out of 82 diarrhoeic samples, 4 samples (1 human, 2 sheep and goat and 1 cattle) were found to be infected with salmonella spp. Out of 883 non-diarrhoeic samples, 36 samples (12 human, 19 sheep and goat, camels and 4 cattle) were found to be infected with salmonella spp.

Prevalence of Salmonella isolates in fish, poultry meat, table eggs, fresh camel milk and wild pigeon was (0 %, 4.1%, 2.1%, 0%, 11.6%, and 2.7%) respectively table (1) and fig. (1). the prevalence rate salmonella isolates was significantly higher ($P<0.05$) in wild pigeons than in fish and camel milk. Prevalence infection in table eggs was 2.1% (11 isolates out of 506).

From table (2) and fig. (2) The prevalence of Typhoid and non-typhoid salmonellosis were 46.1% and 53.8 % respectively. Isolated Salmonella spp. were Salmonella subsp.1, S. arizonae subsp.3A, Salmonella subsp.5, Salmonella typhimurium, Salmonella typhi, Salmonella paratyphi A and Salmonella pullorum (37.7%, 3.3%, 4.9%, 24.6%, 8.2%, 1.6% and 19.7%) respectively table (1). The human typhoid isolates were Salmonella typhi and Salmonella paratyphi A. The higher salmonella isolates in sheep and goat were Salmonella subsp.1 while in cattle were Salmonella typhimurium. Salmonella pullorum infection was isolated from table eggs and poultry meat including the viscera (specially the liver).

Table (1): prevalence of Salmonella isolates in samples of human and animals.

samples	Number sampled	Number positive (%)
Human	532	13 (2.4)
Sheep	330	21 (6.4)
Camels	44	1 (2.3)
Cattle	59	5(8.5)
Fish	85	0 (0)
poultry meat	120	5 (4.1)
table egg	506	11 (2.1)
fresh camel milk	13	0 (0)
wild pigeons	43	5 (11.6)
Total	1732	61 (3.5)

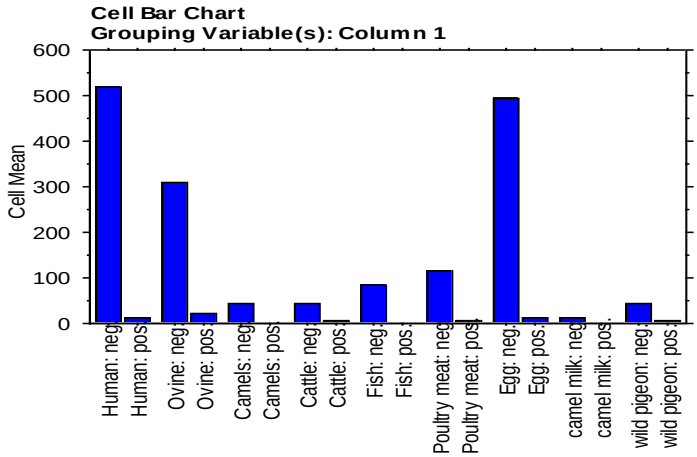

Fig. (1) Infected and non-infected Salmonellae sp. In human and animal (sheep, camels, cattle, fish, poultry meat, table eggs and wild pigeons). Probabilities of P<0.05

Table (2): Salmonellae sp. isolates in human and animal samples in Najran, Saudi Arabia

Salmonellae isolates	Animal faeces			Human stool	Poultry meat	Table egg	Wild Pigeons	TOTAL %
	Sheep	Camel	cattle					
Salmonella subsp.1	17		1		5			23 (37.7)
S. arizonae subsp.3A				2				2 (3.3)
Salmonella subsp.5				3				3 (4.9)
Salmonella typhimurium	4	1	4	1			5	15 (24.6)
Salmonella typhi				5				5 (8.2)
Salmonella paratyphi A				1				1 (1.6)
Salmonella pullorum				1		11		12 (19.7)
Total	21	1	5	13	5	11	5	61

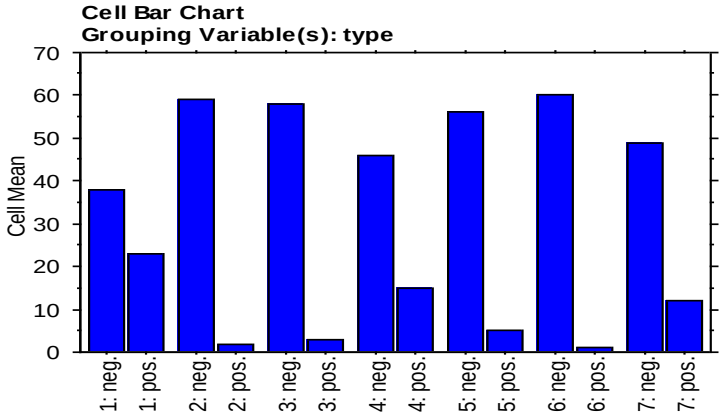

Fig. (2) Salmonellae sp. Isolates prevalence (Salmonella subsp.1 (1) , S. arizonae subsp.3A (2), Salmonella subsp.5 (3), Salmonella typhimurium (4), Salmonella typhi (5), Salmonella paratyphi A (6), Salmonella pullorum (7). Probabilities of P<0.05.

Isolated salmonella sp. were significantly higher sensitive (P<0.05) to Ciprofloxacin (5µg) (97.1 %) thanall antibiotic disc table (3) and fig. (3). Isolated salmonella sp. were highest resistance to Choloramphenicol (10 µg) (88.6%) and Nalidixic acid (30 µg) (82.9 %). table (3). Salmonella subsp.1 and Salmonella subsp.5 were highly sensitive to Ceftriaxone (30 µg) then to Ciprofloxacin (5µg). The S. arizonae subsp.3Awas highly sensitive to

Norfloxacillin (10 µg) then to Ciprofloxacin (5µg). However the others salmonella isolates were highly sensitive to Ciprofloxacin (5µg).fig. (4). The highest inhibition zone was recorded in Ciprofloxacin (5 µg), Ceftriaxone (30 µg) and Norfloxacillin (10 µg) with (26.4 ± 4.7 mm, 21.2 ± 5.6 mm and 20 ± 6 mm respectively) fig. (5). While The lowest inhibition zone was recorded in Nalidixic acid (30 µg), Choloramphenicol (10 µg) and Tetracycline (10 µg) with (9.5 ± 6.5 mm, 11.4 ± 6.7 mm and 13.5 ± 6 mm respectively) fig. (5).

Table (3): Antimicrobial susceptibility patterns of Salmonella species in human and animal samples in Najran, Saudi Arabia:

Antibiotic (concentration)	(%) sensitive	(%) resistant
1-Cotrimoxazole (25 µg)	51.4	48.6
2-Choloramphenicol (10 µg)	11.4	88.6
3-Ciprofloxacin (5 µg)	97.1	2.9
4-Nalidixic acid (30 µg)	17.1	82.9
5-Ampicillin (10 µg)	42.8	57.1
6-Gentamicin (10 µg)	68.6	31.4
7-Amikacin (30 µg)	45.7	54.3
8-Tetracycline (10 µg)	22.9	77.1
9-Norfloxacillin (10 µg)	45.7	54.3
10-Ceftriaxone (30 µg)	65.7	34.3
11-Amoxicillin &Clavulinic acid (30 µg)	48.6	51.4
12- cephradine (30 µg)	42.8	57.1

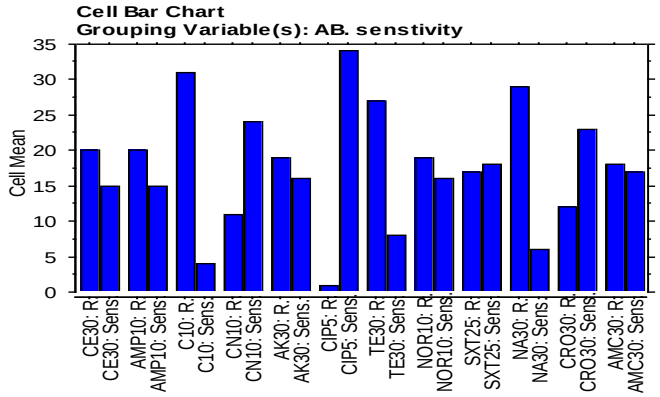

Fig. (3) Sensitivity and resistance of Salmonellae sp. Isolates against antibiotic disc infusion . Probabilities of P<0.05.

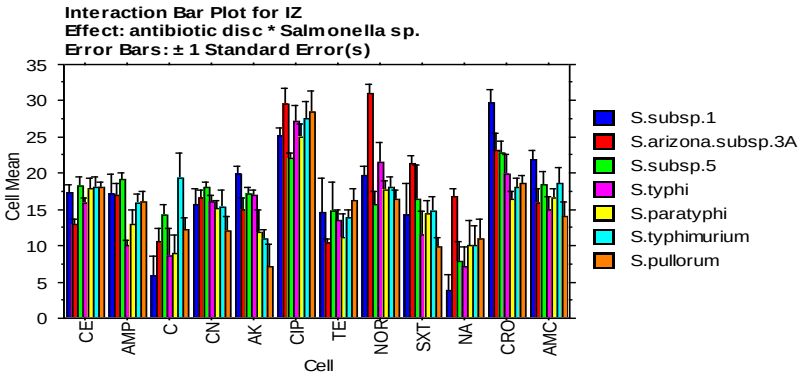

Fig. (4) The highly Sensitive and highly resistance of Salmonellae sp. Isolates against antibiotic disc infusion by detecting inhibition zone. ±1 Standard errors

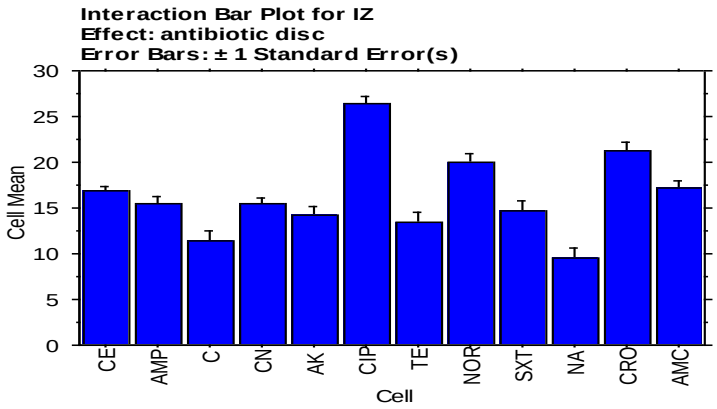

Fig. (5) Inhibition zone of different antibiotic infusion discs against different Salmonellae sp. Isolates. . ±1 Standard errors

Salmonella spp. are documented to be pathogens that cause a spectrum of diseases in humans and animals, including domesticated and wild mammals, reptiles, birds, and insects. Salmonella spp. infections are caused by consumption of contaminated food, person-to-person transmission, waterborne transmission and numerous environmental and animal exposures.

In this study, The overall Salmonella infection prevalence in Najran area, Saudi Arabia was lower (61 out of 1732) (3.5%) compared to previous study done at Jeddah, Saudi Arabia (15 out of 60) (25%) (Iyer et al., 2013). However, the infection in our study was higher compared to study done at Qatif Central Hospital, Saudi Arabia (0.64 %) (Elbashier et al., 2003). This study revealed that both diarrhoeic and non-diarrhoeic samples could harbour Salmonella infection. This confirms that the non-diarrhoeic samples is carrier and the source of infection to human and animals. This result was agreed with (Ojo and Adetosoye, 2009) that revealed that both diarrhoeic and non-diarrhoeic dogs can harbor Salmonella andthe presence of Salmonella in pet dogs makes them a potential source of infection to their human companions.

In our study, the prevalence of non-typhoid salmonellosis was nearly higher than typhoid salmonellosis in Najran area, Saudi Arabia and theses results were agreed with the results obtained by (Somily et al., 2012) in Riyadh, Saudi Arabia where the majority of Salmonella isolates in this study were non-typhi serotypes. In Saudia Arabia for the first time S. arizonae subsp.3A and Salmonella subsp.5 (salmonella bongori) had been isolated in this study. S. entericasubsp. arizonaeis one of the less common subspecies of Salmonella. Like many non-typhoidal salmonellae, it is mostly found in animal species (commonly reptiles) and only occasionally infects humans. Snakes appear to be important carriers of this bacterium, with as many as 78.8% harboring the organism (Habermalz and Pietzsch, 1973). The single exception, subsequently described, is S. bongori, previously known as subspecies V, which by DNA-DNA hybridization is a distinct species (Reeves et al., 1989). Serotypes in S. entericasubspecies II (S. entericasubsp. salamae), IIIa (S. entericasubsp. arizonae), IIIb (S. entericasubsp. diarizonae), IV (S. enterica subsp. houtenae), IV (S. entericasubsp. indica), and S. bongori are usually isolated from cold-blooded animals and the environment but rarely from humans (Farmer et al., 1984).

In our study the S. arizonaesubsp.3A and Salmonella subsp.5 (salmonella bongori) were isolated fromcarrier resident indianpersons in Najran area, Saudi Arabia, furthermore It is known that India's environment filled with snakes and This explains the isolation of S. arizonae subsp.3A. In this study Salmonella subsp.1 in Najran area, Saudi Arabia were isolated from sheep, cattle and poultry. The higher salmonella isolates in sheep and goat were Salmonella subsp.1. In addition, this result was first recorded in Saudi Arabia. These results is also discussed by (Agasan et al., 2002)that observed Strains of newly emerging Salmonella entericasubsp. enterica(subspecies I) serotype 4, 5, 12: I, causing food-borne infection. However, (Porwollik, et al., 2004) found that Subspecies 1 of Salmonella enterica is responsible for almost all Salmonella infections of warm-blooded animals, and (Kingsley et al., 2000) where Sayed that factors which enable Salmonella serotypes to circulate within populations of livestock and domestic fowl. We have identified a DNA region, which is present in Salmonella serotypes commonly isolated from livestock and domestic fowl (S. enterica subspecies I).

Prevalence of Salmonella isolates in fish was 0 % in the our study, in Najran, Saudi Arabia while in Eastern Province of Saudi Arabia Prevalence was 39.9% in frozen retail imported fresh water fish (Elhadi, 2014). The

negative fish result in our study might be due to fish samples were fresh seawater fish and had no history of storage, also were local fish and were not imported.

In the study in Najran area, Saudi Arabia, isolated salmonella sp. were resistance to Choloramphenicol (10 µg) (88.6%), Nalidixic acid (30 µg) (82.9 %), Tetracycline (10 µg) (77.1%) andAmpicillin (10 µg) (57.1%). These results were Agreed with (Malik et al., 1993) inAsir region, southern Saudi Arabia, that found all isolated Salmonella spp. were resistant to tetracycline, ampicillin, and chloramphenicol. Furthermore, (Al Ayed, 2014) in Najran area, Saudi Arabia found that the highest resistance of all non-typhoidal salmonellosis isolates in children were to tetracycline (71.4%), followed by ampicillin (54.8%), chloramphenicol (26.2%) and gentamicin (19%). However, in our study, we found isolated salmonella sp. were significantly higher sensitive ($P<0.05$) to Ciprofloxacin (5µg) (97.1 %) and resistance with 2.9 %. These findings are in agreement with those of a similar study on Prevalence of nontyphoidalSalmonella serogroups and their antimicrobial resistance patterns in a university teaching hospital in Eastern Province of Saudi Arabia in which some of the strains (14.93%) were reported to be resistant to cefotaxime and ciprofloxacin (3%) (Elhadi et al., 2013). Ciprofloxacin and cefotaxime are the antimicrobial agents recommended for treatment of invasive infections due to Salmonella (Threlfall, 2002). Increasing antimicrobial resistance in NTS is a global public health problem that complicates antimicrobial therapy, and is increasingly due to the overuse and misuse of antimicrobial agents in animal feeds (Tsai et al., 2012).

In conclusion, S. arizonae subsp.3A and Salmonella subsp.5 (salmonella bongori) in human had been isolated in the first time in our Najran region and Saudi Arabia. Also the most salmonella isolates in sheep and goat were Salmonella subsp.1. In addition, this result was first recorded in Saudi Arabia. Overall, isolates were multidrug-resistant except Ciprofloxacin was drug of choice to treat the salmonellosis in human and animals specially the invasive isolates. In view of the serious implications associated with drug-resistant Salmonella species, a more deliberate use of antibiotics in both human medicine and animal industry is warranted. Continued surveillanceof antimicrobial resistance and use of antimicrobial agents in food animals is also indispensable. Furthermore, attentions must be taken to wild pigeons that played important role as source of Salmonella typhimurium infection to human, animals and domestic birds. Non-Saudi Residentworkers should be have health certificate and periodically renewed specially Restaurant workers because they were a source of new isolates, serotypes and clones of Salmonella species in Saudi Arabia.

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