Sudan Journal of Science and Technology (2014) 15(2): 40-48

Sudan Journal of Science and Technology
Journal homepage: http://jst.sustech.edu/

Occurrence of *Salmonella species* in the Environment of broiler Poultry Farms of Jabal Awliya Locality, Sudan.

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ARTICLE INFO

Article history
Received: 3 September 2014
Accepted: 26 September 2014
Available online: 1st March 2015

KEYWORDS:
Salmonellosis,
Antimicrobial susceptibility ,
Broiler,
Jabal Awliya Locality

ABSTRACT

The aim of this study was to detect the occurrence of *Salmonella species* in the environment of broiler poultry farms of Jabal Awliya Locality and to investigate the antimicrobial susceptibility of the isolates. A total of 162 samples were collected from water source, drinkers, feed, feeders, litter, dust, faeces, cloacal swabs, and hand swabs from workers from different 18 farms from April 2013 to February 2014. Samples were analyzed by using ISO 6975(2002) and confirmed by using API20E strips. The results showed that the overall prevalence of *Salmonella spp* was 10 (6.2%) recovered from 8(44.4%) farms. Isolates were collected from drinkers1 (10.0 %%), feeders 1(10.0%), faeces 1(10.0%), cloacal swabs 1(10.0%), dust 3(30.0%), and litter 3(30.0%), however, water source, poultry feed, and hand swabs of workers were free from *Salmonella species*. This study showed that all the isolates (100%) were sensitive to ciprofloxacin, cefixime, cefotaxime, and colistin followed by, chloramphenicol (90. %), co-tri moxazole (70.0 %), streptomycin (70.0%), gentamicin (70.0%), ampicillin (30.0%), tetracycline (30.0%), nalidixic acid (30%), while amoxicillin showed the highest resistant antibiotic.
This study was done at the laboratory of the Microbiology department, Faculty of Medical laboratory Sciences, Al-Neelain University.

INTRODUCTION

Salmonellosis is a serious medical and veterinary problem and raises great concern in food industry. Poultry is the most potential source of *Salmonella* food presenting in man (Al-Maliki *et al*., 2012). *Salmonella* can infect poultry flocks via

feed, water, hatching eggs and through environmental factors including birds, insects, rodents and farm workers (Wray and Wray, 2002). Frederick and Huda (2011) reported that the incidence of the infestation of *Salmonella* in poultry houses is more likely to be transmitted to the birds which increase the risk for the exposure of salmonellosis to the humans. They also mentioned that conventional cultural method is one of the most reliable techniques that can be used for isolation and identification of *Salmonella species*. *Salmonella* are Gram-negative, non-spore forming, rod shaped, non-capsulated, motile with peritrichous flagella. They are cytophilic, metabolize glucose to acids, catalase-positive, and oxidase-negative (Alao *et al*., 2012).

Antibiotics have been widely used in the poultry industry to enhance growth and feed efficiency and reduce bacterial diseases. The most commonly used antimicrobial agents for either chemoprophylaxis or therapy for control of bacterial diseases includes sulfadiazine, tetracycline, gentamycin, amoxicillin, neomycin, ciprofloxacin, enrofloxacin, colistin, flumequine, spectinomycin, ampicillin, tylosin, and trimethoprim (Sirdar, 2010). Effective Antimicrobial therapy reduces morbidity and mortality from *salmonella* food poisoning. Without Therapy, illness may last 3-4 weeks and case-fatality rates may exceed 10% (WHO, 2003).

The objectives of this study were to:

-Determine the prevalence of *Salmonella species* in the environment of the broiler poultry farms of Jabal Awliya Locality.

- Investigate antimicrobial susceptibility testing of the isolates to determine resistance pattern by disc diffusion method.

MATERIALS AND METHODS

Study design

Jabal Awliya Locality borders southwards on Gezira Locality, westwards on the White Nile, and eastwards on the Blue Nile. It has a population of 10000 inhabitants. The locality is characterized by Sundus Scheme, along with a number of livestock, poultry, fishing projects, besides farms of vegetables, fruits and fodder production projects.

Eighteen broiler farms located in El-Dekhainat, El-Ghadesya, Wad-El-Agali, El-Fetaih, El-Shegailab, Tayba El-Hassanab, and Arak Salih were selected for this study. Samples were collected from water (source, and drinkers), feed (source, and feeders), dust, litter, cloacal swabs, faeces, and hand swabs from workers during April 2013 to February , 2014 to detect *Salmonella species* using ISO 6579(2002), and API20E strips was also used for confirmation.

Sampling

Twenty five ml of water from source and drinkers was collected by using sterile syringes and added to 225 ml of buffered peptone water (HIMEDIA, M614), Also 25 g was collected from feed source, feeders, faeces, and litter by using sterile spoons and sterile ISO bags and transported in ice bags then added to 225 ml of buffered peptone water. Moistened swabs with buffered peptone water were used to collect samples from dust, cloacae, and handler's hands, and transferred aseptically in to tubes containing 9 ml of buffered peptone water. Samples in buffered peptone water were incubated at 37 ^{o}C±1 for 24 ± 3 hours.

Isolation and identification of *Salmonella spp*

One ml from the above inoculated buffered peptone water was transferred in to10 ml of Muller-Kauffmann tetrathionate novobiocin broth (MKTTn broth-HIMEDIA, M 14961), and incubated at 37 ^{o}C±1 for 24 ± 3 hours. Another 0.1 ml from the same inoculated buffered peptone water was transferred in to10 ml of Rappaport- Vassiliadis broth (MICROMEDIA MN 0070) and incubated at 41.5 ^{o}C±1 for 24 ± 3 hours. Then a

loopful from the inoculated MKTTn broth and RV broth was used to be cultured on both Xylose lysine deoxycholate agar (XLD agar-HIMEDIA, M 031) and Salmonella-Shigella agar (S.S agar-HIMEDIA, M 108) and incubated at 37 ^{o}C±1 ^{o}C for 24 ± 3 hours. Suspected colonies were streaked on pre-dried nutrient agar plates (HIMEDIA, M001), then incubated at 37 ± 1 ^{o}C for 24 ± 3 hours. Microscopic examination to detect Gram-stain and motility was performed from pure culture obtained from nutrient agar as described by Cheesbrough, (1991).Biochemical tests that were used to identify *Salmonella species* were oxidase test (HIMEDIA,DD018), hydrogen sulfide production from Triple sugar iron agar (SHARLAU,-01-192) , urea hydrolysis (christensen)-HIMEDIA, M 112), lysine decarboxylation (HIMEDIA, M376), indole reaction (SHARLAU, 02-568 ,and Voges-Proskaur (glucose phosphate broth - HIMEDIA, M070)were also used as described by Cheesbrough, (1991).
Confirmation was done by using API 20E identification kits (Bio Merieux, Marcy, France).

Antimicrobial susceptibility test:

Antibiotics that were used in this study were: ampicillin, amoxicillin, chloramphenicol, streptomycin, ciprofloxacin, cefixime, cefotaxime, colistin, nalidixic acid tetracycline, co-trimoxazole, and gentamicin. Kirby-Bauer method was performed as described by CLSI, (2006), by using Muller and Hinton agar (HIMEDIA, M1084).Turbidity of the inoculum of the isolates was compared with 0.5 McFarland standards.

RESULTS

The results showed that 10 (6.2%) out of 162 collected samples were positive for *Salmonella spp*. Eight (44.4%) out of 18 farms were contaminated with *Salmonella spp*. There was no Salmonellae isolated from water source, feed source and hand swabs of workers. Also 1(10.0 %) species was isolated from a drinker in El Fetaih. Also 1(10.0%) species was isolated from a feeder in Tayba El-Hassanab. Furthermore 3(30.0%) species were isolated from litter in El-Fetaih, Tayba El-Hassanab, and Arak salih; Also 3(30.0%) species were collected from dust in El- Shegailab, El-Fetaih, and Tayba El-Hassanab. Salmonella were collected from faeces1 (10%) and cloacal swabs 1(10%) from wad El-Agali, and Tayba El-Hassanab respectively (Table1, and table 2).

Antibiotics susceptibility of isolated Salmonella:

This study showed that all the isolates were sensitive to ciprofloxacin, cefixime, cefotaxime, and colistin, followed by, chloramphenicol (90.0%), co-trimoxazole (70.0%), streptomycin (70.0%), gentamicin (70.0%), ampicillin (30.0%), tetracycline (30.0%), nalidixic acid (30.0%), however, amoxicillin showed highly resistance. Also the isolates showed intermediate resistance to gentamycin (20.0%), ampicillin (20.0%), amoxicillin (10.0%), and streptomycin (10.0%), while they showed resistance to amoxicillin (90.0%), tetracycline (70%) nalidixic acid (70.0%), ampicillin (50%) co-trimoxazole (30.0%), streptomycin (20.0%), gentamicin (10.0%), and chloramphenicol (10.0%) (Table, 3).

Table 1: Isolation rate of *Salmonella spp* percentage collected from 18 farms in Jabal Awliya Locality.

Source	Water source	Drinkers	Poultry feed	Feeders	Litter	Dust	Hand swabs	Cloacal swabs	Faeces	Total
Prevalence	0(0.0%)	1(10%)	0(0.0%)	1(10%)	3(30%)	3(30%)	0(0.0%)	1(10%)	1(10%)	10(6.2%)

Table2: Distribution of isolated *Salmonella spp* from Jabal Awliya Locality according to cities

Location	No of farms	No of positive samples	Water source	Drinkers	Feed source	feeder	Litter	Dust	Hand swabs	Cloacal swabs	Faeces	Total
Dekhainat	2	0 (0.0%)	0	0	0	0	0	0	0	0	0	0
Wad agali	2	1 (10.0%)	0	0	0	0	0	0	0	0	1	1
ElFetaih	2	3 (30.0%)	0	1	0	0	1	1	0	0	0	3
Ghadesya	1	0 (0.0%)	0	0	0	0	0	0	0	0	0	0
Shegailab	3	1 (10.0%)	0	0	0	0	0	1	0	0	0	1
Tayba	6	4 (40.0 %)	0	0	0	1	1	1	0	1	0	4
Arak Salih	2	1 (10.0 %)	0	0	0	0	1	0	0	0	0	1
Total	18	10	0	1	0	1	3	3	0	1	1	10

Table 3: Antimicrobial susceptibility of *Salmonella spp* isolated from Jabal Awliya Locality broiler farms

Antimicrobial agent	Code	Concentration on (µg/ml)	S %	I %	R %
Amoxicillin	AMX	10	0(0.0%)	1(10%)	9(90%)
Ampicillin	AMP	10	3(30%)	2(20%)	5(50%)
Ciprofloxacin	CIP	5	10(100%)	0(0.0%)	0(0.0%)
Cefotaxime	CTX	30	10(100%)	0(0.0%)	0(0.0%)
Cefixime	CFM	5	10(100%)	0(0.0%)	0(0.0%)
Colistin	CL	10	10(100%)	0(0.0%)	0(0.0%)
Gentamycin	GEN	10	7(70%)	2(20%)	1(10%)
Chloramphenicol	C	30	9(90%)	0(0.0%)	1(10%)
Streptomycin	S	10	7(70%)	1(10%)	2(20%)
Co-tri moxazole	COT	25	7(70%)	0(0.0%)	3(30%)
Tetracycline	TE	30	3(30%)	0(0.0%)	7(70%)
Nalidixic acid	NA	30	3(30%)	0(0.0%)	7(70%)

DISCUSSION

This study revealed that the overall prevalence of *Salmonella spp* in the environment of broiler poultry farms of Jabal Awliya Locality was 10(6.2%) out of 162 detected samples. This percentage is lower than that of Al-Abadi and Al–Mayah, (2011) who isolated 34(9.2%) out of 370 *Salmonella*. However, this percentage is higher than that of Saad *et al.,* (2007) who isolated 267(5.31%) out of 5028 Salmonella in Saudi Arabia, and Mohammed *et al.,* (2009) who isolated 4 (5%) out of 80 samples collected from the environment of traditional poultry farms in Khartoum North, and Al-Zenki *et al*., (2007) who reported that 5.4% Salmonella were isolated from 2882 samples collected in Kuwait .These differences in overall prevalence of *Salmonella* may be attributed to several risk factors such as the environment, system of management, biosecurity , hygienic status,

and the used methods for isolation of the organism.

This study also showed that the source of drinking water was free from *Salmonella spp* and 1(10%) Salmonellae was isolated from a drinker in El-Fetaih .This result indicates that the contamination does not come from the source of water and confirm that the organism originates either from faeces and secretions of sick birds in the same flock or as a result of the hygienic status of the workers. This result agrees with Alali *et al. (*2010) who reported that there was no Salmonellae isolated from drinking water from both organic broiler farms and conventional farms in Khartoum. However, this result disagrees with El-Hussein *et al.* (2010) who isolated 7(7.23%) out of 97 by using the same method from the source and this may be attributed to the difference in samples number. Yagoub and Ahmed, (2009) who showed contamination of drinking water with *Salmonella* in Khartoum State as they detected 0.5% and this may be attributed to their higher numbers of collected samples, besides Mohammed *et al*., (2009), who reported that 3.8% *Salmonella spp* were isolated from 26 collected samples from drinking water and drinkers in Shambat. Also this result showed lower percentage as compared to that of Renwich *et al.* , (1992) who isolated 63 out of 226 (27.9%) in drinking water in Canada. Also Zaman *et al.* (2012) showed that *Salmonella typhi* represents 28% from isolated *Salmonella spp* collected from 50 samples from tanks and drinkers in Iran.

This study also showed the prevalence of *Salmonella spp* in both litter and dust (30.0%). This result showed higher percentage compared to Al-Abadi and Al-Mayah, (2011) who detected 5 (16.7%) out of 30 Salmonella in sawdust litter, and Mohammed *et al.,* (2009) who reported that 3 (11.1%) out of 27 samples were isolated from litter samples from EL-Halfaya farm(layer), and Al- Nakhli *et al*., (1999) who reported that 8 (2.3 %%) out of 348 were positive for Salmonella from litter. This prevalence showed less percentage compared as with to that of Ibrahim *et al.* (2013) who isolated 8(53.3%) salmonella in Egypt from 15 litter samples. Dust in the poultry houses may also be a hazard, since dust has been recognized as a vehicle of transmission of Salmonella when large numbers of organisms are present (Harbaugh *et al.,* 2006**).** Contaminated dust may also indicate previous infection compared to faeces. This result disagrees with Saad *et al.,* (2007) who isolated 11(10%) out of 110 environmental samples. High prevalence of *Salmonella spp* isolated from litter and dust in this study may be attributed to the capability of the organism to survive up to 26 months in thin layers of litter (Davies and Breslin, 2003), and at least 53 weeks in dust (Davies and Wray, 1996).

Cloacal swabs method is likely to be relatively insensitive compared to the culture of more voluminous faecal material (Kotton *et al*., 2006).This result disagrees with Al-Abadi and Al-Mayah, (2011) who isolated 19% out of 100 cloacal swabs in Basra Province, also Ahmed *et al., (*2014) who detected 12% out of 100 cloacal swabs in Egypt, and Saad *et al*. (2007) who isolated 101(3.31%) out of 3049 samples in Sudi Arabia, this may be attributed to the differences of the strains and the number of the chickens, their susceptibility to be infected with Salmonella, and the system of the management of the farms .This study also revealed that 1 (10.0%) faeces sample was positive for *Salmonella spp* which showed different isolation rate compare to that of Alali *et al.,(*2010) who reported that 10(5.6%) out of 180 were positive for Salmonella in faeces from organic broiler farms, whereas 93(38.8%) out of 240 were

isolated from conventional broiler farms. However, fresh faeces provide an indication of current infection of flocks.

This study revealed that there was no Salmonellae isolated from feed source and this may be attributed to the non uniform distribution of the organism within the samples, besides the effect of stress on the organisms from processing treatments used in feed mills (Zdragas *et al*., 2000), addition to the treatment of feed with formaldehyde which give a false negative result (Carrique-Mas, and Davies,2008).This result disagrees with Saad *et al.,* (2007) who isolated 3(2.91) out of 103 feed samples .

Poultry feeds (10%) can be sources of Salmonella and consequently serve as an indirect cause of human infection by consuming poultry meats and meat products. Feeds are contaminated either from feed mills or on farms during feed formulation, feeding or handling and subsequently spread to poultry mostly through ingestion. Salmonellas have the ability to survive under prolong periods in dry conditions like feeds and may be recycled in all production stages in commercial feed preparation (Whyte *et al*., 2003).

The study also revealed that hand swabs from workers were negative for *Salmonella spp*, this result disagrees with Ibrahim *et al. (*2013) who detected 8(8.9%) salmonella from 90 hand swab workers, and this variants may be related to the difference in the samples size, also it can be an indication of the good hygienic status of the workers, and the effectiveness of the detergents and disinfectants that were used.

In this results all isolates were sensitive to ciprofloxacin, cefotaxime, cefixime, and colistin indicating that these antibiotics are the drugs of choice for the treatment of salmonellosis, followed by chloramphenicol (90.0%), co-tri moxazole (70.0 %), streptomycin (70.0%), gentamicin (70.0%), ampicillin (30.0%), tetracycline (30.0%), nalidixic acid(30%). this results agrees with Fadlalla *et al.* (2012) who showed that all isolates were sensitive to ciprofloxacin and with very low resistance pattern to gentamicin, and Mohammed**,** (2009) who showed high sensitivity of the isolates against ciprofloxacin and gentamicin which indicated that salmonella revealed resistance to gentamicin. This study also showed high resistant to amoxicillin which agrees with Hemen, (2012).

CONCLUSIONS

Water source, feed source and hand workers of Jabal Awliya Locality poultry farms were free from *Salmonella species,* whereas litter and dust were the most highly contaminated sources. Amoxycillin showed the most resistant antibiotic which would lead to treatment failure and probably lead to development of resistant strains of *Salmonella* and that of other isolates.

ACKNOWLDEGEMENTS

This work was supported by the German Academic Exchange Service (DAAD) to the first author to pursue Ph.D. in Microbiology. We are thankful to Mrs. Enas Elsiddig Elfaki for her valuable collaboration. Also we are grateful to the owners, managers, and workers of the farms for allowing us to use their farms and chickens for the study. Also grateful thanks to the Faculty of Medical Laboratory Sciences, Al-Neelain University for their kind permission to use the microbiology lab.

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