



Prevalence and Antimicrobial Resistance of *Salmonella species* Isolated from the Environment of Poultry Farms in Khartoum North Locality, Sudan

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ABSTRACT

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A total of 162 samples were collected from different 18 commercial broiler farms in Khartoum North Locality to detect the prevalence and antimicrobial resistance of *Salmonella species* during the period from May 2013 to February 2014. Samples including (water, feed, dust, litter, cloacal swabs, faeces, and hand swabs from workers), and they were investigated by using ISO 6975: 2002, and confirmed by using API20 E strips. The results showed that 18(11.1%) from 162 samples were found to be contaminated with *Salmonella spp.* These were recovered from 13(72.2%) farms. 1(5.6%), 3(16.7%), 0(0.0%), 3(16.7%), 2(11.1%), 6(33.3%), 2(11.1%), 0(0.0%), and 1(5.6%) were isolated from water source, drinkers, poultry feed, feeders, dust, litter, faeces, cloacal swabs, and hand swabs respectively. All isolates were sensitive to ciprofloxacin (100%), cefixime (100%), and cefotaxime (100%), followed by gentamicin (94.4%), chloramphenicol (88.9%), colistin (83.3), streptomycin (66.7%), co-trimoxazole (66.7%), nalidixic acid (61.1%), ampicillin (55.6%), tetracycline (55.6%), and amoxicillin (5.6%) which showed the highest prevalent resistant antibiotic.

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INTRODUCTION

Many serovars of *Salmonella* become more pathogenic and resistant to multiple drugs and involved in a number

of outbreaks and sporadic cases. The pathogen lives in the intestinal tract of birds, insects, mice, farm animals, other

animals and sometimes in eggs (Coburn *et al.*, 2007). Farm pertaining samples and their environmental conditions including faeces, soil, crevices, dusts, manure, litter, feeders and drinkers increase the rate of contamination (Wales *et al.*, 2006). Environmental sampling has been reported to be a good indicator for the presence of *Salmonella* in poultry flocks (Davies and Breslin, 2001). The spread of salmonellosis is associated with a wide range of sources and transmission pathways including the consumption of contaminated food products from pigs, poultry and ruminants, contaminated drinking water, overseas travel and direct contact with domestic and wild animal faeces via environmental and occupational exposure (Wilson and Baker, 2009).

Antimicrobials are used in broiler poultry to enhance growth and feed efficiency, and to reduce bacterial diseases (Donoghue, 2003). The used antimicrobial classes for chickens treatment include aminoglycosides, tetracycline, beta-lactams, quinolones, macrolides, polypeptides, amphenicoles, and sulphonamides (Stolker and Brinkman, 2005).

Khartoum North is the third largest city in the Republic of Sudan, located on the east bank of the Blue Nile. The total area of the patch described is 455 sq Km. the population of the locality is about 533.700 inhabitants according to 2003 census reached to 800.00 as a result of massive migration. Agriculture and industry are the main activity.

The aim of this study was to detect the prevalence and antimicrobial resistance of *Salmonella species* isolated from the environment (water, feed, dust, litter, cloacal swabs, faeces, and hand swabs

from workers) of poultry farms in Khartoum North Locality, Sudan.

MATERIALS and METHODS

Samples:

A total of 162 samples were collected aseptically from 18 different farms in Khartoum North Locality (Hillat-Koko, Omdoum, Shambat, El-Halfaya, El-Droshab, El-Kadaro, and El-Kabbashi). These included water (source, and drinkers), feed (source, and feeders), dust, litter, cloacal swabs, faeces and handworkers during April 2013 to February 2014 to detect *Salmonella species* following the standard guide lines from (ISO 6579:2002). A volume of 25 ml of water was collected aseptically from both water source and drinkers by using sterile syringes, then added to 225 ml buffered peptone water (HIMEDIA, M614), while a weight of 25 g was collected aseptically 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 buffered peptone water. Also swabs moistened with buffered peptone water were used to collect samples from dust, cloacae, and handler's hands, then were transferred aseptically into tubes containing 9 ml buffered peptone water. Samples were labeled, and sent to the laboratory of the Microbiology Department, Faculty of Medical laboratory Sciences, Al-Neelain University, where samples were incubated at 37 ± 1 °C for 24 ± 3 hours.

Isolation of *Salmonella spp*:

From buffered peptone water, 0.1 ml was transferred into a tube containing 10 ml of Rappaport-Vassiliadis (RV) broth (MICROMEDIA, MN 0070), then incubated at 41.5 ± 1 °C for 24 ± 3 hours. Another 0.1 ml of buffered peptone

water was transferred into a tube containing 10 ml of Muller-Kauffmann tetrathionate novobiocin broth (MKTn) broth (HIMEDIA, M 14961), and incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 3 hours, then, a loop-full from the RV broth and MKTn was transferred and streaked on Xylose lysine deoxycholate (XLD) agar (HIMEDIA, M 031) and Salmonella-Shigella (S.S) agar (HIMEDIA, M 108) and incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 3 hours.

Identification

Microscopic examination:

Suspected colonies were streaked on pre-dried nutrient agar plates (HIMEDIA, M001), incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 3 hours, then Gram-stain and motility test were performed as described by Cheesbrough, (1991).

Biochemical identification and confirmation:

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 v.p test (glucose phosphate broth) (HIMEDIA, M070). Isolates were further confirmed with API 20E identification kits (Bio Merieux, Marcy, France).

Antimicrobial susceptibility test:

Ampicillin, amoxicillin, chloramphenicol, streptomycin, ciprofloxacin, cefixime, cefotaxime, tetracycline, nalidixic acid, colistin, co-trimoxazole, and gentamicin were used in this study (HIMEDIA M1084). Kirby-Bauer method was performed as

described by CLSI, (2006) using Muller Hinton agar (HIMEDIA M1084).

RESULTS

Prevalence of *Salmonella spp* in the environment of Khartoum North broiler farms:

The results showed that 18(11.1%) out of 162 collected samples were positive for *Salmonella spp* recovered from 13(7.2%) farms distributed as 1(5.6%) was isolated from water source in Omdoum, 3(16.7%) were isolated from drinkers in El-Droshab, El-Kadaro, and El-Kabbashi. Also 3(16.7%) were isolated from feeders in Omdoum, Hillat Koko, and El-Drosahab. Furthermore 6(33.3%) were isolated from litter in Hillat Koko, El-Halfaya, El-Droshab, El-Kadaro, and El-Kabbashi. Also 2(11.1%) were collected from dust in Hillat Koko, and El-Kadaro. Also 1(5.6%) was isolated from hand swab of a worker in Shambat, besides 2(11.1%) faeces collected from El-Halfaya, and El-Kadaro. However, there was no *Salmonellae* in samples collected from cloacal swabs and feed source (Table 1, and Figure 1).

Antibiotics susceptibility of isolated *Salmonella*:

This study showed that all the isolates were sensitive to ciprofloxacin, cefixime, and cefotaxime followed by, gentamicin (94.4%), chloramphenicol (88.9%), colistin (83.3), streptomycin (66.7%), co-trimoxazole (66.7%), nalidixic acid (61.1%) ampicillin (55.6%), tetracycline (55.6%), and amoxicillin (5.6%). Also the isolates showed intermediate resistance to amoxicillin, ampicillin, gentamicin, nalidixic acid, and streptomycin (5.6%) for each, while they showed resistance to amoxicillin (88.9%), tetracycline (44.4%), ampicillin (38.9%), co-

trimoxazole (33.3%), nalidixic acid (16.7), and (33.3), streptomycin (27.8%) colistin chloramphenicol(11.1%)(Table 2).

Table 1: Isolation rate of *Salmonella spp* collected from Khartoum North broiler farms(N=162)

Source of sample	No of examined samples	No of positive samples	Prevalence rate(%)
Water source	18	1	5.6
Drinkers	18	3	16.7
Poultry feed	18	0	0.00
Feeders	18	3	16.7
Litter	18	6	33.3
Dust	18	2	11.1
Hand swabs	18	1	5.6
Cloacal swabs	18	0	0.00
Faeces	18	2	11.1
Total	162	18	11.1

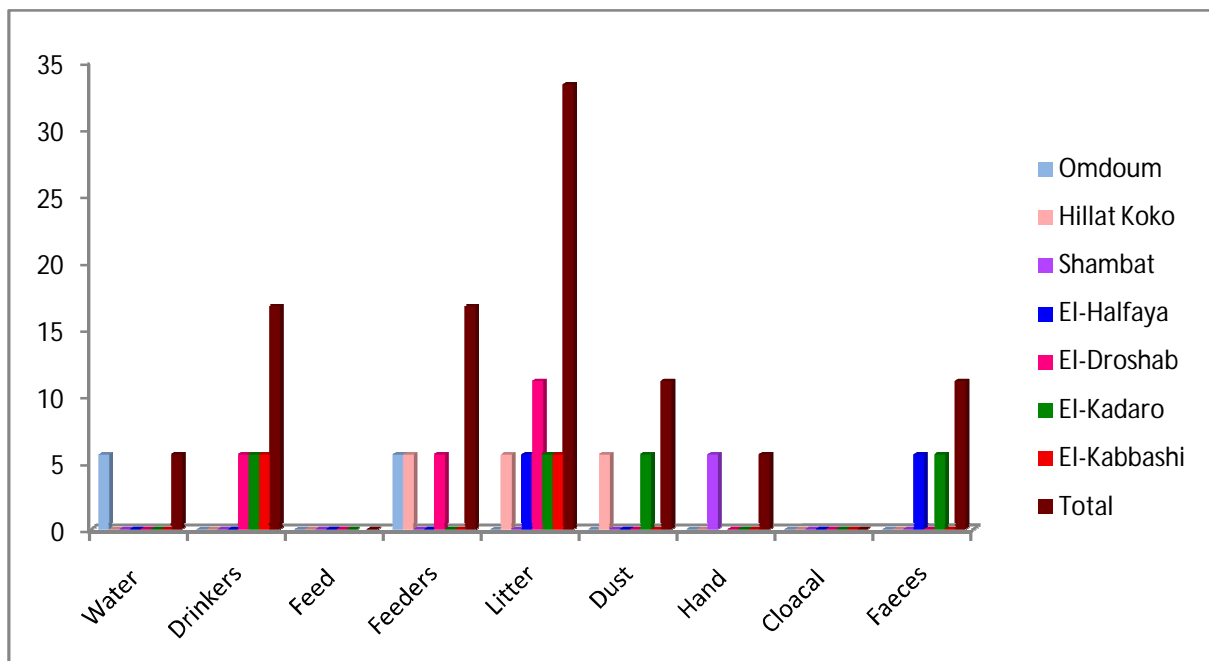


Figure1: Distribution of *Salmonella spp* isolated from 18 poultry farms according to their locations

Table 2: Antimicrobial resistance of *Salmonella spp* isolated from broiler farms in Khartoum North locality(N=18)

Antimicrobial agent	Code	Concentration (mcg/disc)	Sensitive%	Intermediate%	Resistant%
Amoxicillin	AMX	10	1(5.6)	1(5.6)	16(88.9)
Ampicillin	AMP	10	10(55.6)	1(5.6)	7(38.9)
Ciprofloxacin	CIP	5	18(100)	0(0.0)	0(0.0)
Gentamycin	GEN	10	17(94.4)	1(5.6)	0(0.0)
Chloramphenicol	C	30	16(88.9)	0(0.0)	2(11.1)
Streptomycin	S	10	12(66.7)	1(5.6)	5(27.8)
Co-Tri moxazole	COT	25	12(66.7)	0(0.0)	6(33.3)
Tetracycline	TE	30	10(55.6)	0.0	8(44.4)
Nalidixic acid	NA	30	11(61.1)	1(5.6)	6(33.3)
Cefixime	CFM	5	18(100)	0(0.0)	0(0.0)
Cefotaxime	CTX	30	18(100)	0(0.0)	0(0.0)
Colistin	CL	10	15(83.3)	0(0.0)	3(16.7)

DISCUSSION

This study revealed that 18(11.1%) out of 162 samples were positive for *Salmonella spp* in the environment of broiler poultry farms of Khartoum North locality which showed higher percentage compared to Ezdihar (1996) who examined 610 samples from poultry in the Sudan and isolated 45 *Salmonellae* which counted for(7.4%) of the total isolates, 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 Mohammed (2009) who isolated 36(4.9%) out of 733 samples collected from different sources in Khartoum State. Also Al-Zenki *et al.* (2007) who reported that out of 2882 samples collected in Kuwait from the farm, the overall percentage prevalence of *Salmonella* was 5.4%, this may be attributed hygienic measures applied.

The drinking water plays an important role in the transmission of many pathogenic agents, and there have been many reports about water contamination with *Salmonella spp* (Jafari *et al.*, 2006).

In this study 1(5.6%) and 3(16.7%) *Salmonella spp* were isolated from drinking water and drinkers respectively, which confirm that *Salmonellae* may originate either from faeces and secretions of sick birds in the same flock or from water already contaminated by pathogenic organisms. This percentage disagrees with El Hussein *et al.* (2010) who isolated 7(7.23%) out of 97 from drinking water by using the same method this may be attributed to the variation in the numbers of collected samples, and Nayak *et al.* (2003) who reported isolation rate of (10%). Also this result is higher than that of Yagoub and Ahmed, (2009) since they showed less contamination of drinking water with *Salmonella* in Khartoum State tap water and reported that (0.5%) were isolated from tap water in Khartoum State, and this may be attributed to the difference in the isolation methods used. Also Mohammed *et al.* (2009), reported that 1(3.8%) *Salmonella spp* were isolated from 26 collected samples from drinking water and drinkers from broiler

in Shambat. The present results also disagree with Alaliet *et al.* (2010) who reported that no *Salmonella* was isolated neither from 60 water samples from organic broiler farms nor from 80 samples from conventional farms. The present results showed lower percentage than that of Renwich *et al.* (1992) who isolated 63 *Salmonella* out of 226 samples (27.9%) of drinking water in Canada this may be attributed to the higher number of their samples and environmental variations.

This study showed that 6(33.3%) *Salmonella spp* were isolated from litter. Litter expresses the highest frequent source for *Salmonella spp* in which the organism can survive up to 26 months in thin layers of litter (Davies and Breslin, 2003). *Salmonella* from litter can lead to heavy contamination of the bird's feathers and feet which increases the probability to recover the organism from carcasses in poultry processing plants due to fecal shedding onto the litter (Trampel *et al.*, 2000). This study showed higher percentage compared to Mohammed *et al.* (2009) who reported only 3(11.1%) out of 27 samples were isolated from litter samples from EL-Halfaya farm (layer), Also Nayak *et al.* (2003) who isolated (13%), and Al-Nakhli *et al.* (1999) who reported that 8(2.3%) out of 348 were positive for *Salmonella* from litter. This higher percentage obtained in this study may be attributed to the hygienic status of the workers whom 50% of them do not wash their boots or use any disinfectant before entering the farms, due to lack of biosecurity measures of the investigated farms of which 17(94.4%) are open system, whereas 1(5.6) semi closed system, besides 50% of the investigated farms has other animals than chicken. However, this study represents

less percentage than Renwich *et al.* (1992) who isolated 223 out of 226 (98.8%) *Salmonella spp* from the litter in Canada.

This study showed that there was a negative detection for *Salmonella spp* from cloacal swabs which confirm that cloacal swabs are relatively insensitive due to relatively low prevalence of infection in individual birds, and low number of organisms excreted by infected birds in many cases. Moreover, cloacal swabs only obtain a small amount of faeces and *Salmonella* maybe present in low numbers or be non-uniformly distributed in the faeces, this method is likely to be relatively insensitive compared with the culture of more voluminous faecal material (Kotton *et al.*, 2006). This study also revealed that 2(11.1%) fresh faeces samples were positive for *Salmonella spp* which provide an indication of current infection of flocks, however Alali *et al.* (2010) 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. In this study showed that 2(11.1%) were positive for *Salmonella* in dust, this percentage is higher than Nayak *et al.* (2003) who isolated (5%) from environmental swab. Dust in the poultry houses in large amount 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). *Salmonella* has been reported to survive in poultry houses at least 53 weeks in dust (Davies and Wray, 1996). Contaminated dust may also indicate previous infection compared with faeces. Dust is however a more sensitive type of sample for detecting *Salmonella* in

poultry flocks (EFSA, 2007). This is likely to be due to the comparative advantage of *Salmonella* in this type of matrix compared to other Enterobacteriaceae, which do not tend to survive as well in dry conditions (Haysom and Sharp, 2003).

This study also revealed that there was a negative detection for *Salmonella spp* from feed source, while 3(16.7%) *Salmonella spp* were isolated from feeders which disagrees with Veldman *et al.*(1995) who made a survey on poultry feed contamination and found 10% out of 360 samples were contaminated, this may be attributed to the low number of collected samples in this study. Whereas Nayak *et al.* (2003) who isolated 3% and 1% from feed and feeder samples respectively. Complications of isolating *Salmonella* from feed not only has been suggested to stem from the non-uniform distribution of the organism within the samples, but also from the effect of stress on the organisms from processing treatments used in feed mills. (Zdragas *et al.*, 2000). In addition, the treatment of feed with formaldehyde can interfere with detection methods and give a false negative result (Carrique-Mas and Davies, 2008). Poultry feeds can be sources of *Salmonella* and consequently serve as an indirect cause of human infection to people consuming poultry meat 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. *Salmonella* 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).

This study also revealed that 1(5.6%) hand swab was positive for *Salmonella* which confirm the role of poultry in contaminating the hands of poultry handlers with *Salmonella*.

This study also showed that all isolates were sensitive to ciprofloxacin followed by gentamicin, this result agrees with Fadlalla *et al.*(2012) who showed that all isolates were sensitive to ciprofloxacin and with very low resistance pattern to gentamicin, Mohammed (2009) showed high sensitivity of the isolates against ciprofloxacin and gentamicin which give an indication that *salmonella* revealed resistance to gentamicin. The present study showed high resistance to amoxicillin, tetracycline, ampicillin, and co-trimoxazole which also agree with Fadlalla *et al.* (2012).

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