3

Prevalence, Detection and Antimicrobial Resistance Pattern of *Salmonella* in Sudan

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1. Introduction

Infectious microbial diseases constitute a major cause of death in many parts of the world, particularly in developing countries. *Salmonella* has been identified as an important food and water-borne pathogen that can infect human and animals resulting in significant morbidity and mortality (Akkina et al., 1999). *Salmonella* is a facultative anaerobe, Gram-negative rod-shaped, 2 – 3 x 0.4 – 0.6 μm in size and motile by peritrichous flagella except for *S.* Gallinarum and *S.* Pullorum which are immotile. Members of the genus have a % G+C content of 50-53. They are urease and Voges-Proskauer negative and citrate utilizing (Montville and Matthews, 2008).

Salmonellae are typically non-lactose, non-sucrose fermenting but are able to ferment glucose, maltose and mannitol with the production of acid only as in the case of *S.* Typhi and acid with H_2S in the case of *S.* Paratyphi and for most other *Salmonella* serovars (Cruickshank, 1975). Optimum temperature for growth is in the range of 35 – 37°C but some can grow at temperatures as high as 54°C and as low as 2°C (Gray & Fedorka-Cray, 2002). *Salmonella* grow in a pH range of 4 - 9 with the optimum being 6.5 – 7.5. They require high water activity for growth (> 0.94) but can survive at a_w of < 0.2 such as in dried foods. Inhibition of growth occurs at temperatures < 7°C, pH < 3.8 or a_w < 0.94 (Hanes, 2003).

Based on differences in 16S rRNA sequence data, the genus *Salmonella* is divided into two species: *S. enterica* and *S. bongori*. *S. enterica* is further divided into six subspecies: subspecies *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and subspecies *indica* (Popoff & Minor, 2001). Kauffmann-White scheme classifies members of *Salmonella* species according to three major antigenic determinants composed of somatic (O-antigens), flagellar (H-) and virulence (K-) antigens. Agglutination by antibodies specific for the various O-antigens, groups the salmonellae into six serogroups: A, B, C_1, C_2, D and E. Rarely cross reactivity between O-antigens of *Salmonella* and other genera of Enterobacteriaceae do occur. Therefore further classification of serotypes is based on the highly specific H-antigens (Scherer & miller, 2001). H-antigens can be expressed in one of two phases: phase 1 H-antigens are serovar specific while phase 2 antigens are not. However K-antigens are produced by serovars that are

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characterized by extracellular polysaccharide capsules (Hu & Kopecko, 2003; Yousef & Carlstrom, 2003). Currently, Kauffmann-White scheme recognizes 2610 *Salmonella* serovars, the majority (2587) belongs to *S. enterica* while the remaining (23 serovars) are assigned to *S. bongori* (Guibourdenche et al., 2010).

The incidence of typhoid salmonellosis is stable, with comparatively few cases in developing countries. Cases of typhoid salmonellosis are estimated to be in the range of 16-17 million resulting in about 600,000 deaths annually (Pang et al., 1995). However, cases of non-typhoid salmonellosis are increasing worldwide. The World Health Organization (WHO) estimates 1.3 billion cases with 3 million deaths annually. Data on salmonellosis cannot be ascertained in most developing countries because many patients with acute gastroenteritis do not visit a health care provider or do not submit a specimen for laboratory testing (Portillo, 2000; Hanes, 2003; Hu & Kopecko, 2003). An unpublished data of Sudan Ministry of Health indicated that the incidence of salmonellosis has markedly increased. In 2004 these were 43,144 cases and 68 deaths in different hospitals. However, a more accurate figure of salmonellosis is difficult to determine because only large outbreaks are investigated whereas sporadic cases are under-reported.

Since *Salmonella* is closely related to both human and animal health, more rapid and sensitive methods for the identification of this bacterium are required (Schrank et al., 2001). In the regions where enteric fever is common, clinical diagnosis of typhoid fever is inadequate, as the symptoms it causes are non-specific and overlap with those of other febrile illness. Serological tests, predominantly the Widal test, are available but have very low sensitivity and specificity, and no practical value in endemic areas despite their continued use (Levine et al., 1978). Isolation of the causative organism remains the most effective diagnostic method in suspected typhoid fever (Zhou & Pollard, 2010). Standard culture methods for detecting *Salmonella* spp. include non-selective pre-enrichment followed by selective enrichment and plating on selective and differential media (Whyte et al., 2002). These methods take approximately 4-7 days (Harvey & Price, 1979; Perales & Audicana, 1989), so they are considered laborious and time consuming.

In a clinical setting, suspected Enterobacteriaceae are often subjected to biochemical testing to investigate the ability of an isolate to grow upon certain substrates, produce various metabolic products or alter the pH. In the 1970s, efforts were being made to collect together multiple biochemical tests to allow rapid and relatively high throughput identification of clinical bacterial isolates (Lindberg et al., 1974). Numerous test kits of varying accuracy became available for the identification of the Enterobacteriaceae. The API 20E system (BioMérieux, France) was found to be the most reliable, having a 99% correlation with standard biochemical tests and a 94% identification rate (Nord et al., 1974). Over 60 bacterial species can currently be identified with the API20E, with identification extending to the serovar level for Typhi and Paratyphi A among others. However, results of such tests are open to interpretation and require experience and a high level of technical skill to generate reproducible results (Jamshidi et al., 2007). Therefore, several alternative faster methods for the detection of *Salmonella* have been suggested.

Molecular methods have been applied in the identification of *Salmonella* and were found to be very useful in differentiating between species. These methods include pulsed field gel electrophoresis (PFGE) (Mohand et al., 1999; Nair et al., 1994), IS200 fingerprinting (Ezquerra et al., 1993), PCR ribotyping (Lagatolla et al., 1996), ribosomal DNA intergenic spacer amplification and heteroduplex analysis (Jensen and Hubner, 1996), amplification

fragment length polymorphism (Aarts et al., 1998; Nair et al., 2000), automated nuclease PCR assay (Hoorfar et al., 2000) and random amplification of polymorphic DNA (RAPD) (Shangkuan and Lin, 1998). Since all molecular techniques are based on variability of microbial chromosomes or plasmids, then DNA sequencing would appear to be the best approach for differentiating subtypes (Liebana et al., 2001)

Many oligonucleotide primer sets have been described for the detection of *Salmonella* using the PCR technique. These primers include those associated with the invasion genes *inv*A and *inv*E (Stone et al., 1994), histidine transport operon *hit*J (Cohen et al., 1993), *SP*I1 invasion gene *hil*A (Guo et al., 2000), virulence plasmid gene *spv* (Gulig et al., 1993), virulence gene *sip*C (Sharma and Carlson, 2000), enterotoxin gene *stn* (Makino et al., 1999), *omp*C (Amavisit et al., 2001*), spa*Q (Kurowski et al., 2002), *ori*C (Widjojoatmodjo et al., 1992), *fim*A (Swenson et al., 1991), 16S rRNA (Lin and Tsen, 1996), *iro*B (Bäumler et al., 1998) and a repetitive DNA fragment (Jitrapakdee et al., 1995). Recent studies described a serotype-specific PCR method for differentiating *Salmonella* Pullorum from other serotypes using the *rfb*S gene (Desai et al., 2005). Most researchers agree that in the future more serotype-specific PCR assays will be developed.

Resistance of infectious microorganisms to commonly prescribed antibiotics has emerged and spread in both developed and developing countries (Zhao et al., 2003; Ahmed et al., 2000; Grob et al., 1998). This imposes serious constraints on the options available for the treatment of many infections (Kunin et al., 1990). In the case of salmonellae, resistance to tetracyclines or chloramphenicol was first reported in 1961 (Ramsey and Edwards, 1961). Since then, reports on salmonellae resistance to one or more antibiotics have increased substantially and resistance has emerged even to newer more potent antimicrobial agents (Montville and Matthews, 2008; Piddock, 2002). In addition, multidrug resistance in *Salmonella* has become a public health concern (Crump and Mintz, 2010; Asai et al., 2010; Singh et al., 2010).

In the Sudan, as in most other developing countries, resistance and multiple resistance to antimicrobial agents among members of Enterobacteriaceae including some *Salmonella* serovars was found to increase during the last decades (Yagoub et al., 2005; Ahmed et al., 2000; Hassan, 1985, Shears et al., 1988; Musa and Shears, 1998). The sensitivity of *Salmonella* Typhi, *S*. Paratyphi A and *S*. Paratyphi B to ten antibiotics was examined in Sudan (Ahmed et al., 2000). The examined strains were sensitive to all drugs tested except for one *S*. Typhi strain which was resistant to cotrimaxazole; tetracycline and sulfonamide and one *S*. Paratyphi A which was resistant to tetracycline. The sensitivity of *Salmonella* Paratyphi A and *S*. Paratyphi B which were isolated from Sudanese white cheese was tested against 9 antibiotics (Yagoub et al., 2006). ciprofloxacin, chloramphenicol and ofloxacin were the most effective drugs against the tested isolates. The resistance was more frequent to ampicillin, tetracycline, penicillin, gentamicin and co-trimoxazole.

Due to the indiscriminate and injudicious use of antibiotics in human and veterinary medicine as well as for the promotion of growth in food animals, *Salmonella* strains resistant to first line antibiotics will continue to develop at an increasing rate. Therefore updated knowledge of *Salmonella* serotypes resistance patterns is important for the proper selection and use of antimicrobial drugs and for the development of appropriate prescribing policies.

1.2 History of *Salmonella* research in Sudan

In Sudan, the prevalence of *Salmonella* serovars is not well documented, as salmonellae are not routinely isolated and identified. Only a few studies have been reported by few workers.

Horgan (1947) made the first report on *Salmonella* infections in cattle. He investigated a food poisoning outbreak at Wad Madani town and isolated *Salmonella* serovar Dublin from faeces of two persons who fell sick after eating meat. Again the serovar Dublin was isolated from infected calves and from one of the apparently healthy animals (Soliman and Khan, 1959). A survey to ascertain the incidence rate of *Salmonella* infection in animals was made in Khartoum (Khan, 1970). During the survey, 230 *Salmonella* cultures were recovered from different sources belonging to 63 serotypes.

Subsequent *Salmonella* surveys which have been conducted at Khartoum and Malakal added 15 serovars to the list of Sudan. The serovars reported were: *S.* Amager, *S*. Derby, *S*. Kandle, *S*. Reading, *S*. Salford, *S*. Adelaide, *S*. Amersfoort, *S*. Bertin, *S*. Chester, *S*. Mushmar-haemek, *S*. Muenche, *S*. Muensters, *S*. Newport, *S*. Pomona and *S*. Poona (Khan, 1970). In his attempt to assess the quality of fresh meats in Sudan, Sariy Eldin (1971) reported the occurrence of *Salmonella* Wein, *S*. Dublin, *S*. Havana, *S*. Typhimurium, *S*. Senegal and *S*. Braenderup. *S*. Dublin was also isolated from sheep liver (Salih and Ibrahim, 1972). Fifty-eight *Salmonella* strains were isolated from slaughtered chicken in Khartoum North and Omdurman (Yagoub and Mohamed, 1987). The most common serotypes reported were: *S*. Mons, *S*. Amek and *S*. Uganda. The incidence of *S*. Dublin in the mesenteric lymph nodes and faeces of sick calves in Kuku dairy cooperative farm, Omdurman and El Obeid slaughter houses was also reported (Saliem, 1987).

Forty-five *Salmonella* isolates (not serotyped) were isolated from carcasses, liver, spleen, intestinal contents of chickens from a poultry farm in El Obeid (unpublished data). The isolation of *Salmonella enterica* subspecies *enterica* serotype San-Diego from three goats (3.84%) at Omdurman Central Abattoir was reported (El Tom et al., 1999). Recently, *Salmonella* Umbadah plus 19 new serovars were reported from different sources at Khartoum (Hag Elsafi et al., 2009; El Hussein et al., 2010).

2. Materials and methods

2.1 Isolation and identification of *Salmonella*

Salmonellae were isolated and identified according to the techniques recommended by the International Organization for Standardization described by Molla et al. (2004).

For confirmation, presumptive salmonellae were subjected to biochemical tests (Macfaddin, 1980), further identified with API 20E identification kits (Bio Merieux, Marcy, France) and a slide agglutination test was employed thereafter, using a commercially available *Salmonella* polyvalent O (Denkafeiken, Japan) and H antisera (Mast Diagnostic, UK). Presumptive *Salmonella* isolates were shipped to the Public Health Agency, Office International des´ Epizooties (OI´E) Reference Laboratory for salmonellosis, Guelph, Ontario, Canada or to the Egypt Management Central Laboratory for serotyping and phage typing.

2.2 Serotyping and phage typing

For serotyping, the somatic (O) antigens of the *Salmonella* isolates were determined with the slide-agglutination test as described by Ewing (1986), whereas the flagellar (H) antigens were identified by a microtechnique (Shipp and Rowe, 1980) that uses microtitre plates (Poppe et al., 2001). The antigenic formulae of *Salmonella* serovars as listed by Popoff (2001) were used to name the serovars.

The standard phage typing technique described by Anderson and Williams (1956) was used. Strains that did not conform to any recognized phage type were considered atypical (AT). *Salmonella* Enteritidis strains were phage typed according to Ward et al. (1987) with typing phages obtained from the International Centre for Enteric Phage Typing (ICEPT), Central Public Health Laboratory, Colindale, United Kingdom via the National Laboratory for Enteric Pathogens (NLEP), Health Canada, Winnipeg, Manitoba. The phagetyping-scheme and phages for *S*. Typhimurium, developed by Callow (1959) and further extended by Anderson (1964) and Anderson et al. (1977), were obtained from the ICEPT via the NLEP.

2.3 Antimicrobial susceptibility testing

The antimicrobial resistance of the isolates was tested against ten antimicrobial agents by the agar diffusion method with Mueller Hinton agar and antibiotic disks (Hi Media), following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 2000). *E. coli* ATCC 25922 was used for quality control. The categories susceptible or resistant were assigned on the basis of the critical points recommended by the NCCLS (2007).

The antibiotics tested were those commonly used in poultry, animals or in human, they are in mcg/disc: tetracycline (Te) 10; gentamicin (Cn), 10; nalidixic acid (Na) 25; streptomycin (St), 25; co-trimoxazole (Cot), 25; chloramphenicol (C), 30; ciprofloxacin (Cip), 5; ampicillin (Amp), 25; norfluxacin, (Nor), 10 and apramycin (Apr), 15.

2.4 Plasmid profiling analysis

For each isolate a single colony was grown overnight in 1ml LB media at 37^{o}C. The bacterial cells were harvested by centrifugation for 30s in a micro-centrifuge (Sanyo), the supernatant was discarded and the pellet was subjected to Plasmid DNA extraction according to alkaline – detergent method (Dillon et al., 1985). The plasmids extracted were stored at -20^{o}C till used for further analysis. Later, the extracted plasmid for each isolate was analyzed electrophoretically on a 0.8% agarose gel. The gel was then exposed to ultraviolet transillumination and photographed in a gel documentation system (Model GAS Uvitec. Product). One Kb DNA ladder (Invitrogen, Germany) was also used to determine the plasmids sizes.

2.5 Specificity of PCR primer sets

Ten specific primer sets (Invitrogen, Germany) each targeting a different gene were evaluated for their specificity and sensitivity to detect locally isolated *Salmonella* serovars. DNA from each *Salmonella* serovar and non-*Salmonella* strain was extracted according to the boiling – centrifugation method (Soumet et al., 1994). A single colony of a pure nutrient agar culture was grown overnight at 37^{o}C in 1ml Luria – Bertani broth. Bacterial cells were precipitated by centrifugation at 13,000 rpm for 5min in a micro-centrifuge (MSE, MSBo1o.cx2.5, Sanyo, UK). The supernatant was discarded and the pellet was re-suspended in 500µl deionized distilled water. The suspension was boiled for 10min in a water bath then immediately cooled on ice. Extracted DNA was then stored refrigerated at 4oC until used as a template for PCR amplification.

The extracted chromosomal DNA was amplified by an established PCR technique (Sambrook et al., 1989). PCR amplification reactions were carried out in 25 µl total volume

of PCR mixture containing 5 µl of template DNA, 12.5µl of the PCR master mix (Promega) (50 unit/ml Taq DNA polymerase in an appropriate reaction buffer {pH 8.5}, 400 µM each dNTPs and 3mM MgCl2) and 0.1 µM of each of primer pair. DNA was amplified according to reaction conditions published for each primer pair in a thermal cycler (Techne/ Flexigene - biotech).

Appearance of the target band specified for each primer set on the 1.2% agarose gel under specified gel electrophoresis conditions is considered as a positive amplification product.

2.6 Sensitivity of PCR primers

To determine the sensitivity of each PCR primer set, a single colony of a pure culture of *Salmonella* Typhi was grown overnight at 37ºC in 10ml Luria – Bertani broth. After incubation for 24 hours ten-fold dilutions (10 to 10-9) of the broth culture were made. The viable cell count in each dilution was determined using plate count media. For each mixture 100µl was cultured immediately in a plate count media (Somasegaran and Hoben, 1985; Vincent, 1970). The cultures were incubated overnight at 37ºC. The numbers of formed colonies were then counted (each colony is considered to be formed by a single cell). The DNA from each dilution was extracted as previously described and was used as a PCR template for each primer set.

3. Results

3.1 Prevalence

Out of 1921 collected and examined samples; 833 (43.4%) belonged to poultry, 680 (35.4%) to food items, 224 (11.7%) to human faeces, 107 (5.6%) to chlorinated drinking water and 77 (4%) to food animal faeces (Table 1). In Total, 213 (11.09%) *Salmonella* strains belonging to 54 different serovars were isolated. Of these, 210 were members of *S. enterica* subspecies *enterica*,

Source	Total Samples Examined	Total +ve samples	% from Source	% from +ve samples	% from total examined
Food	680	32	4.7	15.0	1.7
Poultry	833	70	8.4	32.8	3.6
Water	107	10	9.3	4.7	0.5
Animal faeces	77	8	10.4	3.8	0.4
Human Faeces from handlers	224	93	41.5	43.7	4.8
Total	1921	213	-	100	11.09

Table 1. Number and percentage of *Salmonella* isolated from food, poultry, water, animal faeces and human

Serogroup	No. of Serovars	Serovars	Phagetype	Source					Total	%
				Food	Poultry	Water	Animal Faeces	Human Faeces		
S. *enterica*	**52**									
O:4(B)	15	Limete		0	0	0	0	2	2	0.94
		Stanleyville		5	12	0	1	0	18	8.45
		Sarajane		0	0	0	0	1	1	0.47
		Massenya		0	0	0	0	2	2	0.94
		Tudu		0	0	0	0	1	1	0.47
		Ituri		0	0	0	0	2	2	0.94
		Lagos		0	0	0	0	3	3	1.41
		Java		0	0	0	0	2	2	0.94
		Typhimurium	2	2	1	3	0	1	7	3.29
		Schwarzengrund		0	0	0	0	2	2	0.94
		Agona		0	0	0	1	5	6	2.82
		Stanley		0	0	0	0	2	2	0.94
		I:4,12:eh:-		0	0	0	1	0	1	0.47
		Paratyphi B		0	0	0	0	6	6	2.82
		Dragona		0	0	0	1	0	1	0.47
Total				**7**	**13**	**3**	**4**	**29**	**56**	**26.29**
O:7(C_1)	12	Menden		0	0	0	0	3	3	1.41
		Isangi		0	0	0	0	2	2	0.94
		Inganda		0	0	0	0	2	2	0.94
		Edinburg		0	0	0	0	4	4	1.88
		Montevideo		0	0	0	0	3	3	1.41
		Kisii		0	0	0	0	2	2	0.94
		Virchow		1	9	0	0	0	10	4.69
		Mbandaka		0	1	0	0	0	1	0.47
		Rissen		0	2	0	0	0	2	0.94
		Livingstone		5	0	2	0	1	8	3.76
		Infantis		0	0	0	0	2	2	0.94
		I:6,7:-:enz		1	0	0	0	0	1	0.47
Total				**7**	**12**	**2**	**0**	**19**	**40**	**18.78**

Serogroup	No. of Serovars	Serovars	Phagetype	Source					Total	%
				Food	Poultry	Water	Animal Faeces	Human Faeces		
S. *enterica*	**52**									
		Kalina		0	0	0	0	5	5	2.35
		Lexington		0	0	0	0	2	2	0.94
		Okerara		0	0	0	0	3	3	1.41
O:3,10(E_1)	7	Amoundernes		0	0	0	0	1	1	0.47
		Muenster		0	3	0	0	0	3	1.41
		Uganda		0	0	0	2	0	2	0.94
		Meleagridis		0	0	0	0	1	1	0.47
Total				**0**	**3**	**0**	**2**	**12**	**17**	**7.98**
		Albany		0	0	0	0	3	3	1.41
		Blockley		4	6	0	0	0	10	4.69
O:8(C_2)	5	Kentucky		0	17	0	0	2	19	8.92
		Hadar		0	6	0	0	0	6	2.82
		Molade		2	2	5	0	0	9	4.23
Total				**6**	**31**	**5**	**0**	**5**	**47**	**22.07**
		II (formarly Ottershaw)		0	0	0	0	2	2	0.94
O:40(R)	3	Saugus		0	0	0	0	3	3	1.41
		Johannesburg		0	0	0	0	3	3	1.41
Total				**0**	**0**	**0**	**0**	**8**	**8**	**3.76**
O:1,3,19(E_4)	2	Senftenburg		4	0	0	0	3	7	3.29
		Umbadah		0	0	0	2	0	2	0.94
Total				**4**	**0**	**0**	**2**	**3**	**9**	**4.23**
O:13(G)	2	Havana		0	2	0	0	0	2	0.94
		Poona		0	3	0	0	0	3	1.41
Total				**0**	**5**	**0**	**0**	**0**	**5**	**2.35**
O:35(O)	2	Alachua		1	6	0	0	0	7	3.29
		Adelaide		4	0	0	0	0	4	1.88
Total				**5**	**6**	**0**	**0**	**0**	**11**	**5.16**

Serogroup	No. of Serovars	Serovars	Phagetype	Source					Total	%
				Food	Poultry	Water	Animal Faeces	Human Faeces		
S. *enterica*	**52**									
O:9(D_1)	2	Enteritidis	21a, Atypical	2	0	0	0	4	6	2.82
		Typhi		0	0	0	0	9	9	4.23
Total				**2**	**0**	**0**	**0**	**13**	**15**	**7.04**
Other	2	I:rough-O:z29:-		0	0	0	0	1	1	0.47
		I:Rough-O:I,z13:1,5		1	0	0	0	0	1	0.47
Total				**1**	**0**	**0**	**0**	**1**	**2**	**0.94**
subsp. *salamae*	1	S. salamae		0	0	0	0	2	2	0.94
subsp. *arizonae*	1	S. arizonae		0	0	0	0	1	1	0.47
Total				**0**	**0**	**0**	**0**	**3**	**3**	**1.41**
Overall Total				**32**	**70**	**10**	**8**	**93**	**213**	**100.00**

Table 2. Prevalence of *Salmonella* serovars in different sources

two were *S. enterica* subspecies *salamae* and one belonged to subspecies *arizonae*. Serovars of *S. enterica* subsp. *enterica* reported here belonged to 10 serogroups as shown in Table 2. Serogroups B (56 isolates), C_1 (40 isolates), E_1 (17 isolates), C_2 (47 isolates) and R (8 isolates) were each represented by 15, 12, 7, 5 and 3 different serovars, respectively. The remaining serogroups (E_4, G, O, D_1 and other) were represented by two serovars each. The predominant serovars were: Kentucky (8.9%), Stanleyville (8.4%), Blockley and Virchow (each 4.7%), Molade and Typhi (each 4.2%), Livingstone (3.8%), Typhimurium, Senftenberg and Alachua (each 3.3%) and serovars Hadar, Agona, Paratyphi B and Enteritidis (2.8% each). The remaining 38 serovars were represented each by less than six isolates. S. Typhimurium isolates were phagetype 2 while five of S. Enteritidis isolates were 21a phenotype and one was an Atypical phenotype.

Table 2 shows that the serovar Typhimurium was isolated from four of five sources examined whereas Stanleyville, Livingstone and Molade were isolated from three of the Sources. The remaining serovars were isolated each from two or only one source. The most common serovar of the poultry isolates (n=70) was *S.* Kentucky (24.3%) followed by Stanleyville (17.1%), Virchow (12.9%) and Blockley, Hadar and Alachua (8.6% each). *S.* Typhi (9 isolates) was the most common (9.7%) serovar among human faeces isolates (n=93) followed by Paratyphi B (6.5%), Agona and Kalina (5.4 % each). Among the food isolates (n=32) *S.* Stanleyville and Livingstone were the most common (15.6%) with 5 isolates each. The most common serovars among water isolates (n=10) and animal faeces isolates (n=8) were *S.* Molade (50%) and Uganda (25%), respectively. To the best of our knowledge, 21 of the serovars reported here were isolated for the first time in Sudan and were all from human source only. These included: *S.* Stanley, Okerara, Sarajane, Limete, Massenya, Edinburg, Isangi, Inganda, Java, Alabany, Kalina, Tudu, Ottershaw, Saugus, Amoundrenes, Lexington, Kisii, Ituri, Lagos, salamae and arizonae.

3.2 Antimicrobial resistance and plasmid analysis

Results in Table 3 show that the highest frequency of resistance observed was to streptomycin (41.3%) followed by tetracycline (31.9%), gentamycin (28.2%), ampicillin (25.4%), nalidixic acid (22.1%), co-trimoxazole (17.4%), ciprofloxacin (8.9%), chloramphenicol (8%), norfloxacin (7.5%) and apramycin (5.6%). All isolates of the serovars Tudu, Montevideo, Meleagridis, Johannesburg, Umbadah, Poona, Kisii and one Rough isolate which were found infrequently (n ranging from 1-3) were susceptible to all of the antibiotics tested. Out of the 37 isolates which showed resistance against co-trimoxazole, 27 (73%) belonged to the serovars Sanleyville (11/18 isolates), Virchow (10/10 isolates) and Typhi (6/9 isolates). Similarly, out of the 68 tetracycline-resistant isolates, 40 (58.8%) belonged to the serovars Stanleyville, Virchow, Blockley and Kentucky, each contributing by 10 isolates. Isolates of S. Blockley (n=10) were all resistant to tetracycline but were fully sensitive to co-trimoxazole, chloramphenicol, ciprofloxacin, ampicillin, norfloxacin and apramycin. All *S.* Kentucky isolates (n=19) were resistant to nalidixic acid but fully sensitive to chloramphenicol. Of the 22 isolates belonging to *S.* Typhi (9), Paratyphi B (6) and Typhimurium (7), 14 were resistant to ampicillin, 6 to gentamycin, 4 to chloramphenicol and 3 to each of ciprofloxacin and nalidixic acid but none to norfloxacin.

Serovar	Antibiotics									
	Te	Cn	Na	St	Cot	C	Cip	Amp	Nor	Apr
Limete (n=2)	1	2	1	2	0	0	1	0	1	1
Stanleyville (n=19)	10	0	1	1	11	0	0	7	0	0
Sarajane (n=1)	1	0	0	0	0	1	0	0	1	0
Massenya (n=2)	2	0	0	0	0	2	2	0	0	0
Tudu (n=1)	0	0	0	0	0	0	0	0	0	0
Ituri (n=2)	0	2	0	2	0	0	0	0	0	0
Lagos (n=3)	0	0	0	3	0	0	0	0	0	0
Java (n=2)	0	2	1	2	0	0	1	0	0	0
Typhimurium (n=7)	0	1	0	1	0	0	0	3	0	2
Schwarzengrund (n=2)	0	0	0	1	0	0	0	0	0	0
Agona (n=6)	0	2	0	0	0	0	0	2	2	2
Stanley (n=2)	2	2	0	2	0	0	0	0	0	0
I:4,12:eh:- (n=1)	1	0	0	0	0	0	0	0	0	0
Paratyphi (n=6)	1	3	1	6	3	1	1	5	0	0
Dragona new variant (n=1)	1	0	0	1	0	0	0	0	0	0
Menden (n=3)	0	0	1	1	0	1	1	2	1	0
Isangi (n=2)	2	1	1	1	0	2	1	2	0	0
Inganda (n=2)	1	2	0	2	0	1	0	2	1	0
Edinburg (n=4)	2	4	0	4	0	4	1	4	1	0
Montevideo (n=3)	0	0	0	0	0	0	0	0	0	0
Kisii (n=2)	0	0	0	0	0	0	0	0	0	0
Virchow (n=10)	10	0	10	0	10	0	0	0	0	0
Mbandaka (n=1)	0	1	1	0	0	0	0	0	0	0
Rissen (n=2)	0	1	1	2	1	0	0	0	0	0
Livingstone (n=8)	0	5	7	2	0	0	0	2	0	0
Infantis (n=2)	2	0	0	0	0	0	0	2	0	0
I:6,7:-:enz (n=1)	1	0	0	0	1	0	0	0	0	0
Kalina (n=5)	0	5	0	5	0	0	0	0	0	2
Lexington (n=2)	0	0	0	1	0	0	0	0	0	0
Okerara (n=3)	0	0	0	3	0	0	0	0	0	0
Amoundernes (n=1)	0	1	0	1	0	0	0	0	0	0
Muenster (n=3)	0	0	0	1	0	0	0	0	0	0
Uganda (n=2)	1	0	0	0	0	0	0	0	0	0

Serovar	Antibiotics									
	Te	Cn	Na	St	Cot	C	Cip	Amp	Nor	Apr
Meleagridis (n=1)	0	0	0	0	0	0	0	0	0	0
Albany (n=3)	0	3	0	2	0	1	2	1	2	1
Blockley (n=10)	10	1	1	1	0	0	0	0	0	0
Kentucky (n=19)	10	12	19	12	1	0	6	6	6	1
Hadar (n=6)	0	0	0	6	0	0	0	3	0	0
Molade (n=9)	3	0	0	3	3	0	0	2	0	0
II (formarly Ottershaw) (n=2)	0	0	0	2	0	0	0	0	0	0
Saugus (n=3)	0	0	0	3	0	0	0	0	0	0
Johannesburg (n=3)	0	0	0	0	0	0	0	0	0	0
Senftenberg (n=7)	0	1	0	0	0	0	0	0	0	1
Umbadah (n=2)	0	0	0	0	0	0	0	0	0	0
Havana (n=2)	2	0	0	0	1	0	0	0	0	0
Poona (n=3)	0	0	0	0	0	0	0	0	0	0
Alachua (n=7)	0	0	0	1	0	0	0	2	0	0
Adelaide (n=4)	0	1	0	1	0	0	0	0	0	1
Enteritidis (n=5)	0	3	0	3	0	1	1	2	1	1
Typhi (n=9)	5	2	2	7	6	3	2	6	0	0
I:rough-O:z29:- (n=1)	0	0	0	0	0	0	0	0	0	0
I:Rough-O:I,z13:1,5 (n=1)	0	0	0	0	0	0	0	1	0	0
S. salamae (n=2)	0	2	0	2	0	0	0	0	0	0
S. arizonae (n=1)	0	1	0	1	0	0	0	0	0	0
Total of resistant isolates	68	60	79	54	37	18	15	54	16	12

Table 3. Number of isolates in different serovars resistant to different antibiotics

There were 164 *Salmonella* isolates belonging to 46 serovars exhibited 55 different resistance patterns. A percentage of 23.5 (50/213) of the isolates displayed a single type of resistance, 17.8% (38/213) showed resistance to two classes of antibiotics (data not shown) and 35.7% (76/213) were multidrug-resistant (resistant to more than two antimicrobials). Resistance patterns, MAR indices and plasmid profiles are presented in Table 4. As shown in this Table, the 76 multidrug-resistant isolates (MDR) exhibited 37 different MDR profiles. Of these profiles, 17, 10, 6 and 4 resulted from resistance to 3, 4, 5 and 6 antimicrobials. All of *S.* Virchow isolates (n=10) showed a resistance pattern of TeNaCot. One isolate of each of *S.* Enteritidis and *S.* Inganda and two isolates of *S.* Paratyphi B shared the pattern CnStAmp. One isolate of each of *S.* Inganda, *S.* Isangi, *S.* Limete and two isolates of *S.* Edinburg showed four different hexa-resistance patterns. Seven isolates of *S.* Kentucky, three of *S.* Typhi and one isolate of each of *S.* ParatyphiB and *S.* Albany had six penta-resistance

patterns. Four isolates of *S*. Kentucky shared the same profile of TeCnNaStAmp while the three isolates of *S*. Typhi shared the pattern TeStCotCAmp.

Twenty-one isolates belonging to ten serovars showed resistance to four antibiotics and exhibited ten resistance patterns. Of these, three patterns (TeCnNaSt, NaCipNorAmp and NaCipNorApr) were shown by eight isolates of *S*. Kentucky. One of the patterns, TeCnNaSt, was displayed by five of the 19 *S*. Kentucky isolates while the three isolates of *S*. Molade and two of *S*. Typhi shared the pattern TeStCotAmp. The other six patterns were shown by eight isolates belonging to the serovars Edinburg (2), Paratyphi B (1), Rissen (1), Java (1), Livingstone (1), Enteritidis (1) and Albany (1).

Of the 76 isolates showing MDR, 42 (55.3%) originated from human sources, 26 (34.2%) from poultry, 3 (3.9%) from each of water and food and 2 (2.6%) from animal sources. Of the 26 poultry isolates showing MDR, 13 belonged to *S*. Kentucky and 9 to *S*. Virchow whereas 17 of the 42 MDR human isolates belonged to *S*. Typhi (7), *S*. Paratyphi (6) and *S*. Edinburg (4). Average of multiple antibiotic resistance indices (MAR indices) indicated that *Salmonella* isolated from water had the highest average MAR index (0.4) followed by human (0.39), poultry (0.37), animal (0.35) and food (0.3).

Pattern	Serovars	Source	MAR index	Plasmid Profile (Kb)
Cn,Nor,Apr	Agona	Human	0.3	ND
Cn,Nor,Apr	Agona	Human	0.3	ND
Cn,Amp,Apr	Albany	Human	0.3	ND
Te,Na,St	Blockley	Poultry	0.3	ND
Cn,St,Cip	Enteritidis	Human	0.3	12.5
Cn,St,Amp	Enteritidis	Human	0.3	12.2
Cn,St,Amp	Inganda	Human	0.3	ND
Te,C,Amp	Isangi	Human	0.3	10.6
Cn,St,Apr	Kalina	Human	0.3	ND
Cn,St,Apr	Kalina	Human	0.3	ND
Cn,St,Apr	Limete	Human	0.3	10.6
Na,St,Amp	Livingstone	Food	0.3	10.6
Te,C,Cip	Massenya	Human	0.3	12.2
Te,C,Cip	Massenya	Human	0.3	10.6, 12.2
St,C,Amp	Menden	Human	0.3	ND
St,Ct,Amp	Paratyphi B	Human	0.3	8.3
St,Ct,Amp	Paratyphi B	Human	0.3	10.6, 12.2
Cn,St,Amp	Paratyphi B	Human	0.3	7.9, 10.6
Cn,St,Amp	Paratyphi B	Human	0.3	10.6

Pattern	Serovars	Source	MAR index	Plasmid Profile (Kb)
Te,C,Nor	Saragane	Human	0.3	12.2
Te,Cn,St	Stanley	Human	0.3	ND
Te,Cn,St	Stanley	Human	0.3	7.9
Te,Na,Cot	Stanleyville	Poultry	0.3	8.3, 12.5
Te,Cot,Amp	Stanleyville	Animal	0.3	1.2, 12.2, 12.5
Te,Cot,Amp	Stanleyville	Food	0.3	1.2, 12.2, 12.5
Te,Cot,Amp	Stanleyville	Poultry	0.3	1.2, 12.5
Cn,Na,Amp	Typhi	Human	0.3	3.5, 8.3
Na,St,Cip	Typhi	Human	0.3	8.3, 12.2, 12.5
Te,Na,Cot	Virchow	Poultry	0.3	ND
Te,Na,Cot	Virchow	Poultry	0.3	ND
Te,Na,Cot	Virchow	Poultry	0.3	ND
Te,Na,Cot	Virchow	Poultry	0.3	ND
Te,Na,Cot	Virchow	Poultry	0.3	ND
Te,Na,Cot	Virchow	Poultry	0.3	ND
Te,Na,Cot	Virchow	Poultry	0.3	ND
Te,Na,Cot	Virchow	Poultry	0.3	ND
Te,Na,Cot	Virchow	Poultry	0.3	ND
Te,Na,Cot	Virchow	Food	0.3	ND
Cn,St,Cip,Nor	Albany	Human	0.4	12.5
Cn,Na,C,Amp	Edinburg	Human	0.4	12.2, 10.6
Cn,Na,C,Amp	Edinburg	Human	0.4	ND
Cn,Na,C,Amp	Enteritidis	Human	0.4	ND
Cn,Na,St,Cip	Java	Human	0.4	12.5
Te,Cn,Na,St	Kentucky	Poultry	0.4	ND
Te,Cn,Na,St	Kentucky	Poultry	0.4	ND
Te,Cn,Na,St	Kentucky	Poultry	0.4	2.9, 3.5
Te,Cn,Na,St	Kentucky	Poultry	0.4	2.9, 5.5
Te,Cn,Na,St	Kentucky	Poultry	0.4	5.5
Na,Cip,Nor,Amp	Kentucky	Poultry	0.4	15.6, 12.5, 12.2
Na,Cip,Nor,Amp	Kentucky	Poultry	0.4	15.6, 12.2
Na,Cip,Nor,Apr	Kentucky	Poultry	0.4	12.2

Pattern	Serovars	Source	MAR index	Plasmid Profile (Kb)
Cn,Na,St,Amp	Livingstone	Animal	0.4	8.3, 12.2
Te,St,Ct,Amp	Molade	Water	0.4	1.2, 2.9, 8.3
Te,St,Ct,Amp	Molade	Water	0.4	1.2, 8.3
Te,St,Ct,Amp	Molade	Water	0.4	1.2, 8.3
Te,St,Ct,C	Paratyphi B	Human	0.4	12.5
Cn,Na,St,Ct	Rissen	Poultry	0.4	12.2
Te,St,Ct,Amp	Tuphi	Human	0.4	12.2, 12.5
Te,St,Ct,Amp	Tuphi	Human	0.4	3.5, 12.2
Cn,St,C,Cip,Nor	Albany	Human	0.5	8.3, 12.2
Cn,Na,St,Cip,Nor	Kentucky	Human	0.5	12.2
Cn,Na,St,Cip,Nor	Kentucky	Human	0.5	5.5, 12.2
Te,Cn,Na,St,Ct	Kentucky	Poultry	0.5	ND
Te,Cn,Na,St,Amp	Kentucky	Poultry	0.5	7.9
Te,Cn,Na,St,Amp	Kentucky	Poultry	0.5	ND
Te,Cn,Na,St,Amp	Kentucky	Poultry	0.5	10.6
Te,Cn,Na,St,Amp	Kentucky	Poultry	0.5	7.9
Cn,Na,St,Cip,Amp	Paratyphi B	Human	0.5	12.2, 12.5
Te,St,Ct,C,Amp	Typhi	Human	0.5	8.3, 12.5
Te,St,Ct,C,Amp	Typhi	Human	0.5	3.5, 8.3, 12.5
Te,St,Ct,C,Amp	Typhi	Human	0.5	8.3, 12.5
Te,Cn,Ct,C,Cip,Amp	Edinburg	Human	0.6	15.6, 10.6
Te,Cn,Ct,C,Cip,Amp	Edinburg	Human	0.6	15.6, 12.5
Te,Cn,St,Ct,C,Amp	Inganda	Human	0.6	12.5
Te,Cn,Na,C,Cip,Amp	Isangi	Human	0.6	12.2, 15.6
Te,Cn,Na,St,Cip,Nor	Limete	Human	0.6	12.2, 12.5

Table 4. Multiple antibiotic resistance profile and MAR indices of individual *Salmonella* isolates

Results of plasmid profile for MDR *Salmonella* isolates is shown in Table 4. Plasmid DNA was detected in 67.1% of the multiple resistant isolates demonstrating nine plasmids which constituted four plasmid profiles among the 37 resistance patterns. The number of plasmids per isolate ranged from 1 – 3 and their sizes ranged from 1.2 - 15.6 Kb. The majority of isolates (31.6%) had two plasmids but 27.6% had a single plasmid while 7.9% of the isolates had three plasmids. MDR *Salmonella* isolates that not harboring plasmids showed ten different resistance patterns which includes all of the antibiotics examined except for ciprofloxacin. Plasmids were not detected in all *S*. Virchow isolates (n=10) which share the

resistance profile TeNaCot, a profile which re-appeared only in one isolate of *S*. Stanleyville. The largest plasmid (15.6 Kb) was detected in two isolates of *S*. Edinburg, two isolates of *S*. Kentucky and one isolate of *S*. Isangi. They were all resistant to ampicillin and ciprofloxacin and showed three different resistance patterns. The smallest plasmid (1.2 Kb) detected in three isolates of each of *S*. Molade and *S*. Stainleyville. These isolates were resistant to tetracycline, co-trimoxazole and ampicillin. It is also clear that, all of the ciprofloxacin resistant isolates were found to contain at least one of the largest plasmids (12.2, 12.5 and 15.6 Kb). The 12.2 Kb plasmid was present, either alone or in combination with plasmids of other sizes, in 15 (40.5%) of the different resistance patterns.

3.3 Detection by PCR

DNA extracted from 213 *Salmonella* strains and 12 closely related non-*Salmonella* strains were used to evaluate the specificity and sensitivity of ten primer sets to detect *Salmonella* sp. using the polymerase chain reaction technique (Table 5). The primer pairs targeting *inv*A, *hil*A, *iro*B, *ori*C, *fim*A, *hit*J and *stn* genes successfully amplified the DNA extracted from all *Salmonella* isolates generating the specific amplicon for each primer, and no amplification products were detected with DNA from non-*Salmonella* strains. These primers were, therefore, recommended as reliable means for simple and rapid PCR – based detection of the locally isolated *Salmonella* serovars.

Primer	*Salmonella* isolates (n=213)		Non-*Salmonella** isolates (n=12)		Sensitivity limit as for *S*. Typhi (CFU/ml)
	No. of +ve	No. of -ve	No. of +ve	No. of -ve	
invA	213	0	0	12	6.07x10
hilA	213	0	0	12	$6.07x10^2$
ompC	203	10	0	12	$6.07x10^2$
iroB	213	0	0	12	$6.07x10^2$
oriC	213	0	0	12	$6.07x10^2$
fimA	213	0	0	12	$6.07x10^2$
16S rDNA	213	0	4	8	$6.07x10^2$
Rep.feg	201	12	5	7	$6.07x10^2$
Hist	213	0	0	12	$6.07x10^2$
Stn	213	0	0	12	$6.07x10^2$

Table 5. Interpretation and follow-up action for PCR-based detection results. * *E. coli*, *Klebsiella* sp., *Klebsiella pneumonia*, *Bacillus subtils*, *Serratia* sp., *Proteus* sp., *Shigella dysenteriae*, *Shigella flexneri*, *Citrobacter freundii*, *Pseudomonas aeroginosa*, *Staphylococcus* sp. (1), *Staphylococcus* sp. (2).

The ompC primer set produced the expected amplicon with the DNA extracted from most of the *Salmonella* isolates, but failed to do so with DNA of *S.* Molade and *S.* Meleagridis. Negative results were also obtained with all non-*Salmonella* strains. The primer set 16S rDNA generated target size amplicons with all *Salmonella* isolates. However, similar amplicons were produced from the DNA of some non-*Salmonella* strains including *Proteus sp., Shigella dysenteriae, Citrobacter freundii* and *Pseudomonas aeruginosa*. The repetitive fragment of DNA primer set failed to amplify the DNA of 2 isolates of each of *S.*

Adelaide, *S.* Senftenberg and *S.* Virchow, and one isolate of *S.* Blockley, *S.* Hadar, *S.* Molade, Poona, *S.* Schwazengrund and *S.* Stanlyville. Moreover, the repetitive fragment of DNA amplified a specific fragment from the DNA of *E. coli, Klebsiella pneumoniae, Bacillus subtilis, Proteus sp.* and *Shigella flexneri*.

4. Discussion

Salmonella was isolated from different sources with an overall prevalence of 11.09%, which is comparable to the isolation frequency (9.2 and 11.2%) reported by El Hussein et al. (2010) in Khartoum State, Sudan and by Mohammad et al. (2006) in Zahedan, Iran respectively. The prevalence percentage reported demonstrates the widespread occurrence and distribution of *Salmonella* in Sudan. This prevalence was clearly higher than the range of 3.34 - 4.0%, reported in the limited studies conducted previously (Soliman and Khan, 1959; Khan, 1962, 1970; Yagoub and Mohamed, 1987; El Tom et al., 1999; Yagoub et al., 2006 and Hag Elsafi et al., 2009). Several studies in other developing countries have reported a higher overall prevalence of *Salmonella* (human, food, and animal) such as 68.2% in Ethiopia, 51.2% in Argentina , 25.9% in Korea, and 72% in Thailand (Cardinale et al., 2003). It is important to recognize that the prevalence and distribution of *Salmonella* serovars varies from region to region (Dominguez et al., 2002; Uyttendaele et al., 1998) and isolation rates depend upon the country where the study was carried out, the sampling plan and the detection limit of the methodology (Roberts, 1982; Uyttendaele et al., 1998). Consequently, it is difficult to make comparisons between *Salmonella* surveillance conducted in different countries. However, the serovars isolated from the various sample types in our survey in Sudan were comparable to the results reported by various investigators in other countries. For example, Baudart et al. (2000) reported the prevalence of *S.* Agona, *S.* Enteritidis, *S.* Infantis, *S.* Mbandaka, *S.* Muenster, *S.* Rissen, *S.* Typhimurium, *S.* Montevideo and *S.* Virchow in different aquatic environments. The same serovars plus *S.* Senftenberg were isolated by Saha et al. (2001) from faecal samples taken from hospitalized diarrhoeal children in India. Similar to this study, Liebana et al. (2001, 2002)and Tamada et al. (2001) reported the isolation of *S.* Mbandaka, *S.* Montevideo and *S.* Livingstone from animal sources. The findings of Delicato et al. (2004) and of Fernandez et al. (2003) were also comparable to our results. They reported the isolation of *S.* Enteritidis, *S.* Infantis and *S.* Typhimurium from human faeces. *S.* Typhimurium and *S.* Enteritidis, in particular, are regarded worldwide as significant pathogenic serovars, with certain phagetypes being associated with serious illness in humans, chickens and animals (Dominguez et al., 2002; Jorgensen et al., 2002; Roy et al., 2002 & Mohammad et al., 2006). The predominant phagetype of *S.* Typhimurium was PT2, while those for *S.* Enteritidis were PT21a and Atypical. In Western Europe, phagetype 4 was generally reported as a dominant phage for S. Enteritidis (Humphrey et al., 1991), while in the United States, PT8 was generally dominant (Hickman-Brenner et al., 1991).

The most predominant serovar in this study was *S.* Kentucky constituting 8.9% of the recovered isolates. The ranking of this serovar among other serovars increased substantially during the past decade in most European countries (Gill et al., 2002; 2004; Gill and Threlfall, 2007; Bonalli et al., 2011). However, this serovar was considered an unsuccessful pathogen because it was rarely associated with human illness (Collard et al., 2007). *S.* Kentucky and *S.* Stanleyville were mostly isolated from poultry samples in comparison with other serotypes (24.3 and 17.1%, respectively). In chickens, it is well established that *S.* Enteritidis is the most

predominant *Salmonella* serovar, followed by *S*. Typhimurium (Mohammad et al., 2006; Suresh et al., 2011). However, no S. Enteritidis and only one isolate of *S*. Typhimurium was isolated from poultry in this study.

Many animal species harbour *Salmonella* and can act as potential reservoirs for human infections. For example, *S*. Menden, *S*. Enteritidis, *S*. Montevideo and *S*. Senftenberg, which were recovered from humans in this study, were previously isolated from different animals in Sudan (Khan, 1970). *Salmonella* may enter the food chain through carcass contamination with animal faeces at slaughter and during processing, or through food or food handlers. However, human infection may also occur through contaminated water, pets, and exotic animals. Measures taken to control these routes of transmission are an effective way of preventing salmonellosis. The collection of prevalence data of *Salmonella* serovars is an important component of a successful epidemiological surveillance for public health management in any country.

Since all human isolates were recovered from stool samples of food handlers, it is likely that food can act as potential reservoirs for salmonellosis epidemics. More strict measures should be implemented in the food industry to curtail the spread of salmonellosis in Sudan. For example, monitoring of restaurant workers should be performed at more frequent intervals rather than the current mandatory annual check-up for renewal of work permits. This should be accompanied by organized training programs involving suppliers of food items and corporate administrators as well as front line restaurant employees. More involvement by public health authorities in surveillance programs is needed to ensure that public safety regulations are properly implemented.

This initial survey has provided useful information about the status of salmonellosis in Khartoum, Sudan. We have demonstrated that *Salmonella* was isolated mostly from humans followed by chickens suggesting that chicken and chicken-food products could be a potential source of salmonellosis in the food chain. The *S*. Kentucky serovar, which has a lesser potential for infection, was common to human, chicken and food items. Furthermore, *S*. Kentucky isolates are known for their resistance to ciprofloxacin, the antibiotic of choice for the treatment of typhoid in Sudan (Bonalli et al., 2011). Thus this phenotype may spread to other serovars which might have greater potential for infection.

The increased level of drug resistance in *Salmonella* has become a public health concern (Pui et al., 2011). As antibiotic usage varies among countries, different resistant phenotypes and genotypes can be expected. Thus monitoring of antimicrobial resistance patterns from different sources and regions is an important issue.

In this study, the rate of resistance to ampicillin (25.4%) and chloramphenicol (8.0%) was comparable to those detected for the same antibiotics in the year 2000. At that time, Ahmed et al. (2000) reported 25.0% and 13.0% resistance to ampicillin and chloramphenicol, respectively. However, the percentage of *Salmonella* isolates resistant to nalidixic acid and ciprofloxacin in Sudan has increased from zero percent (Ahmed et al., 2000) to 22.0 and 8.9%, respectively. The wide resistance to Nalidixic acid is a matter of concern, since nalidixic acid resistance has been associated with a decrease in susceptibility to fluoroquinolones, including ciprofloxacin, which are used to treat salmonellosis in humans (Gorman and Adley, 2004). The human isolates were more resistant to ciprofloxacin (16.1%) than the poultry isolates of which only 5.7% showed resistance to the antibiotic. Our results

are consistent with what has been reported by Tassios et al. (1997), but disagree with the findings of Al-Bahry et al. (2007) who found that isolates of human origin were less resistant than those of chicken. In this study, resistance was 41.3% to streptomycin and 31.9% to tetracycline which is inconsistent with a report of complete susceptibility, to both antibiotics, by Singh et al. (2010) in India. Our results are in line with other investigators who observed high rates of resistance to streptomycin and tetracycline by *Salmonella* isolates (Zhao et al., 2005; Stevens et al., 2006; Dogru et al., 2009; Iseri and Erol, 2010). De Oliveira et al. (2010) attributed the prevalence of resistance to streptomycin and tetracycline to their frequent administration in veterinary medicine.

A lower percentage of resistance was observed for norfloxacin (7.5%), chloramphenicol (8.0%) and ciprofloxacin (8.9%). Lower resistance rates to these drugs were also reported by other workers (Gulsen et al., 2004; Zhao et al., 2006; Lestari et al., 2009). Therefore, these drugs may continue to be the drugs of choice for treatment of human salmonellosis in Sudan. apramycin, to which only 5.6% of *Salmonella* isolates were resistant, can replace the currently used antibiotics (mostly streptomycin and tetracycline) in the treatment of poultry.

Reduced susceptibility to fluoroquinolones and chloramphenicol has also been reported particularly for *S.* Kentucky isolates in many countries of the region such as Ethiopia (Molla et al., 2006), Morocco (Bouchrif et al., 2009) and Tunisia (Turki et al., 2011). Weill and Le Hello (2008) indicated that the acquisition and spread of distinct antibiotic resistance, especially resistance to ciprofloxacin, the drug of choice in Sudan, is associated with the emergence of S. Kentucky.

Turki et al. (2011) reported a correlation between resistance to nalidixic acid and ciprofloxacin. This was not the case in our study; instead, nalidixic acid resistance closely correlates with tetracycline resistance. Strong evidence of cross resistance between chloramphenicol and ampicillin was also observed. Of 17 chloramphenicol resistant isolates, 12 (70.6%) were also resistant to ampicillin. Values of 100%, 85% and 21% cross resistance between these two antibiotics was reported by different investigators (Goldstein et al., 1983; Rowe et al., 1992; Gupta et al., 1990).

This study demonstrated that 35.7% of the *Salmonella* isolates tested (n=213) were multidrug resistant. This level was comparable to that reported by Bouchrif et al. (2009) in Morocco (44%) and Van et al. (2007) in Vietnam (34%) but much lower than the level reported by Thong and Modarressi (2011) in Kuala Lumpur (67%). Among all *Salmonella* isolates from different sources, 37 antibiotic resistance patterns were detected, indicating wide spread multidrug resistance. This result is comparable with the findings of Singh et al. (2010) who identified 24 different resistance patterns among their *Salmonella* isolates. The authors attributed the observed high number in multidrug resistance patterns to the frequent use of antibiotics in the environments from which the isolates originated. It should be emphasized that most of the MDR isolates (n=76) in this study originated from human (42) and poultry sources (26). Nowroozi et al. (2004) demonstrated that indiscriminate use of antibiotics in poultry production has increased the emergence and maintenance of MDR bacteria in the environment. Similarly, Ahmed et al. (2000) attributed the high level of multiple resistance salmonellae among human isolates to the inordinate and irrational use of antimicrobial agents in the Sudan. The authors mentioned a number of factors that have led to the prevalence of antibiotic resistance. The most important among which are the deliberate self-

administration of antibiotics by patients themselves, the wide use of antibiotics due to the high prevalence of infectious diseases, lack of laboratory support in rural areas and selective prescribing due to cost constrains.

Plasmid DNA was demonstrated in 67.1% of the multiple resistant isolates. Our results indicated the association of large integrons (12.2 - 15.6 Kb) with the resistance to ampicillin and/or ciprofloxacin. Certain plasmid sizes may be responsible for resistance to particular antibiotics (White et al., 2001). Resistance to ampicillin in *Salmonella* was mediated by a β–lactamase gene carried on a plasmid (Thong & Modarressi, 2011). Since some of the isolates were resistant to one or more antibiotics and yet did not harbour any plasmid, the antibiotic resistance might be chromosomally mediated or mediated by other mobile elements such as transposons (Yah & Eghafona, 2008). The finding of both plasmid and non-plasmid mediated antibiotic resistance is consistent with other studies (Rodrigue et al., 1992; Ansary et al., 2006). This implies that there is no consistent relationship between the detected plasmid profile and antibiotic resistance patterns. Similar conclusions were previously drawn by Shears et al. (1988) in Sudan and Mamun et al. (1993) in Bangladesh.

In summary, this study confirmed that food, water, poultry and food handlers might act as reservoirs for antimicrobial resistant *Salmonella*. To diminish contamination rates by *Salmonella*, risk reduction strategies such as training of food handlers regarding food safety, institutional periodic focused medical checkups for food handlers, more restrictions on the irrational use of antibiotics, and establishment of standardized monitoring systems for on-going drug resistance surveillance are required. Although the current study addressed a local problem in Sudan where it appears that there is a high incident of antibiotic resistant salmonellae, many other developing countries are faced with a similar situation (Al-Bahry et al., 2007; Turki et al., 2011; Thong and Modarressi, 2011). In addition, dissemination of information on antibiotic resistance is important for development of prescribing polices and for general medical practitioners in a remote area who may not have access to microbiology laboratory back-up and, hence, must depend on prevailing knowledge of antibiotic-resistant *Salmonella*.

Results of the primers pairs targeting *inv*A, *hil*A, *iro*B, *ori*C, *fim*A, *hit*J and *stn* genes indicated their reliability, sensitivity and accuracy for the detection of *Salmonella enterica*. These findings affirm results of the previous studies (Moganedi et al., 2007; Murphy et al., 2007; Narvaneni and Jamil, 2005; Moore and Feist, 2006). It has been shown that *inv*A and *hil*A genes are associated with pathogenicity of *Salmonella* spp. The former gene was reported to be essential for the invasion of epithelial cells by *Salmonella* (Moganedi et al., 2007); consequently, all *Salmonella* isolates studied are capable of invading the epithelial cells of the host (Bajaj et al., 1995). The *hil*A gene proved to be highly conserved among our *Salmonella* serovars. Being unspecific, the repetitive fragment of DNA, 16S rDNA and opmC primers were considered as unsuitable for PCR detection of locally isolated *Salmonella* serovars. The positive results obtained by the primer set 16S rDNA with some non-*Salmonella* strains could be explained by the fact that this primer set is constructed from 16SFl and 16SIII derived from the two regions of 16S rRNA gene. *16S*F1 is the reverse and complementary strand of *16S*I, which has been found to hybridize with *Salmonella* as well as with *Citrobacter* sp., while the *16S*III sequence was able to hybridize with *Klebsiella* and *Serratia* spp., in addition to *Salmonella* (Lin and Tsen, 1996). Similarly, the repetitive DNA fragment PCR assay was found to be unspecific for *Salmonella* as it failed to amplify the DNA of a number of *Salmonella* isolates and positive results were obtained for most of the non- *Salmonella*

strains. Comparably, Ziemer and Steadham (2003) reported the ability of this primer set to amplify the DNA of bacteria commonly associated with intestinal samples.

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