International Scholars Journals

International Journal of Public Health and Epidemiology ISSN 2326-7291 Vol. 9 (3), pp. 001-005, March, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Antimicrobial susceptibility of *Salmonella* serotypes isolated from human and animals in Sudan

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Accepted 23 December, 29

The aim of the present study was to determine the prevalence of multidrug resistance among *Salmonella enterica* serotypes recovered from human and animals in Sudan. A total of 119 *Salmonella* isolates recovered from stools of humans, cattle, camels and poultry were subjected to *in vitro* susceptibility against 10 commonly prescribed antibacterial agents. The test was done by the disc diffusion assay, isolates were categorized as sensitive or resistant based on standardized zones of inhibition and the odd ratio (OR) was performed to measures of degree of association between the two result characteristics of agents. The majority of *Salmonella* serotypes (80.67%) were found resistant to at least one of the tested nine antibacterial agents and 45 isolates (37.82%) were found multidrug resistant (MDR). Human isolates were found to be more resistant than the animal's isolates. Ciprofloxacin, gentamicin and colistin were found to be highly active against the isolates. But the isolates showed high resistance to ampicillin, chloramphenicol, tetracycline, furazolidone and sulfamethoxazole + trimethoprim. The odd ratios (OR) among the isolates to combination of two antimicrobials ranged from 1.1 to 5.75. The highest OR was shown in combination cefalexin-nalidixic acid, which has an OR of 5.75. Salmonella serotypes revealed very high resistance (80.67%) and high MDR (37.82%) rates. *Salmonella* sp. recovered from human exhibited higher rates of resistance than those recovered from animals.

Key words: *Salmonella*, Sudan, antimicrobials, multidrug resistance, odd ratio (OR).

INTRODUCTION

Salmonella enterica infections are the second leading cause of bacterial foodborne illness and approximately 95% of cases of human salmonellosis are associated with the consumption of contaminated products such as meat, poultry, eggs, milk, seafood, and fresh produce. Typically, the disease is self-limiting; however, with more severe manifestations such as bacteremia,. Antimicrobial therapy is often administered to treat the infection (Mead et al., 1999; Foley and Lynne, 2008). Animals are main source of multiple resistant salmonella for human, indicating their significance in the epidemiology of human salmonellosis. Antibiotic resistance has been attributed to genetic and environmental factors (Angulo et al., 2004; Hammerum et al., 2009).

A number of studies have been undertaken in Sudan on Salmonella and salmonellosis notably in animals. Mamoun et al. (1992) isolated 21 Salmonella strains from several poultry farms in three different States in the Sudan. Salmonella eneritidis was detected in 1.43% of raw milk samples (Yagoub and Mohammed, 1987; Yagoub et al., 2005). Yagoub et al. (2006) isolated Salmonella paratyphi A and Salmonella paratyphi B from 6% of the white cheese samples collected from retailer shops and restaurants in Khartoum and Omdurman cities during the period from February to November, 2001. Yagoub (2009) detected Salmonella sp. in 6.2% of fish samples and Hag Elsafi et al. (2009) detected Salmonella sp. in 3.4% of fecal samples collected from in and around

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Khartoum State. S. enterica subspecies was recovered from 9.2% of different raw and cooked food items (El-Hussein et al., 2010). Saeed and Hamid (2010) confirmed the role of food handlers in the spread and transmission of food borne communicable diseases which includes salmonellosis as they detected pathogens in 30.1% of the food handlers.

The present study aimed to determine the prevalence of multidrug resistant strains among Salmonella serotypes isolated from human and animals in Sudan.

MATERIALS AND METHODS

Salmonella isolates

A total of 119 S. enterica isolates belonging to different Salmonella serotypes were included in the study. The strains were isolated from stools of human (n = 87), cattle (n =6), camels (n = 2) and poultry (n = 24). S. enterica serotypes were identified using (O) and (H) anti-sera (VLA Scientific Product Sales, Surrey, UK). Some isolate could not be typed by the available antisera. The serotypes and their sources are shown in Table 2.

Antimicrobial susceptibility testing

The 119 Salmonella serotypes were tested against 10 antimicrobial agents namely: ampicillin, cefalexin, chloramphenicol, ciprofloxacin, colistin, furazolidone, gentamicin, nalidixic acid, sulfamethoxazole + trimethoprim and tetracycline. The antimicrobial susceptibility test was performed by the disk diffusion method with standard antibiotics disks (Oxoid, Basingstoke and Hampshire, England) using Muller-Hinton agar plates as per the National Committee for Clinical Laboratory standards (NCCLS, 2002). The disks are placed on the agar with flamed forceps or a single disk applicator and gently pressed down to ensure contact. Plates are incubated immediately, or within 30 min. If several colonies are seen within a zone of inhibition, the strain was checked for purity and retested. Isolates were categorized as sensitive or resistant based on standardized zones of inhibition.

Odds ratios and sensitivity patterns

The odds ratio (OR) was chosen as the measures of degree of association between the 2 characteristics, that is, resistance to A and B. An OR equal to 1 indicates no association between the two characteristics, ratios greater than 1 indicated a positive association (increased probability of resistance to the 2nd antimicrobial of a 2-way combination). Confidence intervals (95%) for OR were computed as described by Fienberg (1977). Chi-square values were competed to test the null hypothesis OR = 1. The probability of rejecting the null hypothesis was set at 0.05. The continuity correlation was applied to the computation OR, standard error OR and chi-square (Fienberg, 1977).

RESULTS

In vitro antimicrobial susceptibility testing

The results of the in vitro antimicrobial susceptibility testing and presence of multi drug resistance (MDR) among Salmonella serotypes from human and animals are shown in Table 1. Antimicrobial resistance was observed in 93.1% of the human, and 46.8% of the animal isolates. Resistance among Salmonella serotype from human isolates was significantly higher (47.1%) than those from animals (0 to 16.7%). The percentage resistance of Salmonella serotype to the 10 antimicrobial drugs is shown in Table 2. Salmonella choleraesuis revealed the highest MDR (66.6%) whereas the percentages of resistance shown by Salmonella typhi and Salmonella enteritidis is 33.3%.

The frequency of resistance to antimicrobials among isolates from human was higher than that among animal isolates. All isolates from human and animals were susceptible to gentamicin. But strains showed high resistance to ciprofloxacin, tetracycline. Human isolates showed higher percentages than that of animal ones ($P<0.05$) (Table 3). The percentage of resistance to tetracycle was highest among human than animal isolates. All isolates from human and animals were sensitive to ciprofloxacin and with very low resistance pattern to gentamicin. Furazotidone showed a moderately high rate of resistance.

Odds ratio (OR)

The odds ratios for Salmonella serotypes resistance to 2-way antimicrobial is presented in Table 4. The OR among Salmonella serotypes isolates to combination of two antimicrobial ranged from 1.1 to 5.75. The highest OR shown in combination cefalexin-nalidixic acid, which have an OR of 5.75. This means that when a Salmonella isolate is resistant to cefalexin, nalidixic acid resistance is 5.75 times more likely to be observed in the same isolate than when the strain is cefalexin sensitive. Chloramphemicol-gentamicin combination has the lowest odds ratio of 1.1. Other antimicrobial combinations not listed are the ones that statistically have low odds ratios.

DISCUSSION

In the present study, resistance to antimicrobials was observed in 93.1% of the human isolates and 46.8% of the animal isolates with an overall of 80.67% for both sources. Antimicrobial susceptibility test of the 98 isolates of Salmonella revealed that 32.7% were resistant to one or more of the 24 antimicrobials tested. Generally, resistance for 13 different antimicrobial drugs was recognized. The most common resistance was to streptomycin (24/32, 75%), ampicillin (19/32, 59.4%), tetracycline (15/32, 46.9%), spectinomycin (13/32, 40.6%) and sulfisoxazole (13/32, 40.6%). These results reflect some known cross resistance, as exemplified by the high odds ratios, it also reflect the prevalence of a plasmid carrying the 2 resistance genes as reported

Table 1. Antimicrobial resistance and MDR of *Salmonella enterica* isolates recovered from human and animals.

Source	No. of isolates	Resistance		Multiple resistance	
		N	%	N	%
Human	87	81	93.1	41	47.1
Poultry	24	9	37.5	3	12.5
Cattle	6	4	66.7	1	16.7
Camels	2	2	100	0.0	0.0
Total	119	96	80.67	45	37.82

Table 2. Percentage resistance and MDR of *Salmonella* serotype to 10 antimicrobial drugs.

Serotype	Resistance %	Multiple drug resistance
Salmonella typhi	76.2	33.3
Salmonella paratyphi A	66.6	50
Salmonella paratyphi B	79.3	48.3
Salmonella choleraesuis	83.3	66.6
Salmonella enteritidis	58.3	33.3
Non-typeable isolates	80	47.5

before (Davidson and Babish, 1982).

Tetracycline, ampicillin, sulfamethoxazole + trimethoprim, chloramphenicol and furazolidone revealed the highest incidence of resistance. While ciprofloxacin gentamicin and cefalexin showed low to moderate frequencies of resistance in human and animal isolates.

The tetracycline, ampicillin, sulfamethoxazole + trimethoprim and chloramphenicol resistances were observed in 65, 45, 41 and 59 isolates respectively, while all isolates were sensitive to ciprofloxacin. This could be due to exhaustive use of the first group of antimicrobials, while the other group is newly introduced in the medical field, and not used for animal treatment. S. paratyphi B was the serotype with the highest incidence of drug resistance among the human strains, which was observed in 79.3% of the isolates. S. enteritidis was the serotype with the lowest drug resistance frequency, which was observed in 58.3%. It is probable that the high incidence of resistance in S. paratyphi B has resulted from an extensive use of antibiotics, although, it is the more common strains in the human isolates.

The WHO Global Foodborne Infections Network (2011) has no available data about resistance profiles of salmonella in Sudan. In Ethiopia, a significant proportion of Salmonella isolates have developed resistance for a number of anti-salmonella drugs, 32.7% of isolates were found resistant to one or more of the tested antimicrobial agents. The most common resistance was to streptomycin (24/32, 75%), ampicillin (19/32, 59.4%), tetracycline (15/32, 46.9%), spectinomycin (13/32, 40.6%) and sulfisoxazole (13/32, 40.6%) (Zewdu and Cornelius, 2009). In USA, 18.0% isolates from all sources were found resistant to two or more antimicrobials. Resistance to sulfisoxazole, streptomycin, and tetracycline was the most prevalent, whereas resistance to ciprofloxacin was the least. 27.7% isolates from animal feed, dog and environmental swabs were resistant to two or more antimicrobials. Resistance to sulfisoxazole was found increasing, but resistance to tetracycline was decreasing (Kiessling et al., 2007; Foley and Lynne, 2008).

The OR can be used to help in formulating the treatment of diseases. For example, if Salmonella paratyphi B is resistant to ampicillin 14% of the time and tetracycline 24% of the time (Table 3), neither one of these antimicrobials would be a likely choice for the treatment of Salmonella. However, if ampicillin was the 1st antimicrobial to be used and the animal did not respond, then the choice to use different antimicrobials would likely be made. However, if the OR for the combination of ampicillin opposed to tetracycline is 4.7 (Table 4), this mean that S. paratyphi B is 4.7 times more likely to be resistant to tetracycline, than ampicillin. In this case, tetracycline would not be a good antimicrobial to be used. This information can be used in 2 ways - in the treatment of clinical cases and to predict the antimicrobial to which the organism is less likely to be resistant.

Ampicillin, ciprofloxacin cefalexin and sulfamethoxazole + trimethoprim has been reported to be the drugs of choice for treatment of salmonella and other Enterobacteriaceae (Stoycheva and Murdjeva, 2006). The extensive use, use in sub-curative doses that may be

Table 3. Resistance to individual antimicrobial among *Salmonella* serotype from human and animals.

		Antimicrobial resistance									
Source	***Salmonella* serotypes**	**Ampicillin**	**Chloramphenico**	**Cefalexin**	**Ciprofloxaci**	**Colistin**	**Furazolidon**	**Gentamycin**	**Nalidixic acid**	**Sulfamethoxazole + trimethoprim**	**Tetracycline**
Human	*Salmonella typhi*	5	9	9	0	4	13	0	8	7	10
	Salmonella paratyphi A	2	2	1	0	0	3	2	1	2	5
	Salmonella paratyphi B	14	13	13	0	4	18	1	11	15	24
	Salmonella choleraesuis	3	2	3	0	1	4	1	4	2	4
	Salmonella enteritidis	1	2	1	0	1	1	1	1	1	2
	Non-typeable isolates	4	2	5	0	1	11	0	3	6	7
	Total	29	30	32	0	8	50	5	28	33	52
Poultry	*Salmonella e**n**teritidis*	2	2	0	0	0	1	0	1	2	1
	Salmonella heidelberg	0	0	1	0	0	1	0	0	1	1
	*Salmonella **a**msterdam*	1	1	1	0	0	2	0	0	0	1
	Non-typeable isolates	4	6	1	0	1	2	0	1	3	7
	Total	7	9	3	0	1	6	0	2	6	10
Cattle	*Salmonella enteritidis*	1	0	1	0	0	1	0	0	0	1
	Non-typeable isolates	1	1	1	0	0	1	0	1	1	1
	Total	2	1	2	0	0	2	0	1	1	2
Camels	Non-typeable isolates	1	1	1	0	0	1	0	1	1	1
Grand total		45	41	38	0	12	59	5	32	45	65

Table 4. Odds ratios for *Salmonella* sp. resistance to 2-way antimicrobial combinations.

Antimicrobial combination	Odds ratio	Antimicrobial combination	Odds ratio
Ampicillin X Cefalexin	1.14	Cefalexin X gentam**y**cin	3.3
Ampicillin X nalidixic acid	1.26	Cefalexin X nalidixic acid	5.75
Ampicillin X tetracycline	4.7	Cefalexin X tetracycline	2.2
Chloramphenicol X colistin	1.2	Cefalexin X sulfamethoxazole + trimethoprim	1.3
Chloramphenicol X gentam**y**cin	1.1	Furazolidone? X nalidixic acid	2.8
Chloramphenicol X nalidixic acid	1.6	Furazolidon? X tetracycline	1.6
Chloramphenicol X sulfamethoxazole + trimethoprim	1.2	Furazolidone X sulfamethoxazole + trimethoprim	1.2
Chloramphenicol X tetracycline	2.3	Nalidixic acid x sulfamethoxazole + trimethoprim	1.4
Cefalexin X colistin	1.6	Nalidixic acid X tetracycline	2.9
Cefalexin X furazolidone	2.3	Sulfamethoxazole + trimethoprim X tetracycline	1.3

outdated preparation of these antimicrobials, were used in human and animals. In this study, there are some isolates which are resistant to the recommended dose of ampicillin and sulfamethoxazole + trimethoprim. All

isolates are susceptible to ciprofloxacin and cefalexin in recommended doses (20 and 50 mg/kg body weight (bwt) respectively). The prolonged use of ampicillin and sulfamethoxazole + trimethoprim in Sudan might have led to the emergence of resistant isolates. The curative dose for sulfamethoxazole + trimethoprim were found to be above 100 mg/kg bwt for S. enterica which is slightly higher than the recommended dose. All doses below the recommended doses for ampicillin, cefalexin and sulfamithoxazole + trimethoprim are not curative, except ciprofloxacin which showed a dose of 15 mg/kg bwt as a curative dose.

ACKNOWLEDGEMENTS

The study was supported by the Sudan University of Science and Technology (IMT) and the British Council project number: KHT/991/21/Vet (MEH).

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