



## Drug Resistance Pattern of Salmonella Isolated from Poultry dropping in Khartoum North

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### ABSTRACT

Two hundred dropping samples were collected from ten poultry farms throughout Khartoum North. The samples were investigated for the presence of Salmonella. Sixty four Salmonella isolates were obtained and confirmed by biochemical tests, these were 7 isolates of *Salmonella arizonae*, 10 isolates of *Salmonella choleraesuis*, 18 isolates of *Salmonella gallinarum*, 23 isolates of *Salmonella pullorum* and 6 isolates of *Salmonella typhi*.

The results of sensitivity test reflected high antibiotic resistance. *Salmonella* isolates varied in their sensitivity from one antibiotic to another.

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## INTRODUCTION

In the past 60 years, antibiotics have been used critically to fight infectious disease caused by bacteria and other microbes. Antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the Twentieth Century. However, disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. Wound infections, Salmonella outbreaks, gonorrhoea, tuberculosis, pneumonia and septicaemia are just a few of the diseases that have become

hard to treat with antibiotics (Slama *et al.*, 2005).

One part of the problem is that bacteria and other microbes that cause infections are remarkably resilient and have developed several ways to resist antibiotics and other antimicrobial drugs. Another part of the problem is due to increasing use, and misuse, of existing antibiotics in human and veterinary medicine and in agriculture (Angulo *et al.*, 2000). In 1998, in the United States, 80 million prescriptions of antibiotics for human use were filled. This equals

12.500 tons in one year. Animal and agricultural uses of antibiotics are added to human use. Agricultural practices account for over 60% of antibiotic usage in the U.S., so this adds an additional 18,000 tons per year to the antibiotic burden in the environment (Varma *et al.*, 2006). Nowadays, about 70 percent of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment. Some organisms are resistant to all approved antibiotics and can only be treated with experimental and potentially toxic drugs (Calderon and Sabundayo, 2007). In a recent study, 38% of *Salmonella* cases were shown to be resistant to Ciprofloxacin, and an additional 29% of cases were resistant to more than one antibiotic (Khaita *et al.*, 2007).

Microbial development of resistance to antibiotics, as well as economic incentives, has resulted in research and development in the search for new antibiotics in order to maintain a pool of effective drugs at all times. While the development of resistant strains is inevitable, the slack ways of administration and use of antibiotics has greatly exacerbated the process.

Unless antibiotic resistance problems are detected as they emerge, and actions are taken immediately to control them, society could be faced with previously treatable diseases that have become again untreatable, as in the days before antibiotics were developed (Kutateladze and Adamia, 2008). Antimicrobial agents have played an important role in animal production since the 1950's. As livestock and poultry farms have grown and animal density on those farms has increased, the demand for better disease management has increased. The use of antimicrobial agents in animal production has improved animal health and led to higher yields. However, this practice has also contributed to the increased prevalence

of antibiotic-resistant bacteria significant to human health.

Antibiotic-resistant *Salmonella* have been isolated from various food products. Several outbreaks of multidrug-resistant *Salmonella* infections have been documented in the United States, including an outbreak associated with unpasteurized Mexican-style aged cheese, ground beef outbreaks, and an outbreak associated with pasteurized milk. The multidrug-resistant nature of these organisms makes treatment failure more likely (Angulo *et al.*, 2000). The objective of this study was to detect *Salmonella* species present in poultry droppings and their antimicrobial resistance to different antibiotics.

## **MATERIALS AND METHODS**

### **Collection of samples**

A total of 200 droppings samples was collected from ten poultry farms that located in Khartoum North between July and December 2012. The samples were collected in sterile universal bottles and immediately taken to the laboratory for culturing and bacteriological investigation for the presence of *Salmonella* species.

### **Isolation and Identification of *Salmonella***

The selective media used for isolation were: Selenite Cystine broth, Brilliant green agar, Bismuth Sulfide agar and XLD agar (Oxoid) as described by Barrow and Feltham (2003) and Parveen *et al* (2007).

The identification of isolates was carried out as described by Olsen *et al.*, (2004), routinely used biochemical tests were applied including all primary test such as enzymes production (oxidase and catalase) and motility followed by the secondary tests including sugars utilization and fermentation tests (such as glucose, sucrose, lactose), indole MR and VP.

### Antimicrobial Susceptibility test

The antimicrobial susceptibility (phenotype of 68 *Salmonella* isolates) were studied using disc diffusion method, the isolates were cultured in Muller Hinton agar (Oxoid) and incubated at 37C° for 24 hours (Berrang *et al.*, 2009).

The following antibiotics were used, Amikacin (30 mcg), Ceftazidime (30 mcg), Chloramphenicol (30 mcg), Aztreonam (30 mcg), Tetracycline (30 mcg), Piperacillin (100 mcg), Imipenem (10 mcg) and Ciprofloxacin (1 mcg). The zones of inhibition were measured, and the diameters of the zones were recorded to the nearest millimeters.

### RESULTS

The salmonella serotypes isolated from poultry dropping and their percentage are shown in Figure (1).

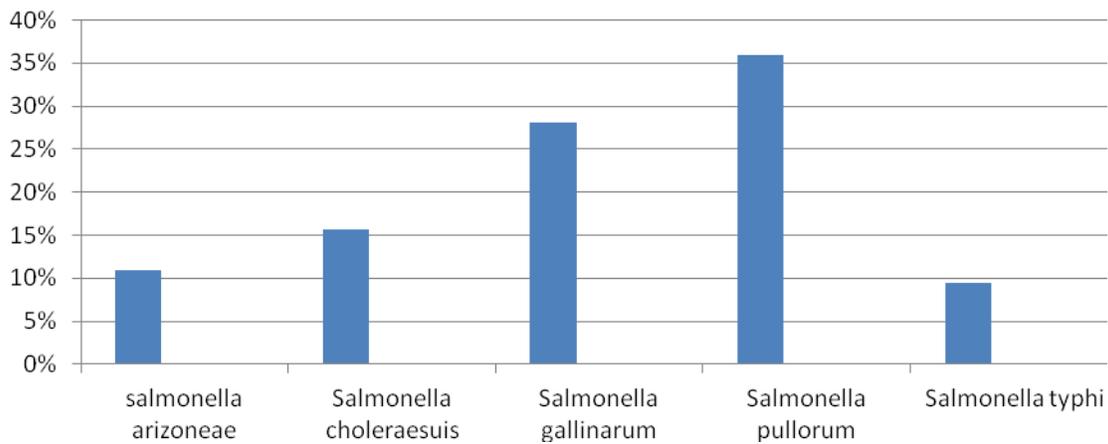
A total of 64 isolates was obtained, they were as follow: 23(35.9%) *S. pullorum*, 18(28%) *S. gallinarum*, 10(15.6%) *S.*

*choleraesuis*, 7(10.9%) *S. arizonae* and 6(9%) *S. typhi* (Collected from ten poultry farms that located in Khartoum North between July and December 2012. The samples were collected in sterile universal bottles and immediately taken to the laboratory for culturing and bacteriological investigation for the presence of *Salmonella* species).

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**Figure 1: Percentage Salmonella Species isolated from poultry droppings**

*Salmonella* species were exposed to different sets of antibiotics, the response was varied with each species and antibiotic, the

levels of response were shown in the Table (1).

**Table 1: The percentage of Resistance of Salmonella species to different antibiotics in terms of percentage**

Antibiotic	<i>S. arizonae</i>		<i>S. choleraesuis</i>		<i>S. gallinarum</i>		<i>S. pullorum</i>		<i>S. typhi</i>	
	No	%	No	%	No	%	No	%	No	%
Amikacin	4	57	6	60	10	55.5	11	47	3	50
Ceftazidime	5	71.4	5	50	6	33.3	6	26	1	16.6
Chloramphenicol	1	14.3	3	30	3	16.7	5	21	2	33.3
Aztreonam	4	57	6	60	11	61	8	34	5	38
Tetracycline	4	57	9	90	9	50	12	52	3	50
Piperacillin	4	57	8	80	8	44.4	15	65	2	33.3
Imipenem	2	28.5	4	40	9	50	10	43.5	3	50
Ciprofloxacin	6	85.7	4	40	7	38.9	10	43.9	5	38

No → Number of Resistant Strains from each Species.

% → Percentage of Resistant Strains from each Species.

## DISCUSSION

*S. pullorum* and *S. gallinarum* are known causes of poultry infections, this elucidated their isolation in high numbers comparable to other Salmonellae isolates while *S. typhi* is known to be adapted to humans mainly and does not occur in other animals as pathogens due to the presence of capsule protein in contrast to other species (Jantsch *et al.*, 2011).

Drug resistance now a day become a problem, because many types of bacteria exhibit resistance to many types of antibiotics used to treat diseases, associated with human health.

Prescription of antibiotics in Sudan depends mainly on clinical diagnosis only and sometimes without physician supervision.

The resistance is due to wrong antibiotic usage or irrational use of drugs. Bacteria may acquire drug resistance due to chromosomal mutation, transformation, conjugation or transduction. Antibiotic-resistance likely also emerged as bacteria began producing compounds in order to

survive in their environment, and competing species found ways to counteract these compounds (Matthew *et al.*, 2007).

In this study 50 % of *S. gallinarum*, 52% of *S. pullorum* were resistant to Tetracycline, 44 % of *S. gallinarum*, 65% of *S. pullorum* were resistant to Piperacillin.

The results of this study are in accordance with other studies investigating phenotypic drug resistance pattern (Holmberg *et al.*, 1984).

Ciprofloxacin is recommended for treatment of Salmonellosis (Kiessling *et al.*, 2007), however in this study Ciprofloxacin resistance is encountered in 50% of the total Salmonella isolates, 38.9 % of *S. gallinarum* and 43.5% of *S. pullorum* isolates. However in the United States a study showed that 10% of Salmonella isolates were likely resistant to ciprofloxacin (Bauer *et al.*, 2006). The high resistance rates to Ciprofloxacin encountered in this study could be attributed to the misuse of antibiotics by poultry farmers.

Chloramphenicol should not be prescribed for use unless by physicians or veterinarians because of its side effects, 21% of the total isolates, 16.7 % of *S. gallinarum*, 21.7% of *S. pullorum* were found to be resistant. Since 1996, the National Antimicrobial Resistance Monitoring System (NARMS) has identified increasing numbers of Salmonella isolates resistant to Chloramphenicol and Tetracycline. These isolates also have decreased susceptibility or resistance to Ceftriaxone, an antimicrobial used to treat serious infections (NARMS, 2006). N.F Martin *et al.* reported in Canada in 2004, several Salmonella with Multi Drug Resistant (MDR), which also resist many types of antibiotics.

The transmission of multidrug-resistant (MDR) Salmonella in broiler chicks under selective-pressure was studied. An MDR Salmonella strain had significantly increased transmission when chicks were treated with tetracycline because of the Tet M. a gene that confers tetracycline's resistance that is very common, demonstrating that antimicrobial use influences transmission of antimicrobial-resistant pathogens in poultry (Bauer *et al.*, 2006). This study concluded that sensitivity and resistance of Salmonella isolates to antibiotics seems to assume an increasing rate.

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