

Evidence of carriage of antibiotic resistant *Salmonella* species of public health and veterinary significance in the intestines of house crows(*Corvus splendens*) in Tanzania

ABSTRACT

Background

The Indian house crow, *Corvus splendens* (Vieillot) was introduced in Zanzibar in Tanzania by the British and immigrants from India in 1897 to help clean the town. The crow is responsible for polluting the environment, water sources and human surroundings by their droppings and the rubbish they carry. This behavior has led to concern that, the crows may be responsible for the spread of certain pathogens including *Salmonella* and their persistence in the environment. Given the zoonotic potential of *Salmonella*, the main aim of this study was to investigate the occurrence of *Salmonella* infections in Indian house crow and, in particular, to determine if the isolates were similar to those associated with disease in livestock or humans.

Methods

Indian house crows crossing the Mabibocompound of the National Institute for Medical Research (NIMR) in Dar es Salaam city in Tanzania were lured with meat and blood baits to land into the Crow live-trap. A total of 100 house crows were captured, humanely sacrificed and small and large intestines were obtained by using aseptic techniques for microbiological investigations. Preliminary identification of *Salmonella* was based on colonial characteristics on selective media and microscopic examination of smears following Gram staining. Confirmation of *Salmonella* species was done by biochemical tests. Antibiotic susceptibility testing was done by using the disc diffusion method on Mueller Hinton agar.

Results and discussion

Eight isolates were identified by standard microbiological techniques as *Salmonella* spp. (6% suggestive of *Salmonella gallinarum* and 2% suggestive of *S. typhi*). All

isolates were found to be susceptible to ciprofloxacin but resistant to amoxicillin. Lower levels of susceptibility were noted for chloramphenicol and ceftriaxone. Our results demonstrate the presence of antibiotic resistant *Salmonella* spp. in the Indian house crows' population and provide an assessment of potential public and poultry health risks to the high-density human and poultry populations within the coastal area.

Conclusion

The reduced antibiotic susceptibility of *S.typhi* and *S.gallinarum* has veterinary, clinical and public health consequences associated with transmission of antimicrobial drug resistant *Salmonella* in poultry and humans. The risk of transmission of antibiotic resistant *Salmonella* to humans and poultry provides further rationale for the public action on eradicating the house crows.

Keywords: *Salmonella*, antibiotic resistance, susceptibility, Tanzania

1. INTRODUCTION

The Indian house crow, *Corvus splendens* (Vieillot), is one of the world's most invasive bird species, causing disturbance to more than 25 countries throughout the Indian Ocean, Arabian Peninsula and South East Asia [1]. Bijlsma and Meininger reported that the crow originated from Asia, but adaptively colonized many other parts of the world ranging from Malaysia to South Africa [2, 3]. It was first reported on the island of Zanzibar in East Africa in 1897 whereby it was introduced by the British and immigrants from India to help clean the town [4]. The Indian house crow presents great ecological flexibility and by the 1950s, it had spread to the large part of the East African coast and it continues to expand its range. This bird species has been causing problems to the natural biodiversity of the regions, as well as impacting upon human health, tourism, infrastructure, and general development [1]. The crows have been found to be responsible for the reduction of small reptiles and amphibians and indigenous crows [5]. In addition they kill poultry, prey on the chicks and eggs of native birds and harass other birds, animals and insects [5].

The population of house crows in Tanzania mainland has increased significantly since 1980's as the species adapted well in the new environment and has no natural enemies

[6]. The birds are called the world most destructive crows and are frequently observed in close contact with water sources, reserve water tanks and they keep picking unattended human food and crops. Their abundance is closely linked to human population size, due to the expanding amount of rubbish generated [7]. The crows are responsible for polluting the environment, water sources and human surroundings by their droppings and the rubbish they carry. This behavior has led to concern that, the crows may be responsible for the spread of certain pathogens including *Salmonella* and their persistence in the environment [4].

Several studies have shown that a number of serovars of *Salmonella enterica* exist in wild birds, and it has been suggested that wild birds might be playing a significant role in the epidemiology of human and livestock salmonellosis [8]. However, little is known about the existence of *Salmonella* strains in Indian house crows and human and livestock associated strains. The present study was therefore aimed at investigating the occurrence of *Salmonella* infections in Indian house crow and, in particular, to elucidate if the isolates were similar to those associated with disease in livestock or humans.

2.0 MATERIALS AND METHODS

2.1 Indian house crow collection

Indian house crows were captured at the Mabibo compound of the National Institute for Medical Research (NIMR) in Dar es Salaam city in Tanzania. This specific site was chosen based on convenience as most of the researchers involved in this study are based there. Indian house crows crossing the compound were lured with meat and blood baits to land into the crow live-trap. A total of 100 house crows were captured and used in this study. This sample size considered both the available funds and the study duration.

2.2 Isolation of *Salmonella* spp.

The birds were euthanized by an overdose of pentobarbitone sodium (50 mg/kg body weight) and then dissected immediately. While occluded by forceps small and large intestines were aseptically removed from the abdominal cavity for

100 microbiological investigations. Intestinal samples from each bird were placed in a
101 separate container. About 1cm of the opened intestine of each sampled bird was
102 placed in bijou bottle containing 10 ml of sterile biological peptone water and
103 incubated overnight at 37°C. A loopful of intestinal contents from biological peptone
104 water was streaked on solid differential and selective media (MacConkey) agar (Oxoid
105 Ltd, Basingstoke, UK) and incubated at 37°C for 24 hours. Colonies suggestive of
106 salmonella (colorless, translucent to opaque, irregular edge colonies which are non-
107 lactose fermenters) were sub-cultured on Salmonella-Shigella agar (Oxoid Ltd,
108 Basingstoke, UK) in duplicate. Pure *Salmonella* colonies from *Salmonella*-Shigella
109 agar (colorless edge colonies with black centre) were then subjected to identification.

110

111 **2.3 Identification of Salmonella isolates**

112 Preliminary identification of *Salmonella* was based on colonial characteristics on
113 selective media and microscopic examination of Gram stained
114 smears. *Salmonella* suspected colonies were picked up using a sterile wire loop, added
115 to a drop of normal saline and smeared on a microscopy glass slide. Gram stain was
116 performed and gram-negative rod shaped organisms were identified as suggestive of
117 *Salmonella* species. Confirmation of *Salmonella* species was done by biochemical
118 tests using the Analytical Profile Index (API-20E) system according to manufacturer's
119 instructions (bioMérieux, inc, Darham, USA). Nutrient agar slant and butt were
120 utilized for determination of production of specific metabolites such as gas, acid or
121 alkaline solution.

122

123 **2.4 Antibiotic susceptibility testing**

124 Antibiotic susceptibility testing was done by using the disc diffusion method
125 on Mueller Hinton (MH) Agar (Oxoid Ltd, Basingstoke, UK) using *C. jejuni* NCTC
126 11168 for quality control purposes. Bacterial suspensions were prepared in a sterile
127 normal saline and adjusted to a turbidity equivalent to 0.5 McFarland standards
128 (1.5×10^8 cells/ml). Sterile swab was used to inoculate the suspension by evenly
129 streaking on prepared Mueller Hinton agar plate and allowed to stay for 3-5 minutes
130 to allow drying. Sterile forceps were used to place discs on the inoculated plates and
131 incubated at 37°C for 24 hours. The isolates were tested for susceptibility to
132 ceftriaxone, amoxicillin, ciprofloxacin and chloramphenicol (Oxoid Ltd,

133 Basingstoke,UK), which are drugs of choice for treatment of Salmonella infections.
134 After 48 hours of incubation, the diameters of inhibition zones were measured.
135 Interpretation of results was guided by both standardized tables supplied by the
136 National Committee on Clinical Laboratory Standards (currently known as Clinical
137 and Laboratory Standards Institute) (NCCLS, 2002)and manufacturer's
138 instructions.

139

140 **2.5 Ethics statement**

141 The trapping of Indian house crows was done using a live trap provided to NIMR by
142 the Ministry of Natural Resources and Tourism under the crows' eradication program.
143 The crows' eradication program has permission of the government of the United
144 Republic of Tanzania through the Ministry of Natural Resources and Tourism to
145 capture and humanely kill crows in Dar es Salaam city in Tanzania.Crows trapped in
146 the program are being killed so that to reduce their population density. Permission to
147 use the captured crows for research was provided by the Department of veterinary
148 Medicine and Public Health in accordance with Tanzania Wildlife Research Institute
149 (TAWIRI)*Guidelines for Conducting Wildlife Research* (2001) and in compliance
150 with Tanzania Veterinary Act number 16 of 2003 (Veterinary regulations;
151 Government notice no. 389 of 2005), following a mandate of the Sokoine University
152 of Agriculture (SUA) to conduct disease investigations in wildlife in different
153 protected and unprotected areas in Tanzania. All biological samples were collected in
154 order to perform research on presence of *Salmonella* species in house crows.

155

156 **3.0 RESULTS**

157 **3.1 Occurrence of enteric *Salmonella* in sampled house crows**

158 *Salmonella* spp. were found in 8 out of the 100 intestinal samples of captured Indian
159 house crows tested, which represents a prevalence of 8%. Using the API-
160 20E system (Table 1) the isolates exhibited biochemical profiles suggestive
161 of *Salmonella* spp, the tests indicate the probable occurrence of two different
162 serotypes i.e. *S.gallinarum* being the predominant accounting for 6 (6%) of the isolates
163 and *S.typhi* accounted for 2 (2%) samples of Indian house crows.

164

Table 1 here

165 **3.2 Antibiotic susceptibility of isolated enteric *Salmonella***

Table 2 shows all the 8 *Salmonella* isolates detected were susceptible to ciprofloxacin. More than half (75%) of isolates (6 out of 8) were resistant to chloramphenicol, of which 5 were *S.gallinarum* and 1 was *S.typhi*. High resistance was also noted for Ceftriaxone, whereby 7 out of 8 isolates (87.5%) were resistant, of which 5 were *S.gallinarum* and 2 were *S.typhi*. All the 8 *Salmonella* isolates were resistant to amoxicillin.

Table 2 here

4.0 DISCUSSION

In this study we have isolated *Salmonella* species indicating most probably two serovars of *Salmonella*, *S. gallinarum* and *S. typhi* from the intestines of apparently healthy Indian house crows. Our results are comparable to the findings reported in 2004 by Millán *et al.*, who observed that 8.5% of wild birds including crows were harboring *Salmonella* spp [9]. Furthermore, our results correlate with those of Agasi *et al.*, who reported the isolation of *Salmonella* spp. from cloacal swabs taken from two out of 30 apparently healthy crows in Japan [10]. Contrary to these findings, other studies conducted elsewhere [11, 12] couldn't isolate *Salmonella* from the crows. The isolation of the two serovars of *Salmonella* (*S. gallinarum* and *S. typhi*) from the intestines of Indian house crows in this study confirms the role of these birds as potential carriers of enteric *Salmonella* for both humans and animals [13, 14]. *S. typhi* is the causative agent of human typhoid fever [15] while *S. gallinarum* causes fowl typhoid in birds [16]. Occurrence of *S. gallinarum* in chickens [17, 18] and *S. typhi* in humans [19-21], in the country, has been reported in previous studies.

One of the important symptoms of *Salmonella* infection in humans is the occurrence of fever. However, fever is among the most common syndromes prompting persons to seek healthcare in Tanzania and other countries. It follows true that, the numerous causes of fever (febrile illness) are often difficult to distinguish by mere clinical presentation. Although malaria is easily ruled out by blood film examination or a malaria rapid diagnostic test, salmonellosis requires serological testing. Recently, increasing *Salmonella* infection in humans has been detected by blood culture in different parts of Tanzania and other countries [19, 20]. The outbreaks described in northern Tanzania, the complications of intestinal perforation and neurologic manifestations provided clue of typhoid fever as the possible cause whereby in the

199 follow up analysis a much larger group of patients with undifferentiated fever was
200 found positive for typhoid fever [19, 20].

201

202 The behavior of Indian house crows of keeping in close contact and their interaction
203 with human environment indicates their potential as vehicles of transmission of
204 *Salmonella* infections in Tanzania. The risk of enteric *Salmonella* infections originating
205 from crows is heightened due to the fact that their population is expanding both in the
206 country and in neighboring countries [17].

207

208 It has been reported by several authors that infections with *S. typhi* are restricted to
209 humans [22, 23] and that there exists no animal or environmental reservoirs for the
210 bacterium [24]. Experimental infections with *S. typhi* have however been reported in
211 higher primates [25]. The observation that this bacterium infected crows in the present
212 study could be an attribute of the use of an identification technique with low
213 discriminatory ability thereby leading to misidentification. Another possibility could
214 be that this study has hit upon a major discovery in *Salmonella* biology by identifying
215 a new host for this particular species.

216

217 The present study revealed the highest antibiotic sensitivity level of 100% shown by
218 the isolates to ciprofloxacin which was in agreement with the previous report of
219 Zahreiet *al.*, 2005 [26]. This signifies continual use of ciprofloxacin as a drug of
220 choice for treatment of infections caused by these organisms. In addition, the present
221 study also revealed the prevalence of antibiotic resistance among
222 *Salmonella* isolates. The isolated strains were highly resistant to amoxycillin,
223 ceftriaxone and chloramphenicol. The highest resistance level of 100% was observed
224 for amoxycillin, followed by ceftriaxone (87.5%) and lastly, chloramphenicol (75%);
225 rendering them ineffective for the treatment of infections caused by these strains. The
226 possible sources of antibiotic resistant *Salmonella* species could be humans and
227 animals as overuse and misuse of antibiotics both in veterinary [27, 28] and medical
228 fields [29], which are key attributes in development of antibiotic resistance among
229 different microbes, have been reported in the country. In turn, Indian house crows
230 which are ubiquitous scavengers could be an important factor in spread of antibiotic
231 resistant salmonellosis both to humans and animals over the distances they cover by

their droppings. In the One Health Initiative, the problem of antibiotic resistance in which house crows now seem to take part, collective efforts from public health and veterinary professionals are needed for its mitigation.

The limitations of the current study included the following i) sampling of the crows was conducted in a limited area making the sample less representative of the city crow population ii) adoption of an identification technique with low discriminatory power which could lead to miss-classification of the isolates into serovars.

5.0 Conclusion

From our results we can conclude that, the Indian house crow could be the source of *Salmonella* (*S. gallinarum* and *S. typhi*) infections in humans and animals. The observed antibiotic resistance among crow derived salmonella isolates is of particular interest since it suggests that the crows might contribute to spread the antibiotic resistant Salmonellosis in humans and animals thereby complicating treatment of such cases. Based on the findings from this study, it is advisable that people should avoid contacts with crows and maintain good hygienic practices, treat drinking water and improve sanitation so as to avoid acquiring infections originating from crows. Crow eradication programs should be strengthened so as to stem the risk of transmission of antibiotic resistant *Salmonella* from these wild birds to human and animals.

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348 **Table 1:** API- Biochemical indications of *Salmonella* strains

349

Test	<i>Salmonella gallinarum</i>	<i>Salmonella typhi</i>
ONPG	-	-
ADH	-	-
LDC	+	+
ODC	+	-
CIT	+	-
H ₂ S	+	+
URE	-	-

TDA	-	-
IND	-	-
VP	-	-
GEL	-	-
GLU	+	+
MAN	+	-
INO	-	-
SOR	-	-
RHA	-	-
SAC	-	-
MEL	+	+
AMY	-	-
ARA	+	-

Key: ONPG = β -galactosidase, ADH = arginine dihydrolase, LDC = lysine decarboxylase, ODC = ornithine decarboxylase, CIT = Citrate, H_2S = hydrogen sulfide, URE = urea agar, TDA = tryptophan deaminase, IND = indole, VP = acetoin production test, GEL = gelatinase test, GLU = glucose assimilation test, MAN = mannitol assimilation test, INO = inositol assimilation test, SOR = sorbitol assimilation test, RHA = rhamnose assimilation test, SAC = saccharose assimilation test, MEL = melibiose assimilation test, AMY = amygdaline assimilation, ARA = arabinose assimilation test

Table.2 Antibiotic susceptibility of isolated *Salmonella* strains

Antibiotic	Concentration per disc (μ g)	Susceptible strains (N)	Resistant strains (N)
1. Ciprofloxacin	10	<i>S. gallinarum</i> = 6 <i>S. typhi</i> = 2	<i>S. gallinarum</i> = 0 <i>S. typhi</i> = 0
2. Chloramphenicol	30	<i>S. gallinarum</i> = 1 <i>S. typhi</i> = 1	<i>S. gallinarum</i> = 5 <i>S. typhi</i> = 1
3. Ceftriaxone	30	<i>S. gallinarum</i> = 1 <i>S. typhi</i> = 0	<i>S. gallinarum</i> = 5 <i>S. typhi</i> = 2
4. Amoxycillin	30	<i>S. gallinarum</i> = 0 <i>S. typhi</i> = 0	<i>S. gallinarum</i> = 6 <i>S. typhi</i> = 2