

Evidence of carriage of antimicrobial 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, 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 crows and to determine if the isolates were similar to those associated with disease in livestock or humans.

Methods

Indian house crows were lured with meat and blood baits to land into the Crow live-trap set at the Mabibocompound of the National Institute for Medical Research (NIMR) in Dar es Salaam city in Tanzania. A total of 100 house crows were captured, humanely sacrificed and small and large intestines were obtained by using aseptic techniques for microbiological investigations. Culture technique was employed to detect the presence of *Salmonella* in intestinal contents; and preliminary identification of the isolates 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. Antimicrobial 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 antimicrobial resistant *Salmonella* spp. in the Indian house crows' population and provide an indication of potential public and poultry health risks associated with these birds in the coastal area.

Conclusion

The occurrence of antibiotic resistant *S. Typhi* and *S. Gallinarum* among Indian house crows has both veterinary and public health consequences as they may be transmitted to poultry and humans. This therefore provides further rationale for the public action on eradicating the house crows.

Keywords: *Salmonella*, antibiotic resistance, crows, 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; particularly the human and livestock associated strains. The present study was therefore aimed at investigating the occurrence of *Salmonella* infections in Indian house crows and 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. The crows were lured with meat and blood baits to land into the crow live-trap set within the compound. 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 microbiological investigations. Intestinal samples from each bird were placed in a separate container. About 1 cm of the opened intestine of each sampled bird was placed in a bijoux bottle containing 10 ml of sterile biological peptone water and

100 incubated overnight at 37°C. A loopful of intestinal contents from the biological
101 peptone water was streaked on solid differential and selective media (MacConkey)
102 agar (Oxoid Ltd, Basingstoke, UK) and incubated at 37°C for 24 hours. Colonies
103 suggestive of *Salmonella* (colorless, translucent to opaque, irregular edge colonies
104 which are non-lactose fermenters) were sub-cultured on *Salmonella*-Shigella agar
105 (Oxoid Ltd, Basingstoke, UK) in duplicate. Pure *Salmonella* colonies from
106 *Salmonella*-Shigella agar (colorless edge colonies with black centre) were then
107 subjected to identification.

108

109 **2.3 Identification of *Salmonella* isolates**

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

120

121 **2.4 Antimicrobial susceptibility testing**

122 Antimicrobial susceptibility testing was done by using the disc diffusion method
123 on Mueller Hinton (MH) Agar (Oxoid Ltd, Basingstoke, UK) using *C. jejuni* NCTC
124 11168 for quality control purposes. Bacterial suspensions were prepared in a sterile
125 normal saline and adjusted to a turbidity equivalent to 0.5 McFarland standards
126 (1.5×10^8 cells/ml). Sterile swab was used to inoculate the suspension by evenly
127 streaking on prepared Mueller Hinton agar plate and allowed to stay for 3-5 minutes
128 to allow drying. Sterile forceps were used to place discs on the inoculated plates which
129 were then incubated at 37°C for 48 hours. The isolates were tested for susceptibility to
130 ceftriaxone, amoxicillin, ciprofloxacin and chloramphenicol (Oxoid Ltd, Basingstoke,
131 UK), which are drugs of choice for treatment of *Salmonella* infections. After 48 hours
132 of incubation, the diameters of inhibition zones were measured. Interpretation of

133 results was guided by both standardized tables supplied by the National Committee
134 on Clinical Laboratory Standards (currently known as Clinical and Laboratory
135 Standards Institute) (NCCLS, 2002) and manufacturer's instructions.

136

137 **2.5 Ethics statement**

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

152

153 **3.0 RESULTS**

154 **3.1 Occurrence of enteric *Salmonella* in sampled house crows**

155 *Salmonella* spp. were found in 8 out of the 100 intestinal samples of captured Indian
156 house crows tested, which represents a prevalence of 8%. Using the API-
157 20E system (Table 1) the isolates exhibited biochemical profiles suggestive of two
158 different *Salmonella* serotypes, i.e. *S. Gallinarum* being the predominant accounting for
159 75.0% of the isolates and *S. Typhi* which accounted for 25.0% samples of Indian house
160 crows.

161

Table 1 here

162 **3.2 Antimicrobial susceptibility profiles of isolated enteric *Salmonella***

163 Table 2 shows all the 8 *Salmonella* isolates detected were susceptible to ciprofloxacin.
164 More than half (75%) of isolates (6 out of 8) were resistant to chloramphenicol, of
165 which 5 were *S. Gallinarum* and 1 was *S. Typhi*. High resistance was also noted for

166 Ceftriaxone, whereby 7 out of 8 isolates (87.5%) were resistant, of which 5 were
167 *S. Gallinarum* and 2 were *S. Typhi*. All the 8 *Salmonella* isolates were resistant to
168 amoxicillin.

169 *Table 2 here*

170 **4.0 DISCUSSION**

171 In this study we have isolated *Salmonella* species, most probably *S. Gallinarum* and *S.*
172 *Typhi*, from 8.0% of the intestines of collected apparently healthy Indian house
173 crows. Our results are comparable to the findings reported in 2004 by Millán *et al.* [9]
174 who observed that 8.5% of wild birds including crows were harboring *Salmonella* spp.
175 Furthermore, our results correlate with those of Agasiet *et al.* [10] who reported the
176 isolation of *Salmonella* spp. from 6.7% of the cloacal swabs taken from apparently
177 healthy crows in Japan (n=30). Contrary to these findings, other studies conducted
178 elsewhere [11, 12] couldn't isolate *Salmonella* from the crows. The isolation of
179 the two serovars of *Salmonella* (*S. Gallinarum* and *S. Typhi*) from the intestines of
180 Indian house crows in this study confirms the role of these birds as potential carriers
181 of enteric *Salmonella* for both humans and animals [13, 14]. *S. Typhi* is the causative
182 agent of human typhoid fever [15] while *S. Gallinarum* causes fowl typhoid in birds
183 [16]. Occurrence of *S. Gallinarum* in chickens [17, 18] and *S. Typhi* in humans [19-21],
184 in the country, has been reported in previous studies. The risk of enteric *Salmonella*
185 infections originating from crows is heightened due to the fact that their population is
186 expanding both in the country and in neighboring countries [17].

187
188 One of the important symptoms of *Salmonella* infection in humans is the occurrence
189 of fever. However, fever is among the most common syndromes prompting persons to
190 seek healthcare in Tanzania and other countries. It follows true that, the numerous
191 causes of fever (febrile illness) are often difficult to distinguish by mere clinical
192 presentation. Although malaria is easily ruled out by blood film examination or a
193 malaria rapid diagnostic test, salmonellosis requires serological testing. Recently,
194 increasing *Salmonella* infection in humans has been detected by blood culture in
195 different parts of Tanzania and other countries [19, 20]. The outbreaks described in
196 northern Tanzania, the complications of intestinal perforation and neurologic
197 manifestations provided clue of typhoid fever as the possible cause whereby in the

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

200

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

209

210 The present study revealed the highest antimicrobial sensitivity level of 100% shown
211 by the isolates to ciprofloxacin which was in agreement with the previous report of
212 Zahreiet *al.*, 2005 [26]. This signifies continual use of ciprofloxacin as a drug of
213 choice for treatment of infections caused by these organisms. However, the present
214 study also revealed the prevalence of resistance to different antimicrobial agents among
215 the obtained *Salmonella* isolates. The isolates were highly resistant to amoxicillin
216 (100%), ceftriaxone (87.5%) and chloramphenicol (75%); suggesting that they
217 are ineffective for the treatment of infections caused by these strains in the study area.

218 The possible sources of antimicrobial resistant *Salmonella* species could be humans
219 and animals as overuse and misuse of antimicrobials both in veterinary [27, 28] and
220 medical fields [29], which are key attributes in development of antibiotic resistant
221 among different microbes, have been reported in the country. In turn, Indian house
222 crows which are ubiquitous scavengers could be an important factor in spread of
223 antimicrobial resistant salmonellosis both to humans and animals over the distances
224 they cover by their droppings. In the One Health Initiative, the problem of
225 antimicrobial resistance in which house crows now seem to take part, collective efforts
226 from public health and veterinary professionals are needed for its mitigation.

227

228 The limitations of the current study included the following i) sampling of the crows
229 was conducted in a limited area making the sample less representative of the city crow
230 population ii) adoption of an identification technique with low discriminatory power

231 which could lead to miss-classification of the isolates into serovars.

232

233 **5.0 Conclusion**

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

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

Test	<i>Salmonella</i> Gallinarum	<i>Salmonella</i> Typhi
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, H₂S = 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 Antimicrobial susceptibility of isolated *Salmonella* strains

Antimicrobial agent	Concentration per disc (µg)	Susceptible strains (N)	Resistant strains (N)
1. Ciprofloxacin	10	S. Gallinarum = 6 S. Typhi = 2	S. Gallinarum = 0 S. Typhi = 0
2. Chloramphenicol	30	S. Gallinarum = 1 S. Typhi = 1	S. Gallinarum = 5 S. Typhi = 1
3. Ceftriaxone	30	S. Gallinarum = 1 S. Typhi = 0	S. Gallinarum = 5 S. Typhi = 2
4. Amoxycillin	30	S. Gallinarum = 0 S. Typhi = 0	S. Gallinarum = 6 S. Typhi = 2