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Table of Contents

Journal Articles

Review Articles

Case Reports

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Journal Articles

OCCURRENCE OF ANTIBIOTIC RESISTANT *SALMONELLA* AND *CAMPYLOBACTER* IN WILD BIRDS

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SUMMARY

Salmonella and *Campylobacte*r are well recognised as important zoonotic foodborne pathogens. This study was undertaken to detect the occurrence of *Salmonella* and *Campylobacter* in a population of wild birds and to determine the antibiotic susceptibility of the isolates. A total of 68 fresh faecal samples were collected from wild birds in four areas (Bangi, Kepong, and two areas in Serdang). One (1.47%) faecal sample was positive for *Salmonella* spp. from a pigeon in Kepong and the isolate was resistant to erythromycin and tetracycline. Six (8.82%) faecal samples were positive for *Campylobacter* spp, three (50%) were from pigeons in Serdang and another three (50%) were pigeons from Kepong. All isolates were identified as *Campylobacter jejuni* and were resistant to trimethoprim-sulfamethoxazole (100%), followed by cefotaxime (83.3%), tetracycline (33.3%) and ampicillin (16.7%). The presence of antibiotic resistant *Salmonella* spp. and *Campylobacter* spp. in wild birds poses a public health risk because they may transmit these antibiotic resistant pathogens to farm animals and spread them in the environment.

Keywords: Salmonella spp.*, Campylobacter* spp.*, antibiotic resistant, wild birds*

INTRODUCTION

A number of studies reported that birds are often healthy carriers of a wide range of disease agents (bacteria, viruses, parasites, fungi) including those that are zoonotic and also they are hosts to a number of disease vectors (Abulreesh *et al.*, 2007). Given their ability to fly freely and some over great distances during migration, these birds can spread these pathogens in the environment including grazing pastures, park areas, surface waters and also to animals in the farms. Among the pathogens that may be carried by these wild birds that pose human health risks are highly pathogenic avian influenza (HPAI) virus, West Nile Fever virus, *Chlamydophila psittaci, Salmonella* and *Campylobacter* (Abulreesh *et al.*, 2007; Dhama *et al.,* 2008). It has also been reported that *E. coli* O157 which can cause enterohaemorrhagic infections in humans and *Helicobacter canadensis*, an emerging zoonotic pathogen, have been recovered from wild birds and wild geese (Waldenstrom *et al.*, 2003).

Antibiotic resistant bacteria as well as pathogens such as *Salmonella* and *Campylobacter* are reported to occur commonly in wild birds (Dhama *et al.,* 2008). These birds could have been exposed to the environments containing such resistant bacteria and may act as important reservoirs of antibiotic resistant bacteria. Few studies have been conducted on *Salmonella* and *Campylobacter* in wild birds in Malaysia and their susceptibility to antibiotics. *Salmonella* and *Campylobacter* are common foodborne pathogens. In United States, they are the main causes of foodborne diseases and frequently associated with handling or consumption of raw or undercooked poultry products. The organisms are frequently isolated from farm animals, in particular pigs and poultry. These organisms may contaminate raw meat during processing. The aim of this study was to determine the occurrence of *Salmonella* and *Campylobacter* in wild birds and the antibiotic susceptibility of the isolates.

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MATERIALS AND METHODS

Samples collection

A total of 68 faecal samples were collected from four areas in Selangor including Bangi (18 samples), Kepong (16 samples) and two areas in Serdang (21 and 18 samples). Fresh faecal samples were collected by placing clean plastic sheets under trees and on the grounds where birds gather in large numbers around the area to rest or search for food. Each faeces was collected as soon as it dropped onto the plastic sheet. It could not be ascertain if there were droppings from the same bird, however, it was assumed that probably a bird would not or seldom defaecate twice at the same spot. Two swabs were taken for each faecal dropping. One swab was placed into a sterile bottle containing buffered peptone water (BPW) (Oxoid®) for pre-enrichment and the other in Cary-Blair (Oxoid®) transport medium. Samples were then transported to Veterinary Public Health Laboratory within 1-2 h in an icebox after collection for isolation of *Salmonella* and *Campylobacter*.

Salmonella isolation and identification

The faecal samples collected in the BPW were incubated for 24 h at 37˚C. One ml of each pre-enriched culture was transferred to a Rappaport Vasiliadis (RV) broth (Oxoid®) and incubated at 42˚C for 24 h. One loopful of each enriched culture (in RV broth) was then streaked onto Xylose-Lysine-Deoxycholate (XLT4) agar (Merck®) and incubated at 37˚C for 24-48 h under aerobic condition. The typical *Salmonella* colonies appeared black or black-centered with a yellow periphery. The presumptive *Salmonella* colonies were subcultured onto XLT4 agar and incubated for 18-24 h at the same temperature to obtain pure cultures. Three colonies were picked for biochemical tests which included reactions to

urease test, triple sugar iron (TSI) agar and lysine iron agar (LIA). Slide agglutination test (SAT) was done to the isolates using *Salmonella* Polyvalent 'O' and 'H' Antisera A-S.

Campylobacter isolation and identification

Each faecal sample that was collected in Cary-Blair transport medium (Oxoid®) was streaked directly onto modified charcoal cefoperazone deoxycholate (mCCDA) agar (Oxoid®) incorporated with CCDA selective supplement (Oxoid®). The plates were placed in an anaerobic jar under microaerophilic condition (5% oxygen, 10% carbon dioxide, and 85% nitrogen) generated by using gas generating pack (BD Campy Pack) and incubated at 42˚C for 48 h. Presumptive identification of *Campylobacter* was done based on colony morphology (irregular shape, confluent growth along the streaking line, grey and slightly raised), motility (curved, slender rod with corkscrew, darting movement observed on hanging drop motility test) and shape of the organism (Gram-negative, curved, or comma shaped, S or gull-wing shape). The presumptive *Campylobacter* spp. colonies were subcultured onto Columbia Blood Agar (Oxoid®) with 5% defibinated horse blood added and incubated 42˚C for 48 h to obtain pure cultures. The isolates were subjected to biochemical tests which included oxidase, urease, indoxyl acetate hydrolysis and hippurate hydrolysis tests.

Antibiotic susceptibility test

The antibiotic susceptibility test on the isolates was done using disc diffusion method as suggested by the Clinical and Laboratory Standards Institute (CLSI) (2013). Loopfuls of each pure culture were mixed with 2 ml nutrient broth (Oxoid®) until turbidity visually comparable to 0.5 McFarland standards was obtained. A sterile swab was dipped into the inoculum suspension. The excess suspension was removed from the swab and then the swab was streaked evenly onto the surface of Mueller Hinton agar (Oxoid®) in three overlapping directions. For *Campylobacter* isolates, Mueller Hinton agar (Oxoid®) incorporated with defibrinated horse blood was used. The antibiotic-impregnated discs were placed on the inoculated agar surface using a disc dispenser and patted gently with a sterile forceps to ensure complete contact.

Six different antibiotic discs used for *Salmonella*: ampicillin AMP (10 µg), ceftriaxone CRO (30 µg), ciprofloxacin CIP (5 µg), gentamicin CN (10 µg), erythromycin E (15 µg), and tetracycline TE (30 µg) and the plates were incubated at 37°C for 24 h. For *Campylobacter* isolates, ampicillin AMP (10 µg), cefotaxime CTX (30 µg), ciprofloxacin CIP (5 µg), erythromycin E (15 µg), tetracycline TE (30 µg), and trimethoprim- sulfamethoxazole STX (1.25/23.75 µg) were used and the plates were incubated at 42°C for 48 h microaerobically. The zones of inhibition were measured and read against the Zone Diameter Interpretive Standard to report the isolate as susceptible (S), intermediate (I) or resistant (R) to each of the antibiotic tested.

RESULTS

The wild birds sampled consisted mainly of pigeons and crows. Other wild birds commonly reported in Malaysia included sparrows, starlings and mynahs but were not present at the time of sampling. The occurrence of *Salmonella* spp. and *Campylobacter* spp. in the faeces of the wild birds sampled are presented in Table 1.

Table 1. Frequency of *Salmonella* spp. and *Campylobacter* spp. isolated from wild birds

Location	No. of samples	No. of *Salmonella* spp. isolated	No. of *Campylobacter* spp. isolated
Serdang 1 (pigeon)	13	0	3
Serdang 2 (pigeon)	21	0	0
Bangi (crow)	18	0	0
Kepong (pigeon)	16	1	3
Total	68	1 (1.47%)	6 (8.82%)

DISCUSSION

This study found only one (1.47%) faecal sample was *Salmonella* positive from a pigeon in the Kepong area. The finding was similar to other studies, that is, low percentage of birds was affected with *Salmonella*. The study by Ganapathy *et al.,* (2007) on 40 cloacal swab samples from house crows in Selangor found none (0%) were positive for *Salmonella* spp. A study in Japan isolated *Salmonella* from 3.9% (17/436) of faeces of feral pigeons collected from public areas (Tanaka *et al.*, 2005). Craven *et al.* (2000) reported the faecal droppings from wild birds around four farms in Georgia, USA were found positive for *Salmonella* spp., ranging from 0% to 33% while Andres *et al.* (2012) reported birds captured in 31 locations around pig farms in Spain were 3.5% *Salmonella*. The wild birds that were exposed to contaminated environment can be readily infected. According to Tizard (2004), salmonellosis occurs most commonly in those birds that feed on the ground or on food subject to faecal contamination, or those that drink contaminated water. However, it could probably be that the birds in this study were less exposed to *Salmonella*-contaminated environment.

About 8.8% of the faecal samples were positive for *Campylobacter* spp. All isolates were identified as *C. jejuni*. The finding of the study was rather low compared to other studies; Ganapathy *et al.* (2007) found a high prevalence of *Campylobacter* spp. in crows at 25% (20/79) while Saleha *et al.* (2001) reported 18% (23/127) in birds (mainly mynahs and sparrows) caught around five chicken farms. Chuma *et al*. (2000) identified 30 *C. jejuni*, 20 *C. coli* and four *C. lari* using multiplex PCR assay on 507 sparrow faeces collected on the roof of a

building on their university campus in Kagoshima, Japan over a 6-month period. The study by Craven et al (2000) in Georgia, United States reported 0–50% positive for *C. jejuni* while Colles *et al.* (2008) found 37% (351 out of 954) of faecal samples from starlings in a university campus in Wytham, United Kingdom were *Campylobacter*-positive and 30% of the isolates were *C. jejuni*. Similar to *Salmonella* spp., wild birds may get infected with *Campylobacter* from the environment. The occurrence of *Campylobacter* spp. was seen higher in pigeon and none in crow in this study was possibly because of the small number of crows. In Norway, the highest isolation rate for *C. jejuni* was from crows (89.8%), followed by gulls and domestic pigeons (Kapparud and Rosef, 1983).

The *Salmonella* isolate was resistant to two antibiotics, erythromycin and tetracycline. According to Gopee *et al*. (2000), antimicrobial resistance in bacteria isolated from wild animals is uncommon. However, *Salmonella* isolated from wildlife that have close contact with human and farm animals' environment have been documented to exhibit multi-resistance to antibiotics. This is because the antibacterial agents are widely used in animal and human populations and consequent spread of resistant organisms in the environment. For *Campylobacter* spp., the results revealed that all isolates (100%) were resistant to trimethoprim- sulfamethoxazole, 83.3% were resistant to cefotaxime followed by 33.3% to tetracycline and the least resistant, 16.7%, was to ampicillin. Four isolates (66.6 %) were resistant to three (CTX-AMP-SXT) and four (TE-CTX-AMP-SXT) antibiotics, indicating their multi-resistance. It is reported that multidrug resistance in *Campylobacter* is not rare. Moreover, *Campylobacter* have developed a number of resistance mechanisms to different antibiotics; for examples, the resistance to macrolides is associated with target modification and active efflux, resistance to tetracyclines is acquired by horizontal gene transfer, to quinolones is mediated by point mutations (Luangtongkum *et al.*, 2009) and to penicillins by intrinsic resistance and β-lactamase production (Li *et al.*, 2007).

Antibiotic resistant *Campylobacter* and *Salmonella* are found to colonise the intestinal tracts of wild birds and are shed in the faeces. The organisms are spread to the environment through the birds' droppings which may be a risk to farm animals as well as to public health because of the potential of coming in contact, directly or indirectly, with the organisms.

REFERENCES

Abulreesh, H.H., Goulder, R. and Scott, G.W. (2007). Wildbirds and human pathogens in the context of ringing and migration. Ringing and Migration. 23: 193-200.

Andres, S., Vico, J.P., Garrido, V., Grillo, M.J., Samper, S., Gavin, P., Herrera-Leon, S. and Mainar-Jaime, R.C. (2012). Epidemiology of subclinical salmonellosis in wild birds from an area of high prevalence of pig salmonellosis: phenotypic and genetic profiles of *Salmonella* isolates. Zoonoses and Public Health. 60: 355–365.

Chuma, T., Hashimoto, S. and Okamoto, K. (2000). Detection of thermophilic *Campylobacter* from sparrows by multiplex PCR: the role of sparrows as source of contamination of broilers with *Campylobacter*. Journal of Veterinary Medical Science. 62: 1291 -1295.

Clinical and Laboratory Standards Institute (CLSI) (2013). Performance Standards for Antimicrobial Disk and Diffusion Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard. 4th ed. Vol. 33 (7). Wayne, USA.

Colles, F.M., Dingle, K.E., Cody, A.J. and Maiden, M.C.J. (2008). Comparison of *Campylobacter* populations in wild geese with those in starlings and free range poultry on the same farm. Applied and Environmental Microbiology. 74: 3583-3590

Craven, S.E., Stern, N.J., Line, E., Bailey, J.S., Cox, N.A. and Fedorka-Cray, P. (2000). Determination of the incidence of *Salmonella* spp., *Campylobacter jejuni* and *Clostridium perfringens* in wild birds near broiler chicken houses by sampling intestinal droppings. Avian Diseases. 44: 715–720

Dhama, K., Mahendran, M. and Tomar, S. (2008). Pathogens transmitted by migratory birds: threat perceptions to poultry health and production. International Journal of Poultry Science. 7: 516–565.

Ganapathy, K., Saleha, A.A., Jaganathan, M., Tan, C.G., Chong, C.T., Tang, S.C., Ideris, A., Dare, C.M., and Bradbury, J.M. (2007). Survey of *Campylobacter, Salmonella* and Mycoplasmas in house crows (*Corvus splendens*) in Malaysia. The Veterinary Record. 160: 622–624.

Gopee, N.V., and Adesiyun, A.A. (2000). A longitudinal study of *Escherichia coli* strains isolated from captive mammals, birds, and reptiles in Trinidad. Journal of Zoo and Wildlife Medicine, 31: 353–360.

Kapparud, G. and Rosef, O. (1983). Avian wildlife reservoir of *Campylobacter fetus* subsp. *Jejuni*, *Yersinia* spp. and *Salmonella* spp. in Norway. Applied and Environmental Microbiology. 45: 375–380.

Li, X.Z., Mehrotra, M., Ghimire, S. And Adewoye, L. (2007). B-lactam resistance and β-lactamases in bacteria of animal origin. Veterinary Microbiology. 121 :197–214.

Luangtongkum, T., Jeon, B., Han, J., Plummer, P., Logue, C.M. and Zhang, Q. (2009). Antibiotic resistance in *Campylobacter* : emergence, transmission and persistence. Future Microbiology. 4 : 189–200.

Saleha, A.A., Christopher, J.R.G., Aini, I. and Ganapathy, K. (2001) Occurrence of *Campylobacter* in free-flying wild birds. Proceeding of 2nd International Congress /13th VAM Congress and CVA-Australasia/Oceania Regional Symposium, Kuala Lumpur, pp.70 -71.

Tanaka, C., Miyazawa, T., Watarai, M. and Ishiguro, N. (2005). Bacteriological survey of feces from feral pigeons in Japan. Journal of Veterinary Medical Science. 67: 951-953

Tizard, I. (2004). Salmonellosis in wildbirds. Seminars in Avian and Exotic Pet Medicine, 13: 50-66.

Waldenstrom, J., On, S.L.W., Ottvall, R., Hasselquist, D., Harrington, C.S. and Olsen, B. , (2003). Avian reservoirs and zoonotic potential of the emerging human pathogen *Helicobacter Canadensis*. Applied and Environmental Microbiology. 69: 7523–7536.