Journal Articles

OCCURRENCE OF ANTIBIOTIC-RESISTANT ESCHERICHIA COLI AND SALMONELLA SPP. IN PSITTACINE BIRDS IN SELECTED PETTING ZOOS IN KLANG VALLEY, MALAYSIA

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SUMMARY

Today, psittacine birds are gaining popularity in petting zoos for entertainment and educational purposes. However, the birds may be carriers or reservoirs of a number of pathogens which may have zoonotic risk. Therefore, this study was carried out to determine the antibiotic resistance of *Escherichia coli* and *Salmonella* spp. in psittacine birds. Fresh faecal samples were collected from 40 apparently healthy birds which had frequent contact with the public in three petting zoos located in Klang Valley area. *E. coli* was isolated from 15 fresh faecal samples (37.5%) and *Salmonella* spp. was isolated from 3 (7.5%) of the samples. Salmonellosis and *E. coli* infection in humans is generally contracted via consumption of contaminated food of animal origin or via contact with animals, environment or manure, thus people touching animals without properly cleaning their hands are at risk of getting infected. Antibiotic susceptibility test was done and 33.3% (5 out of 15) of *E. coli* isolates were found resistant to Ampicillin; 26.7% (4 out of 15) resistant to Streptomycin; 20% (3 out of 15) resistant to Ciprofloxacin and Tetracycline; 13.3% (2 out of 15) resistant to Chloramphenicol, Nalidixic acid and Sulphonamides; 6.7% (1 out of 15) resistant to Cefotaxime, Ceftiofur, Gentamycin and Norfloxacin. None of the *E. coli* isolates were found resistant to Amoxicillin/Clavulanic acid. While for *Salmonella* spp., all of the isolates were resistant to Amoxicillin/Clavulanic acid and Ampicillin, while none were found resistant to Ciprofloxacin, Gentamycin and Norfloxacin. 66.7% of *Salmonella* isolates (2 out of 3) were resistant to Cefotaxime and Ceftiofur; 33.3% (1 out of 3) resistant to Chloramphenicol, Nalidixic acid, Streptomycin, Sulphonamides and Tetracycline. Multidrug-resistant *E. coli* was at 20% (3 out of 15) whereas multidrug-resistant *Salmonella* spp. was 66.7% (2 out of 3). Multidrug-resistant *E. coli* and *Salmonella* spp. pose a serious threat to the public, as both are capable of causing severe gastroenteritis in humans. Thus, proper precautionary steps should be taken seriously by both the petting zoos and visitors to ensure the bird handlers and visitors are protected.

Keywords: psittacine birds, Escherichia coli, Salmonella spp., antibiotic resistance

INTRODUCTION

In animal recreational parks, parrots are reared for educational and entertainment purposes. However, parrots and other birds can carry or may be reservoirs for many pathogens that may be zoonotic in nature, such as *Escherichia coli* (*E. coli*) and *Salmonella* spp. (Pontes et al., 2018). *E. coli* is a Gram negative, facultative anaerobe and non-spore forming bacillus (Parija, 2012). Virulent strains of *E. coli* are responsible for most diarrhoea, meningitis, septicaemia and urinary tract infections in children worldwide (Makvana and Krilov, 2015).

Certain strains of *E. coli* are important pathogens that can cause illness in humans and animals, affecting the urinary and digestive tracts, blood, and central nervous system (Schaechter, 2009). It consists of a diverse group of bacteria, in which pathogenic *E. coli* strains are categorized into six different pathotypes, including enterotoxigenic *E. coli* (ETEC), diffusely adherent *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) and shiga toxin-producing *E. coli* (STEC). These six pathotypes are also referred to as diarrheagenic *E. coli* as they are associated with diarrhoea (Centers for Disease Control and Prevention, 2020). Shiga toxin-producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC) are two pathotypes of human diarrheagenic *E. coli* that affect birds and pose a potential zoonosis risk (Farooq et al., 2009; Gioia-Di Chiacchio et al., 2016). Enteropathogenic *E. coli* (EPEC) is one of the prominent causes of high child mortality rate in developing countries. (Farooq et al., 2009; Gioia-Di Chiacchio et al., 2016). The infection is transmitted primarily via faecal-oral route, when people consume contaminated food or water, such as raw or undercooked food and food contaminated with faeces (Centers for Disease Control and Prevention, 2020). Hence, it is possible to get infected after touching the surroundings of petting zoos or animal exhibition areas.

Salmonella spp. is a Gram-negative, flagellated, facultative anaerobic bacilli characterized by O, H, and Vi antigens (Parija, 2012). *Salmonella* gastroenteritis results in sudden onset of diarrhoea, fever, abdominal cramps, and occasionally nausea and vomiting (Centers for Disease Control and Prevention, 2020). It is estimated to cause 93.8 million cases of gastroenteritis yearly worldwide with 155,000 deaths (Majowicz et al., 2010). *Salmonella* spp. is

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primarily an intestinal bacterium; however, it may be also present in the environment such as water, soil, raw meat and offal, animal feed, and vegetable material subjected to faecal contamination (Quinn et al., 2011). *Salmonella* spp. can infect a wide range of host animals, including mammals, birds and reptiles. They may present in animals without causing clinical signs, or can cause a wide range of mild to serious salmonellosis infections (Rogers, 2011). They are excreted mainly in faeces and faecal oral route is the main route of infection to be transmitted. Non-typhoidal salmonella transmits disease mainly via contaminated food. Incubation period for gastroenteritis type depends on the bacteria loads, but signs are usually seen within 6 to 48 hours after consuming contaminated food. Nausea, vomiting, diarrhoea and abdominal pain are the common signs (Giannella, 1996). *E. coli* and *Salmonella* spp. may be present in psittacine birds without the birds showing any symptoms which may pose a potential hazard to the public. More worrying is if the organisms carry multidrug-resistant (MDR) genes, which can cause a serious health issue in humans.

There are relatively few studies on the potential zoonotic pathogens in open animal parks or zoo animals (Stirling et al., 2007). This would be the first report to document the occurrence of these two bacteria in psittacine birds in petting zoos in Malaysia. This study is important due to the increased incidence of *E. coli* and *Salmonella* spp. infections worldwide. The infections are more likely to occur via contact with contaminated feathers and environment. Therefore, this study investigated the occurrence of antibiotic-resistant *E. coli* and *Salmonella* spp. in psittacine birds in selected petting zoos in Klang Valley area, Malaysia.

MATERIALS AND METHODS

Samples collection

Fresh faecal samples from 40 psittacine birds of 18 species (African Grey Parrot (n=3), Black Palm Cockatoo (n=1), Blue and Gold Macaw (n=9), Blue Fronted Amazon Parrot (n=2), Eclectus Parrot (n=2), Galah Cockatoo (n=2), Green Wing Macaw (n=2), Hahn's Macaw (n=1), Harlequin Macaw (n=1), Medium Sulfur-Crested Cockatoo (n=3), Patagonian Conure (n=3), Red-Bellied Macaw (n=1), Rose-Ringed Parakeet (n=1), Scarlet Macaw (n=1), Sun Conure (n=2), Umbrella Cockatoo (n=4), Yellow-Collared Macaw (n=1) and Yellow-Naped Amazon (n=1)) were collected from three petting zoos located in Klang Valley area. These consisted of 16 birds from Petting Zoo A, 11 birds from Petting Zoo B and 13 birds from Petting Zoo C. Two faecal swabs were obtained from each bird. One swab was placed in a bottle containing Buffered Peptone Water (BPW) (Oxoid) for the isolation of *Salmonella* spp., whereas another swab was put into a plain transport tube for the isolation of *E. coli*. Then, the swabs were transported in ice-packed cool box to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia within two to three hours.

Isolation and Identification Procedures for Escherichia coli

Each faecal swab sample in a plain transport tube was streaked onto Chromocult® Coliform Agar (Merck) and all plates were incubated aerobically at 35°C for 24 hours. Each suspected colony (dark-blue to violet) was coated with a drop of KOVACS' indole reagent. The presence of *E. coli* was confirmed if the colonies turned to cherry-red colour after a few seconds (Merck, 2005).

Isolation and Identification Procedures for Salmonella spp.

Each faecal swab sample that was placed in BPW during sampling was pre-enriched by incubating at 37°C for 18 hours under aerobic condition. Then, 0.1ml of each pre-enriched broth sample was transferred into 10ml of Rappaport Vassidalis (RV) (Oxoid) enrichment broth which was then incubated at 42°C for 24 hours for enrichment stage. One loopful of each culture from RV enrichment broth was streaked onto Xylose Lysine Deoxycholate (XLD) agar (Oxoid) and Brilliant Green Agar (BGA) (Oxoid). All inoculated plates were then incubated at 37°C for 24 hours under aerobic condition (International Organization for Standardization, 2017). Biochemical tests which included Triple Sugar Iron (TSI), Lysine Iron Agar (LIA) and SIM (Sulfide, Indole, Motility) were done to identify the presumptive *Salmonella* spp. which were then confirmed serologically by Slide Agglutination Test (SAT) using *Salmonella* polyvalent "O" and "H" antisera A-S.

Antibiotic Susceptibility Test (AST)

The isolates of *Salmonella* spp. and *E. coli* were subjected to antibiotic susceptibility test using disc diffusion test method. A loopful of each bacterial suspension was spread onto Muller Hinton Agar (MHA) (Oxoid) plate, let dry and tested against 12 antibiotics which were Amoxicillin/Clavulanic Acid (20µg), Ampicillin (10µg), Cefotaxime (30µg), Ceftiofur (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Gentamycin (10µg), Nalidixic Acid (30µg), Norfloxacin (10µg), Streptomycin (10µg), Sulphonamides (300µg) and Tetracycline (30µg). Six antibiotic discs were placed on MHA plate using an automatic disc dispenser. All plates were incubated aerobically at 37°C for 24 hours (Hudzicki, 2009). Each diameter of zone of inhibition was measured using a digital calliper and interpreted as sensitive, intermediate or resistant based on Clinical and Laboratory Standards Institute (CLSI, 2020).

RESULTS

From a total of 40 faecal samples from psittacine birds, 15/40 samples were positive for *E. coli* with an overall occurrence rate was 37.5%. *Salmonella* spp. isolation found positive in 3/40 samples with an overall occurrence rate was 7.5%. Table 1 presents the occurrence of *E. coli* and *Salmonella* spp. in the petting zoos.

Figure 1 and 2 showed the overall antibiotic susceptibility of *E. coli* and *Salmonella* spp. isolates respectively. 33.3% (5 out of 15) of *E. coli* isolates were found resistant to Ampicillin; 26.7% (4 out of 15) resistant to Streptomycin; 20% (3 out of 15) resistant to Ciprofloxacin and Tetracycline; 13.3% (2 out of 15) resistant to Chloramphenicol, Nalidixic acid and Sulphonamides; 6.7% (1 out of 15) resistant to Cefotaxime, Ceftiofur, Gentamycin and Norfloxacin. None of the *E. coli* isolates was found resistant to Amoxicillin/Clavulanic acid. While for *Salmonella* spp., all of the isolates were resistant to Amoxicillin/Clavulanic acid and Ampicillin, while none were found resistant to Ciprofloxacin, Gentamycin and Norfloxacin. 66.7% of *Salmonella* isolates (2 out of 3) were resistant to Cefotaxime and Ceftiofur; 33.3% (1 out of 3) resistant to Chloramphenicol, Nalidixic acid, Streptomycin, Sulphonamides, and Tetracycline. Multidrug-resistant *E. coli* was at 20% (3 out of 15) whereas multidrug-resistant *Salmonella* spp. was 66.7% (2 out of 3).

Table 1. Occurrence of *E. coli* and *Salmonella* spp. in psittacine birds in the three petting zoos.

Petting Zoos	No. of samples	No. of positive samples (%) *E. coli*	No. of positive samples (%) *Salmonella spp.*
A	16	8	0
B	11	4	1
C	13	3	2
Total	40	15 (37.5%)	3 (7.5%)

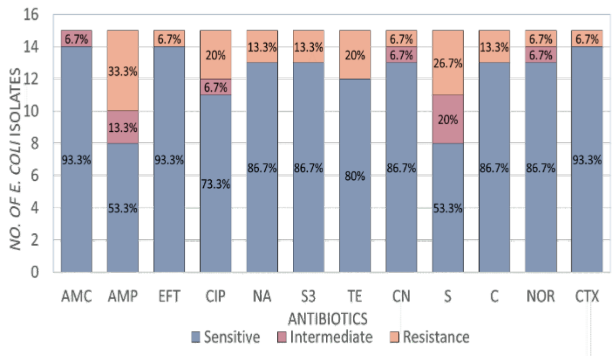

Figure 1. Antibiotic Susceptibility of *E. coli* isolates.
AMC, Amoxicillin/Clavulanic Acid, AMP, Ampicillin, EFT, Ceftiofur, CIP=Ciprofloxacin, NA=Nalidixic Acid, S3=Sulphonamides, TE=Tetracycline, CN=Gentamycin, S=Streptomycin, C=Chloramphenicol, NOR=Norfloxacin, CTX=Cefotaxime

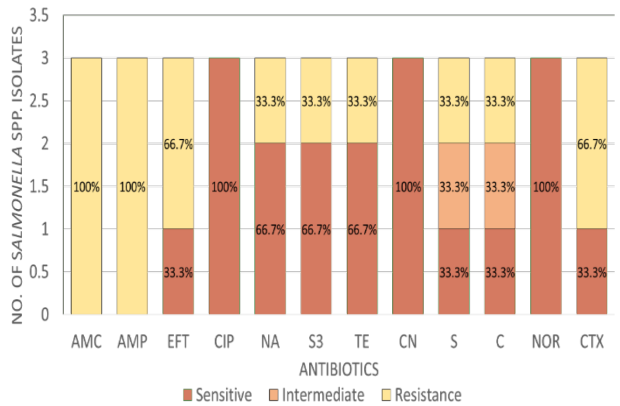

Figure 2. Antibiotic Susceptibility of *Salmonella* spp. isolates.
AMC=Amoxicillin/Clavulanic Acid, AMP=Ampicillin, EFT=Ceftiofur, CIP=Ciprofloxacin, NA=Nalidixic Acid, S3=Sulphonamides, TE=Tetracycline, CN=Gentamycin, S=Streptomycin, C=Chloramphenicol, NOR=Norfloxacin, CTX=Cefotaxime
Chloramphenicol, Nalidixic acid, Streptomycin, Sulphonamides, and Tetracycline.

DISCUSSION

From this study, the occurrence of *E. coli* in psittacine birds was 37.5% (15/40). Little is known about the natural gut microbiota of wild birds (Hidasi et al., 2013), a few studies suggest that the presence of *E. coli* in gastrointestinal tract (GIT) of parrots is indicative of pathogenic or opportunistic infection, suggestive that the birds might be under stressful condition. GIT of healthy parrots is mainly colonized by gram-positive bacilli, it is unusual to see Gram negative bacteria which is potentially pathogenic in their GIT (Doneley, 2009). Isolation of *E. coli* from psittacine birds is infrequent and positive *E. coli* from birds with enteritis should be considered as possible pathogens (Graham and Graham, 1978). Bowman and Jacobson found that 16/40 of the healthy psittacine birds were positive for *E. coli*, these bacteria are known to be potential pathogens under stressful condition (Bowman and Jacobson, 1980). However, the role of *E. coli* as commensals in healthy psittacine remains controversial (Siqueira et al., 2017). Study by Bangert and colleagues found that *E. coli* is normal in low number of healthy parrots (Bangert et al., 1988); Flammer and Drewes reported that *E. coli* was found in 31% of clinically healthy birds (Flammer and Drewes, 1988); while studies by Harrison and McDonald showed that gram negative bacteria should not be present in parrots with healthy and balanced diet (Harrison and McDonald, 1996). Thus, we can say that, isolation of *E. coli* from healthy birds is not impossible but would not usually be seen in large number of healthy individuals, and presence of *E. coli* always indicates potential pathogens for the birds. Unlike in the case of most mammals and domestic birds, where *E. coli* can be found in large number in the individuals.

The occurrence of *Salmonella* spp. in psittacine birds (N=40) showed that there were three positive isolates (7.5%). As mentioned in the report by Siqueira et al. (2017), *Salmonella* spp. is not common in intestinal tracts of psittacine. As such the presence of the bacteria in these birds could pose health hazard to the public, particularly via faecal oral route, causing gastroenteritis. Although the occurrence of *Salmonella* isolates in this study was low, it is of concern because of the public health significance.

The variation in the percentage of bacteria isolation among different petting zoos has not been further investigated. The differences are believed to be due to the differences in management practice, different diet structures, and stress level. For example, the hygiene condition, frequency of contact with people, dietary changes, antibiotic usage, environmental stress and age of the birds, etc. However, the background of the zoos and each of the birds has not been investigated in this study. These parrots are sometimes put in open area for exhibition and photography purposes. They are hence given a chance to have close contact with the public and other animals such as wild birds, in which these individuals might be the source of transmission of the bacteria. Wild pigeons invading zoo enclosures looking for food and water can potentially transmit diseases or even acquire pathogens from other animals (Sanches et al., 2017). However, the strains of the isolates are not being studied in this experiment, as not all the strains of *E. coli* and *Salmonella* spp. pose a zoonotic risk. This result could mean that there is a potential zoonotic risk of these bacteria infecting humans.

Multidrug-resistance (MDR) is defined as acquired resistance to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012). A total of 20% (3 out of 15) of MDR *E. coli* and 66.67% (2 out of 3) of MDR *Salmonella* isolates were isolated. These MDR organisms pose a risk of being acquired by humans. Antibiotic resistance results in the failure of certain antibiotics to control or kill the bacteria, thus reducing the options of treatment. This has complicated the treatment process, causing infections to become difficult or impossible to treat. Resistant infections result in more costly health care options due to longer duration of illness, additional tests and more expensive drugs. As psittacine birds are becoming popular in zoos and animal parks, and people often have close contact with them, thus increasing the risk of transmitting these AMR strains to the public. This is more severe when these bacteria gain resistance towards more categories of antimicrobials. Especially person with compromised immune system, children as well as the elderly when they come in close contact with the birds, may be at risk of being infected, with possibility of experiencing severity of the disease, limited treatment options and costly medical treatment.

CONCLUSION

From this study, the occurrence of *E. coli* was 37.5% and *Salmonella* spp. was 7.5% in psittacine birds from three petting zoos in Klang Valley area, Malaysia. The study also found presence of antimicrobial resistance in both *E. coli* and *Salmonella* spp. isolates and multidrug-resistance in five isolates. This result indicates that parrots housed in zoos might be carriers of bacteria that could be harmful to humans. The presence of these organisms poses a potential zoonotic risk to the people who have direct or indirect contact with the birds. Salmonellosis and *E. coli* infection in humans is generally contracted through consumption of contaminated food of animal origin or contact with animals, environment or manure, thus people touching animals without properly cleaning their hands are at risk of getting infected. In addition to that, proper sanitation procedures after having contact with the animals have not been emphasized in these places. At the same time, the birds may acquire these bacteria from the environment, feed and the handlers. The zoos should always keep an eye on the birds that showing clinical signs, and take action to isolate and provide treatment, birds that are unhealthy should not come into close contact with other birds and visitors. Thus, both animal parks and the public must take necessary precautionary steps, it is recommended that the zoos and animal parks always emphasize on the hygiene steps and provide more hand washing facilities at convenient spots to ensure that hand washing practice is being done after handling the birds.

CONFLICT OF INTEREST

None of the authors of this paper has any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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