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Prevalence and Antimicrobial Resistance of *Escherichia coli, Staphylococcus aureus* and *Salmonella* spp. from cloacal swabs of quail

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Food-borne pathogens have increased throughout the world and continue to be a major public health concern. The present study was performed to determine the prevalence of *Salmonella* spp*., Staphylococcus aureus* and *Escherichia coli* from cloacal swabs of Japanese quails at two different farms in Kemaman, Terengganu and to determine antimicrobial susceptibility pattern against eight selected used antibiotics. A total of 83 strains of *E. coli*, 37 strains of *S. aureus* and 1 strain of *Salmonella* spp. were isolated from 100 samples after thoroughly characterized by standard culture method and biochemical tests. The isolates were subjected to antimicrobial resistance test against 8 antibiotics using Kirby-Bauer disk diffusion method. Antibiogram study showed that *E. coli* isolates were highly resistance to cephazolin (68.67%), erythromycin (66.26%) and chloramphenicol (65.06%) while *S. aureus* highly resistance towards amoxicillin (89.19%) and erythromycin (75.68%). The result shows only one positive *Salmonella spp*. (2%) that is resistance to tetracycline, doxycycline and cephazolin. Therefore, good personnel hygiene in processing and handling of quails and their products is very important to ensure healthy quail. Excessive use or misuse of antibiotics should be avoided in order to reduce the emergence of multiple antibiotic resistant strains in bacteria.

Keywords: *Escherichia coli*, *Staphylococcus aureus, Salmonella* spp., Japanese quails, cloacal swabs, antimicrobial resistance

INTRODUCTION

Quails are a small avian species in Phasianidae family and a migratory bird which migrate between Asia and Europe that are now used for commercial eggs and meat production. Quails meat is an important source of vitamin B6, niacin, thiamin, pantothenic acid and riboflavin (Hamad et al., 2012) making them suitable for commercial production. There are billions of bacteria that can be found in poultry feces. The bacteria present can be categorized into the normal flora, the opportunistic microorganism and the pathogenic ones (Roy et al., 2017). The type of microorganism present in the quail cloacal and intestinal tract are affected by several factors such as the farm management, presence of pathogens, immunity status of the quail or the health status of the quail (Calvert, 2012). Thus, the production of quails can be affected by the presence of pathogenic bacteria such as *Staphylococcus aureus, Salmonella* spp*. and Escherichia coli* where it can cause food-borne illness to the consumer.

E. coli infections in poultry can resulted in several symptoms which include egg peritonitis, omphalitis, cellulitis, swollen head syndrome and

colisepticaemia (Roy et al., 2017). *S. aureus* can cause bumble foot disease to the avian animals (Shareef et al., 2009) and poses health risk to the consumers by production of enterotoxin (Kadariya et al., 2014) leading to Staphylococcosis. Whereby *Salmonella* spp. can cause a wide range of illnesses such as Salmonellosis which affect all species of the animals and human population (Animal Health Organization, 2010). Thus, antibiotics are important to treat the infection. This study was carried out to determine the prevalence of *Escherichia coli, Staphylococcus aureus* and *Salmonella* spp. from cloacal swabs of quails and the antimicrobial susceptibility pattern against eight selected antibiotics.

MATERIALS AND METHODS

Sample collection

A total of 100 cloacal swab samples were collected randomly from two quail farms (Albakry and Surada farm) located at Kemaman, Terengganu. All samples were taken using sterile cotton swabs and immerse a swab of fecal specimen into a falcon tube of 7 ml of Cary Blair medium (Oxoid Ltd, UK). This medium was used to prevent drying of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Samples were kept in the ice box containing ice pack and transported to Microbiology Laboratory for further analysis.

Isolation of *Escherichia coli*

After enrichment in brilliant green broth, a loopful of inoculum from broth was streaked onto Eosin Methylene Blue (EMB) agar medium (Hi-media, India) for 24 h at 37 ºC (Cheesbrough, 1985). The green metallic colony morphology with characteristics of *E. coli* was sub-cultured onto nutrient agar to obtain the pure culture with homogenous colonies. The pure isolates were kept in slant agar at 4 ºC for further analysis.

Isolation of *Staphylococcus aureus*

A loopful of inoculum from enriched samples were streaked onto Vogel and Johnson (VJ) agar and incubated at 37 ºC for 24 h. Then, bacteria colonies on VJ agar were further inoculated onto Mannitol Salt (MS) agar as selective medium to promote the growth of *S. aureus* and incubated for 24 h at 37 ºC (Shareef et al., 2009; Hamad et al., 2012; Palanisamy and Bamaiyi 2015). The pure isolates were kept in slant agar at 4 ºC for further analysis.

Isolation of *Salmonella* spp.

The bottles containing swabs in Selenite Cystine Broth (Hi-media) were incubated aerobically at 37 ºC for 24 h. Then, a loopful of each broth culture were streaked onto the selective medium, Xylose Lysine Deoxycholate agar (XLD) (Hi-media), and the plates were incubated at 37 ºC for 24 h. The isolates with typical *Salmonella* spp. morphology in which colonies is pink to red with black center on XLD agar were picked and subculture on XLD agar to obtain pure cultures and incubated at 37 ºC for 24 h. The pure isolate was kept in slant agar at 4 ºC for further analysis.

Gram staining

Gram staining was done to differentiate whether the bacteria is Gram positive or Gram negative by observing their morphology such as color, shape and size of colonies. The representative *E. coli*, *S. aureus* and *Salmonella* spp. colonies were characterized microscopically using Gram's stain according to the method described by James and Natalie (2005). Briefly, the cells were heated, fixed and the stain with the following procedures: Crystal Violet (1 min), Iodine-Lugol (1 min), Decolourization (1 min), and Safranin (1 min). Microscopic examination was done under microscope with high power objectives 100X using immersion oil.

Biochemical test

The biochemical tests including oxidase test, catalase test and Triple sugar iron (TSI) tests were carried out for identification of *Escherichia coli* and *Salmonella* spp. while coagulase test, oxidase test and catalase test were used to identify *Staphylococcus aureus.*

Antimicrobial sensitivity test

The antibiotic sensitivity patterns of all *E. coli*, *S. aureus* and *Salmonella* spp. isolates were performed by standard disc diffusion method (Saifullah et al., 2016) and interpreted as susceptible, intermediate and resistant as described by Clinical and Laboratory Standards Institute (CLSI, 2014). This antibiotic disc diffusion (CLSI 2014) test had been done in Muller- Hinton agar (Hi-Media, India). The isolates are tested for resistance against erythromycin (15 µg), cephazolin (30 µg), gentamycin (10 µg), tetracycline (30 µg), doxycycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (10 µg), sulphamethoxazole (30 µg), sulfisoxazole (25 µg), streptomycin (25 µg) and amikacin (30 µg) which commonly used antibiotics against these

pathogenic bacteria. Sterile swab was used to inoculate the plates. Then, swab was spread gently on Muller Hinton (MH) agar (Hi-Media, India) plates from side to side to obtain uniform inoculums. Antibiotics discs were aseptically placed on the inoculated plates. The plates were inverted and incubated at 37 °C. After 24 h of incubation and the zone of inhibition diameters were measured (mm).

Statistical analysis

All data were recorded into a Microsoft Excel 2016. The quail data were analyzed using Chi square analysis for independence statistical analysis using *E. coli*, *S. aureus* and *Salmonella* spp. infections as dependent variables. P-value less than 0.05 was considered for statistical significance.

RESULTS

Prevalence of *Salmonella spp., E. coli, S. aureus*

The overall prevalence of *Salmonella* spp., *E. coli* and *S. aureus* were 83 and 37%, respectively in different farms at Kemaman, Terengganu as shown in Table 1.

Cultural, staining and motility characteristics

Typical *E. coli* on EMB agar appear as metallic sheen greenish black colonies. On XLD agar, *Salmonella* spp. appear colonies of pink to red with black center while *S. aureus* colonies were appeared as black, convex shiny colonies surrounded by a yellow zone due to ability to reduce tellurite to metallic tellurium (Chong 2014).

Biochemical test

All *E. coli* and *Salmonella* spp. isolates were catalase, TSI positive but oxidase negative while all *S. aureus* isolates were catalase and coagulase positive but oxidase negative.

Antibiogram profiles

Antibiotic sensitivity tests of quail *Salmonella* spp. *E. coli* and *S. aureus* isolates revealed as sensitive, intermediate and resistant profiles which are shown in Table 2, 3 and 4. For example, a Chloramphanicol (30 µg), the diameter of zone of inhibition is scaled as resistance (≤12), intermediate (13 to 17) and susceptible (≥18) in the antimicrobial disc used.

Table 1: Prevalence of *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus* from both farms at Kemaman, Terengganu

Farm	No. of samples	*Salmonella* spp.	*E. coli*	*S. aureus*
Surada	50	1 (2%)	44 (88%)	23 (46%)
Al-Bakry	50	0 (0%)	39 (78%)	14 (28%)

Table 2: Susceptibility pattern of all the positive *Salmonella* spp. towards different types of antibiotics

Types of antibiotics	No. of positive *Salmonella* spp. Surada Farm (1)		
	Resistance	Intermediate	Susceptible
Amoxycillin	0 (0%)	0 (0%)	1 (2%)
Ampicillin	0 (0%)	0 (0%)	1 (2%)
Cephazolin	0 (0%)	0 (0%)	1 (2%)
Chloramphenicol	1 (2%)	0 (0%)	0 (0%)
Ciprofloxacin	0 (0%)	0 (0%)	1 (2%)

Table 3: Susceptibility pattern of all the isolate *E. coli* to different antibiotics

Types of antibiotics	No. of positive *Escherichia coli*					
	Al-Bakri Farm (39)			Surada Farm (44)		
	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible
Amikacin	0 (0)	0 (0)	39(100%)	0 (0)	1 (2.27%)	43 (97.72%)
Amoxicillin	13 (33.33%)	3 (7.69%)	23(58.97%)	10 (22.72%)	7 (15.90%)	27 (61.36%)
Cephazolin	26 (66.66%)	11 (28.20%)	2 (5.12%)	31 (70.45%)	12 (27.27%)	1 (2.27%)
Chloramphenicol	26 (66.66%)	0 (0)	13 (33.33%)	28 (63.63%)	0 (0)	16 (36.36%)

Table 4: Overall percentage of antibiotic sensitivity profile of *S. aureus* isolated from quail's cloaca.

Antimicrobial class	Antimicrobial Agent	No. of isolates (%)		
		Resistance	Intermediate	Susceptible
β-lactams	Amoxicillin	89.19%	2.70%	8.10%
Cephalosporin	Cephazolin	32.43%	18.91%	48.64%
Penicols	Chloramphanicol	24.32%	37.84%	64.86%
Quinolones	Ciproflaxacin	2.70%	5.41%	91.89%

rone level with an anti-inflammatory properties. According to Holt (2013) these forced molting practices will increase chicken susceptibility to some pathogenic bacteria such as *Salmonella enteritidis* with probability of infection increase by 100 to 1000 times. Hence, according to Golden et al., (2008) showed that forced molting by fasting in farm management practices may cause severe health problems to the consumers. The studied by Palanisamy and Bamaiyi (2015) at one of the quails farm at Kota Bharu Kelantan after the great flood happened on December 2014 to January 2015, found 10 out of 90 cloacal swab sample were positive with *Salmonella* spp. Isolates of *Salmonella* spp. were obtained in 7 out of 45 from the 3 weeks old chick and 3 out of 45 from 2-month-old birds. As compared to Dipineto et al., (2004), there were no Salmonella was isolated from quails between three flocks in Italy and California.

The previous study by Zihadi et al., (2018), *Salmonella* was detected from 10 out of 75 total cloacal swab sample from three quail farms because Salmonellosis cases is one of the most concern in human and animal health in Bangladesh. These are due to variety climatic conditions that cause the spreading of the organism through the environment. In this study, *Salmonella* spp. was resistant towards tetracycline and erythromycin but sensitive towards ciprofloxacin and imipenem (Zihadi et al., 2018) compared with current study, *Salmonella* spp. was also resistant towards tetracycline. From Uddin et al., (2018), for poultry study showed *Salmonella* was resistant towards tetracycline, neomycin, ampicillin and novobiocin. From Palanisamy and Bamaiyi (2015) study, the antimicrobial resistance of *Salmonella* spp. was resistant towards Ampicillin only. However, low number of *Salmonella* isolate in this study was resistant towards chloramphenicol, doxycycline and sulphamethoxazole.

Escherichia coli

According to Sackey et al., (2001), *Escherichia coli* have been an important microorganism responsible for food poisoning. This study found a high prevalence of *E. coli* (83%) in Japanese quail in Kemaman, Terengganu. The result in this study is similar to Jakaria et al., (2012) where the prevalence of *E. coli* was 78.86% in cloacal samples of poultry. In this present study, the quails used for this study were mostly affected by *E. coli*. It was also found that the prevalence of *E. coli* infection (88%) in Surada Jaya Farm was higher than the Al-Bakri farm (78%) in commercial broilers quail farm. The differences might be due to different environments, feed and management conditions (Derakhshantar and Ghanbarpour, 2002).

From the antibiotic susceptibility test, it was observed that *E. coli* were highly sensitive to amikacin (30 µg), gentamycin (10 µg) and doxycycline (30 µg). Amikacin has been introduced recently in poultry farm followed with rationale use of this drug may prevent development of resistant isolates of *E. coli* in future (Rahman et al., 2018). The results were similar with Malaysian poultry where *E. coli* isolates shows resistance to tetracycline and gentamicin with the range between 11-95% (Apun et al., 2008). Other studies have shown *E. coli* in poultry was highly resistance towards chloramphenicol, tetracycline, gentamicin and erythromycin (Rahman et al., 2008).

Staphylococcus aureus

Prevalence of *S. aureus* isolated in this study was similar with Olateru et al., (2018) in which 43% of *S. aureus* was reported that obtained from rectal swab of chicken. In contrast, Bhedi (2016) that reported the prevalence of *S. aureus* from cloacal swab was 6.0%. *S. aureus* was also found tracheal swabs, skin swabs and meat swab at 14, 14 and 10%, respectively. While Geidam et al., (2012) reported 77.2% of *S. aureus* isolated from 3 different poultry farm in Selangor by skin and

feather swabs.

Staphylococcus is commonly carried in the skin, nasopharynx, cloaca, boils, and part of the normal microflora (Ekhaise et al., 2008; Geidam et al., 2012) and normally *S. aureus* is present in small quantities. So hygienic practices and good management system is important in order to control that pathogenic bacteria from spread. It is observed that both farms (Albakry and Surada farm) lack biosecurity and have poor management system resulting in a higher percentage of prevalence *S. aureus* than the previous study like Bhedi (2016). Out of 37 coagulase positive *S. aureus*, the higher isolation was from Surada farm (62.2%) where lack of space for quail's growth could be one of the factors of *S. aureus* spread.

Most of the isolates were found sensitive to ciprofloxacin (10 µg); Albakry farm (85.8%) and Surada farm (95.7%), respectively. *S. aureus* isolates were resistant against amoxicillin followed by erythromycin for both farms. The amoxicillin (100% and 82.7%) were similar with Shareff et al., (2009) where out of 29.1% *S. aureus* positive, 100% were resistance for β-lactam antibiotics such as ampicillin and amoxicillin. While from the study of β-lactam resistance among coagulase positive *Staphylococci* isolates from broiler chicken that conducted by Bakheet et al., (2018) showed all isolates were resistance to oxacillin (100%), cefoxitin (93.8%), penicillin (90.1%) and amoxicillin (87.6%). In another study, Pondit et al., (2018) reported 75% *S. aureus* isolates from quail egg samples were resistant to amoxicillin, and penicillin. In addition, the previous study showed variety pattern of antimicrobial resistance especially towards β-lactam antibiotic which become a serious concern in public health and food safety. Therefore, it is important to continually monitor antibiotic resistance of clinical isolates in order to control and treat the *S. aureus* infection; and also, the organism have propensity to acquire antimicrobial resistance.

CONCLUSION

In conclusion, the *E. coli, S. aureus* and *Salmonella* spp. present in quail show antimicrobial resistance towards a number of antibiotics tested in this study. Thus, careful and systematic usage of antibiotics in quail farming is important to reduce the emergence of resistance strains bacteria.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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