REPORT ON *Salmonella* ISOLATION AND ANTIBIOTICRESISTANCE OF *Escherichia coli* ISOLATED FROM POULTRY SAMPLES FROM FARMS IN MALAYSIA FROM 2011 TO 2014

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ABSTRACT

Salmonella and *Escherichia coli* are both important bacteria that affect the livestock industry in Malaysia. Both pathogens are Gram-negative and ubiquitous in the environment, making them difficult to control and equally important due to their zoonosis, causing foodborne diseases to humans. This study involves testing of Salmonella from 19693 samples collected from poultry farms and antibiotic resistance testing done on *E. coli* samples isolated from poultry farms in Malaysia from 2011-2014. Salmonella testing was done using conventional microbiological method. From the 19693 samples processed, 11.6% were found to be positive for Salmonella (3.0% *Salmonella* Enteritidis, 1.3% *Salmonella* Typhimurium and 7.3% unidentified serotypes grouped as *Salmonella* species). Antibiotic resistance testing of *E. coli* isolated from poultry samples showed high resistance levels (>80%) to tilmicosin (94.2%), amoxicillin/clavulanic acid (92.9%) and amoxicillin (85.4%), whereas few other antibiotics tested such as quinolones and sulpha are moderately resistant. Prudent use of antibiotics should be practiced by all livestock industries in order to control both Salmonella and *E. coli* which pose a threat to human food chain either by causing foodborne illnesses or antibiotic-resistance issues.

Keywords: *Salmonella*, *E. coli,* antibiotic-resistance

INTRODUCTION

Salmonella is a global public health concern as it is one of the major causes of foodborne diseases in the world. Salmonella infects both humans and animals. The Salmonella serotypes isolated from farms have also been found to cause illness in humans, suggesting a Salmonella transmission from animals to humans via the food chain (Li *et al*., 2013). Poultry is believed to be the principle food reservoir responsible for salmonellosis (Rusul *et al*., 1996), particularly non-typhoid human Salmonella infections (Tsai and Hsiang, 2004). Therefore, information on the prevalence of Salmonella in poultry is important in order to devise effective preventive and control measures. The present study was carried out to evaluate Salmonella isolated from poultry farms in Malaysia over a three-year period of 2011-2014.

MATERIALS AND METHODS

A total of 19693 samples submitted to Vet Food Agro Diagnostics (M) Sdn.Bhd. from 2011 to 2014 were tested for the presence of Salmonella. The samples were collected from various broiler, breeder and layer poultry farms in Pahang, Kedah, Penang, Perak, Selangor, Negeri Sembilan, Melaka, Johor and Sabah. Sample types include organs, embryos, egg shell and fluff, cloacal and faecal swabs, feed, water, eggs, chick box liners, litter and environmental swabs. Samples were transported to the laboratory under refrigerated conditions and tested on the day of receipt. The samples were tested for Salmonella based on the microbiological method described by Department of Veterinary Services (DVS, 2008) with some modifications. The

samples were subjected to non-selective pre-enrichment, selective enrichment, selective plating, biochemicaltests and serotyping. Serotyping was conducted with polyvalents 'O', 'H' and six other antisera to identify mainly *Salmonella* Enteritidis and *Salmonella* Typhimurium. The other unidentified serotypes were grouped as *Salmonella* species.

RESULTS AND DISCUSSION

Table 1. Salmonella from poultry farms (2011-2014)

Sample Type	*Salmonella enteritidis*	*Salmonella Typhimurium*	*Salmonella* species	Total positive	No. of samples
Organs	396 (7.9%)	21 (0.4%)	203 (4.1%)	62 (12.4%)	4990
Eggs	5 (1.0%)	-	13 (2.7%)	18 (3.7%)	489
Cloacal/ Faecal Swabs	55 (2.0%)	65 (2.3%)	238 (8.5%)	358 (12.7%)	2813
Environ-mental Samples	126 (1.8%)	117 (1.7%)	701 (10.0%)	944 (13.4%)	7033
Feed	6 (0.2%)	36 (1.3%)	214 (7.5%)	256 (8.9%)	2867
Water	8 (0.5%)	9 (0.6%)	62 (4.1%)	79 (5.3%)	1501
TOTAL	**596 (3.0%)**	**248 (1.3%)**	**1431 (7.3%)**	**2275 (11.6%)**	**19693**

Out of the 19693 samples tested from 2011 to 2013, 11.6% were found to be positive for Salmonella (3.0% *S. enteritidis*, 1.3% *S. typhimurium* and 7.3% *Salmonella*species). The highest percentage of positive samples was from environmental samples (13.4%) followed by cloacal/faecal swabs (12.7%), and organs (12.4%). As indicated by the results, environmental sampling is a good indicator for the presence of Salmonella in poultry flocks. Salmonella transmission from the environment to flocks may be aggravated by unhygienic farming activities such as improperly cleaned or disinfected poultry houses, overcrowding and lack of biosecurity measures in the farm (Frederick *et al.*, 2011). Therefore, there is a need to introduce and implement proper hygiene control measures in poultry farms to reduce or prevent Salmonella from entering the food chain.

ANTIBIOTIC RESISTANCE OF *E. coli*

Antibiotics are widely used in veterinary and human medicine for therapy and control of bacterial infections. In livestock, antibiotics are widely used to treat *E. coli* which causes enteric diseases to livestock and secondary complication from other diseases. Antibiotics may be continuously administered to commercial livestock such as poultry and pigs as either antimicrobial growth promoters or prophylaxis treatment; leading to enhanced selection of resistant bacteria in livestock (van den Bogaard *et al*., 2001) which may subsequently contribute to antibiotic resistance in human acquired through the human food chain (Sayah *et al*., 2005). Hence, antibiotic usage is considered the most important factor for the emergence, selection and dissemination of antibiotic-resistant bacteria (Sayah *et al.,* 2005). As such, it is

important for livestock producers to pick the correct antibiotics and the correct dosage for treatment in order to limit the incidence of antibiotic-resistant bacteria especially multiresistant bacteria or "superbugs". This study was conducted to evaluate the resistance of *Escherichia coli* isolated from local commercial livestock in 2011-2014 on ten different antibiotics.

MATERIALS AND METHODS

Samples submitted to Vet Food Agro Diagnostics (M) SdnBhd from 2011 to 2014 were analysed for *E. coli* via microbiological methods using selective media such as MacConkey agar and Chromocult Coliform Agar (Merck). Sample types include organs, feces and swabs from poultry sources. The isolated *E. coli* were subsequently tested for antibiotic resistance using Kirby-Bauer agar diffusion method. The ten antibiotics evaluated in this study are amoxicillin, amoxicillin/clavulanic acid, ciprofloxacin, enrofloxacin, norfloxacin, colistin, florfenicol, fosfomycin, tilmicosin and trimethoprim sulfamethoxazole.

RESULTS AND DISCUSSION

Table 2. Antibiotic resistance of *E. coli* isolated from poultry sources in 2011-2014

Antibiotic	No. (%) of resistant strains	Total tested
Amoxicillin (10 µg and 25 µg)	76 (85.4%)	**89**
Amoxicillin/clavulanic acid (20/10 µg)	39 (92.9%)	**42**
Ciprofloxacin (5 µg)	4 (44.4%)	**9**
Enrofloxacin (5 µg)	58 (53.7%)	**108**
Norfloxacin (10 µg)	28 (46.7%)	**60**
Colistin (10 µg and 50 µg)	2 (2.0%)	**98**
Florfenicol (30 µg)	40 (60.6%)	**66**
Fosfomycin (50 µg and 200 µg)	40 (41.2%)	**97**
Tilmicosin (15 µg)	49 (94.2%)	**52**
Trimethoprim sulfamethoxazole (1.25+23.75 µg)	26 (63.4%)	**41**

Result from antibiotics resistance test done on *E.coli*, show highest resistance on Tilmicosin (94.5%). This trend tallies with farm heavy usage of this antibiotic as an alternative to replace older Macrolide like Erythromycin and Tylosin. Both Amoxicillin and Amoxicillin with Clavulanic acid was highly resistance as well with 85.4% and 92.9% respectively. This resistance was due to continuous usage in the farm as prophylaxis treatment rather than therapeutic treatment, due to their cheaper cost as compare to other antibiotics. Other antibiotics like Florfenicol, Trimethoprim sulfamethoxazole and Enrofloxacin recorded fair level of resistance. This was due to more cautious usage of those 3 ingredients due to Florfenicol only limited to respiratory treatment, and Trimethoprim sulfamethoxazole which creates swollen kidney after treatment and higher cost of Enrofloxacin. As usual, colistin recorded lowest in resistance, 2.0% due to its molecule that are not absorbed into the body systems and only maintains at gastro-intestinal level. Prudence use of antibiotics is not new propaganda heard in emergence and spread of resistant bacterial strains like *Camplylobactersp*, *Escherichia coli* and *Enterococcus sp* from poultry products to consumers which put human at risk to new strains of bacteria that resist antibiotic

treatment (Apata, 2009). Therefore a focus should be done on continuous surveillance of the antibiotics resistance from poultry industry due to sub-therapeutic dose of antibiotics given, and possibility of shifting to nutraceutical treatments such as probiotics and essential oils as alternative antibiotics treatments.

REFERENCES

Blanco JE, Blanco M, Mora A and Blanco J. 1997. Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. Journal of Clinical Microbiology 35 (8): 21842185.

D.F. Apata 2009. Antibiotic Resistance in Poultry. International Journal of Poultry Science 8(4):404-408.

Department of Veterinary Services. 2008. Salmonella MVK/BACT 01.

Frederick A., and N. Huda. 2011. Salmonellas, Poultry House Environments and Feeds: A Review. *Journal of Animal and Veterinary Advances* 10 (5): 679-685.

Li R., J. Lai, Y. Wang, S. Liu, Y. Li, K. Liu, J. Shen and C. Wu. 2013. Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China. *International Journal of Food Microbiology* 163:14-18.

Rusul G, J. Khair, S. Radu, C. T. Cheah, and R. Md Yassin. 1996. Prevalence of *Salmonella* in broilers at retail outlets, processing plants and farms in Malaysia. *International Journal of Food Microbiology* 33: 183-194.

Sayah RS, Kaneene JB, Johnson Y and Miller R. 2005. Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. Applied and Environmental Microbiology 71 (3): 1394-1404.

Tsai H. J. and P. H. Hsiang. 2005. The prevalence and antimicrobial susceptibilities of *Salmonella* and *Campylobacter* in ducks in Taiwan. *Journal of Veterinary Medical Science* 67(1): 7-12.

Van den Bogaard AE, London N, Driessen C and Stobberingh EE. 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. Journal of Antimicrobial Chemotherapy 47: 763-771.