Malaysian Journal of Microbiology, Vol 11(2) Special Issue 2015, pp. 109-115
http://dx.doi.org/10.21161/mjm.12114

Malaysian Journal of Microbiology
Published by Malaysian Society of Microbiology
(In SCOPUS since 2011)

Antimicrobial resistance of *Escherichia coli*, *Salmonella* and enterococci isolated from surface of conventional broiler eggs, "*Kampung*" chicken eggs and carrying trays from wet markets in Selangor, Malaysia

Aw Yoong Kit, Loong Mei-Jean, Yue Chii Xiang, Lee Kok Ming, Ng Sian Yee, and Lee Sui Mae*

School of Science, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 46150, Bandar Sunway, Selangor, Malaysia.
Email: lee.sui.mae@monash.edu

ABSTRACT

Aims: Bacteria on chicken egg surfaces can be potential sources of food borne diseases. The aim of this study was to determine the prevalence of *E. coli*, *Salmonella* and enterococci on the surface of conventional broiler eggs, "*Kampung*" chicken eggs and carrying trays and to determine the antimicrobial resistant profile of these isolates.
Methodology and results: Conventional broiler eggs, "*Kampung*" chicken eggs and carrying trays were sampled randomly from nine wet markets in Selangor, Malaysia. The surface of the eggs and carrying trays were swabbed and *E. coli, Salmonella* and enterococci were isolated using selective agars. Antimicrobial susceptibility testing (AST) was performed on the isolates against different antimicrobials via disk diffusion test. A large proportion of *E. coli* isolates (>50% of isolates from conventional broiler eggs and "*Kampung*" chicken eggs) was resistant to chloramphenicol and tetracycline whereas enterococci (>60% of isolates from conventional broiler eggs and "*Kampung*" chicken eggs) isolates were resistant to tetracycline and erythromycin. *Salmonella* isolates were found to be susceptible to all of the antimicrobials tested except for tetracycline. There was also presence of isolates showing multiple resistances in this study. *E. coli* isolates (8.8%) from the surface of "*Kampung*" chicken eggs were resistant against 10 different antimicrobials whereas 17.8% of the enterococci isolates from the surface of "*Kampung*" chicken eggs were resistant to 11 different antimicrobials.
Conclusion, significance and impact of study: The presence of multiple-antimicrobial resistant bacteria especially on the surface of "*Kampung*" chicken eggs that are ready to be sold to consumers is a serious concern. However, further study has to be conducted to determine the ultimate source of the bacterial contamination before specific food safety measures can be introduced.

Keywords: Antimicrobial resistance, chicken eggs, *Escherichia coli*, *Salmonella*, enterococci

INTRODUCTION

According to the Department of Veterinary Sciences (2014), eggs are consumed widely among Malaysians. In Malaysia, 10.3 million eggs were produced and 314 eggs were consumed per capita in 2011. In order to keep up with demand, most eggs are produced by the poultry industry through semi-intensive or intensive farming (Aini, 1990). Production of eggs through these methods include raising layers in crowded cages to increase production of eggs, supplementing feeds with growth hormones to accelerate growth and antimicrobials to prevent diseases (Aini, 1990; Aarestrup, 1999).

Overuse of antimicrobials in poultry farming is one of the main factors contributing to the rise of antimicrobial resistance in microbial pathogens (Wegener, 2003). Musgrove *et al.* (2006) isolated *Escherichia coli* and *Salmonella* resistant to multiple antimicrobials such as tetracycline, gentamycin and sulphamethoxazole from commercial chicken eggs. Schwaiger *et al.* (2010) also found *Enterococcus* spp. with high antimicrobial resistance against erythromycin, fosfomycin and clindamycin from chicken eggs under conventional and organic raising methods. In another study by Singh *et al.* (2010), it was reported that all the *Salmonella* isolated from chicken eggs were resistant to bacitracin, polymyxin-B and colistin. These antimicrobials are commonly used in treating human infections and the rise of antimicrobial resistance to these antimicrobials can result in serious consequences. ARB related infections limits treatment options as current treatments can fail to treat the infection, resulting in increased mortality rate (Fauci and Marston, 2014). Chicken eggs and the carrying trays can serve as a vehicle to facilitate transport of highly resistant pathogens (Suresh *et al.*, 2006).

There are generally two types of chicken eggs in the market in Selangor, Malaysia; the conventional broiler eggs which are usually brown in colour, and the free-

***Corresponding author**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

raised "*Kampung*" chicken eggs which are usually white in colour (Aini, 1990). "*Kampung*" chicken eggs are considered a premium commodity as it is regarded as more nutritious compared to conventional broiler eggs (Oh, 1987). It is also said that "*Kampung*" chickens are raised free-range and not fed with conventional commercial feeds containing growth hormones and antimicrobials (Aini, 1990). In this study, *Escherichia coli*, *Salmonella* and enterococci were isolated from the surface of conventional broiler eggs, "*Kampung*" chicken eggs and carrying trays. The antimicrobial resistance profile of the isolates was determined and compared between conventional broiler eggs, "*Kampung*" eggs and on the carrying tray.

MATERIALS AND METHODS

Sample collection

Conventional and "*Kampung*" chicken eggs were collected randomly using systemic sampling method. Six eggs represented one sample. Three sets of chicken eggs were sampled from each of nine wet markets around Selangor and Kuala Lumpur. A total of 27 samples with a total of 243 conventional broiler eggs and "*Kampung*" chicken eggs were collected. Three egg carrying trays per wet market (n = 27) were also obtained randomly.

Swabbing

The entire surface of each egg was swabbed using sterile cotton swabs moisten with sterile buffered peptone water (BPW, Merck, Germany) and placed into 10 mL BPW as pre-enrichment (six eggs in total). The content was vortexed. Two milliliters of the culture was transferred into sterile Bijoux bottle and incubated aerobically at 42 °C for 24 h for selective pre-enrichment of enterococci and the remaining 8 mL of the culture was incubated aerobically at 37 °C for 24 h for general pre-enrichment.

Isolation of microbes

Isolation of *E. coli, Salmonella* and enterococci were performed as described in USFDA (2006) and Zhang *et al.* (2011). Prevalence of bacteria was calculated using the formula below:

$$\text{Prevalence}(\%) = \frac{\text{No.of positive sample}}{\text{Total number of sample}} \times 100\%$$

Escherichia coli

Serial dilution of the 8 mL culture for general pre-enrichment was performed using BPW. One hundred microliters of each dilution was spread-plated onto Chromocult® coliform agar ES (CCA, Merck, Germany) and incubated aerobically at 37 °C for 24 h. *E. coli* form dark blue colonies. Up to 15 random positive colonies were picked using Harrison's disk method to be sub-cultured to pure colonies. Gram stain, catalase and oxidase tests were performed and colonies which are Gram negative rods, catalase and oxidase negative are presumptive for *E. coli*.

Salmonella spp.

One milliliter and 0.1 mL of the 8 mL culture for general pre-enrichment were added into 9 mL of selenite-cysteine broth (SC, Merck, Germany) and 9.9 mL of Rappaport-Vassiliadis broth (RV, Merck, Germany) respectively. The SC culture was incubated aerobically at 37 °C for 24 to 48 h and the RV culture was incubated aerobically at 42 °C for 24 to 48 h. At 24 and 48 h of incubation, both SC and RV culture were streaked onto brilliant green agar (BGA, Oxoid, UK) and xylose lysine desoxycholate agar (XLD, Merck, Germany) and incubated at 37 °C for 24 h. *Salmonella* form pink colonies surrounded by pink medium on BGA and red colonies with black center on XLD. All presumptive isolates were stabbed into triple sugar iron slant (TSI, Merck, Germany) and incubated aerobically at 37 °C for 24 h. TSI slants with pink top, yellow butt and blackening of medium showed presumptive *Salmonella* isolates. The presumptive *Salmonella* isolates were streaked onto Tryptone Soy Agar (TSA, Merck, Germany) and incubated aerobically at 37 °C for 24 h for further confirmation. Absence of swarming motility showed presumptive *Salmonella* isolates. Gram stain, catalase and oxidase test were performed and colonies which are Gram negative rods, catalase and oxidase negative are presumptive for *Salmonella*.

Enterococci

Serial dilution of the 2 mL culture selective pre-enrichment for enterococci was performed using BPW. One hundred microliters was spread plated onto bile aesculin azide Agar (BAA, Merck, Germany) and incubated aerobically at 37 °C for 24 h. Enteroccocis form colonies with black halo. Up to 15 random positive colonies were picked using Harrison's disk method to be sub-cultured to pure colonies. Gram stain and catalase test were performed and colonies which are Gram positive cocci and catalase positive are presumptive for enterococci.

Antibiotic susceptibility test (AST)

AST was performed using disk diffusion method as described in the Clinical and Laboratory Standard Institute (CLSI) document M02-A10 (CLSI, 2009). The type of antimicrobials used was determined according to the presumptive identity of the isolate with reference to the CLSI M02-A10 as shown in Table 1. All antimicrobial disks were obtained from Oxoid, UK. Vancomycin resistance of enterococci was reconfirmed via agar diffusion method using 32 µg/mL of vancomycin (Nacalai Tesque, Japan) as described in the CLSI document M02-A10 (CLSI, 2009).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 1: The type antimicrobial disks used in determining the AST of *E. coli*, *Salmonella* spp. and enterococci isolates (amount in parentheses).

E. coli and *Salmonella* spp.	Enterococci
Nitrofurantoin (300 µg)	Ampicillin (10 µg)
Piperacillin-tazobactam (100/10 µg)	Erythromycin (15 µg)
Trimethoprim-sulphamethoxazole (25 µg)	Tetracycline (30 µg)
Chloramphenicol (30 µg)	Teicoplanin (30 µg)
Kanamycin (30 µg)	Chloramphenicol (30 µg)
Ciprofloxacin (5 µg)	Ciprofloxacin (5 µg)
Meropenem (10 µg)	Linezolid (30 µg)
Imipenem (10 µg)	Nitrofurantoin (300 µg)
Tetracycline (30 µg)	Quinupristin-dalfopristin (15 µg)
Gentamicin (10 µg)	Trimethoprim-sulphamethoxazole (25 µg)
Cefotaxime (30 µg)	Vancomycin (30 µg)
Ampicillin (10 µg)	
Amoxycillin-clavulanic acid (20/10 µg)	
Aztreonam (30 µg)	

RESULTS AND DISCUSSION

Prevalence of bacteria on the surface of conventional broiler eggs, "*Kampung*" chicken eggs and carrying tray

Prevalence of enterococci on the surface of both conventional and "*Kampung*" chicken eggs was found to be the highest compared to both *E. coli* and *Salmonella* spp. (Table 2). This high prevalence of enterococci from the surface of chicken eggs was expected as enterococci are part of the normal intestinal flora of chickens (Lauderdale *et al.*, 2007). High enterococci prevalence was also shown by Schwaiger *et al.* (2010) which was found to be 52.5%. The surface of the carrying trays was also tested and both *E. coli* and enterococci were isolated with 22.2% and 48.2% prevalence respectively. However, *Salmonella* spp. had the lowest prevalence on the surface of conventional and "*Kampung*" eggs. No *Salmonella* was found on the carrying trays. These results are in agreement with Singh *et al.* (2010). Their *Salmonella* prevalence level from the surface of conventional broiler eggs was 2.14%. In another study by Suresh *et al.* (2006), their *Salmonella* prevalence was found to be 5.9%. This showed that *Salmonella* prevalence is relatively low on the surface of chicken eggs. *E. coli* prevalence was 42% as shown by Akond *et al.* (2009) which were higher than the result of this study. Differences between prevalence rates of each study could be due to different handling methods and also geographical region.

Table 2: The prevalence of *Escherichia coli*, *Salmonella* spp. and enterococci on the surface of conventional broiler eggs, "*Kampung*" chicken eggs and carrying trays.

	Conventional (n=27)	"*Kampung*" (n=27)	Carrying Trays (n=27)
Escherichia coli	18.5	11.1	22.2
Salmonella spp.	3.7	3.7	0
Enterococci	63.0	59.3	48.2

Antimicrobial susceptibility

Escherichia coli

AST results for *E. coli*, *Salmonella* and enterococci are shown in Table 3 to Table 5. Both *E. coli* from conventional broiler eggs and "*Kampung*" chicken eggs showed high proportion being resistant to chloramphenicol, trimethoprim-sulphamethoxazole and tetracycline (Table 3). Isolation of these resistant *E. coli* could be due to widespread use of these antimicrobials as growth promoters and for prophylaxis in chickens. Usage of these antimicrobials had been previously reported in Europe (Castanon, 2007) but the antimicrobial usage in such context in Malaysia has never been formally stated and reported. It is encouraging that all the isolates (both commercial broiler eggs and "*Kampung*" chicken eggs) were still susceptible to meropenem, piperacillin-tazobactam and aztreonam as these are newer generation of antimicrobials used.

A larger proportion of *E. coli* isolated from the surface of conventional broiler eggs are resistant against nitrofurantoin and trimethoprim-sulphamethoxazole as compared to *E. coli* isolated from the surface of "*Kampung*" chicken eggs (17.5% and 57.9% respectively for conventional broiler eggs and 11.8% and 20.6% respectively for "*Kampung*" chicken eggs) (Table 3). However, *E. coli* isolated from the surface of "*Kampung*" chicken eggs showed larger proportion being resistant against kanamycin, ciprofloxacin, cefotaxime, ampicillin, amoxicillin-clavulanic acid and ceftazidime when compared to isolates from conventional broiler eggs. This was an interesting observation as "*Kampung*" chicken is mostly regarded as free-range and not fed with antimicrobials. More than 80% of the "*Kampung*" chickens

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

were raised under free-range conditions in Malaysia (Aini, 1990). It is unclear exactly how these antimicrobial resistant arised in "*Kampung*" chicken. It could be due to external introduction from the environment via faeces of other animals or cross contamination during handling and transportation. Interestingly, the isolates were found to be resistant to third generation cephalosporins such as cefotaxime and ceftazidime that are used in treating Gram negative infections in humans. This could be due to cross resistance where the exposure of another similar antimicrobial results in resistance towards antimicrobials of similar classes which in this case would be the production of extended spectrum β-lactamases (Livermore and Brown, 2001).

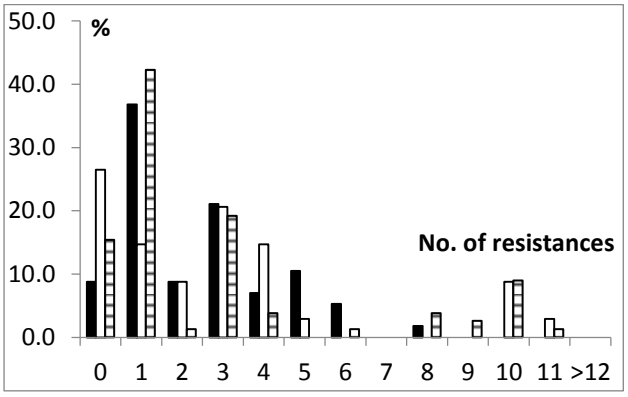
Table 3: Antimicrobial susceptibility pattern of *Escherichia coli* isolates.

Antibiotic	*Escherichia coli*		
	Conventional (n=57)	"*Kampung*" (n=34)	Tray (n=78)
Nitrofurantoin	17.5	11.8	14.1
Piperacillin-tazobactam	0	0	1.3
Trimethoprim-sulphamethoxazole	57.9	20.6	35.9
Chloramphenicol	49.1	50.0	39.7
Kanamycin	0	11.8	20.5
Ciprofloxacin	5.3	17.6	19.2
Meropenem	0	0	0
Imipenem	0	2.9	0
Tetracycline	71.9	73.5	57.7
Gentamicin	1.8	0	1.3
Cefotaxime	3.5	11.8	16.7
Ampicillin	24.6	58.8	41.0
Amoxycillin-clavulanic acid	8.8	14.7	18.0
Aztreonam	0	0	1.3
Ceftazidime	1.8	8.8	15.4

Number indicates the percentage of isolates being resistant towards that particular antimicrobial.

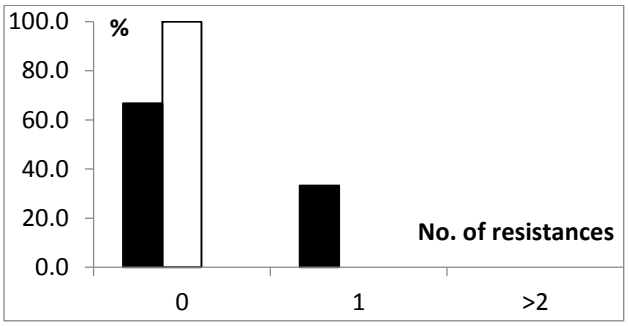
Escherichia coli isolated from carrying trays showed similar proportion of antimicrobial resistance as compared to conventional and "*Kampung*" chicken eggs. Carrying trays are commonly used to carry both conventional and "*Kampung*" chicken eggs, and contributes towards the cross contamination of different microbial populations. This also suggests the potential of carrying trays as a reservoir for ARB.

Looking at the multiple antimicrobial resistances of *E. coli*, it could be seen that there were isolates which were resistant to more than two antimicrobials which was frequently associated with resistant towards tetracycline and ampicillin. Isolates from "*Kampung*" chicken eggs were also found to be resistant to 10 (8.8%) and 11 (2.9%) different antimicrobials (Figure 1). Multiple resistant *E. coli* from chicken eggs had been reported by Schwaiger *et al.* (2008). This could be due to that "*Kampung*" chickens were exposed to antimicrobials during the raising process. However, as this study was done at the point of sale, further work on the farm-point detection has to be done for further confirmation.



■ : Conventional, □ : "*Kampung*", ▤ : Carrying trays

Figure 1: Percentage of *E. coli* isolates which exhibits multiple antimicrobial resistances.



■ : Conventional, □ : "*Kampung*". No Salmonella isolates were recovered from carrying trays.

Figure 2: Percentage of *Salmonella* isolates which exhibits multiple antimicrobial resistances.

Salmonella spp.

All *Salmonella* spp. isolated were susceptible to the 15 antimicrobials, with exception of one isolate being resistant only to tetracycline (Table 4 and Figure 2). This could be due to the low prevalence of *Salmonella* on the surface of eggs which resulted in small number of *Salmonella* isolates. As the numbers of *Salmonella* isolates were low, the AST results may not accurately reflect the true diversity of antimicrobial resistance pattern of *Salmonella* isolates on chicken eggs. However, other studies have shown that *Salmonella* from chickens was resistant against several common antimicrobials such as kanamycin and trimethoprim-sulphamethoxazole including multiple resistant *Salmonella* (Boonmar *et al.*, 1998; Cui *et al.*, 2005).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 4: Antimicrobial susceptibility pattern of *Salmonella* spp. isolates.

Antibiotic	*Salmonella* spp.		
	Conventional (n=3)	"*Kampung*" (n=5)	Tray (n=0)
Nitrofurantoin	0	0	0
Piperacillin-tazobactam	0	0	0
Trimethoprim-sulphamethoxazole	0	0	0
Chloramphenicol	0	0	0
Kanamycin	0	0	0
Ciprofloxacin	0	0	0
Meropenem	0	0	0
Imipenem	0	0	0
Tetracycline	33.3	0	0
Gentamicin	0	0	0
Cefotaxime	0	0	0
Ampicillin	0	0	0
Amoxycillin-clavulanic acid	0	0	0
Aztreonam	0	0	0
Ceftazidime	0	0	0

Number indicates the percentage of isolates being resistant towards that particular antimicrobial.

Enterococci

A large proportion of enterococci isolates was resistant to tetracycline, erythromycin and quinupristin-dalfopristin (>40% of isolates from conventional broiler eggs and "*Kampung*" chicken eggs) (Table 5). This again could be due to the overuse of antimicrobials in poultry farming as explained earlier. It is important to note that there was a larger proportion of isolates from "*Kampung*" chicken eggs being resistant to those antimicrobials including ampicillin and linezolid as compared to conventional broiler eggs.

Table 5: Antimicrobial susceptibility pattern of enterococci isolates.

	Enterococci		
	Conventional (n=103)	"*Kampung*" (n=90)	Tray (n=85)
Ampicillin	2.9	20.0	1.2
Erythromycin	43.7	63.3	35.3
Vancomycin	0	0	0
Tetracycline	70.9	92.2	62.4
Teicoplanin	2.9	17.8	2.4
Chloramphenicol	21.4	42.2	11.8
Ciprofloxacin	29.1	32.2	32.9
Linezolid	14.6	35.6	12.9
Nitrofurantoin	7.8	28.9	8.2
Quinupristin-dalfopristin	64.1	63.3	30.6
Levofloxacin	28.2	30.0	15.3

Number indicates the percentage of isolates being resistant towards that particular antimicrobial.

No vancomycin resistant enterococci were isolated in this study. However, there are enterococci resistant to linezolid and a higher proportion was actually "*Kampung*" chicken eggs isolates (14.6% conventional vs. 35.6% "*Kampung*"). Linezolid is a new class of antimicrobials from the class oxazolinidone usually used to treat very serious Gram positive infections resistant to other antimicrobials. Linezolid is also used to treat infections by vancomycin resistant enterococci. Similarly, there were also enterococci isolates resistant to quinopristin-dalfopristin (Table 5). Quinopristin-dalfopristin is a streptogramin antimicrobial used to combat antimicrobial resistant Gram positive infections, such as methicillin resistant *Staphylococcus aureus* and vancomycin resistant enterococci infections. It remains unclear how these isolates attained resistance to the linezolid and quinupristin-dalfopristin. However, this posed a great threat as the emergence of vancomycin resistant enterococci in poultry may also acquire linezolid and/or quinupristin-dalfopristin resistance too which will limit the already scarce options the healthcare industry have in treating such infections.

Isolates from carrying trays showed similar results in harbouring ARB. This again showed the function of carrying trays as a transport medium as well as an important site for cross contamination to happen as carrying trays are usually reused.

Enterococci isolates in this study also showed multiple resistances against antimicrobials especially against tetracycline and quinupristin-dalfopristin (Table 5 and Figure 3). Multiple resistant enterococci were often reported with association to poultry isolates (Radu *et al.*, 2001; Schwaiger *et al.*, 2010; Obeng *et al.*, 2012). However, in this study, there were enterococci isolates which were resistant to 11 out of 12 antimicrobials used and 17.8% of "*Kampung*" chicken isolates showed such resistances which was much higher than the 2.9% from isolates of conventional broiler eggs. The only antimicrobial these isolates were not resistant to was vancomycin.

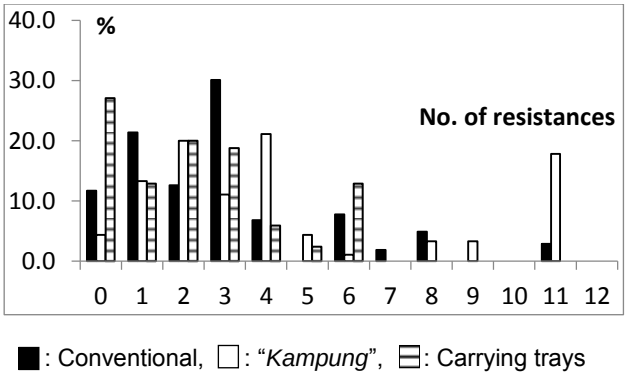


Figure 3: Percentage of enterococci isolates which exhibits multiple antimicrobial resistances.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

There were some limitations to this study. Firstly, samples were obtained from wet markets around Selangor, Malaysia and therefore this is a study on the point of sale. The presence of ARB could be from cross contamination in the production plant and human handling during transportation and sales. Therefore, this study cannot pinpoint the source of the ARB found. Secondly, the small sample size (nine wet markets) is insufficient to draw a conclusion on the presence of ARB in Malaysia. However, presence of ARB on chicken eggs is of concern especially to the consumers which highlights the importance of proper handling of chicken eggs. Risk of cross contamination on food (especially uncooked food) from chicken eggs can be minimized by washing hands and utensils.

Future work should concentrate on performing sampling on the farms, processing plant and markets to locate the source of ARB. However, this is the first study to our knowledge on ARB on conventional broiler eggs, "*Kampung*" chicken eggs and carrying trays in Selangor, Malaysia. With the presence of ARB, more control measures should be in place to combat the rise of antimicrobial resistance.

CONCLUSIONS

Even though this study was not able to locate the source of ARB prevalence, the presence of ARB on conventional broiler eggs, "*Kampung*" chicken eggs and carrying trays should be taken into serious consideration in regards to the sterility of fresh poultry produce in Malaysian markets. It is clear that the imminent rise of ARB poses a huge threat to human health and also the healthcare industry. Therefore, stricter control of antimicrobial usage in poultry farming can very well decrease or at least slow down the emergence of ARB.

ACKNOWLEDGEMENTS

We acknowledge Monash University Malaysia for the financial support.

REFERENCES

Aarestrup, F. M. (1999). Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. *International Journal of Antimicrobial Agents* **12(4), 279-285.**

Aini, I. (1990). Indigenous chicken production in South-east Asia. *World's Poultry Science Journal* **46(1), 51-57.**

Akond, M. A., Hassan, S. M. R., Alam, S. and Shirin, M. (2009). Antibiotic resistance of *Escherichia Coli* isolated from poultry and poultry environment of Bangladesh. *American Journal of Environmental Science* **5(1), 47-52.**

Boonmar, S., Bangtrakulnonth, A., Pornruangwong, S., Samosornsuk, S., Kaneko, K. and Ogawa, M. (1998). Significant increase in antibiotic resistance of *Salmonella* isolates from human beings and chicken meat in Thailand. *Veterinary Microbiology* **62(1), 73-80.**

Castanon, J. I. R. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science* **86(11), 2466-2471.**

CLSI. (2009). *CLSI document M02-A10*: CLSI.

Cui, S., Ge, B., Zheng, J. and Meng, J. (2005). Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. *Applied and Environmental Microbiology* **71(7), 4108-4111.**

Department of Veterinary Services, Malaysia. (2014). Malaysia: Per capita comsumption of livestock products 2004-2013. http://www.dvs.gov.my/documents/10157/a5a97b67-daf9-4662-b253-1dc18d88f7ec [Retrieved on 4/6/2014]

Fauci, A. S. and Marston, H. D. (2014). The perpetual challenge of antimicrobial resistance. *JAMA* **311(18), 1853-1854.**

Lauderdale, T., Shiau, Y., Wang, H., Lai, J., Huang, I. W., Chen, P., Chen, H., Lai, S., Liu, Y. and Ho, M. (2007). Effect of banning vancomycin analogue avoparcin on vancomycin-resistant enterococci in chicken farms in Taiwan. *Environmental Microbiology* **9(3), 819-823.**

Livermore, David M. and Brown, Derek, F. J. (2001). Detection of β-lactamase-mediated resistance. *Journal of Antimicrobial Chemotherapy* **48(suppl 1), 59-64.**

Musgrove, M. T., Jones, D. R., Northcutt, J. K., Cox, N. A., Harrison, M. A., Fedorka-Cray, P. J. and Ladely, S. R. (2006). Antimicrobial resistance in *Salmonella* and *Escherichia coli* isolated from commercial shell eggs. *Poultry Science* **85(9), 1665-1669.**

Obeng, A. S., Rickard, H., Ndi, O., Sexton, M. and Barton, M. (2012). Comparison of antimicrobial resistance patterns in enterococci from intensive and free range chickens in Australia. *Avian Pathology* **42(1), 45-54.**

Oh, B. T. (1987). Malaysia: economic importance. *Newcastle Disease in Poultry: A New Food Pellet Vaccine. Australian Centre for International Agricultural Research (ACIAR) Monograph* **(5), 83-85.**

Radu, S., Toosa, H., Rahim, R. A., Reezal, A., Ahmad, M., Hamid, A. N., Rusul, G. and Nishibuchi, M. (2001). Occurrence of the vanA and vanC2/C3 genes in *Enterococcus* species isolated from poultry sources in Malaysia. *Diagnostic Microbiology and Infectious Disease* **39(3), 145-153.**

Schwaiger, K., Schmied, E. M. V. and Bauer, J. (2008). Comparative analysis of antibiotic resistance characteristics of Gram-negative bacteria isolated from laying hens and eggs in conventional and organic keeping systems in Bavaria, Germany. *Zoonoses and Public Health* **55(7), 331-341.**

Schwaiger, K., Schmied, E. M. V. and Bauer, J. (2010). Comparative analysis on antibiotic resistance characteristics of *Listeria* spp. and *Enterococcus* spp. isolated from laying hens and eggs in conventional and organic keeping systems in Bavaria, Germany. *Zoonoses and Public Health* **57(3), 171-180.**

Singh, S., Yadav, A. S., Singh, S. M. and Bharti, P. (2010). Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Research International* **43(8), 2027-2030.**

Suresh, T., Hatha, A. A. M., Sreenivasan, D., Sangeetha, N. and Lashmanaperumalsamy, P. (2006). Prevalence and antimicrobial resistance of *Salmonella* Enteritidis and other salmonellas in the eggs and egg-storing trays from retails markets of Coimbatore, South India. *Food Microbiology* **23(3), 294-299.**

USFDA. (2006, 03/16/2013). FDA's bacteriological analytical manual. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm114664.htm [Retrieved on June 2012]

Wegener, H. C. (2003). Antibiotics in animal feed and their role in resistance development. *Current Opinion in Microbiology* **6(5), 439-445.**

Zhang, J., Massow, A., Stanley, M., Papariella, M., Chen, X., Kraft, B. and Ebner, P. (2011). Contamination rates and antimicrobial resistance in *Enterococcus* spp., *Escherichia coli*, and *Salmonella* isolated from “No antibiotics added”–labeled chicken products. *Foodborne Pathogens and Disease* **8(11), 1147-1152.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538