UNIVERSITI PUTRA MALAYSIA

DETECTION OF ANTIBIOTIC RESIDUES AND ISOLATION OF ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* FROM CHICKEN MEAT AND CHICKENS IN MALAYSIA

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By

TIN TIN MYAING

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

January 2003

DEDICATED TO MY PARENTS,

MY HUSBAND AND MY SONS

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

DETECTION OF ANTIBIOTIC RESIDUES AND ISOLATION OF ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* FROM CHICKEN MEAT AND CHICKENS IN MALAYSIA

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Chairman: Associate Professor Dr. Saleha Abdul Aziz

Faculty: Veterinary Medicine

Public health is intrinsically related to food animal production. Antibiotic residues in the food of animal origin and antibiotic resistant bacteria threaten human health. There is an increase in population and the demand for chicken meat in Malaysia is also increasing. Not much data is available on antibiotic residues and antibiotic resistant *E. coli* from chickens and foods of animal origin in Malaysia.

In this study, a total of 400 chicken meat samples were subjected to antibiotic residues screening tests. The prevalence of antibiotics residues in chicken meat was between 11.1% to 21.7%, using three microbial growth inhibition tests; namely, fast antimicrobial screening test (FAST), *Bacillus stearothermophilus* disc assay (BSDA) and a commercial test kit (TAT) with reference to four plate test (FPT). The test performances were evaluated on sensitivity, specificity, positive predictive value and negative predictive value. The sensitivity of these tests ranged

from 55.6 to 65% and specificity, from 82 to 90.6%. Kappa agreement was between 0.5 to 0.8. Based on the above performance parameters, as well as the cost, simplicity and incubation period, BSDA is a screening test of choice.

A total of 182 *E. coli* isolates from these chicken meat samples were found resistant to twelve antibiotics; vancomycin (99.4%), trimethoprim (98.9%), nalidixic acid (97.2%), tetracycline and cephradine (96.7%), ampicillin (94.5%), enrofloxacin (83.5%), erythromycin (82.9%), ciprofloxacin (81.3%), cefoperazone (80.2%), chloramphenicol (74.4%) and kanamycin (68.6%). Forty-six antibiotypes and nine antibiogroups were observed.

Escherichia coli isolated from antibiotic residues positive samples and antibiotic residues negative samples were tested for antibiotic susceptibility to twelve antibiotics. A higher percentage of antibiotic resistance was observed in *E. coli* isolates from antibiotic residues positive samples compared to those from antibiotic residues negative samples. 58.3% and 29.2% of *E. coli* isolates from antibiotic residues positive samples and 25% and 17.1% of *E. coli* isolates from antibiotic residues negative samples were resistant to 12 and 11 antibiotics, respectively.

Plasmid isolation was conducted in 132 of the *E. coli* isolated. Plasmid occurrence rate of 81.8% were observed in this study with high diversity of plasmids profiles among *E. coli* isolates from different sources. The number of plasmids ranged from 0 to 8 and the sizes of plasmids ranged from 1.2 MDa to 118.6 MDa. Forty-five different plasmid profiles were observed. No apparent correlation was found between the plasmid profiles of the strains and their antibiotic resistance patterns.

In another study, the occurrence of antibiotic resistant *E. coli* was determined in four flocks where in three flocks, chickens were given commercial feed containing antibiotics and in one flock the feed given were without antibiotics. *Escherichia coli* isolates from chickens given feed without antibiotics showed low resistance to all antibiotics even at one day old. The screening of antibiotic residues was done in 20 of these chickens at the age of 42 days old. The occurrence of antibiotic residues was 10% in chickens given feed containing antibiotics. It was observed that *E. coli* isolates from the antibiotic residues positive samples were also resistant to 12 antibiotics while those from antibiotic residues negative samples were resistant to 2-8 antibiotics. This observation requires further investigation.

Klebseilla spp., *Pseudomonas* spp. and *E. coli* were isolated from the feeds and water of the flock where chickens given feed without antibiotics. All these bacteria were resistant to 4-10 antibiotics. Antibiotic resistant *E. coli* was observed in day-old chicks without any selective pressure, such as even when no antibiotic was added to the ration. Thus, proliferation of antibiotic resistant *E. coli* is less dependent on the use of antibiotics/antimicrobials in poultry farms and most likely that chickens obtain antibiotic resistant *E. coli* from the environment.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat untuk ijazah Doktor Falsafah.

MENGESAN RESIDU ANTIBIOTIK DAN PENGASINGAN *ESCHERICHIA COLI* YANG TAHAN TERHADAP ANTIBIOTIK DARIPADA DAGING AYAM DAN AYAM DI MALAYSIA

Oleh

TIN TIN MYAING

Pengerusi: Profesor Madya Dr. Saleha Abdul Aziz

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Kesihatan awam berkait rapat dengan pengeluaran haiwan ternakan. Kehadiran patogen bawaan makanan dan juga dalam makanan mengancam kesihatan manusia. Terdapat peningkatan dalam populasi ayam dan permintaan daging ayam juga meningkat. Data yang berkaitan dengan residu antibiotik and *E. coli* tahan antibiotik dalam ayam adalah kurang di Malaysia.

Dalam kajian ini, sejumlah 400 sampel daging ayam telah diuji kehadiran residu antibiotik. Prevalens residu antibiotik dalam daging ayam adalah antara 11.1% hingga 21.7%, dengan menggunakan tiga ujian perencatan pertumbuhan mikrobia, iaitu "fast antimicrobial screening test (FAST), *Bacillus stearothermophilus* disc assay (BSDA) commercial test kit (TAT)" dengan dirujuk kepada "four plate test (FPT)". Prestasi ujian yang dinilai meliputi sensitiviti, spesifisiti, nilai ramalan positif dan nilai ramalan negatif. Sensitiviti ujian tersebut berkisar antara 55.6 hingga 65% dan spesifisiti daripada 82 hingga 90.6%.

Persetujuan kappa adalah antara 0.5 hingga 0.8. Berdasarkan parameter prestasi diatas, serta kos, kemudahan dan tempoh pengeraman, didapati BSDA adalah yang terpilih sebagai ujian penyaringan.

Sejumlah 182 isolat *E. coli* yang diasingkan daripada sampel daging ayam didapati tahan terhadap 12 jenis antibiotik- vankomisin (99.4%), trimetoprim (98.9%), asid nalidik (97.2%), tetrasiklin dan sefaridin (96.7%), ampisilin (94.5%), enrofloxasin (83.5%), eritromisin (82.9%), ciprofloxasin (81.3%), cefoparazon (80.2%), khlorampenikol (74.4%) dan kanamisin (68.6%). Terdapat 46 antibiotik dan sembilan kumpulan antibio.

Esherichia coli yang diasingkan daripada sampel yang positif dan yang negatif residu untuk diuji ketahanan terhadap dua belas jenis antibiotik. Keputusan ujian menujukkan peratusan *E. coli* tahan antibiotik yang tinggi daripada sampel yang positif untuk antibiotik berbanding dengan sampel yang negatif untuk residu antibiotik. Terdapat 58.3% dan 29.2% *E. coli* daripada sampel positif untuk residu antibiotik tahan terhadap 12 dan 11 antibiotik manakala 25% dan 17.1% *E. coli* daripada sampel negatif untuk residu antibiotik yang tahan terhadap 12 dan 11 antibiotik.

Pengasingan plasmid dilakukan ke atas 132 strain *E. coli* Kadar kehadiran plasmid adalah 81.8% dan terdapat kepelbagaian tinggi dalam profil plasmid di antara isolat *E. coli* yang diperoleh daripada pelbagai sumber Bilangan plasmid berkisar antara 0 hingga 8 dan saiz plasmid berada pada julat 1 2 MDa hingga 118 6 MDa. Empat puluh lima profil plasmid yang berbeza telah diperhatikan. Tiada korelasi dijumpai antara profil plasmid strain *E. coli* dengan pola ketahanan antibiotik.

Pada kajian selanjutnya, kewujudan *E. coli* tahan antibiotik ditentukan dalam empat kelompok ayam yang mana tiga kelompok ayam diberi makanan dengan penambahan antibiotik dan dalam satu kumpulan makanan tiada penambahan antibiotik. *E. coli* yang diasingkan daripada ayam yang diberi makanan tanpa penambahan antibiotik menunjukkan ketahanan yang rendah terhadap semua antibiotik, walaupun pada umur satu hari. Penyaringan residu antibiotik terhadap 20 sampel pada umur 42 hari telah dilakukan. Terdapat 10% positif untuk residu antibiotik. Juga didapati bahawa *E. coli* yang diasingkan daripada sampel positif untuk residu antibiotik didapati tahan terhadap 12 antibiotik, sedangkan ayam daripada sampel negatif untuk residu antibiotik negatif tahan terhadap 2-8 antibiotik. Pemerhatian ini perlu dikaji.

Klebsiella spp., Pseudomonas dan *E. coli* diasingkan dalam makanan dan air daripada ayam diberi makanan tanpa penambahan antibiotik. Semua bakteria ini didapati tahan terhadap 4-10 antibiotik. *Esherichia coli* tahan antibiotik didapati pada anak ayam umur satu hari, tanpa ada sebarang tekanan pemilihan seperti tiada antibiotik ditambah pada makanan. Oleh itu, perkembang biakan *E. coli* tahan antibiotik kurang bergantung pada penggunaan antibiotik dalam ladang ayam dan diperkirakan bahawa ayam peroleh *E. coli* tahan antibiotik daripada persekitaran.

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