Isolation and Identification of non-plasmid Multidrug Resistant E.coli from Poultry Wastes in Chittagong Region, Bangladesh

Muhammad Shahjalal Khan1, Naznin Akhtar2, Muhammad Ehteshamul Haque1, Abanti Barua3, Tasneem Chowdhury4 and RomelMullick1 and Abu Sayeed Mohammad Mahmud5*

1Department of Microbiology, University of Chittagong, Chittagong, Bangladesh
2Tissue Banking and Biomaterial Research Unit, Atomic Energy Research Establishment, Dhaka, Bangladesh
3Industrial Microbiology Research Division, Bangladesh Council of Scientific and Industrial Research, Chittagong, Bangladesh

Abstract

In two branches of poultry culture; small local ones and big industrial ones, tetracycline is a common antibiotic, which has been taken as a standard antibiotic in this study. 20 isolates were taken from big poultry farms like Agha Ltd and DENM Poultry. 10 isolates were taken from small local poultry farms like Rahat Poultry and Star Poultry. After collection of samples, total numbers of bacteria with and without tetracycline were counted. In both cases numerous bacterial growths were observed. The normal dose of tetracycline is 30 µg/ml which failed extremely to regulate high bacterial growth. Two dilutions (10^3 and 10^4) of sample 1, 2, 3 and 4 were taken and allowed to grow at different concentrations of tetracycline like 30,60 and 100 µg/ml, where bacterial growth was observed. High concentration of antibiotics for example, above 100 µg/ml may be harmful to humans and animals. After performing sensitivity test against other commonly used antibiotics in poultry, it was found that isolated tetracycline-resistant E. coli were 100% resistant to penicillin and erythromycin, 100 sensitive to imipenem, 93.34% resistant to tetracycline, 23.03% resistant to gentamycin and 53.33% resistant to chloramphenicol. These indicated the multidrug resistant property of isolates. Subsequent agarose gel electrophoresis showed no plasmid DNA band in the gel indicating non-existence of any bacterial plasmid and also proved that the observed resistance was chromosomal gene-mediated or at least not plasmid mediated.

Keywords: E. coli; Non-plasmid; Multi-drugs resistant; Poultry wastes

Introduction

The hope ushered by the discovery of antimicrobials has been tainted by the emergence of bacterial strains which are able to resist this therapeutics. Due to the use and misuse of antimicrobials in the last few decades, today's clinically important bacteria are not only single drug resistant but also multiple antibiotics resistant. These multidrug resistant bacteria are increasing public health hazard all over the world [1]. Antimicrobial susceptible bacteria are substantially less responsible for causing infections compared to the antimicrobial-resistant bacteria which actually cause infections leading to higher rates of morbidity and mortality [2]. The reason behind this high rate is that, these antimicrobial-resistant microorganisms are resistant to conventional treatment and can cause serious infection resulting in prolonged illness and greater death risk. Annually, about 440,000 new cases of Multidrug-resistant Tuberculosis (MDR-TB) are reported, causing no less than 150,000 deaths. In most malaria-endemic countries, widespread resistance to earlier generation antimalarial medicines, such as, chloroquine and sulfadoxine-pyrimethamine is seen [3]. Over the past decade, intercontinental spread of methicillin resistant Staphylococcus aureus [4] and penicillin resistant Streptococcus pneumonia [5], has progressed and has given rise to concerns about increasing resistance of Salmonella typhi [6]. It has proved the parochial approach to be a failure. Most antibiotic use is in two areas: in humans in the community, and in animals for growth promotion and prophylaxis. 20-50% human uses of antibiotics are unnecessary and 40-80% agricultural uses of antibiotics are highly questionable [7]. In the Southern Netherlands, almost 80 percent of raw chicken supplied by the grocery stores was found to be containing multidrug-resistant bacteria. When these germs were compared with the specimens collected from hospital patients, researchers found that, the predominant resistant genes were identical [8]. Antimicrobial resistance has been recognized by the World Health Organization (WHO) as a global problem that calls for global response. Keeping the problem in view, WHO issued the global principles for the containment of antimicrobial resistance in animal intended for food. After some recommended interventions, the WHO global strategy for the containment of antimicrobial resistance will hopefully enable local authorities to reduce the spread of resistance and slow down its emergence in diverse setting [9,10]. These guidelines recommend prudent use of antimicrobials and the establishment of surveillance programmes for antimicrobial consumption and resistance and further research as well.

Collection of sample

Samples were collected from four poultry farms
1) Agha Poultry Ltd, Roufabad, Hathajari, Chittagong
2) DENM Poultry Farm, North Fatehabad, Chittagong
3) Star Poultry, University of Chittagong campus area
4) Rahat Poultry, Mogoltuli, Chittagong

Sample-1 (Agha Poultry) and Sample-2 (DENM Poultry) are big commercial poultry farms. Sample-3 (Star Poultry) and Sample-4

*Corresponding author: Abu Sayeed Mohammad Mahmud, Industrial Microbiology Research Division, Bangladesh Council of Scientific and Industrial Research, Chittagong, Bangladesh, Tel: 01748700196; E-mail: sayedism@gmail.com

Received November 11, 2013; Accepted February 05, 2014; Published February 07, 2014

Citation: Khan MS, Akhtar N, Haque ME, Barua A, Chowdhury T, et al. (2014) Isolation and Identification of non-plasmid Multidrug Resistant E.coli from Poultry Wastes in Chittagong Region, Bangladesh. J Bacteriol Parasitol 5: 182. doi: 10.4172/2155-9597.1000182

Copyright: © 2014 Khan MS, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
(Rahat Poultry) are small local poultry farms. Samples collected from each of these poultries were a) raw feces from the inside of the farms, b) feces from the open fields beside the farms which were thrown away as waste products.

Transportation of the sample

After collection the samples were placed in a sterile ice-bag containing ice and were transported to the laboratory of Department of Microbiology, University of Chittagong.

Processing of samples

Samples were allowed to reach room temperature and then 10 gm of fresh fecal sample was mixed with 90ml of sterile normal saline and shook to form homogenous mixture. All samples were mixed by vigorous shaking.

Bacteriological count

All the bacteriological enumerations were carried out by pour plate method. In this case total number of bacteria and total number of resistant bacteria were counted [11].

Total Viable Count (TVC) with and without antibiotic

1 ml of from 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵ dilutions were poured into different sterile Petri plates. The Nutrient agar media (temperature 45°C) were poured into each petri-plate. After solidification, the plates were incubated at 37°C for 24 hours at inverted position. After 24 hours, plates with 30-300 bacterial colonies were counted.

There is a difference between total viable count without antibiotic and total viable count with antibiotic. In case of total viable count with antibiotic, antibiotics (30 µg/ml tetracycline) were mixed to the sterilized media (temperature 45°C) and were shaken well before plating.

Transferring single colonies from NA plates to EMB agar media

Single colonies were picked up randomly by sterile tooth picks from plates (with different concentration of Tetracycline). The colonies were then streaked on individual EMB agar containing 30 µg/ml tetracycline. The EMB plates were incubated at 37°C for 24 hours.

Transferring to broth culture

After incubation, presence of growth with green metallic sheen was observed on the EMB plates. One loopful from such growths was transferred randomly to 3 ml of nutrient broth (in 10ml screw cap tubes) containing 30 µg/ml tetracycline samples. 30 such growths (10 from sample-1, 10 from sample-2, 5 from sample-3 and 5 from sample-4) were transferred patching from all of the samples. The 30 culture tubes were then allowed for incubation at 37°C for 24 hours with loose capping and vigorous shaking of over 250 rpm.

Identification of the Isolated E. coli

Microscopic examination of morphology bacteria

The size, shape, arrangements and Gram reactions of the 24 hour bacterial cultures were observed in a microscopical field [12].

Conventional biochemical test for the identification of E. coli

Conventional Biochemical tests were carried out for the identification of E. coli. The tests are- Indole test, Methyl-red test, Voges-proskauer test, Citrate test and Motility test. Tetracycline (30 µg/ml) was present in all biochemical tests.

Antimicrobial susceptibility of the microorganisms to antibiotics

The standard disc diffusion method also known as Kirby Bauer method [13] was used for the in vitro determination of the sensitivity to the antimicrobial agents.

Antibiotic disc used

Antibiotics were chosen so that some of them were used during sample collection (e.g. tetracycline), some of them were continuously used in the poultry in addition to the running antibiotics, some of them were moderately or rarely used in poultry farms, some of them were not used (e.g. Imipenem and Gentamycin) (Table 1).

Plate preparation

A cotton swab was dipped in the suspension prepared in compliance with McFarland solution, excess fluid was removed by pressing and rotating the cotton bar inside the wall of the tube just above the fluid level. Then the swab was streaked over the surface of the Muller-Hinton agar medium to obtain uniform inoculums and some plates were also prepared by pour plate method.

Preparation and application of the disc to the plates

The discs were then placed on the surface of the seeded plates at appropriate spatial arrangement by using a sterile forceps. Then the plates were inoculated at 37°C for 24 hours and observed for the clear zone of inhibition.

Observation of clear zone of inhibition

After incubation the zones of complete inhibition were measured by using MD8 Scan Zone Reader.

Plasmid isolation

Plasmid extraction procedure was carried out following the protocol developed by ICDDR,B. The extracted plasmid was then isolated using a horizontal 1% Agarose Gel Electrophoresis technique.

Preparation of the sample

The pure cultures were transferred to 10 ml screw cap tubes containing 3 ml Luria Bertani (LB) broth with 30 µg/ml tetracycline. The broths were then incubated at 37°C with loose capping and vigorous shaking (200 rpm) for overnight. Then inoculums were transferred to another 3 ml LB broth at a 1:200 ml rate containing same concentration of tetracycline and incubated for 4-6 hours at 37°C with loose capping and vigorous shaking (200 rpm). After sufficient growth

| Antibiotics name | Symbol | Concentration of antibiotics applied |
|------------------|--------|--------------------------------------|
| Tetracycline     | T      | 30 µg                               |
| Gentamycin       | G      | 10 µg                               |
| Imipenem         | I      | 10µg                                |
| Chloramphenicol  | C      | 30 µg                               |
| Penicillin       | P      | 10 µg                               |
| Erythromycin     | E      | 15 µg                               |

Table 1: Six antibiotics that were tested against the E. coli isolates using standard disc.
with slight turbidity the incubation stopped and the cells were prepared for extraction.

**Plasmid extraction**

1.0ml of overnight culture was taken in an Eppendorf’s tube (1.5ml) and cells were collected by centrifugation for 7 minutes at 12,000 rpm. The supernatant was discarded and the pellet was thoroughly suspended in 100 μl of solution I and the solution was kept at room temperature (32°C) for 10 min.

Then 200 μl of solution II (lysis solution) was added and mixed gently by inverting the tube for a few times. After that 150 μl of ice-cold solution III (neutralizing solution) was added and mixed vigorously by vortexing for a few seconds. The tubes were kept on ice for 5 minutes. The mixture was then centrifuged at 12,000 rpm for 15 minutes to pellet the chromosomal DNA. The clear supernatant (approximately 95% ethanol (800 μl) were added in each tube and vortexed for a few seconds to mix well. It was then kept in room temperature for about 20 minutes for DNA precipitation. The precipitated DNA was collected by centrifugation for 15 minutes at 12,000 rpm. The supernatant was discarded and the pellet was dried in a drier at 45°C for 20 minutes. Finally the dried DNA was dissolved in 30 μl TE buffer and kept at 4°C.

**Separation of plasmid DNA by agarose gel electrophoresis**

Plasmid DNA was separated by horizontal electrophoresis in 1% agarose slab gels in a Tris-Acetate EDTA (TAE) buffer at room temperature at 80 volt (50 mA) for 3 h, briefly, 30 μl of plasmid DNA solution was mixed with 3 μl of tracking dye (Appendix) and was loaded into the individual well of the gel. The gel (5mm thick) was stained with 0.5 µg/ml of ethydium bromide for 15 min at room temperature and then distilled with distilled water for 10 min.

**Results**

**Bacterial enumerations**

**Total count of bacteria with and without antibiotics (tetracycline):**

Total number of bacteria (without antibiotics) in the samples collected from Agha Ltd, Demn poultry (big commercial poultries) and Rahat poultry, Star poultry (small local poultries) were counted and the results were given in Table 2 and presented in the Figure 1. The numbers of total bacteria differ from sample to sample. Total average count of the fecal wastes collected from a small local poultry -Rahat poultry (32°C) for 10 min.

| Sample | Dilution | No. of Colony | No. of microorganisms/ml | Average |
|--------|----------|---------------|--------------------------|---------|
| Sample – 1 AGHA | 10² 10⁴ 10⁶ | Too Numerous 93 103 10⁴ | × 830000 330000000 | 11276666.67 |
| Sample – 2 DEMN | 10² 10⁴ 10⁶ | Too Numerous 91 100 10⁴ | 910000 47000000 | 15970000 |
| Sample – 3 STAR | 10² 10⁴ 10⁶ | Too Numerous 142 92 10⁴ | 1620000 92000000 | 31140000 |
| Sample – 4 RAHAT | 10² 10⁴ 10⁶ | Too Numerous 153 102 10⁴ | 1530000 102000000 | 34510000 |

**Table 2: Total Count of Bacteria without Antibiotics (tetracycline).**

**Total resistant bacterial count in the samples 1,2,3,4.**

The second highest count (31140000/ml) was also from a small local poultry -Rahat poultry (sample-3). Total average count of sample- 1(big commercial poultry-Agha Ltd.) and sample-2 (big commercial poultry-Demn poultry) were 11276667/ml and 15970000/ml respectively. The highest count (from small local poulty Rahat poultry) was 3.07 times greater than that of lowest count (from a big commercial poultry-Agha Poultry). In total bacterial count with antibiotics (tetracycline) of same sample (sample-4, Star poultry, small local poultry) showed commercial poultry-Agha Ltd., sample-2 (big commercial poultry-Demn poultry) were 11276667/ml and 15970000/ml respectively. The highest count (from small local poultry Rahat poultry) was 3.07 times greater than that of lowest count (from a big commercial poultry-Agha Poultry). In total bacterial count with antibiotics (tetracycline) of same sample (sample-4, Star poultry, small local poultry) showed...
highest bacterial count (3980000) and sample-1 (Agha, Big commercial poultry farm) exhibited the lowest bacterial count (8000/ml). The highest one was 497.5 times greater than lowest one. It is important to note that the amount of tetracycline resistant bacteria in local poultries (sample-1 and 2) is much higher than that of sample 3 and 4 (Figure 2 and Table 3-5).

### Isolation and identification of tetracycline resistant *E. coli*

A total of 30 individual colonies of *E. coli* were isolated and were characterized according to the biochemical properties. Following figures show the characteristic metallic sheen on EMB agar plate of the isolates and the biochemical properties (Figures 3-7).

### Antimicrobial Susceptibility

#### Antimicrobial susceptibility patterns of the isolates

Six antibiotics were tested against the *E. coli* isolates using standard disc.

1. Tetracycline (T, 30 µg)
2. Gentamycin (G, 10 µg)
3. Imipenem (I, 10 µg)
4. Chloramphenicol (C, 30 µg)
5. Penicillin (P, 10 µg)
6. Erythromycin (E, 15 µg)

After performing sensitivity test it was found that isolated tetracycline-resistant *E. coli* were 100% resistant to penicillin and erythromycin, 100% sensitive to imipenem, 93.34% resistant to

---

**Table 5:** Bacterial count (dilution $10^4$) with different concentration of tetracycline.

| Sample | Dilution | Concentration of tetracycline ($\mu$/ml) | No. of Colony | No. of Bacteria/ml |
|--------|----------|-----------------------------------------|--------------|--------------------|
| 1      | $10^4$   | 30                                      | 33           | $33 \times 10^4$   |
|        | $10^4$   | 60                                      | 11           | $11 \times 10^4$   |
|        | $10^4$   | 100                                     | 3            | $3 \times 10^4$    |
| 2      | $10^4$   | 30                                      | 53           | $53 \times 10^4$   |
|        | $10^4$   | 60                                      | 24           | $24 \times 10^4$   |
|        | $10^4$   | 100                                     | 6            | $6 \times 10^4$    |
| 3      | $10^4$   | 30                                      | 91           | $91 \times 10^4$   |
|        | $10^4$   | 60                                      | 33           | $33 \times 10^4$   |
|        | $10^4$   | 100                                     | 11           | $11 \times 10^4$   |
| 4      | $10^4$   | 30                                      | 30           | $102 \times 10^4$  |
|        | $10^4$   | 60                                      | 60           | $43 \times 10^4$   |
|        | $10^4$   | 100                                     | 100          | $13 \times 10^4$   |

**Figure 3:** Tetracycline (30 µg/ml) Resistant *E. coli* on EMB.

**Figure 4:** Citrate Test.

**Figure 5:** VP Test.

**Figure 6:** MR Test.
Other most common type of antibiotics like penicillin, impenem, chloramphenicol, erythromycin and gentamycin were used to observe multi-drug resistance. 20 isolates were taken from big poultry farms like Agha Ltd and Denn Poultry. 10 isolates were taken from small local poultry farms like Rahat Poultry and Star Poultry. After collection of sample, total number of bacteria with and without antibiotics was counted. In both cases numerous bacterial growths were observed. The normal dose of tetracycline is 30 µg/ml which failed extremely to regulate high bacterial growth. The samples labeled with number 1, 2, 3, and 4 were allowed to grow at different concentrations of tetracycline (30, 60 and 100 µg /ml) where bacterial growth was observed. After performing sensitivity test against other commonly used antibiotics in poultry, it was found that isolated tetracycline-resistant E. coli were 100% resistant to penicillin and erythromycin, 100% sensitive to imipenem, 93.34% resistant to tetracycline, 23.03% resistant to gentamycin and 53.33% resistant to chloramphenicol. These indicated the multidrug resistant property of isolates. A statistically significant [12] Increase in antibiotic resistance was observed among outpatient and inpatient isolates of E. coli. Subsequent Agarose Gel Electrophoresis showed no plasmid-DNA band in the gel indicating non-existence of any bacterial plasmid proving that observed resistance was chromosomal gene-mediated or at least not plasmid mediated. Observation of the multi-drug resistance character of poultry fecal isolates is a terrible warning to natural environment [15,16]. The poultry feces used by farmers as manure can poison the crop. Poultry feces is also used as a common feed for fish, so these fish containing multi-drug resistant culture of bacteria like E. coli can be deadly for humans and animals, that is, for any fish eaters. Antibiotics resistance in bacteria associate with food animals and the use of antibiotics for agricultural purposes, particularly for growth enhancement, contributed to the increased prevalence of antibiotic-resistant bacteria. Our finding proposed that proper antibiotics should be used at proper doses to avoid the development of multi-drug resistant bacteria. To perform these, skilled workers...
| Antibiotics | Concentration (µg/ml) | Zone of inhibition (mm) | Remarks |
|-------------|-----------------------|-------------------------|---------|
| Penicillin  | 10 µg                 | 2                       | R       |
| Gentamicin  | 10 µg                 | 14                      | R       |
| Erythromycin| 15 µg                 | 0                       | R       |
| Tetracycline| 30 µg                 | 0                       | R       |
| Chloramphenicol | 30 µg         | 0                       | R       |
| Imipenem   | 10 µg                 | 29                      | S       |
| Penicillin  | 10 µg                 | 0                       | R       |
| Gentamicin  | 10 µg                 | 18                      | R       |
| Erythromycin| 15 µg                 | 9                       | R       |
| Tetracycline| 30 µg                 | 10                      | R       |
| Chloramphenicol | 30 µg         | 20                      | S       |
| Imipenem   | 10 µg                 | 41                      | S       |
| Penicillin  | 10 µg                 | 0                       | R       |
| Gentamicin  | 10 µg                 | 15                      | R       |
| Erythromycin| 15 µg                 | 6                       | R       |
| Tetracycline| 30 µg                 | 11                      | R       |
| Chloramphenicol | 30 µg         | 0                       | R       |
| Imipenem   | 10 µg                 | 32                      | S       |

Antibiotics Concentration (µg/ml) Zone of inhibition (mm) Remarks
---
Penicillin 10µg 0 R
Gentamicin 10 µg 8 R
Erythromycin 15 µg 2 R
Tetracycline 30 µg 16 I
Chloramphenicol 30 µg 0 R
Imipenem 10 µg 22 S
Penicillin 10µg 0 R
Gentamicin 10 µg 24 S
Erythromycin 15 µg 0 R
Tetracycline 30 µg 0 R
Chloramphenicol 30 µg 3 R
Imipenem 10 µg 36 S
Penicillin 10µg 0 R
Gentamicin 10 µg 27 S
Erythromycin 15 µg 6 R
Tetracycline 30 µg 11 R
Chloramphenicol 30 µg 9 R
Imipenem 10 µg 29 S
Penicillin 10µg 0 R
Gentamicin 10 µg 19 S
Erythromycin 15 µg 3 R
Tetracycline 30 µg 9 R
Chloramphenicol 30 µg 0 R
Imipenem 10 µg 27 S
Penicillin 10µg 5 R
Gentamicin 10 µg 20 S
Erythromycin 15 µg 0 R
Tetracycline 30 µg 13 R
Chloramphenicol 30 µg 19 S
Imipenem 10 µg 41 S
Penicillin 10µg 0 R
Gentamicin 10 µg 16 S
Erythromycin 15 µg 9 R
Tetracycline 30 µg 6 R
Chloramphenicol 30 µg 0 R
Imipenem 10 µg 24 S
Penicillin 10µg 0 R
Gentamicin 10 µg 24 S
Erythromycin 15 µg 3 R
Tetracycline 30 µg 22 S
Chloramphenicol 30 µg 19 S
Imipenem 10 µg 24 S
Penicillin 10µg 0 R
Gentamicin 10 µg 23 S
Erythromycin 15 µg 9 R
Tetracycline 30 µg 0 R
Chloramphenicol 30 µg 9 R
Imipenem 10 µg 26 S
Penicillin 10µg 0 R
Gentamicin 10 µg 24 S
Erythromycin 15 µg 11 R
Tetracycline 30 µg 0 R
Chloramphenicol 30 µg 25 S
Imipenem 10 µg 22 S
with sound knowledge of antibiotics are essential. For personal-small poultry farm, the related individuals should take training on the use of antibiotics. The waste of poultry should be disposed off properly to avoid the spread of drug-resistant bacteria in the environment.

References

1. Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10: S122-129.

2. D’Agata EM, Dupont-Rouzyel M, Magal P, Olivier D, Ruan S (2008) The impact of different antibiotic regimens on the emergence of antimicrobial-resistant bacteria. PLoS One 3: e4036.

3. Antimicrobial resistance (2013) Media centre. World Health Organisation.

4. Ayliffe GA (1997) The progressive intercontinental spread of meticillin-resistant Staphylococcus aureus. Clin Infect Dis 24 Suppl 1: S74-79.

5. Hermans PW, Sluitjer M, Dejsirilert S, Lemmens N, Elzenaar K, et al. (1997) Molecular epidemiology of drug-resistant pneumococci: towards an international approach. Microb Drug Resist 3: 243-251.

6. Rowe B, Ward LR, Threlfall EJ (1997) Multidrug-resistant Salmonella typhi: a worldwide epidemic. Clin Infect Dis 24 Suppl 1: S106-109.

7. Wise R, Hart T, Cars O, Stremlens M, Helmut R, et al. (1998) Antimicrobial resistance. Is a major threat to public health. BMJ 317: 809-810.

8. Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, et al. (2011) Extended-spectrum β-lactamase genes of Escherichia coli in chicken meat and humans, The Netherlands. Emerg Infect Dis 17: 1216-1222.

9. WHO (2000) Global Principles for the Containment of Antimicrobial Resistance in animals intended for food. World Health Organisation, Geneva, Switzerland.

10. Oliver SP, Murinda SE, Jayarao BM (2011) Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: a comprehensive review. Foodborne Pathog Dis 8: 337-355.

11. Al-Tawfiq J A (2006) Increasing Antibiotic Resistance Among Isolates of Escherichia coli Recovered From Inpatients and Outpatients in a Saudi Arabian Hospital. Infect. Control. Hosp. Epidemiol 27: 748-753.

12. Gyles CL (2008) Antimicrobial resistance in selected bacteria from poultry. Anim Health Res Rev 9: 149-158.

13. Miles TD, McLaughlin W, Brown PD (2006) Antimicrobial resistance of Escherichia coli isolates from broiler chickens and humans. BMC Vet Res 2: 7.

14. Mathew AG, Cissell R, Liamthong S (2007) Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathog Dis 4: 115-133.

15. van den Bogaard AE, Stobberingh EE (2000) Global Principles for the Containment of Antimicrobial Resistance in animals intended for food. World Health Organisation, Geneva, Switzerland.

16. Mathew AG, Cissell R, Liamthong S (2007) Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathog Dis 4: 115-133.

17. van den Bogaard AE, Stobberingh EE (2000) Global Principles for the Containment of Antimicrobial Resistance in animals intended for food. World Health Organisation, Geneva, Switzerland.

18. WHO (2000) Global Principles for the Containment of Antimicrobial Resistance in animals intended for food. World Health Organisation, Geneva, Switzerland.

19. Mathew AG, Cissell R, Liamthong S (2007) Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathog Dis 4: 115-133.

20. van den Bogaard AE, Stobberingh EE (2000) Global Principles for the Containment of Antimicrobial Resistance in animals intended for food. World Health Organisation, Geneva, Switzerland.

21. Mathew AG, Cissell R, Liamthong S (2007) Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathog Dis 4: 115-133.

22. van den Bogaard AE, Stobberingh EE (2000) Global Principles for the Containment of Antimicrobial Resistance in animals intended for food. World Health Organisation, Geneva, Switzerland.

23. Mathew AG, Cissell R, Liamthong S (2007) Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathog Dis 4: 115-133.

24. van den Bogaard AE, Stobberingh EE (2000) Global Principles for the Containment of Antimicrobial Resistance in animals intended for food. World Health Organisation, Geneva, Switzerland.

25. Mathew AG, Cissell R, Liamthong S (2007) Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathog Dis 4: 115-133.

26. van den Bogaard AE, Stobberingh EE (2000) Global Principles for the Containment of Antimicrobial Resistance in animals intended for food. World Health Organisation, Geneva, Switzerland.

27. Mathew AG, Cissell R, Liamthong S (2007) Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathog Dis 4: 115-133.

28. van den Bogaard AE, Stobberingh EE (2000) Global Principles for the Containment of Antimicrobial Resistance in animals intended for food. World Health Organisation, Geneva, Switzerland.