

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

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Prevalence of Antibiotic Resistance among *Escherichia coli* from Different Sources in Malaysia

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Abstract: Antimicrobial agent resistance has been recognized as an emerging worldwide problem in both human and animals, antimicrobial agent use is considered the most important factor for the emergence, selection and dissemination of antimicrobial agent-resistant bacteria, intrinsically either acquires the resistance gene from other bacterial environment or development of pumping out mechanism. The aim of this study was to generate baseline data on the prevalence of antimicrobial resistance in *Escherichia coli* isolates from different sources. Seventy *E. coli* isolates from humans and environments were tested for susceptibility to 10 antimicrobial agents by diffusion method. Resistance was found in 61.2% of the isolates. The most prevalent resistances were to kanamycin and tetracycline (81.4%), followed by chloramphenicol (75.7%) and gentamicin, (74.3%). The low prevalent were to cefetoxin (44.3%), norofluoxacin (27.1%) and ciprofluoxacin (24.3%). This study showed the distribution of antimicrobial agent resistance in *E. coli* isolates from a variety of sources and analysis of such patterns of resistance may prove to be useful beyond simple description. Regarding to the concern of water quality and environmental contamination by human and agricultural waster have increased, it has become increasingly important to develop low-cost screening tools that can be used to identify the most probable source of contamination.

Key words: *Escherichia coli*, antibiotic resistance, Malaysia

Introduction

Antimicrobial agents are the most important in the treatment of bacterial infections and thus the worldwide increase in antibiotic-resistant bacteria is of major concern and antibiotic use is suggested to be a major risk factor for the development of antibiotic resistance (Neu, 1992; Witte, 1998), along with other factors like the use of antibiotics in animals and humans for control of bacterial infections and may be incorporated into commercial livestock and poultry feed at subtherapeutic doses for growth promotion. In addition, it may contribute to antimicrobial agent resistance in humans acquired through the human food chain (Witte, 1998; Barton, 1998), poor hygienic conditions and overcrowded living conditions. On the other hand, concerns have been raised about the contamination of surface water with resistant bacteria from livestock operations and human septage. Hagedorn *et al.* (1999) found that livestock contributed more than humans to fecal coliform contamination of surface water and livestock access to surface water parallel with increase the fecal coliform levels by an average of 94%. Antibiotic-resistant bacteria have been isolated from a variety of sources, such as hospitals, domestic sewage, drinking water, rivers and lakes (Kasper and Burgess, 1990; McKeon *et al.*, 1995;

Boon and Cattnach, 1999). The development of resistance is that bacteria in the guts of humans and animals are subjected to different types, concentrations and frequencies of antimicrobial agents. *Escherichia coli* is the main aerobic commensally bacterial species in the gut flora, unlike other microorganism *E. coli* able to acquires resistance easily and is commonly found in many different animal species, therefore it is a good bioindicator model for surveillance studies of Antimicrobial Resistance (AR) (Von Baum and Marre, 2005). Several studies have addressed the prevalence of resistant *E. coli* isolated from the stool (Bonten *et al.*, 1992), the urinary tract (Barrett *et al.*, 2000), or blood (Fluit *et al.*, 2000). However, these studies have varied widely in methodology, in sources and type of population. The main objective of this study, the prevalence and distribution of resistance to antimicrobial drugs among *E.coli* from different sources was investigated.

Materials and Methods

Sources of sampling: Seventy isolates of *E. coli* were collected from five different sources (20 clinical, 10 marine, 10 river, 10 food and 20 animal).

Isolation of *E. coli* from water samples: The membrane filtration method was carried out according to instruction by the United States Environmental Protection Agency (United States Environmental Protection Agency, 1986) to isolate *E. coli* from water samples. In this procedure, water samples were filtered through a sterile, white, grid-marked, 47-mm-diameter membrane (pore size, $0.45 \pm 0.02 \mu\text{m}$), which retained bacteria. After filtration, the membrane containing the bacteria was placed on a selective differential medium (Chromocult Coliform Agar) (CCA, Merck, Germany) (United States Environmental Protection Agency, 1986; Dufour *et al.*, 1981) and incubated at 35°C for 2 h to resuscitate the injured or stressed bacteria and then at 44°C for 22 h. The filter was transferred to CCA agar. After overnight incubation *E. coli* colonies turned pink or purple on these media.

Identification of *E. coli* from various sources: Standard methods were used for the enrichment, isolation, identification and biochemical confirmation of *E. coli* isolates (Clesceri *et al.*, 1998). Upon arrival at the laboratory, culture samples, colonies picked from CCA (surface water samples) were placed in tubes with Luria Broth LB, (Oxoid Ltd., Basingstoke, United Kingdom) and incubated at 35°C for 24 h. Approximately $10 \mu\text{L}$ of the turbid broth was streaked onto MacConkey agar and incubated at 35°C for 18 to 20 h. The MacConkey agar plate was examined for red colonies that precipitated bile and had a dark red center. Bacteria from the broth were transferred into tubes for biochemical confirmation by indole, methyl red, voges-proskauer and simmons citrate tests on API E20 (Biomeru. Au). Only the bacterial isolates that were confirmed to be *E. coli* based on the results of the biochemical tests were selected for antimicrobial agent sensitivity testing. Confirmed isolates were inoculated into new normal saline tubes and incubated until the turbidity was 0.5 McFarland standards (approximately 2 to 3 h).

Antimicrobial agent susceptibility testing: Once a single *E. coli* isolate was isolated and identified from each sample collected, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial agent sensitivity profiles of the *E. coli* isolates (National Committee for Clinical Laboratory Standards, 1997; National Committee for Clinical Laboratory Standards, 1999) for 10 antimicrobial agents ampicillin (10 g), Chloramphenicol (30 μg), sulfmethaxol-trimethoprim (5 μg), tetracycline (5 μg), gentamicin (10 μg), kanamycin (30 μg), cefotaxime (30 μg), norfloxacin (10 μg), ciprofloxacin (10 μg), nalidixic acid (5 μg). These antimicrobial agents were chosen based on their importance in treating human or animal *E. coli* infections and their use as feed additives to promote growth in animals and because of their ability

to provide diversity for representation of different antimicrobial agent classes (Krumperman, 1983). The broth was diluted in normal saline solution to a density of 0.5 McFarland turbidity standards and Diluted broths were poured onto Mueller-Hinton agar plates. After air, drying, antibiotic discs were placed 30 mm apart and 10 mm away from the edge of the plate. Plates were incubated at 37°C for 18 to 20 h. The zone of inhibition and resistance was measured, recorded and interpreted according to the recommendation of the disc manufacturer. The breakpoints used to categorize isolates as resistant or not resistant to each antimicrobial agent were those recommended by the (National Committee for Clinical Laboratory Standards, 1999). *E. coli* ATCC 25922 (American Type Culture Collection) was used for quality control.

Data analysis: Associations between different sources and antimicrobial agent resistance (resistant or not resistant) were expressed as ANOVA methods was used to test for significant differences between species groups performed using SPSS, version 14.5 software. (SPSS, Inc., Chicago)

Results

All of the isolated *E. coli* showed resistance to one or more antibiotics and a pattern of multiple drug resistance was observed among seventy isolates was processed, from which (61.2%) *E. coli* isolates were retrieved for antimicrobial agent resistance profiling. The highest rates of resistance were against, tetracycline and kanamycin were the most commonly reported antimicrobial agent (81.4% overall), followed by chloramphenicol (75.7%), gentamicin (74.3%), ampicillin (72.9%). This study showed the distribution of antimicrobial agent resistance in *E. coli* isolates from a variety of sources and analysis of such patterns of resistance may prove to be useful beyond simple description. While the lowest levels of resistance were seen with ciprofloxacin and norfloxacin respectively.

Discussion

A phenotypic approach for strain identification and strain diversity determination was include, as the approach remains an accepted methods and is compared for the genotypic approach developed in this study. The strain diversity was determined through the antibiotic susceptibility disc diffusion assay of *E. coli* isolates from different sources. The level of antibiotic-resistant *E. coli* observed by (Kaspar *et al.*, 1990) was lower, at 26-46%, in urban water and rural water, (Ogan and Nwiika, 1993) made similar observations. (McKeon *et al.*, 1995) who observed an 87% resistance to at least one antibiotic for the non-faecal and faecal coliforms isolated from groundwater, respectively while, (Parveen *et al.*, 1997)

observed that more than 80% of the *E. coli* strains isolated from municipal waste and river and estuarine water displayed antibiotic resistance this was higher than our findings. Our study is lower than that reported by (McKeon *et al.*, 1995) who observed an 87% resistance to at least one antibiotic for the non-faecal and faecal coliforms isolated from groundwater and (Parveen *et al.*, 1997) who observed that more than 80% of the *E. coli* strains isolated from municipal waste and river and estuarine water displayed antibiotic resistance. (O'Brien, 1987) reported an incidence for sulfonamide resistance of between about 85%, ampicillin resistance 72%, tetracycline resistance 60% and trimethoprim resistance between 4% while in our study the highest number of AR bacteria was observed for (tetracycline 81.4%, kanamycin 81.4%, chloramphenicol 75.7%, gentamicin 74.3% and ampicillin 72.9%). The total number of isolates showing resistance (tetracycline 81.4%, kanamycin 81.4%), were, as expected, abundant in all the isolates, respectively. Resistance to tetracycline may be conserved in bacterial populations over time, regardless of selection pressure, which might result in an overall increase in resistance over time. The important factors that attributed to prevalence of antibiotic resistance in a population is the antibiotic uses as the major factor. While the another important factor contributing to the development of antibiotic resistance is the dissemination of resistant bacteria or resistance genes from a resistant donor to a susceptible host, which subsequently can either become colonized by these bacteria and/or these bacteria might transfer their resistance genes to bacteria belonging to the intestinal flora of the new host during their passage through the intestinal tract. Additionally, the antibiotic resistance for one and more drugs exhibited by *E. coli* in this study could have been the result of independent, simultaneous development of resistance to different agents or could have been the result of co-selection of resistance determinants. (Rankin *et al.*, 2002) and (Winokur *et al.*, 2001) found that in *Salmonella*, cephalosporin resistance is co-transferred with additional resistance markers for chloramphenicol, sulfamethoxazole - trimethoprim and tetracycline and that transfer of the plasmid containing the gene, designated CMY-2, between *Salmonella* and *E. coli* isolates from food animals and humans. Therefore, given this situation, exposing a bacterial population to one antimicrobial agent may result in resistance to other agents without any prior exposure. The presence of tetracycline, kanamycin and chloramphenicol resistance in *E. coli* from a variety of sources agrees with findings of other studies on the antimicrobial agent resistance of *E. coli* from a variety of different sources throughout the world (Erskine *et al.*, 2002; Klein and Bulte, 2003; Schlegelova *et al.*, 2002; Schroeder *et al.*, 2002). The patterns of resistance to the

antimicrobial agents may be due to widespread and lengthy use of tetracycline and other type of antibiotics. Since kanamycin 85%, tetracycline 80% and Chloramphenicol 75% are naturally derived compounds, bacteria can be exposed to these agents in nature and outside any human use for disease treatment, for prophylaxis, or for livestock growth promotion. The sulfamethoxazole and trimethoprim (62.9%), the use of this agent was restricted for food animals in the 1980s after a potential threat to human health from residues in foods of animal origin and they are currently approved for use in treating calf scours. These drugs are broad-spectrum antimicrobial agents with a history of more than 50 years of veterinary use. Although resistance to sulfonamides is widespread and cross-resistance between sulfonamides is complete, they are considered to be of limited use in treatment of ruminants (Prescott *et al.*, 2000). Sulfonamide use for growth promotion in swine is controversial because of persistent problems with volatile residues in swine carcasses (Prescott *et al.*, 2000; Food Safety and Inspection Service, 2002). The lowest levels of resistance (increased susceptibility) found in this study were the levels of sensitive to the quinolone (ciprofloxacin 24.3% and norfloxacin 27.1%) show high susceptibility of *E. coli* to this group respectively except the first generation nalidixic acid (68.6%). Not surprisingly, it is believed that reduced susceptibility will lead to increased incidence in resistance (McDonald *et al.*, 2001). The use of fluoroquinolones (ofloxacin and nalidixic acid) has been restricted since the 1990s, after the rapid emergence of resistance to fluoroquinolones after the introduction of enrofloxacin into poultry production in Europe (Engberg *et al.*, 2001). In addition to restrictions on their use, fluoroquinolones were introduced into clinical medicine only 20 years ago, making them relatively new antimicrobial agents and animal populations do not have a long history of exposure to these drugs compared to the history of exposure to other agents, such penicillin or tetracycline. That resistant organisms can be found in places receiving little human impact should not be surprising, since antibiotics are natural products. However, the evidence suggests that human and agricultural activity have a greater impact on the levels of resistant organisms in all environments, from rivers to pastures and between humans influence most rivers largely. Therefore, antibiotic resistant bacteria in those rivers may derive from wastewater effluent as discussed above. In Korea, 53.6% of coliform isolates from the Sumjin river were resistant to at least one antibiotic, e.g., 21.3% to quinolones (Park *et al.*, 2003). Antimicrobial agents are also provided in water to prevent diseases in poultry flocks and in milk replaces to prevent diseases in calves. Antibiotic resistant organisms from the human gastrointestinal tract, as well as unabsorbed antibiotics, can enter the environment via

sewage. Both the resistant microorganisms and antibiotic residues are excreted, entering the sewage system. Although most people consider the environment to be generally safe from contamination with untreated sewage, beaches occur frequently where leakage or overflow into groundwater or natural waters occurs (Harwood *et al.*, 2000). Antibiotic bacteria can be found in almost every place on earth. Moreover, although levels are higher in areas impacted by agriculture and humans, nevertheless, still resistant organisms in far reaches where human impacts are negligible. This should not be surprising since antibiotics are natural microbial products. Those bacteria that live in the environment are subjected to numerous antagonistic effects from plants, fungi and bacteria that produce antimicrobial chemicals in an effort to compete and survive. Ultimately, the use of antibiotics leads to increased antibiotic resistance in humans, animals and the environment.

Conclusion: The distribution of antimicrobial resistant *E. coli* isolates in all sources studied based on phenotypic findings reinforced the wide dissemination of *E. coli* strains. As concerns about water quality and environmental contamination by human and agricultural waste have increased, it has become increasingly important to develop low-cost screening tools that can be used to identify the most probable source of fecal contamination.

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