ORIGINAL RESEARCH

Molecular characterization and antimicrobial resistance patterns of *Salmonella* spp. and *Escherichia coli* of laying chicken

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ABSTRACT

Multidrug resistant (MDR) Gram-negative bacteria are the most common causes of diseases in commercial poultry, and antibiotic resistance of these organisms is often plasmid mediated. In Bangladesh such types of data are very much scarce. In this study, the antibiogram profile of *Salmonella* spp and *E. coli* isolates from 20 either clinically affected or dead laying chicken obtained from 10 commercial layer farms was performed. And MDR pattern was determined by using 7 common antimicrobials followed by isolation of plasmids to correlate between plasmids and drug resistance. Of these tested samples, 70-100% of both *Salmonella* Spp and *E. coli* were resistant to β-lactam antibiotics (ampicillin, amoxicillin, and penicillin) cephalexin and cotrimoxazole while 60-90% isolates of both species were susceptible to both ciprofloxacin and gentamicin. Isolates of both *Salmonella* spp and *E. coli* contain plasmids above 10 kbp size which might contain MDR genes. This is the first report on the characterization of plasmids found in both *Salmonella* spp and *E. coli* isolates obtained from a significant number of commercial layer farms (N=10) in Chittagong District, Bangladesh. The gathered information furthers our understanding of the mechanisms of drug resistance in specific region related to other parts of the country and world. The large plasmids might be potential factors for dissemination of antibiotic resistance genes regionally.

Key Words: Chicken, Drug resistance, *E. coli*, *Salmonella* spp. Plasmid

Introduction

Antimicrobial resistance in bacterial pathogens has drawn much attention as one of the greatest threats to public health globally (Levy and Marshall, 2004). It is equally important to both human and veterinary medicine. Although, in veterinary medicine practices, commercial poultry production in most of the developing countries like Bangladesh has been playing an important role as a growing industry, hygienic standards are not strictly followed and enforced. Thus many food borne microorganisms enter into the human food chain notably *Salmonella* spp and *E. coli*. These are the common microbial flora of gastrointestinal tract of poultry, human being and other animals and sometimes these become pathogenic to both (Jawetz et al., 1984; Levine, 1987; Steve et al., 2005). Consequently, antimicrobials are used as therapeutic agents in clinically affected livestock, as prophylactic and sometimes as growth promoters. And these are the most important factors promoting the emergence, selection and dissemination of antibiotic resistant microorganisms in both veterinary and human medicine (Witte, 1988; Neu, 1992). Moreover, especially in poultry industry, indiscriminate use of antimicrobials for different purposes leads to emergence of multidrug resistance bacteria and caused high fatality rate especially in immune-compromised individuals (Holmberg et al., 1984). Therefore, antimicrobial resistance patterns in both human and animals are imperative to characterize for control and prevention of spreading multidrug-resistant bacterial strains (Duijkeren et al., 2003). A quiet number of pathogenic microorganisms are found in poultry of which both *Salmonella* spp and *E. coli* is common food borne pathogens. Unlike other parts of the world, the genetic features of the drug resistance in Gram-negative *Enterobactiraceae* from specific locations such as Bangladesh especially in greater Chittagong district have not been studied due to constrained resources.

Dissemination of antimicrobial resistance genes among bacterial strains depends on several factors, and plasmid-mediated horizontal transfer of multidrug resistance genes has been considered as one of the most important mechanisms for obtaining drug resistance by the microbes (Zhao, et al., 2010; Martinez and Baquero, 2002; Davies and Davies, 2010). Earlier studies shows that the genes found in many Gram-negative bacilli encode A, B, and D β-lactamases that mediate resistance to various β-lactam antibiotics have been found on plasmids (Navon-Venezia et al., 2006; Poirel et al., 2010; Carattoli et al., 2012). Moreover, plasmids conferring resistance to quinolones and/or aminoglycosides have been reported (Carattoli, 2009; Miro et al., 2010). In this study, we investigated the susceptibility patterns of commonly used 7 different antimicrobials to *Salmonella* spp and *E. coli* isolated followed by plasmids isolation from both antimicrobial resistant *Salmonella* spp. and *E. coli* to identify correlations between plasmids and drug resistance attained these isolated organisms.

Materials and methods

Sample collection

Either dead or clinically affected layer chicken were collected from 10 commercial layer farms from a previously selected 30 commercial layer farms under Chittagong District. After sacrifice and postmortem examination, liver samples were collected from the suspected chicken and subjected to various biological tests.

Isolation of bacteria and bacteriological analysis

The samples were analyzed within 2-6 hours of collection. The different bacteriological culture media such as Nutrient Agar (NA), Nutrient Broth (NB), *Salmonella-Shigella* Agar, Brilliant Green Agar, Eosin Methylene Blue Agar, Mc Conkey Agar and Deoxycholate Hydrogen Sulphide Lactose Agar were prepared separately.

For sub culturing, the colonies of the NA media were inoculated in the selective media by looping for the identification of *Salmonella* spp. and *E. coli* from the different samples and were incubated at 37°C for overnight. On the other hand, the samples of the NB media were inoculated to all the selective media by looping from the different samples. Samples were inoculated into the selective media and incubated at 37°C for overnight.

Determination of multidrug resistance pattern

Bacterial susceptibility to different antimicrobial agents was measured in vitro by employing the modified Kirby-Bauer (Bauer et al., 1966)

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method by measuring zone sizes (in mm). Commercially available antibiotics discs (Becton Dickinson, USA) were used for the test. The antibiotics discs used in this study included ciprofloxacin (5μg), penicillin (10μg), ampicillin (10μg), and gentamicin (10μg). E. coli ATCC 25922 was used as control. By the standard method of inoculation, the top of a single and well-isolated colony was touched with a sterile loop and the growth was inoculated into 2 ml of Mueller–Hinton broth. The broth culture was then allowed to incubate at 37°C for 4 hours to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to 0.5 McFarland standard and then a sterile cotton swab was dipped into the adjusted suspension within 15 minutes and excess broth was purged by pressing and rotating the swab firmly against the inside of the tub to ensure complete contact with the agar surface. Even distribution of sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. The plates were then allowed to dry for 3 to 5 minutes. Antibiotics impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Even distribution of discs and minimum distance of 24 mm from center to center were ensured. Five discs (four antibiotics discs and one blank disc as control) were placed in each petri dish. Within 15 minutes of the application of the discs, the plates were inverted and incubated at 37°C. After 16 to 18 hours of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to the interpretation table of the Becton Dickinson Microbiology Company, USA.

Plasmid isolation
Plasmid DNA was extracted according to the alkaline lysis method (Tahilkdet al., 2002). The molecular weight of the plasmid DNA was determined by comparison to the electrophoretic mobility of plasmids of known molecular weight using E. coli PDK-9 (Haider et al., 1989).

Results
A total number of 20 bacterial isolates from either clinically affected or dead laying chicken of both Salmonella spp. (n=10) and E. coli (n=10) were tested for their antimicrobials susceptibility, using the agar disk diffusion methods (Table 1). The antimicrobial sensitivity of both Salmonella spp and E. coli were categorized as strong sensitive, moderate sensitive, weakly sensitive and resistant. Of the tested samples, E. coli (100%) was resistant to ampicillin, penicillin and ceftriaxone; 40% E. coli was resistant to both ciprofloxacin and gentamicin; and 80% E. coli was resistance to cephalexin. On the other hand, Salmonella spp. showed resistance to ampicillin (100%), amoxicillin (90%), penicillin (90%), ceftriaxone (80%), cephalexin (70%) ciprofloxacin (20%) and gentamicin (10%), respectively.

Table 1. Antimicrobial susceptibility patterns of Salmonella spp. and E. coli of chicken

<table>
<thead>
<tr>
<th>Antimicrobial agents (μg)</th>
<th>Strongly sensitive %</th>
<th>Moderate sensitive %</th>
<th>Weakly sensitive %</th>
<th>Resistant %</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella spp.</td>
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<td>Salmonella spp.</td>
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<td>Salmonella spp.</td>
<td>E. coli</td>
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<tr>
<td>Ampicillin</td>
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<td>100</td>
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<tr>
<td>Amoxicillin</td>
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<td>10</td>
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<td>Penicillin</td>
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<td>Ceftriaxone</td>
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<td>Ciprofloxacin</td>
<td>80</td>
<td>50</td>
<td>-</td>
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<td>10</td>
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<tr>
<td>Gentamicin</td>
<td>90</td>
<td>50</td>
<td>10</td>
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<tr>
<td>Cephalexin</td>
<td>10</td>
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Discussion
To our knowledge, this is the first study shows correlation between MDR patterns and molecular size of plasmids obtained from both Salmonella spp. and E. coli isolates from commercial chicken in Chittagong region. In this study we examined antibiotic resistance patterns in Salmonella spp. and E. coli isolates from either clinically affected or dead laying hen obtained from commercial layer farms. Of the tested samples, both Salmonella spp. and E. coli isolates were highly resistant to β-lactam antibiotics (ampicillin, amoxicillin and penicillin), however, Tricia et al. (2006) observed both ampicillin (85.7%) and amoxicillin (96.4%) were susceptible to E. coli obtained from broiler chicken compared with the other antimicrobial agents used in this study, these dissimilarities might be due to long term use ampicillin and amoxicillin in layer feed as preventive measure, and low levels of resistance were also observed for ciprofloxacin and gentamicin as well. Among these tested samples, in the current study, 10-20% Salmonella spp. and 40% E. coli isolates were resistant to ciprofloxacin and gentamicin, respectively. The prevalence of gentamicin resistance in the present study does not coincide with the earlier study, who reported 100% avian isolates were susceptible to gentamicin (Tricia et al., 2008). In the previous study showed 20.6% and 29% of E. coli isolates of poultry were resistant to amoxicillin and amoxicillin, respectively (Tricia et al., 2006). But in our study, E. coli showed 100% resistance to ampicillin and 90% resistance to amoxicillin. Likewise, Salmonella spp. showed similar type of resistance to both ampicillin and amoxicillin. Although, ciprofloxacin and gentamicin were highly susceptible to Salmonella spp. and E. coli isolates in broiler chicken (Tricia et al., 2006), only 60% of E. coli isolates showed sensitivity to ciprofloxacin. Resistance of the tested antimicrobials in layer chicken could be due to repeated exposure in their lifetime.

Fig. 1. Plasmids isolates of E. coli from laying chicken in 1.5% agarose gel electrophoresis. L=DNA Ladder, S1-S10= Sample 1–Sample 10: Plasmids isolated from laying chicken.

Fig. 2. Plasmid isolates of Salmonella spp. from laying chicken in 1.5% agarose gel electrophoresis. L=DNA Ladder, S1-S10= Sample 1–Sample 10: Plasmids isolated from laying chicken.

There is strong evidence that the use of antimicrobial agents can lead to the emergence and dissemination of resistant Salmonella spp. and E. coli (David et al., 2001; Tricia et al., 2006) which can then be passed on to people via food or through direct contact. The present study revealed the patterns of multidrug resistance by both Salmonella spp. and E. coli...
isolates of laying chicken to ampicillin (100%/100%), amoxicillin (90%/90%), penicillin (90%/100%) cotrimoxazole (80%/100%) and cephalixin (70%/80%) which are in agreement with the findings of Chowdhury et al., 2009. To deal with multi-drug resistant organisms, it is usually recommended that potentially synergistic antimicrobials combinations would be useful. Both Salmonella spp. and E. coli were strongly susceptible to both ciprofloxacin and gentamicin but the same isolates were 100% resistant to ampicillin. Interestingly, both organisms showed a similar resistance patterns and possess more than 10 kbp plasmid sizes whereas Steve et al., 2005 found that Salmonella spp and E. coli isolates from meat based fast food contains more than 15 kbp plasmids. It can be conferred that plasmid encoded resistance genes to antimicrobials is a significant public health concern in our country since there are possibilities to transfer of resistant genes between bacteria and natural habitats.

Conclusion
In conclusion, 70-100% of both Salmonella spp. and E. coli were resistant to β-lactam antibiotics (ampicillin, amoxicillin, and penicillin), cephalixin and cotrimoxazole while Steve H et al., 2005 found that isolates from meat based fast food contained more than 10 kbp plasmids. Further study is warranted to identification of genes responsible for antimicrobial resistance.

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Conflict of interest
The authors have declared no conflict of interest

References


