CONTAMINATION OF SOME BACTERIA ISOLATED FROM CHICKEN MEAT IN RETAIL MARKETS IN HANOI AND EXAMINATION OF THE ANTIMICROBIAL RESISTANCE ABILITY OF SALMONELLA AND E.COLI STRAINS ISOLATED

Tình trạng ô nhiễm vi sinh vật trên thịt gà bày bán tại một số chợ trên địa bàn thành phố Hà Nội và kiểm tra khả năng kháng kháng sinh của một số chủng E.coli và Salmonella phân lập được

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Tóm tắt

Với mục đích tìm hiểu tình trạng ô nhiễm một số loại vi sinh vật trong thịt gà bán tại các chợ và khả năng kháng kháng sinh của các chủng vi khuẩn E. coli và Salmonella phân lập được, nghiên cứu được tiến hành với tổng số 86 mẫu thịt gà lấy tại các chợ địa bàn thành phố Hà Nội. Kết quả cho thấy 72.09% mẫu không đạt chỉ tiêu Coliforms; 67.69% mẫu không đạt chỉ tiêu tổng số vi khuẩn hiệu khi; 62.79% mẫu không đạt chỉ tiêu Salmonella; 59.30% mẫu không đạt chỉ tiêu E.coli; 55.81% mẫu không đạt chỉ tiêu St. aureus. Streptomycin bị 60.47% các chủng E.coli phân lập được từ thịt gà kháng lại; tiêp đến là sulfonamide (59.30%); neomycin (56.98%); trimethoprim/sulpha methoxazole (54.65%); gentamicin ván kanamycin (53.49%); ampicillin (46.51%); colistin (41.86%); ciprofloxacin (40.7%); norfloxacin bị các chủng E.coli phân lập được kháng lại ít nhất (32.56%). Streptomycine bị các chủng Salmonella phân lập được kháng lại nhiều nhất (79.63%); 68.52% chủng kháng lại trimethoprim/Sulpha methoxazole; 66.67% kháng lại sulfonamide; 53.70% kháng lại gentamycin và neomycin; 50.0% kháng lại kanamycin; 51.85% kháng lại ampicillin; 44.44% kháng lại colistin, norfloxacin và ciprofloxacin bị 33.33% các chủng Salmonella kháng lại.

Từ khóa: Coliforms, E. coli, kháng kháng sinh, salmonella, staphylococcus aureus, thịt gà.

SUMMARY

The aim of study is to examine the contamination bacteria and the antimicrobial resistance of Salmonella and E.coli strains isolated in poultry meat collected in retail markets in Hanoi. The research was carried out with 86 poultry meat samples to establish food hygienic conditions. The result showed that 67.69% were unacceptable based on aerobic total bacteria standard, and 72.09%, 62.79%, 59.30%, 55.81% did not reach coliforms, salmonella, E.coli and St. aureus criteria standards, respectively. Streptomycin were resisted by 60.47% E.coli strains isolated from meat poultry and with sulfonamide (59.30%); neomycin (56.98%); trimethoprimsulphamethoxazole (54.65%); gentamicin ván kanamycin (53.49%); ampicillin (46.51%); colistin (41.86%); ciprofloxacin (40.7%); norfloxacin were the least resisted. Streptomycin were most highly resisted by 60.47% E.coli strains isolated, 68.52% were resistant to trimethoprimsulfamethoxazole; 66.67% were resistant to sulfonamide; 53.70% were resistant to gentamicin and neomycin; 50.0% were resistant to kanamycin; 51.85% were resistant to ampicillin; 44.44% were resistant to colistin; norfloxacin and ciprofloxacin were resisted by 33.33% Salmonella strains.

Key words: Antibiotic resistant, coliforms, E.coli, poultry meat, Salmonella, Staphylococcus aureus.

1. INTRODUCTION

Food-borne disease has been one of the major concerns in each country as well as in the world. Each year, it was estimated approximately 76 million food-borne illnesses, 325,000 cases hospitalization and 5,000 mortal cases were related to food in the United States (Mead et al, 1999). In Vietnam, there were more than 3 million food poisoning cases annually, losses of more than 200 million USD (WHO, 2006).
Nowadays, Hanoi population is more than 6 million and thousands of travellers per day, foodstuff consumption is very large, especially animal products. However, there are a few intensive slaughter-houses in Hanoi and many free private slaughter-houses instead. These slaughterhouse’s activities could not be controlled and they are considered risks of food poisoning. According to WHO 2006, among botulism patients, 90.0% were caused by contamination from the slaughter process and 10.0% were caused by morbid livestock.

Besides, Vietnam is a developing country with abuse of antibiotics in animal husbandry and it may cause antimicrobial resistance of bacteria from animals. Van de Boogard et al. (2000) and Schroeder et al. (2002) proved that antibiotic resistance of bacteria isolated from humans was transferred from antibiotic resistant bacteria in animal.

The purpose of this study is to detect the contamination bacteria of retail poultry meat in retail markets in Hanoi and examined antimicrobial resistance of *Salmonella* and *E.coli* strains isolated to warn customers about the emergence of food poisoning.

2. MATERIALS AND METHODS

2.1. Study design

During the period from January 2009 to June 2009, poultry meat samples were collected from a number of markets in Hanoi to analyze microbiological characteristics and examine antimicrobial resistance of *Salmonella* and *E.coli* isolated.

2.2. Methods

*Microbial analysis*

- Detection and enumeration Coliforms and *E.coli* per 1 gram meat based on Vietnam Standard No. 5155 – 1990.
- Detection and enumeration *Sta.aureus* per 1 gram meat based on Vietnam Standard No. 5156 – 1990.
- Detection *Salmonella* per 1 gram meat based on Vietnam Standard No. 5153 – 1990.

*Antibiotic susceptibility tests.*

*E.coli* and *Salmonella* isolated were incubated in BHI (Oxoid, England) at 37°C for 2 - 8 hours till turbid at 0.5 Macfarland, then edmix with Mueller – Hinton agar (Difco, Great Britain) with proportion 1/10; the agar was put into diameter Petri dishes. The amounts of antimicrobial agents on the papers were as follows: ampicillin 10 μg; colistin 10 μg; gentamicin 10 μg; kanamycin 30 μg; neomycin 30 μg; streptomycin 10 μg; nalidixic acid 30 μg; ciprofloxacin 5 μg; norfloxacin 10 μg; sulfanamide 30 μg; trimethoprim/sulphamethoxazole 25 μg (Oxoid, Anh). Proper distance between two papers is 25mm at least. To dry at room- temperature for 2-3 minutes then incubated at 37°C for 24 hours. The result follows standard recommendations by NCCLS (2006).

*Data analysis*

Using software Minitab 14 version.

3. RESULTS AND DISCUSSION

3.1. Microbial contamination of poultry meat in retail market

It is important to determine the aerobic total criterion which is used as hygienic indicator in the slaughter-process. In total of 86 samples, the number of bacteria was 52.4 x10⁶ CFU/g in maximum and 0.12 x10⁶ CFU/g in minimum; the others ranged from 0.18x10⁶  CFU/g to 47.8 x10⁶ CFU/g. There were some samples that were higher than 10 times the regular standard, the percentage of unacceptable samples was 69.77%.

This result was higher than the result obtained by Tran Thi Nhai (2005) and Nguyen Van Ton (2005) with 54.65% and 58.4% unacceptable samples of poultry meat in Hanoi. In Morocco, Conhen, N et al (2007) found 29.2% meat poultry samples over national standards.

Coliforms, including *E.coli* and Enterobacteriaceae originated from animal and human feces, land, water..., is considered as indicator hygienic quality. Examination of Coliforms is to estimate hygienic status of foodstuff.

From table 1, the sample with maximum contamination was 0.18 x10⁵ CFU/g and minimum was 7.5 x10⁵ CFU/g. It was lower than the study of Nguyen Van Ton (2005) with 100% poultry meat sample in supermarkets and retail markets was unacceptable.
Table 1. Microbial contamination result of poultry meat from retail markets in Hanoi

<table>
<thead>
<tr>
<th>Microbial Criteria</th>
<th>Result</th>
<th>Assess</th>
<th>Acceptable</th>
<th>Unacceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min (CFU/g)</td>
<td>Max (CFU/g)</td>
<td>Range (CFU/g)</td>
<td>n</td>
</tr>
<tr>
<td>Aerobic bacteria</td>
<td>0.12 x 10⁶</td>
<td>52.4 x 10⁶</td>
<td>0.18 x 10⁶ - 47.8 x 10⁶</td>
<td>26</td>
</tr>
<tr>
<td>Coliforms</td>
<td>0.18 x 10⁷</td>
<td>7.50 x 10⁷</td>
<td>0.21 x 10⁷ - 6.9 x 10⁷</td>
<td>24</td>
</tr>
<tr>
<td>E.coli</td>
<td>0.21 x 10⁵</td>
<td>1.84 x 10⁵</td>
<td>0.36 x 10⁵ - 1.53 x 10⁵</td>
<td>35</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>0.41 x 10⁵</td>
<td>6.12 x 10⁵</td>
<td>0.57 x 10⁵ - 4.24 x 10⁵</td>
<td>38</td>
</tr>
<tr>
<td>Salmonella</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>32</td>
</tr>
</tbody>
</table>

(Note: assess Salmonella acceptable or not base on present or absent in 25g food)

According to WHO (2006), there was 25% diarrhea in foodborne illness caused by food infected with E.coli. In this study, 100% of samples were infected with E.coli. There was 0.21x10⁷ CFU/g in maximum and 1.84x10⁵ CFU/g in minimum, mean of 0.36 x 10⁵ CFU/g – 1.53x10⁵ CFU/g. Proportion of unacceptable sample was 59.3%, lower than some results announced. Tran Thi Hanh et al (2004) found 68.75% samples of poultry meat sold in Hanoi’s market. In America, 38.7% meat poultry samples in Washington infected with E.coli (Cuiwei Zhao et al. 2001). Cohen N (2007) indicated 48.4% samples infected with E.coli in Moroco, 22.4% among these was unacceptable.

Sta.aureus is toxic bacterium, one of reasons caused food poisoning. Based on Vietnam Standard, 55.81% samples did not reach the standard. Contamination level of Sta.aureus was quite high, maximum 0.41 x 10⁵ CFU/g and minimum 6.12 x 10⁵ CFU/g. To Lien Thu (2006) obtained 96.0% Sta.aureus contaminated. This situation demonstrated that food safety in Vietnam has been alarming. In other countries, proportions of Sta.aureus contamination are much lower 10.9% in Moroco (Cohen N et al, 2007), 30.3% in Croatia (Lidija Kozacinski et al, 2006)

Salmonella is considered a major cause of foodborne illness in human and infected into the digestive system from eating low quality animal products. Following Vietnam standard, it is required that Salmonella be absent in 25 gram food to ensure human health. The percentage of unacceptable samples is 62.79%. Our result was much higher than the other results by Huong et al (2006), Phan et al (2005) with 48.9% and 45.07% Salmonella prevalence in meat poultry in Hanoi and Mekong area. Sunpetch Angkititrakul et al (2002) researched meat poultry from retail markets and supermarkets announced that 75% samples was contaminated with Salmonella. In Denmark, percentage of import poultry meat contaminated with Salmonella is 11.8% (Skov Marianne. N et al (2007)).

The status of low hygienic conditions in slaughter was the main reason of the results mentioned above. For example, fowl was killed on narrow space in market, depluming and washing intestine in one basin. Therefore, meat product was easily contaminated with Enterobacteria through slaughtering process. Moreover, meat products was sold irrationally, sarcodic and viscus sold on the same table without separation.

3.2. Antibiotic susceptibility of E.coli isolated

Antibiotic resistance of bacteria isolated from animal has been consideration of worldwide. The humans infected with antibiotic resistant bacteria through the food chain lead to difficulty in treatment. ((Elizabeth Barclay, 1998).

Totally 86 E. coli strains isolated from meat poultry was tested for susceptibility with 10 kinds of antibiotics. The result in table 2 showed that 60.47% were resistant to streptomycin, to sulfonamide (59.30%); neomycin (56.98%); trimethoprim/Sulphamethoxazole (54.65%); gentamicin and kanamycin (53.49%); ampicillin (46.51%); colistin (41.86%) and ciprofloxacin (40.7%); norfloxacin was the least resistance with 32.56%. This result is similar to the result by Van Thi Thu Hao et al (2007) indicated that there was an upward trend in antibiotic resistance of E. coli, 83.8% E. coli isolated from meat was resistant to at least one kind of antibiotic. In many areas over the world, E. coli strains had high resistance to older generations of antibiotic and started resistant to most of newer kinds of antibiotic. (Sahm et al., 2001; Turnidge et al., 2002; Farrell et al, 2003; Karlowsky et al, 2006).
Contamination of some bacteria isolated from chicken meat in retail markets in Hanoi...

Table 2. Result of antibiotic resistance of E. coli isolated

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Concentration</th>
<th>S (Sensitive)</th>
<th>I (Intermediate)</th>
<th>R (Resistant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disc. content (μg/dish paper)</td>
<td>Antibiotic diameter (mm)</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>AM</td>
<td>10</td>
<td>≥ 17</td>
<td>41</td>
<td>47.67</td>
</tr>
<tr>
<td>Cl</td>
<td>10</td>
<td>≥ 11</td>
<td>46</td>
<td>53.49</td>
</tr>
<tr>
<td>G</td>
<td>10</td>
<td>≥ 15</td>
<td>31</td>
<td>36.05</td>
</tr>
<tr>
<td>K</td>
<td>30</td>
<td>≥ 18</td>
<td>33</td>
<td>38.37</td>
</tr>
<tr>
<td>N</td>
<td>30</td>
<td>≥ 17</td>
<td>27</td>
<td>31.39</td>
</tr>
<tr>
<td>S</td>
<td>10</td>
<td>≥ 15</td>
<td>25</td>
<td>29.07</td>
</tr>
<tr>
<td>CIP</td>
<td>5</td>
<td>≥ 21</td>
<td>51</td>
<td>59.30</td>
</tr>
<tr>
<td>Su</td>
<td>5</td>
<td>≥ 17</td>
<td>53</td>
<td>61.63</td>
</tr>
<tr>
<td>NOR</td>
<td>10</td>
<td>≥ 17</td>
<td>53</td>
<td>61.63</td>
</tr>
<tr>
<td>SXT</td>
<td>25</td>
<td>≥ 16</td>
<td>29</td>
<td>33.72</td>
</tr>
</tbody>
</table>

Total 86 E. coli strains were examined.

* Note: Ampicillin (AM); Colistin (Cl); Gentamicin (G); Kanamycin (K); Neomycin (N); Streptomycin (S); Ciprofloxacin (CIP); Norfloxacin (NOR); Sulfonamide (Su); Trimethoprim/Sulphamethoxazole (STX); Tetracycline (TE)

Table 3. Result of antibiotic resistance of Salmonella was isolated

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Concentration</th>
<th>S (Sensitive)</th>
<th>I (Intermediate)</th>
<th>R (Resistant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disc. content (μg/dish paper)</td>
<td>Antibiotic diameter (mm)</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>AM</td>
<td>10</td>
<td>≥ 17</td>
<td>20</td>
<td>37.04</td>
</tr>
<tr>
<td>Cl</td>
<td>10</td>
<td>≥ 11</td>
<td>23</td>
<td>42.59</td>
</tr>
<tr>
<td>G</td>
<td>10</td>
<td>≥ 15</td>
<td>18</td>
<td>33.33</td>
</tr>
<tr>
<td>K</td>
<td>30</td>
<td>≥ 18</td>
<td>24</td>
<td>44.44</td>
</tr>
<tr>
<td>N</td>
<td>30</td>
<td>≥ 17</td>
<td>21</td>
<td>38.89</td>
</tr>
<tr>
<td>S</td>
<td>10</td>
<td>≥ 15</td>
<td>11</td>
<td>20.37</td>
</tr>
<tr>
<td>CIP</td>
<td>5</td>
<td>≥ 21</td>
<td>34</td>
<td>62.96</td>
</tr>
<tr>
<td>Su</td>
<td>5</td>
<td>≥ 17</td>
<td>14</td>
<td>25.93</td>
</tr>
<tr>
<td>NOR</td>
<td>10</td>
<td>≥ 17</td>
<td>31</td>
<td>57.41</td>
</tr>
<tr>
<td>SXT</td>
<td>25</td>
<td>≥ 16</td>
<td>13</td>
<td>24.07</td>
</tr>
</tbody>
</table>

Total 54 E. coli strains was examined

3.3. Antibiotic susceptibility of Salmonella isolated

Identify the susceptibility of 10 kinds of antibiotics with 54 Salmonella strains isolated from meat poultry. The result in table 3 pointed out that 79.63% were resistant to streptomycin, 68.52% were resistant to trimethoprim/sulfamethoxazole; 66.67% were resistant to sulfonamide; 53.70% were resistant to gentamicin và neomycin; 50.0% were resistant to kanamycin; 51.85% were resistant to ampicillin; 44.44% were resistant to colistin, norfloxacin and ciprofloxacin was resisted by 33.33% Salmonella strains.

In Vietnam, Van Thi Thu Hao et al. (2007) determined the proportion of antibiotic resistance of Salmonella isolated from meat was very high: 50% were resistant to at least one antibiotic, 20.9% were resistant to at least three antibiotics. Nguyen Thi Nguyet et al. (2006) obtained percentage resistance
to tetracycline (82.4%), ampicillin (76.5%), sulfonamide (64.7%), cloramphenicol (58.8%) and trimethoprim/sulfamethoxazole (52.9%), lower with cefoxitine (5.9%), gentamycin and cefalotin (23.5%). In Spain, Juan J. Carraminana et al. (2004) indicated that Salmonella strains isolated from meat poultry were highly resistant to common antibiotic such as sulfadiazine (96.2%), neomycin (53.4%), tetracycline (21.8%), and streptomycin (11.3%).

The antibiotic resistance of these Salmonella and E.coli strains was not surprising. According to Nguyen Thi Hoa Ly (2004), there were 26 antibiotics remained in poultry such as gentamycin, norfloxacin, cloramphenicol, streptomycin, and amoxicilin...

Abuse of antibiotics in animal rose for food and veterinary respect caused remains of antibiotics in meat, which increased antimicrobial resistance.

4. CONCLUSIONS

Among 86 samples of meat poultry meat, the percentage of sample which was unacceptable for each microbial criteria was 67.69% for Aerobic total criteria, 72.09% for Coliforms criteria and 59.3%, 62.79%, 55.81% were unacceptable for E.coli, Salmonella and St. aureus standard, respectively.

Streptomycin was resisted by 60.47% E. coli strains isolated from meat poultry and with sulfonamide (59.30%); neomycin (56.98%); trimethoprim/sulfamethoxazole (54.65%); gentamicin và kanamycin (53.49%); ampicillin (46.51%); colistin (41.86%); ciprofloxacin (40.7%); norfloxacin were least resisted.

Streptomycin was most highly resisted by 79.63% Salmonella strains isolated from poultry meat, 68.52% were resistant to trimethoprim/sulfamethoxazole; 66.67% were resistant to sulfonamide; 53.70% were resistant to gentamicin and neomycin; 50.0% were resistant to kanamycin; 51.85% were resistant to ampicillin; 44.44% were resistant to colistin; norfloxacin và ciprofloxacin were resisted by 33.33% Salmonella strains.

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