Screening of Antibiotic Residues in Poultry Liver, Kidney and Muscle in Khartoum State, Sudan

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(Received: February 15, 2014; Accepted: April 08, 2014)

Abstract - This study was conducted at Khartoum State – Sudan to screen for antibiotic residues in different poultry tissues (liver, kidney and muscle). A total of 221 tissue sample was obtained from slaughtered animals. Three plate test method was used with 3 species of bacteria viz Escherichia .Coli, Staphylococcus aureus and Bacillus subtilis. After screening for antibiotic residues, the total number of positive samples was 27%. The highest percentage of positive samples was detected in the muscle tissue (29.2%) and the least was in the kidney. Chi square analysis showed an association between the liver and kidney, kidney and muscle when using E. Coli and between liver and kidney when Bacillus Subtilis was used. It was concluded from this study that, when screening for residues using liver or kidney E. Coli or Bacillus subtilis can be used with the same efficiency since they both give almost the same result taking in consideration other factors such as type of drug screened for and the sensitivity of the bacteria. It is recommended that the awareness of the farmers regarding the misuses of drugs and their effect on human health should be promoted. It is also necessary to establish a routine screening programme for antibiotic residues by the appropriate authorities.

Index terms: Antibiotic residues, Disc assay, Poultry tissues, Khartoum.

I. INTRODUCTION

Antibiotics are being excessively used in the different animal farms for various purposes: such as prophylactic, prevention and control of disease, and as growth stimulant. Under circumstances when the with drawl periods of drug is not applied, antibiotic residues may appear in the different animal products such as milk, eggs, meat as reported by various studies[1-4]. The continuous exposure to antibiotics has been found to lead to serious health problems in humans -being the main consumers of animal products- such as allergies, spreading of drug-resistant microorganisms, carcinogenic effect and potential harmful effect on human intestinal micro flora [1,5 and 6]. Screening of animal products for veterinary drugs began mainly with the dairy industry to overcome problems related to fermentative dairy production, from 1970s regulatory screening of slaughtered animals was started [7] Two types of analytical methods are commonly used, the screening methods that includes microbiological tests (Disc assay, modified Premi and Delvotest methods) [3,4,8] and the confirmatory methods using more complex and advanced techniques such as ELISA and HPLC [9].

The microbial inhibition tests were the earliest test used and are still in use because they are inexpensive and can cover entire antibiotic spectrum but are less specific than other tests. In Sudan, the poultry industry is largely increasing. The use of antibiotic as a routine practice is non avoidable to prevent economic loss due to diseases and consequent mortality. No routine screening programme for slaughtered animals is practiced in the country.

The main objectives of this study are:
1. To screen for the presence of antibiotics in liver, kidney and muscle as important poultry tissues
2. To study the relation between different bacteria species namely Escherichia Coli, Staphylococcus aureus and Bacillus subtilis and poultry tissues under investigation.

II. MATERIALS AND METHODS

Sample collection
A total of 221 different tissue samples were collected from different poultry farms in Khartoum State during the period from April 2013 to June 2013. The percentage of different samples was as follows: liver samples were 43%, muscle was 45% and the kidney was 12% (Fig. 1). About 1-2 g of each tissue sample was obtained from freshly slaughtered animals. The samples were stored in ice till transported to the laboratory at the College of Veterinary Medicine- University of Bahri for further analysis.

Antibiotic detection
The three plate test method was used. Three species of bacteria were used: viz Escherichia .Coli ATCC25922, Staphylococcus aureus ATCC29213 and Bacillus Subtilis DSM618.

Disc assay method
Sterilized nutrient agar plates were inoculated with a loop full of a freshly prepared suspension of each bacteria. An incision was made in the tissue sample, then using a clean sterile forceps, a sterile paper disc was placed into the incision and left until it was soaked and then the disc was transferred and placed on the agar surface. The plates were then inverted and incubated at 37-38°C for 18-24 hours. The presence of antibiotic residues in the sample was indicated by the presence of inhibition zone of a diameter of 2mm or more. Absence of antibiotic residues was indicated by the absence of inhibition zone around the growth or the presence of a zone of less than 2mm [9,10].
Figure 1. Percentage of different tissues collected from farms

Statistical analysis
Chi-square test was used to study the statistical association between the different tissues and bacteria.

III. RESULTS AND DISCUSSION

Antibiotic residue detection
From a total of 221 tissue sample screened for antibiotic residues 27% of the samples tested positive for residues while 73% were negative. When the different tissue samples were analyzed, the positive samples were 28.3%, 29.2% and 21.4% for liver, muscles and the kidneys samples respectively (Fig.2)

Bacterial analysis
The results of the three bacterial species analysis are shown in Figures 3, 4 and 5
When Bacillus Subtilis was used as the test bacteria, the highest percentage of positive sample was detected for muscle, followed by the liver and the least was for the kidney (Fig.3)
Figure 3. Total number of positive and negative samples using *Bacillus Subtilis*.

On the other hand when *E. Coli* was used a different trend was observed where the highest percentage of the positive samples was detected for liver followed by that of the muscle (Fig. 4).

Figure 4. Percentage of positive and negative samples using *E. Coli*.

As far as the *Staphylococcus aureus* is concerned the highest percentage was observed for the muscle followed by that of the kidney and the least percentage of positive samples was seen for the liver (Fig. 5).
The association between the different tissues and bacteria was analysed using chi square test as shown in Tables 1, 2, 3 and 4. Chi Square test showed a significant difference between muscle - liver, and liver - kidney but no difference between muscle - kidney when E. Coli was used. When *Staphylococcus aureus* was used there was no significant difference between the response of the different tissues to it. As for Bacillus the difference was significant between the liver - kidney only.

**Table 1**

Chi-Square Tests between Liver and Kidney using *Bacillus Subtilis*  

<table>
<thead>
<tr>
<th>Value Method</th>
<th>Value</th>
<th>D f</th>
<th>Asymp. Sig. (2-sided)</th>
<th>Exact Sig. (2-sided)</th>
<th>Exact Sig. (1-sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>7.721</td>
<td>1</td>
<td>.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuity Correction*</td>
<td>5.578</td>
<td>1</td>
<td>.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Likelihood Ratio</td>
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<td>.007</td>
<td></td>
<td></td>
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<tr>
<td>Fisher's Exact Test</td>
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<td></td>
<td></td>
<td>.010</td>
<td>.010</td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>42</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.67.  
b. Computed only for a 2x2 table

**Table 2**

Chi-Square Test between Kidney and Muscle using *E. Coli*  

<table>
<thead>
<tr>
<th>Value Method</th>
<th>Value</th>
<th>D f</th>
<th>Asymp. Sig. (2-sided)</th>
<th>Exact Sig. (2-sided)</th>
<th>Exact Sig. (1-sided)</th>
</tr>
</thead>
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<tr>
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<td>.027</td>
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<tr>
<td>Likelihood Ratio</td>
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<td>.006</td>
<td>.022</td>
<td>.012</td>
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<tr>
<td>Fisher's Exact Test</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>20</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

a. 3 cells (75.0%) have expected count less than 5. The minimum expected count is 4.05.  
b. Computed only for a 2x2 table
**Discussion**

Recently, a vast number of studies are being carried out to investigate the presence of different veterinary drugs including antibiotic residues in animal products due to the side effects they impose on the health of human consumers. The main objective of this study was to screen for the presence of antibiotic residues in poultry meat. In a previous study, it was shown that antibiotics (namely tetracycline and penicillin) are used extensively in poultry farms either for prevention (50% of visited farms) or for treatment (37.5%) in Khartoum State [3]. As a consequence, there is a strong possibility that antibiotic residues may appear in poultry products especially with the lack of knowledge between farm owners about withdrawal periods of drugs and to avoid the economic losses, poultry products can be distributed in the market regardless of the withdrawal period.

The data obtained in this study showed that 27% of tested tissue samples gave a positive result for the presence of antibiotics compared to 73% that were negative. Other studies also showed the presence of residues in poultry meat, such as those in Turkey where 45.7% of samples were positive for quinolone [11], and 8.33% in Belgium [12] were positive for doxycycline and amoxicillin. Another investigation carried out in different areas of the Dominican Republic showed that poultry meat contains quinolone residues with different concentrations [13]. In Egypt, it was reported that 34% of tested broiler fillets were positive for Oxytetracycline [14].

There is a discrepancy about which tissue to select when testing for antibiotics. The main edible part is the muscles and to a lesser extent liver and kidney. In this study high positive samples were detected in muscle and liver (11.47% and 10%, respectively) compared to 24.6% in the kidneys for poultry tissues in Khartoum [15]. The higher results obtained under the present study may be attributed to the extensive use of antibiotics by farm owners. Higher residual of quinolones were found in liver and kidneys compared to muscles and eggs [16]. Similar results were also reported when using *Bacillus subtilis* as test organism where high percentage of positive samples of 39.4%, 275 and 20.6% were found in liver, kidney and muscle respectively [17].

The levels of antibiotic residues may vary within the different tissues. When choosing muscle for example, there remains a choice between breast or thigh muscle. In this study the breast muscle was chosen. According to previous studies, there was no significant difference of antibiotics residues between the different sections of breast tissues [18] and also there was no significant difference of tetracycline levels between muscle of the thigh and wing [19].

When examining for antibiotic residues, several species of bacteria are used as test organisms. In this study three different bacteria were used namely *E. coli*, *Bacillus Subtilis* and *Staphylococcus aureus*. The highest number of positive samples in liver tissue was found using *E. Coli* (33%). As for muscle samples the highest positive samples was detected when using *Bacillus subtilis* (31.7%) and *Staphylococcus aureus* (28.6%). These results can be affected by the sensitivity of the various bacteria towards the different antibiotics. It has been reported that B- lactam antibiotics gave only inhibition zones with *Micrococcus luteus* while tetracyclines can be detected up to the MRL level with *B. cereus*, and fluoroquinolones with *E. coli* and all these three groups of antibiotics can be detected using one plate seeded with *Bacillus subtilis* [12]. In this study, *Bacillus subtilis* and *Staphylococcus aureus* detected more positive samples (26.2% and 26% respectively) when compared to *E. Coli*. This agrees with
earlier report that *Bacillus subtilis* can detect more positive samples than *E. Coli*[20]. It has been reported that when using Bacillus as a test organism, muscle tissue cannot be used as test material for screening oxytetracycline, enrofloxacin and ciprofloxacin residues, while penicillin G can be screened from muscle tissue[4] .

The results obtained in this study accords with this observation since *Bacillus Subtilis* detected the highest positive muscle samples and penicillin are among the most commonly used drugs in the country. When using agar diffusion tests, a positive test needs to be confirmed by other methods. If a kidney sample gives a positive agar diffusion test result, the antimicrobial concentration in a muscle sample from the same carcass should be also checked by other tests [21].

According to statistical results (Tables 1-4), there was a significant difference when using *E.Coli* between muscle -liver and liver - kidney but the difference was not significant between kidney-muscle. When using *Bacillus subtilis* a significant difference was observed between liver - kidney only.

This result suggests that when testing for antibiotic residues using liver or kidney *E.Coli or Bacillus subtilis* can be used with the same efficiency since they both give almost the same result taking in consideration other factors such as type of drug screened for and the sensitivity of the bacteria. On the other hand, any tissue can be used with *Staphylococcus aureus* since there was no significant difference between all tissues studied.

IV. CONCLUSION

Screening of poultry meat showed detectable levels of antibiotic residues which may indicate the widespread misuses of antibiotic in poultry farms and the lack of awareness of farmers regarding the recommended withdrawal periods of drugs. The statistical analysis showed that *E. Coli* and *Bacillus subtilis* can be used with the same efficiency when testing for antibiotic residues in liver or kidney.

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Higher Education and Scientific Research – Sudan for funding this study.

REFERENCES


