Prevalence and antibiotic resistance of *Pasteurella multocida* isolated from chicken in Ado-Ekiti metropolis

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Abstract

*Pasteurella multocida* is a poultry bacterial pathogen causing fowl cholera in chicken. The prevalence and antibiotic susceptibility of *P. multocida* isolates from freshly dead chicken were determined. Ninety seven (97) freshly dead chicken from 23 different farms were analyzed for the presence of *P. multocida*. Swabs of the trachea and the liver of the necropsied chicken were activated on buffered peptone water and later cultured on blood agar and MacConkey agar. Pure culture of organisms were subjected to cultural and biochemical characterization. In vitro susceptibility of the pure isolates of *P. multocida* against 12 antimicrobial agents was determined using disk diffusion method. Twelve isolates of *P. multocida* were recovered from the chicken, with a prevalence of 12.4%. Nine of the isolates were recovered from the trachea and three from the liver. All the 12 isolates recovered from the trachea were multi-resistant to the antibiotics used in this research. The antibiogram showed that all the isolates resisted ampicillin, amoxicillin/clavulinate, doxycycline and tylosine. Nitrofurantoin and gentamycin had the best antimicrobial activity with 25% and 50% resistance respectively. The resistance of other antibiotics are: Ofloxacin 75%, Ciprofloxacin 83.3%, Enrofloxacin 75%, Furasol 66.7%, Ceftazidime 91.7% and Cefuroxime 66.7%. This result showed that there is an emergence of multi-resistance in *P. multocida*, therefore it is important to carry out sensitivity test before administration of antibiotics in order to control fowl cholera.

Keywords: Antibiotics; Fowl Cholera; Pasteurella multocida; Resistance.

1. Introduction

Fowl cholera is a highly contagious disease caused by *Pasteurella multocida*. This organism affects a broad host range of birds both domestic and wild birds. It causes high mortality that incur significant economic losses in commercial and backyard poultry production [15]. It spreads by contamination of feed or water, by oral or nasal discharges from infected birds. Migratory birds have been reported as a major source of fowl cholera [8].

Although, vaccines are given to birds against fowl cholera, yet fowl cholera has remain one of the main causes of loss in poultry [12]. *P. multocida* is also responsible for atrophic rhinitis in swine, snuffles in rabbit, septicaemia haemorhagica ovis in goat, pneumonia in cattle and haemorrhagic septicaemia in cattle and buffalo, showing that *P. multocida* is not host specific [1], [18]. Antibiotics are used to a large extent for the treatment of fowl cholera. However, prolong and pervasive use of antibiotics has resulted in *P. multocida* acquiring resistance to most of the commonly used antimicrobials [2].

Antibiotic resistance of *P. multocida* isolates varies according to the host animal, specie, time, geographical origin and antimicrobial pre-treatment of the animal [7]. Multi-resistance pathogenic bacteria in food-producing animals and environmental sources is recognized as a global problem for public health [6], [21]. Multiple antibiotics are often recommended for the treatment of fowl cholera in Nigeria [10]. However, there is little information about multiple drug resistance of *P. multocida* as well as the prevalence of the pathogen in poultry; therefore this research is to document the results of multi-resistance *P. multocida* in Ekiti States, South Western Nigeria.

2. Methodology

Samples were collected from twenty three farms between January and June 2015, transported to the microbiology laboratory within two hours. The samples (ninety seven freshly dead chicken) were necropsied, swabs were collected aseptically from the trachea and the liver for bacteria isolation.

2.1. Bacteriology

The swabs collected from both the liver and the trachea were activated in buffered peptone water for 5 hours at 37°C. A loop full of the activated organisms in the buffered peptone water were inoculated onto MacConkey agar (Biomark) and sheep blood agar by streaking. The plates were incubated at 37°C for 24 hours in an incubator (Royalcare England. DNP 9022A). The appearance of a zone of erythrocyte lysis around or under bacterial colonies indicated hemolysis on sheep blood agar.

2.2. Cultural and biochemical characterization

*Pasteurella multocida* isolates were selected based on the cultural characteristics on blood agar. The morphological appearance was also determined. Further confirmation was done by biochemical...
tests some of which are: motility, catalase, oxidase, H₂S production, nitrate, urease, indole, methyl red, Voges-Proskauer and citrate use tests [9].

2.3. Antimicrobial drug sensitivity test

In vitro susceptibility of the identified Pasteurella multocida isolates against antimicrobial agents was determined by the standard disk diffusion procedure. The organisms were standardized using McFarland standard at the absorbance of 450nm. The samples were inoculated on Muller-Hinton agar. The following antimicrobial agents were tested: Cefazidine (CAZ 30 μg), Cefuroxime (CRX 30 μg), Gentamicin (GEN 10 μg), Ciprofloxacin (CPR 5 μg), Ofloxacin (OFL 5 μg), Nitrofurantoin (NIT 300 μg), Ampicillin (AMP 10 μg), Amoxicillin/Clavulinate (AUG 30 μg), Enrofloxacin (ENR10μg), Furasol (FUR 10 μg), Tylosin (TLY 10 μg) and Doxycycline (DOX10 μg). Following the application of antimicrobial discs, the plates were incubated at 37 °C for 24 h in an incubator (Royalcare England. DNP 9022A). The diameters of antimicrobial discs, the pathogens were incubated at 37 °C for 24 h in an incubator (Royalcare England. DNP 9022A). The diameters of the zones of inhibition were measured (millimetres) and were compared to internationally accepted standard to determine the susceptibility or resistance of the isolate [17].

3. Results

Pasteurella multocida were detected in 12 of the 97 cases investigated with a prevalence of 12.4%. Extended phenotypic and biochemical characterization confirmed the isolates as P. multocida. The P. multocida isolates produced small, round, greyish, smooth, mucoid glistening and dewdrop-like colonies on blood agar plates and were Gram-negative coccobacilli. The strains did not grow on McConkey agar and were non-haemolytic on blood agar. Biochemical testing showed that all strains were urease negative, oxidase, citrate, indole and catalase positive. All the strains fermented galactose, fructose, D-glucose, D-mannitol and sucrose, while no reaction was recorded for inositol, raffinose and salicin. The observation made on the samples revealed that most of the P. multocida isolated from the chicken were resistant to most group of antibiotics as shown in table 1.

Nitrofurantoin was found to be the most effective antibiotic with a resistance of (3) 25%, while the organism form (12) 100% resistance to ampicillin, amoxicillin/clavulinate, doxycycline and tylosine. Table 2 showed the antibiotic resistance pattern of the P. multocida isolates.

### Table 1: Percentage (%) Antibiotic Resistant of Pasteurella multocida Isolated from Chicken Trachea and Liver.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>AMP</th>
<th>AUG</th>
<th>OFL</th>
<th>TLY</th>
<th>CPR</th>
<th>ENR</th>
<th>DOX</th>
<th>FUR</th>
<th>GEN</th>
<th>NIT</th>
<th>CAZ</th>
<th>CRX</th>
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<tbody>
<tr>
<td>Trachea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=9</td>
<td>(9)</td>
<td>(9)</td>
<td>(6)</td>
<td>(9)</td>
<td>(8)</td>
<td>(7)</td>
<td>(9)</td>
<td>(6)</td>
<td>(3)</td>
<td>(2)</td>
<td>(8)</td>
<td>(5)</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>66.7</td>
<td>100</td>
<td>66.7</td>
<td>100</td>
<td>66.7</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>n=3</td>
<td>(3)</td>
<td>(3)</td>
<td>(3)</td>
<td>(2)</td>
<td>(2)</td>
<td>(3)</td>
<td>(2)</td>
<td>(3)</td>
<td>(1)</td>
<td>(3)</td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td>100</td>
<td>83.3</td>
<td>75</td>
<td>100</td>
<td>66.7</td>
<td>50</td>
<td>25</td>
<td>91.7</td>
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<td>n=12</td>
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<td>(12)</td>
<td>(10)</td>
<td>(9)</td>
<td>(12)</td>
<td>(8)</td>
<td>(3)</td>
<td>(11)</td>
<td>(11)</td>
<td>(8)</td>
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</table>

Key: Ampicillin (AMP), Amoxicillin/Clavulinate (AUG), Ofloxacin (OFL), Tylosin (TLY), Ciprofloxacin (CPR), Enrofloxacin (ENR), Doxycline (DOX), Furasol (FUR), Gentamicin (GEN), Nitrofurantoin (NIT), Cefazidine (CAZ) and Cefuroxime (CRX).

### Table 2: Antibiotic Resistance Pattern of P. Multocida Isolated from Chicken Trachea and Liver.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No of isolates</th>
<th>Percentage resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP, AUG, OFL, TLY, CPR, ENR, DOX, FUR</td>
<td>4</td>
<td>33.3</td>
</tr>
<tr>
<td>ENR, DOX, FUR, TYL</td>
<td>7</td>
<td>58.3</td>
</tr>
<tr>
<td>AMP, AUG, CRX, CAZ</td>
<td>8</td>
<td>66.7</td>
</tr>
<tr>
<td>GEN, CRX, NIT, CAZ</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td>TLY, AMP, DOX, AUG</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>CAZ, CRX, GEN</td>
<td>5</td>
<td>41.7</td>
</tr>
<tr>
<td>FUR, ENR, CPR</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>NIT, OFL</td>
<td>2</td>
<td>16.7</td>
</tr>
</tbody>
</table>

Key: Ampicillin (AMP), Amoxicillin/Clavulinate (AUG), Ofloxacin (OFL), Tylosin (TLY), Ciprofloxacin (CPR), Enrofloxacin (ENR), Doxycline (DOX), Furasol (FUR), Gentamicin (GEN), Nitrofurantoin (NIT), Cefazidine (CAZ) and Cefuroxime (CRX).

4. Discussion

The occurrence of fowl cholera in commercial poultry birds had been reported as the major concern in the poultry industry by other workers [13], [14], and [16]. In this study, 12.4% isolation rate of P. multocida doubles the level reported by Mbutia et al. [14] who recorded a rate of 6.2%. This difference may be due to the number of samples, method of isolation, presence of stress and age of birds sampled. Isolation of P. multocida in the trachea and liver of chicken was earlier reported by Dashe et al. [10].

The antibiotic resistance of the P. multocida in this study showed a high resistance to the tested antibiotics compared to reports of previous studies; Everlon et al. [11] reported antibiotic resistance of P. multocida to range between 1.5 and 5.2% in isolates from chicken while Dashe et al. [10] reported resistance ranging between 6.7 and 46.7%. In other various reports [2], [15], [20] it was found that the resistance level is not as high as found in this research. This may have been the result of misuse of antibiotics by most of the farmers in the territory even before they report to the clinic. Generally, farmers report cases in their farms after they have tried all the possible means, which often include heavy usage of antibiotics before making any clinical report.

The high resistance of P. multocida isolates to ampicillin, amoxicillin/clavulanate, doxycycline and tylosin has highlighted that prevention and therapeutic effect on avian P. multocida strains in Ekiti, Nigeria should no longer be expected from these antibiotics. The multidrug resistance of P. multocida is presumably attributed to the use of antibiotics as additives in poultry feed, extensive and pervasive use of antimicrobial agents by poultry farmers and Veterinary practitioners [4], [10]. Arora et al. [2] also recorded that injudicious use of antibiotics in poultry has contributed remarkably in the resistance of P. multocida. Another possible reason for the multiple resistance of P. multocida could be attributed to the proliferation of fake or sub-standard drug in Nigeria [10].

The result observed in the resistance of isolates of P. multocida from freshly dead chicken is similar to what was reported for Escherichia coli and Salmonella sp. Atere et al. [5] and Atere [3] reported a high resistant level in the E. coli and Salmonella isolates respectively, which was attributed to misuse of antibiotics before clinical reports. Antimicrobial resistance in P. multocida has been linked to small plasmids [11]. The coexistence of antibiotic resistant strains in these small plasmids has resulted in P. multocida isolates that are multi-resistant [19]. In this study, nitrofuratoin and gentamycin showed the highest sensitivity, this may have been based on the fact that these antibiotics are not readily used in poultry.
5. Conclusion

With the increase in antibiotic resistance found in *P. multocida*, it is therefore recommended that antibiotic sensitivity test should be carried out before treatment; this will go a long way in the control of fowl cholera. It is also of great importance to encourage farmers to consult veterinary clinics (services), avoid self-prescription and carry out laboratory tests before any administration.

References


