Demonstration of antibiotic resistance genes *strA*, *blaTEM*, *tetA*, *tetC* and *sul2* in *Avibacterium paragallinarum*


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*Avibacterium paragallinarum* causes a highly contagious disease in chickens called infectious coryza. We have previously isolated *A. paragallinarum* from diseased chickens and demonstrated their virulence in experimental infection in different poultry species. In the present study, the isolates were screened for selected resistance genes for clinically relevant antibiotics. The study demonstrated resistance genes for sulphamethoxazole, tetracycline, streptomycin and ampicillin resistance in the isolates. Multiple resistance and resistance genes to streptomycin (*strA*), ampicillin (*blaTEM*), tetracycline (*tetC* and *tetA*) and sulphamethoxazole (*sul2*) in isolates of *A. paragallinarum* are reported. The demonstration of these genes in *A. paragallinarum*, similar to what has been demonstrated in other respiratory pathogens, is a concern for potential horizontal spread of multiple drug resistance leading to treatment failures in different respiratory diseases.

Key words: Antibiotic resistance genes, *Avibacterium paragallinarum*.

INTRODUCTION

*Avibacterium paragallinarum* (Basonym; [*Haemophilus paragallinarum*]) causes a highly contagious disease in chickens called infectious coryza, characterized by respiratory distress and in rare cases systemic disease (Blackall et al., 2005). Increasing prevalence of resistance has been reported in many animal pathogens in different regions of the world due to mainly production factors (Byarugaba, 2004). *A. paragallinarum* is not an exception and laboratory studies have reported resistance in these bacteria (Lu et al., 1983; Reece and Coloe, 1985; Blackall, 1988; Blackall et al., 1989; Takahashi et al., 1990; Prasad et al., 1999). Antimicrobial use is the single most important factor responsible for increased antimicrobial resistance although data in developing countries is still limited. However, there is much misuse of drugs in many of these countries without adherence to withdrawal periods and often in underdoses.

Phenotypic resistances against streptomycin, tetracycline and neomycin have been confirmed in this pathogen (Blackall et al., 1989), however only few studies of the resistance genes in these pathogens have been reported. Hsu et al. (2007) recently demonstrated plasmid mediated multidrug-resistance against streptomycin, sulphonamide, kanamycin, and neomycin. Ridley and Threlfall (1998) have previously demonstrated similar multi-drug resistance in gram negative organism...
of MDR Salmonella enterica serovar Typhimurium phage type DT104. Similarly multidrug resistance mechanisms are becoming frequently reported in related genera of the family Pasteurellaceae (Kehrenberg et al., 2003; Kehrenberg and Schwarz, 2001). High-level multidrug resistance is normally associated with mobile genetic elements (plasmids, transposons or integrons) that encode specific resistance genes (Hall, 1997). We have previously isolated A. paragallinarum from diseased chickens in Uganda (Byarugaba et al., 2007a) and demonstrated their virulence in poultry of different species (Byarugaba et al., 2007b). Some of these isolates were from farms that had received earlier treatment, the aim of the molecular basis for resistance to antibiotics could help in minimizing the resistance and assist in the rational use of antimicrobial drugs during treatment, the aim of the present study was to determine the genes involved in phenotypic resistance in A. paragallinarum isolates from Uganda.

MATERIALS AND METHODS

Bacterial strains

Strains of A. paragallinarum characterized in the present study were isolated from chickens showing signs of infectious coryza in Uganda (Byarugaba et al., 2007a) and their virulence to chickens demonstrated (Byarugaba et al., 2007b). They consisted of five Page’s serovar C isolates. The isolates were maintained on TM/SN medium consisting of 1% Biosate peptone (BBL), 1% NaCl, 0.1% Starch (Sigma), 0.05% glucose, 1.5% Noble agar base (Difco) supplemented with 5% (vol/vol) oleic albumin complex (Sigma), 0.0005% (vol/vol) thiamine (Sigma), 1% (vol/vol) heat inactivated chicken serum, and 0.003% (wt/vol) reduced nicotinamide adenine dinucleotide (NADH) (Sigma) (Eaves et al., 1989).

Antimicrobial susceptibility testing

The isolates were tested for susceptibility to ampicillin, tetracycline, streptomycin, chloramphenicol, sulphamethoxazole and neomycin by the agar dilution method to determine their minimum inhibitory concentrations. Isolates were grown overnight in TM/SN broth (agar omitted) and diluted to 10^5 CFU/ml in TM/SN broth and seeded on TM/SN agar containing doubling dilutions of the antibiotics (128–0.5 mg/ml). Breakpoints values were as recommended by Blackall et al. (1989).

Detection of resistance genes

The presence of genes conferring resistance to ampicillin (blaTEM), streptomycin (strA and aadA genes), sulphamethoxazole (sul1 and sul2 genes), and tetracycline (tet (A), tet(B), tet(C) and tet(G) genes) was investigated by PCR with primers and conditions according to Aarestrup et al. (2003). The primer pair sequences used for detection of the various genes was as follows:

- **BlaTEM**: 5'-ATGAGATTTCAACATTTCG-3' (blaTEM)
- **catA**: 5'-ACCACCTGTAAATCGTACGAG-3' (catA)
- **ApHA**: 5'-GCTATCCGCTATGCTGGGCG-3' (aphA-2)
- **StrA**: 5'-CCAATCGCAGATAGAAGGCC-3' (strA)
- **AadA**: 5'-ATCTCTTCGGGCGATTGTG-3 (aadA)
- **sul1**: 5'-CTCGATGAGGACCCGCGGC-3 (sul1)
- **sul2**: 5'-CCGGCTCAAGGAGATGATTG-3 (sul2)
- **tet(A)**: 5'-GAATTCCGAGGATGCGTGG-3 (tetA)
- **tet(B)**: 5'-CTCTGATGAGGAGCCGCGC-3 (tetB)
- **tet(C)**: 5'-GGTGGAAGGTTCAAGGGC-3 (tetC)
- **tet(G)**: 5'-GCAGCGAAAGCGTATTGGC-3 (tetG)

All resistant isolates were also examined for the presence of plasmids using the QIA prep Spin Minprep Kit (QIAGEN, Germany).

RESULTS AND DISCUSSION

Antimicrobial resistance in the strains included was confirmed. The three strains Apg-3, Apg-4 and Apg-5 showed resistance to ampicillin (A), streptomycin (S), tetracycline (T) and sulphamethoxazole (Su) (ASTSu profile) but varied in MIC values (Table 1) suggesting

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (μg/ml) for the different isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apg-01</td>
</tr>
<tr>
<td>Ampicillin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 (S)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2 (S)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.25 (S)</td>
</tr>
<tr>
<td>Neomycin</td>
<td>1 (S)</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td>2 (S)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>32 (R)</td>
</tr>
</tbody>
</table>

S = susceptible; R = resistant.

Table 1. Minimum inhibitory concentrations (MICs) of six antimicrobial drugs tested against A. paragallinarum strains isolated in Uganda.
Table 2. Resistance genes in antimicrobial resistant *A. paragallinarum* isolates from infectious coryza.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Gene</th>
<th>Apg-01</th>
<th>Apg-02</th>
<th>Apg-03</th>
<th>Apg-04</th>
<th>Apg-05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td><em>blaTEM</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Streptomycin</td>
<td><em>strA</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>aadA</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td><em>sul1</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>sul2</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tetracycline</td>
<td><em>tet(A)</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>tet(B)</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>tet(C)</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>tet(G)</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- = Not detected, + = detected.

![Image of gel](image)

**Figure 1.** Detection of tetracycline (*tetC* and *tetA*) and sulphamethoxazole (*sul2*) resistance genes among *A. paragallinarum* strains. Lanes 1 and 16: Direct Load™ Wide Range DNA Marker; Lane: 2,3,4,5 and 6 correspond to isolates Apg-01, Apg-02, Apg-03, Apg-04 and Apg-05 tested for *tetC* genes; lanes: 7,8,9,10 and 11 are the same isolates in the same order tested for *sul2* gene while lanes 12,13,14 and 15 are isolate Apg-01, Apg-03, Apg-04 and Apg-05 tested for *tetA* gene. Isolates Apg-03, Apg-04 and Apg-05 are positive for *tetC*, *sul2* and *tetA* with corresponding band size of 1200, 200, 400 bp respectively. The band sizes for the genes for *blaTEM* and *strA* (gel not shown here) were 850bp and 250bp respectively.

that different resistance genes could be responsible for the resistance in the different strains. Isolate Apg-01 had an MIC value of 32 towards tetracycline while Apg-02 was fully sensitive with all MIC values below the breakpoint.

Based on the detected resistance profiles, the presence of selected resistance genes was investigated by PCR. Resistance genes *strA*, *blaTEM*, *tetA*, *tetC* and *sul2* were detected (Table 2). *blaTEM*, *sul2*, *tetA* and *tetC* were demonstrated in all three isolates showing the resistance profile ASTSu (Apg-3, Apg-4, Apg-5), accounting for the genetic background for AT and Su.

However, *strA*, conferring resistance to streptomycin, was only detected in Apg-3 and Apg-4. The reason for streptomycin resistance in Apg-5 could be due to other resistance mechanisms other than the *aadA* gene that was not detected at all. The genes *aadA*, *sul1*, *tet (B)* and *tet(G)* genes were not demonstrated. Figure 1 shows
tetA, tetC and sul-2 genes demonstrated by PCR.

Previous studies have reported phenotypic resistance against streptomycin, tetracycline and neomycin in *A. Paragallinarum* (Blackall et al., 1989). Plasmids were not demonstrated in such strains (Blackall, 1988) suggesting that the genetic elements responsible for this resistance are most likely carried on the chromosomes. However plasmid mediated resistance and resistance genes for sulphonamide, kanamycin, streptomycin and neomycin have since been demonstrated by Hsu et al. (2007). Plasmids were not demonstrated in our isolates indicating that the resistance genes could be chromosome mediated.

This study has demonstrated multi drug resistance in *A. paragallinarum*. Similar multidrug resistance types are frequently reported in other genera of the family *Pasteurellaceae* (Kehenberg et al., 2003; Kehenberg and Schwarz, 2001). Kehenberg and Schwarz (2001) have showed that sul-2 and strA genes are widely distributed among epidemiologically unrelated isolates of the genera *Pasteurella* and *Mannheimia* often with clustering of genes for sulphonamide, chloramphenicol and streptomycin resistance (*sul2-catA3-strA*), as well as *sul2-strA* or *strA-sul2* genes alone on structurally different plasmids and on the chromosome of *Pasteurella* and *Mannheimia*. In the present study both sul2 and strA were detected in two isolates suggesting they could be occurring in similar clusters as was reported in *P. multocida*. The similarity of occurrence of multidrug resistance genes observed in the present study with those of related respiratory pathogens may suggest some horizontal exchange of these resistance genes in the respiratory system that may result in treatment failures arising from unrelated infections. To our knowledge this is the first report the occurrence of resistance genes in *A. paragallinarum*.

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**REFERENCES**


