International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (5), pp. 001-004, May, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

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

Byarugaba D. K.[1]*, Minga U. M.[2], Gwakisa P. S.[3], Katunguka-Rwakishaya E.[4], Bisgaard M.[5], Christensen H.[5] and Olsen J. E.[5]

[1]Department of Veterinary Microbiology and Parasitology, School of Veterinary Medicine, Makerere University, Kampala, Uganda.
[2]The Open University of Tanzania, Dar Es Salaam, Tanzania.
[3]Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, Sokoine University of Agriculture, Chu Kikuu, Morogoro Tanzania.
[4]Makerere University Graduate School, Makerere University, Kampala, Uganda.
[5]Department of Pathobiology, Faculty of Life Sciences, University of Copenhagen, 4 Stigbolen DK-1870 Frederiksberg C, Denmark.

Accepted 11 March, 2019

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 under doses.

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

*Corresponding author. E-mail: dkb@vetmed.mak.ac.ug. Tel: 256-41-531169. Fax: 256-41-531173.

Table 1. Minimum inhibitory concentrations (MICs) of six antimicrobial drugs tested against *A. paragallinarum* strains isolated in Uganda.

Antibiotic	MIC (µg/ml) for the different isolates				
	Apg-01	Apg-02	Apg-03	Apg-04	Apg-05
Ampicillin	0.5 (S)	1 (S)	16 (R)	32 (R)	64 (R)
Chloramphenicol	2 (S)	1 (S)	2 (S)	1 (S)	0.5 (S)
Streptomycin	0.25 (S)	0.5 (S)	32 (R)	32 (R)	16 (R)
Neomycin	1 (S)	2 (S)	2 (S)	4 (S)	4 (S)
Sulphamethoxazole	2 (S)	4 (S)	64 (R)	64 (R)	32 (R)
Tetracycline	32 (R)	2 (S)	>64 (R)	>64 (R)	64 (R)

S = susceptible; R = resistant.

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 isolated were from farms that had received earlier treatment without good response. As understanding 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 sensitivity 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 (*bla*$_{TEM}$), 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:

*Bla*TEM: 5-ATGAGTATTCAACATTTCCG-3 (*bla*TEM)
5-ACCAATGCTTAATCAGTGAG-3 (*bla*TEM)
catA1: 5-CGCCTGATGAATGCTCATCCG-3 (*catA1*)
5-CCTGCCACTCATCGCAGTAC-3 (*catA1*)
AphA: 5-GCTATTCGGCTATGACTGGGC-3 (*aphA-2*)
5-CCACCATGATATTCGGCAAGC-3 (*aphA-2*)
StrA: 5-CCAATCGCAGATAGAAGGC-3 (*strA*)
5-CTTGGTGATAACGGCAATTC-3 (*strA*);
AadA: 5-ATCCTTCGGCGCGATTTTG-3 (*aadA*)
5-GCAGCGCAATGACATTCTTG-3(*aadA*)
sul1: 5-CTTCGATGAGAGCCGGCGGC-3(*sul1*)
5-GCAAGGCGGAAACCCGCGCC-3′(*sul1*)
sul2: 5-GCGCTCAAGGCAGATGGCATT-3′(*sul2*)
5-GCGTTTGATACCGGCACCCGT-3′ (*sul2*)
tet(A): 5′-GTAATTCTGAGCACTGTCGC-3′(*tet*(A)
5′-TGCCTGGACAACATTGCTT-3 (*tet*(A)
tet(B) 5′-CTCAGTATTCCAAGCCTTTG-3 (*tet*(B)
5-ACTCCCCTGAGCTTGAGGGG-3(*tet*(B)
tet(C) 5-GGTTGAAGGCTCTCAAGGGC-3 (*tet*(C)
5-CCTCTTGCGGGAATCGTCC-3*(tet*(C)
tet(G) 5-GCAGCGAAAGCGTATTTGCG-3(*tet*(G)
5-TCCGAAAGCTGTCCAAGCAT-3 (*tet*(G).

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 2. Resistance genes in antimicrobial resistant *A. paragallinarum* isolates from infectious coryza.

Antibiotic	Gene	Isolates				
		Apg-01	Apg-02	Apg-03	Apg-04	Apg-05
Ampicillin	*bla*$_{TEM}$	-	-	+	+	+
Streptomycin	*strA*	-	-	+	+	-
	aadA	-	-	-	-	-
Sulphamethoxazole	*sul1*	-	-	-	-	-
	sul2	-	-	+	+	+
Tetracycline	*tet(A)*	-	-	+	+	+
	tet(B)	-	-	-	-	-
	tet(C)	-	-	+	+	+
	tet(G)	-	-	-	-	-

- = Not detected, + = detected.

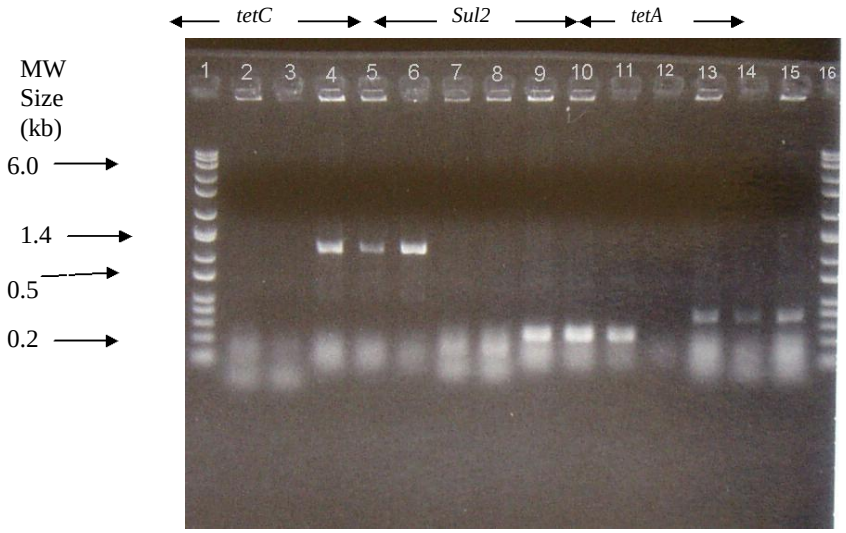

Figure 1. Detection of tetracycline (*tetC* and *tetA*) and sulphamethoxazole (*sul2*) resistance genes among *A. paragallinarum* strains. Lanes 1 and 16: Direct Load ^{TM} Wide Range DNA Marker; Lane: 2,3,4,5 and 6 correspond to isolates Apg-01, Apg-02, Apg-03, Apg-04 and Apg-05 tested for *tetC* genes; 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 t*etA* with corresponding band size of 1200, 200, 400 bp respectively. The band sizes for the genes for *bla*TEM and *str*A (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 break-point.

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 mechnsisms 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* (Kehrenberg et al., 2003; Kehrenberg and Schwarz, 2001). Kehrenberg 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*

ACKNOWLEDGEMENT

This work was supported by the Danish International Development Agency (DANIDA) under the Enhancement of Research Capability (ENRECA) project for Improvement of Health and Production of Chickens in Africa (IHEPRUCA).

REFERENCES

Aarestrup FM, Lertworapreecha M, Evans MC, Bangtrakulnonth A, Chalermchaikit T, Hendriksen RJ, Wegener HC (2001). Antimicrobial susceptibility and occurrence of resistance genes among *Salmonella enterica* serovar *Weltevreden* from different countries. J. Antimicrob. Chemother., 52: 715-718.

Blackall PJ (1988). Antimicrobial drug resistance and the occurrence of plasmids in *Haemophilus paragallinarum*. Avian Dis., 32(4): 742-747.

Blackall PJ, Eaves LE, Rogers DG (1989). Biotyping of *Haemophilus paragallinarum* isolates using hemagglutinin serotyping, carbohydrate fermentation patterns, and antimicrobial drug resistance patterns. Avian Dis., 33: 491-496.

Blackall PJ, Christensen H, Beckenham T, Blackall LL, Bisgaard M (2005). Reclassification of *Pasteurella gallinarum, [Haemophilus] paragallinarum*, *Pasteurella avium* and *Pasteurella volantium* as *Avibacterium gallinarum* gen. nov., comb. nov., *Avibacterium paragallinarum* comb. nov., *Avibacterium avium* comb. nov. and *Avibacterium volantium* comb. nov. Int. J. Syst Evol. Microbiol., 55(1): 353-362.

Byarugaba DK (2004). A view on antmicrobial resistance in developing countries and responsible risk factors. Int. J. Antimicrob. Agents., 24(2) 105-110.

Byarugaba DK, Minga UM, Gwakisa PS, Katunguka-Rwakishaya E, Bisgaard M, Olsen JE (2007a). Investigations on the occurrence of *Avibacterium paragallinarum* infections in Uganda. Avian Dis., 51(2): 534–539.

Byarugaba DK, Minga UM, Gwakisa PS, Katunguka-Rwakishaya E, Bisgaard M, Olsen JE (2007b). Virulence characterisation of *Avibacterium paragallinarum* isolates from Uganda. Avian Pathol., 36(1): 35-42.

Eaves LE, Rogers DG, Blackall PJ (1989). Comparison of hemagglutinin and agglutinin schemes for the serological classification of *Haemophilus paragallinarum* and proposal of a new hemagglutinin serovar. J. Clin. Microbiol., 27(7): 1510 -1513.

Hall RM. (1997) Mobile gene cassette and integron: Moving resistance genes in gram-negative bacteria. Ciba Found Symp., 207: 192-202.

Hsu YM, Shieh HK, Chen WH, Sun TY, Shiang JH (2007). Antimicrobial susceptibility, plasmid profiles and haemocin activities of *Avibacterium paragallinarum* strains. Vet Microbiol., 124(3-4): 209-218.

Kehrenberg C, Schwarz S (2001). Occurrence and linkage of genes coding for resistance to sulfonamides, streptomycin and chloramphenicol in bacteria of the genera *Pasteurella* and *Mannheimia*. FEMS Microbiol Lett., 205(2): 283 -290.

Kehrenberg C, Tham NT, Schwarz S (2003). New plasmid-borne antibiotic resistance gene cluster in *Pasteurella multocida*. Antimicrob Agents Chemother., 47(9): 2978 -2980.

Lu YS, Lin DF, Tsai HJ, Tsai KS, Lee YL, Lee T (1983). Drug sensitivity test of *Haemophilus paragallinarum* isolated in Taiwan. Taiwan J. Vet. Med. Anim. Husb., 41: 73-76.

Prasad V, Murthy KK., Murthy PR, Vakani P, Krishna A, Murthy K, and Radhakrishna MP (1999). Antibiotic sensitivity studies on *Haemophillus paragallinarum* isolated from chickens. Indian Vet. J., 76: 253-254.

Reece RL Coloe PJ (1985). The resistance to anti-microbial agents of bacteria isolated from pathological conditions of birds in Victoria, 1978 to 1983. Aust. Vet. J., 62(11): 379 -381.

Ridley A Threlfall EJ (1998). Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonella typhimurium* DT104. Microb. Drug Resis., 4: 113-118.

Takahashi I, Yoshida T, Honma Y, Saito E (1990). Comparison of the susceptibility of *Haemophilus paragallinarum* to ofloxacin and other existing antimicrobial agents. J. Japan Vet. Med. Assoc., 43: 187-191.