Brazilian Journal of Microbiology 44, 1, 161-164 (2013)
ISSN 1678-4405

Research Paper

Identification and antimicrobial susceptibility patterns of *Pasteurella multocida* isolated from chickens and japanese quails in Brazil

Everlon Cid Rigobelo[1], Patrick Joseph Blackall[2], Renato Pariz Maluta[3], Fernando Antonio de Ávila[4]

[1]Faculdade de Ciência Animal, Universidade Estadual Paulista "Júlio de Mesquita Filho", Dracena, SP, Brazil.
[2]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St. Lucia, Australia.
[3]Faculdade de Microbiologia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, SP, Brazil.
[4]Departamento de Patologia Veterinária, Universidade Estadual Paulista "Júlio de Mesquita Filho" Jaboticabal, SP, Brazil.

Submitted: December 20, 2011; Approved: July 2, 2012.

Abstract

A study was performed to verify the presence of *Pasteurella multocida* in eight different poultry groups of 90 birds each. Groups I to IV were chickens (I being > 6 weeks of age with a history of respiratory illness, II > 6 weeks of age and free of respiratory illness, III < 6 weeks of age with respiratory illness and IV being < 6 weeks of age and with no respiratory illness. Groups V to VIII had the matching characteristics of Groups I to V but consisted of Japanese Quails. The *P. multocida* isolation rate from the groups was as follows; Group I 56/90 (62.3%) Group II 18/90 (20.0%), Group III 12/90 (13.3%), Group IV 3/90 (3.33%), Group V 8/90 (8.88%), Group VI 2/90 (2.22%) Group VII 2/90 (2.22%) and Group VIII 1/90 (1.11%). These isolation rates were not significantly different within the groups of a bird type but the overall chicken isolation rate was significantly higher than the quail isolation rate ($p < 0.01$). All isolates were examined for their sensitivity to four antimicrobial agents. The results showed only low levels of resistance to the agents tested. The highest level of resistance detected was to cephalothin (5.1% of isolates) followed by amikacin (3.4%).

Key words: *Pasteurella multocida*, antimicrobial resistance, chicken, quails.

Introduction

Pasteurella multocida is a common cause of respiratory tract infections in domestic and wild birds (Rimler and Glisson 1977; Hunter & Wobeser, 1980). As an example, the mortality rate due to fowl cholera is estimated to be 5.3% of non-hunting mortalities in North American waterfowl (Stout and Cornwell, 1976). *P. multocida* can cause peracute, acute and chronic infections that can be associated with high mortalities (Christensen & Bisgaard, 2000).

Fowl cholera in quails was first reported by Hinshaw and Emlen (1943). After that first report others have described the diseases in several quail species (Myint and Carter, 1988; Miguel *et al.*, 1998) indicating that quail are highly susceptible to *P. multocida* with a mortality that can reach 13%.

Antimicrobials resistance of bacteria to drugs has become a growing problem in both human and veterinary medicine (Levy, 1998). Many studies have suggested that the use of antibiotics in animal husbandry is a driving force for the selection of resistant strains in certain pathogenic species (Witte, 1998). A large increase of the emergence of multidrug-resistant pathogens has been occurring over the past 10 to 15 years (Shea, 2003).

The potential transfer of antibiotic resistance from animals to humans through the use of antibiotics in animal

Send correspondence to E.C. Rigobelo. Faculdade de Ciência Animal, Universidade Estadual Paulista "Júlio de Mesquita Filho", Rodovia Comandante João Ribeiro de Barros, SP 294, km 651, 17.900-000 Dracena, SP, Brazil. E-mail: everlonagro@yahoo.com.br.

production has been a controversial issue (Witte, 1998). This potential transfer has been implicated as a cause of treatment failure, prolongation of illness and death, and increased costs of treatment (Kelly *et al.*, 2004). In Spain, an increase in oxytetracycline resistance from 1.6 to 14.4% between 1987 and 2004 in *P. multocida* strains isolated from pigs has been shown (San Millan *et al.*, 2009). In Brazil, there appears to have been no publications examining the level of antibiotic resistance in avian isolates of *P. multocida* to date.

In the current study, we have examined the carrier rate of *P. multocida* in two different bird types (chickens and Japanese quails), with groups differing in age (< 6 weeks of age and > 6 weeks of age) and health status (with clinical signs of respiratory disease and free of clinical signs of respiratory disease). We have then examined the antimicrobials resistance profile of the *P. multocida* isolates against four antimicrobial agents.

Materials and Methods

Study flocks

A total of eight flocks on two different farms in the region of Dracena, in the west of São Paulo State, Brazil were included in this study. Each flock was around 900 birds. Flocks I to IV consisted of chickens. In Flock I, the birds were over 6 weeks of age and the flock had a history of clinical respiratory illness. In Flock II, the birds were also over 6 weeks of age but had no history of respiratory illness and no birds showed any clinical signs of respiratory illness. In Flock III, the birds were less than 6 weeks of age and the flock had a history of clinical respiratory illness. In Flock IV, the birds were less than 6 weeks of age but had no history of respiratory illness and no birds showed any clinical signs of respiratory illness. Flocks V to VIII consisted of Japanese quails and had the same distribution of age and respiratory illness as the chicken flocks (V – over 6 weeks of age and with respiratory illness; VI – over 6 weeks of age and free of clinical signs of respiratory illness; VII – less than 6 weeks of age and with respiratory illness; VIII – less than 6 weeks of age and free of clinical signs of respiratory illness).

Antibiotic treatment

When the chickens and quails presented fowl cholera symptoms they received antibiotic treatment with one or more of the following agents - sulfonamides, tetracyclines (oxytetracycline and doxycycline), neomycin and quinolones (norfloxacin). All antibiotics were provided over five consecutive days in the water. Only the birds that presented clinical signs were treated.

Sampling

Within each Flock, a single sampling was performed with 90 birds being sampled in each Flock. With Flocks I, III, V and VII, the samples were taken from birds showing clinical signs of respiratory illness. The birds from Flock I formed Group I, from Flock II Group II and so on. Within each flock, birds were individually sampled by inserting a sterile cotton-tipped applicator onto the pharynx. The swabs were subsequently placed in 2 mL tryptophan broth (Difco) or veal infusion broth (Difco) and kept on ice during transport.

Bacterial isolation

Each swab was streaked on a selective medium (Tryptose blood agar base (Difco) to which 5% citrated bovine blood, 0.02% bacitracin and 1% neomycin were added). After overnight incubation at 37 °C under aerobic conditions, colonies morphologically resembling those of *P. multocida* were subcultured.

Bacterial characterization

Initially all isolates thought to be likely to be *P. multocida* were confirmed as being non-motile, Gram-negative rods, which were facultatively anaerobic, fermented D-glucose without gas formation and were catalase and oxidase positive (Muhairwa *et al.*, 2000). All these isolates were subsequently characterized by standard phenotypic methods as described by Bisgaard *et al.* (1991).

Susceptibility testing

In vitro susceptibility testing was performed by a standardized disk diffusion method (CLSI 2008). *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 served as quality control strains.

The following four antimicrobial agents were tested: cephalothin (CFL, 30 μg); amikacin (AMK, 30 μg), tetracycline (TET, 30 μg) and ampicillin (AMP, 10 μg). The results were interpreted as sensitive, intermediate and resistant using the CLSI guidelines (CLSI 2008).

Statistical analysis

A Chi-square test was performed to determine the significance of the results. The software used was the R version 2.12.0 (The R Foundation for Statistical Computing).

Results

The suspect *P. multocida* isolates produced small, glistening, mucoid and dewdrop-like colonies on blood agar plates, and were Gram-negative coccobacilli. Biochemical testing showed that all strains were urease negative and oxidase and catalase positive. The strains did not growth on McConkey agar and were non-haemolytic on blood agar. All the strains fermented galactose, fructose, D-glucose, D-mannitol and also sucrose, while no reaction with rhamnose, inositol, raffinose, and salicin was observed. All strains were positive in the indole test and non-reactive in the citrate, urease and gelatin tests.

The prevalence of *P. multocida* in different groups of chickens and Japanese quails and the distribution of *P. multocida* isolates are shown in Table 1. Interestingly *P. multocida* was isolated from groups containing healthy birds as follows: 18 of 90 chickens more than six weeks old (Group II), 2 of 90 apparently healthy chickens less than six weeks old (Group VI); 3 of 90 apparently healthy Japanese quails more than six weeks old (Group IV) and 1 of 90 apparently healthy Japanese quails less than six weeks old (Group VIII). It is noticeable that the colonization rate of healthy chickens and quails increased with age (Table 1). The number of isolates from chickens was significantly greater than in quails (89 compared to 13 isolates, $p < 0.01$) and also in birds older than six week (84 compared to 18 isolates, $p < 0.01$) (Table 1). There was no statistical difference between the Groups I to IV and also there was no statistical difference between Group V to VIII ($p > 0.01$).

The results of the antimicrobial resistance testing is shown in Table 2. The QC strains always gave results within the expected range. In both groups, there was no statistically significant difference between the age groups. In general, there was little antibiotic resistance in the isolates, with the greatest level of resistance being around 5% to cephalothin in both age groups of both types of birds.

Discussion

Antimicrobial resistance in *P. multocida* has been linked to small plasmids (Rosenau *et al.*, 1991). The coexistence and spread of these small plasmids has resulted in *P. multocida* isolates that are multiresistant (San Millan *et al.*, 2009) or showing resistance to ampicilin (Rosenau *et al.*, 1991), tetracycline (Kehrenberg *et al.*, 2001) and streptomycin (Wu *et al.*, 2003).

It has been suggested by Kehrenberg *et al.* (2008) that the spread of resistance amongst *P. multocida* isolates is due to the horizontal transfer of plasmids rather than the clonal dissemination of a resistant *P. multocida* isolate. Further, Kehrenberg *et al.* (2008) suggest that the fact that *P. multocida* isolates with a resistance plasmid remain susceptible to a range of other commonly used antibiotics may explain why these isolates are not more commonly encountered.

Prevalence and extent of antimicrobial resistance in a population is strongly correlated with antibiotic usage because selection and dissemination of resistant bacteria are greatly increased by the pressure exerted by these drugs. As a consequence, resistance is most commonly found where heavy use of antibiotics and appreciable host-to-host contact occurs; therefore, sites of intensive farming constitute a large reservoir of antibiotic-resistant bacteria (Murray 1992). In this situation, resistant microorganisms are easily disseminated within units via fecal contact, promoting contamination of the water used by animals or contamination of the soil environment (Teuber, 2001).

In the present study, the antimicrobial resistance level was low, but this may be due to the particular strains isolated, not acquiring resistance or undergoing selection pressure. As clinically ill birds were routinely treated with

Table 1 - Isolation of *P. multocida* in the different study groups.

Group	Species	History	Age in weeks	Nº of Isolates	Percentage of swabs positive
I	Chicken	Respiratory illness	> 6	56	62.2
II	Chicken	No respiratory illness	> 6	18	20.0
III	Chicken	Respiratory illness	< 6	12	13.3
IV	Chicken	No respiratory illness	< 6	3	3.3
V	Quails	Respiratory illness	> 6	8	8.9
VI	Quails	No respiratory illness	> 6	2	2.2
VII	Quails	Respiratory illness	< 6	2	2.2
VIII	Quails	No respiratory illness	< 6	1	1.1

Table 2 - Results of antimicrobial sensitivity testing of all 102 *P. multocida* isolates.

Bird source	% Resistance to the indicated antimicrobial agent			
	Amikacin	Ampicillin	Cephalothin	Tetracycline
Chickens > 6 weeks old (n = 73)	3.4	1.6	5.2	1.8
Chickens < 6 weeks old (n = 15)	3.0	1.4	4.9	1.5
Quail > 6 weeks old (n = 11)	4.2	1.6	4.9	1.7
Quail < 6 weeks old (n = 3)	1.6	1.3	4.8	1.6
All (n = 102)	3.4	1.6	5.1	1.6

antibiotics, there was certainly exposure to antibiotics in the flocks suffering from clinical fowl cholera.

Broadly similar results of a low level of antimicrobial resistance have been reported in a study that looked at 80 isolates from diseased chickens during the period ranging from 2001 to 2003 in the United States (Huang *et al.* 2009). In that study, Huang *et al.* (2009) found the resistance level to ampicillin and cephalotin to be less than 5% with a slightly higher level of resistance to tetracycline (6.2%).

Other studies have reported a much higher level of antimicrobial resistance in poultry *P. multocida* isolates. Selliey *et al.* (2009) in a study of 56 isolates from poultry in Hungary reported that 15% of isolates were resistant to tetracycline. Shivachandra *et al.* (2004) also reported much higher levels of resistance (ampicillin 23.58%; amikacin 55.28% and to tetracycline 24.39%) in study involving one hundred and twenty-three strains of *Pasteurella multocida* obtained from outbreaks of fowl cholera from different avian host and various geographical regions of India.

In conclusion, the present study demonstrated the presence of *P. multocida* in apparently healthy birds. However, the majority of isolates showed low resistance to the antimicrobials tested, suggesting that in two farms analyzed, the antimicrobial resistance of *P. multocida* strains isolated from chicken and Japanese quails was of little concern.

Acknowledgments

The authors thanks the Fapesp by financial support.

References

CLSI (2008) Performance standards for antimicrobial disks and dilution susceptibility tests for bacteria isolated from animals; Approved Standard. 3rd edition. Clinical and Laboratory Standards Institute, Wayne.

Hinshaw WR, Emlen JT (1943) Pasteurellosis in California Valley quail. Cornell Vet 33:351-353.

Huang TM, Lin TL, Wu CC (2009) Antimicrobial susceptibility and resistance of chicken *Escherichia coli*, *Salmonella* spp., and *Pasteurella multocida* isolates. Avian Dis 53:89-93.

Hunter B, Wobeser G (1980) Pathology of experimental avian cholera in mallard ducks. Avian Dis 24:403-414.

Kehrenberg C, Salmon SA, Watts JL, Schwarz S (2001) Tetracycline resistance genes in isolates of *Pasteurella multocida*, *Mannheimia haemolytica*, *Mannheimia glucosida* and *Mannheimia varigena* from bovine and swine respiratory disease: intergeneric spread of the *tet*(H) plasmid pMHT1. J Antimicrob Chemother 48:631-640.

Kehrenberg C, Wallmann J, Schwarz S (2008) Molecular analysis of flofenicol-resistant *Pasteurella multocida* isolates in Germany. J Antimicrob Chemother 62:951-955.

Kelly L, Smith DL, Snary EL, Johnson JA, Harris AD, Wooldrige M, Morris Jr JG (2004) Animal growth promoters: To ban or not to ban? A risk assessment approach. Int J Antimicrobiol Agents 24:205-212.

Levy SB (1998) The challenge of antibiotic resistance. Sc Am 278:46-53.

Miguel B, Wang C, Maslin WR, Keirs RW, Glisson JR (1998) Subacute to chronic fowl cholera in a flock of Paraof Breeder Quail. Avian Dis 42:204-208.

Murray BE (1992) Problems and dilemmas of antimicrobial resistance. Pharmaco 12:S86-S93.

Myint A, Carter GR (1988) Fowl cholera in quail in Burma. Trop Anim Health Prod 20:35-36.

Rimler RB, Glisson JR (1977) Fowl cholera. In: Calnec BW, Barnes HJ, Beard CW, MacDougald LR, Saif YM (eds) Diseases of Poultry. 10th edition. State University Press, Ames, pp. 143-159.

Rosenau A, Labigne A, Escande F, Courcoux P, Philippon A (1991) Plamid mediated ROB-1 beta-lactamase in *Pasteurella multocida* from a human specimen. Antimicrob Agents Chemother 35:2419-2422.

San Millan A, Escudero JA, Gutierrez B, Hidalgo L, Garcia N, Montserrat L, Dominguez L, Zorn GB (2009) Multiresistance in *Pasteurella multocida* is mediated by coexistence of small plasmids. Antimicrob Agents Chemother 53:3399-3404.

Shea KM (2003) Antibiotic resistance. What is the impact of agricultural uses of antibiotics on children health? Pediatrics 112:253-258.

Shivachandra SB, Kumar AA, Biswas A, Ramakrishnan MA, Singh Vijendra P, Srivastawa SK (2004) Antibiotic sensitivity patterns among Indians strains of avian *Pasteurella multocida*. Trop Anim Health Prod 36:743-750.

Stout J, Cornwell BG (1976) Nonhunting mortality of fledged North American waterfowl. J Wildlife Dis 40:681-693.

Teuber M (2001) Veterinary use and antibiotic resistance. Curr Opin Microbiol 4:493-499.

Witte W (1998) Medical consequences of antibiotic use in agriculture. Scienc 279:996-997.

Wu JR, Shieh HK, Shien JH, Gong SR, Chang PC (2003) Molecular characterization of plasmids with antimicrobial resistant genes in avian isolates of *Pasteurella multocida*. Avian Dis 47:1384-1392.