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Antimicrobial resistance in bacteria associated with porcine respiratory disease in Australia

Denise Ann E. Dayao,[1]Justine S. Gibson,[1] Patrick J. Blackall[2] and Conny Turni[2]

[1]The University of Queensland, School of Veterinary Science, Gatton, Qld, 4343, Australia

[2]The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, EcoSciences Precinct, DuttonPark, Qld, 4102, Australia

Corresponding Author: Denise Ann E. Dayao; Mailing Address: Level 2A EcoSciences Precinct, Boggo Road, DuttonPark, QLD 4102, Australia; Phone: +61 7 3255 4304; Fax: +61 7 3846 0935; Email: d.dayao@uq.edu.au

Abstract

The porcine respiratory disease complex greatly affects the health and production of pigs. While antimicrobial agents are used to treat the respiratory infections caused by bacterial pathogens, there is no current information on antimicrobial resistance in Australian pig respiratory bacterial isolates. The aim of this study was to determine the antimicrobial resistance profiles, by determining the minimum inhibitory concentration of nine antimicrobial agents for 71 *Actinobacillus pleuropneumoniae*, 51 *Pasteurella multocida* and 18 *Bordetella bronchiseptica* cultured from Australian pigs. The majority of *A. pleuropneumoniae* isolates were resistant to erythromycin (89%) and tetracycline (75%). Resistance to ampicillin (8.5%), penicillin (8.5%) and tilmicosin (25%) was also identified. The *P. multocida* isolates exhibited resistance to co-trimoxazole (2%), florfenicol (2%), ampicillin (4%), penicillin (4%), erythromycin (14%) and tetracycline (28%). While all the *B. bronchiseptica* isolates showed resistance to beta-lactams (ampicillin, ceftiofur and penicillin), some were resistant to erythromycin (94%), florfenicol (6%), tilmicosin (22%) and tetracycline (39%). The incidence of multiple drug resistance (MDR) varied across the species – in *B. bronchiseptica*, 27.8% of resistant isolates showed MDR, while 9.1% of the resistant isolates in *A. pleuropneumoniae*, and 4.8% in *P. multocida* showed MDR. This study illustrated that Australian pig strains of bacterial respiratory pathogens exhibited low levels of resistance to antimicrobial agents commonly used in the pig industry.

Keywords: Porcine respiratory disease; antimicrobial susceptibility testing; antimicrobial resistance

1. Introduction

The porcine respiratory disease complex (PRDC), one of the most significant problems affecting health and production in the pig industry worldwide, is described as a multifactorial pneumonic state resulting from the interaction of bacteria, viruses and stresses caused by management, environment and genetic conditions (Opriessnig et al., 2011). A range of bacterial pathogens is associated with the initiation and progress of PRDC, with *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Pasteurella multocida* and *Bordetella bronchiseptica* having significant roles (Fablet et al., 2011; Opriessnig et al., 2011).

The use of antimicrobial agents, beta-lactams (ampicillin, penicillin and cephalosporins) (except for *B. bronchiseptica*), co-trimoxazole (sulfonamide and trimethoprim combination), florfenicol, macrolides (erythromycin, tilmicosin and tulathromycin) and tetracyclines remains the best treatment option to control PRDC (Karriker et al., 2013). The usage of antimicrobial agents has the potential to select for antimicrobial resistance (Barton et al., 2003). Resistance to antimicrobials commonly used to treat PRDC have been detected previously in porcine respiratory disease pathogens from many countries (Vicca et al., 2004; de la Fuente et al., 2007; San Millan et al., 2009; Tang et al., 2009; Chander et al., 2011; Kucerova et al., 2011; Nedbalcová et al., 2013).

In the past, antimicrobial resistance in Australia was reported in *A. pleuropneumoniae* (Eaves et al., 1989) and *P. multocida* (Stephens et al., 1995). However, no information exists for *B. bronchiseptica*. Thus, this study aimed to determine the antimicrobial susceptibility of *A. pleuropneumoniae*, *P. multocida* and *B. bronchiseptica* Australian isolates against antimicrobial agents used for bacterial respiratory pathogens.

2. Materials and Methods

The bacterial isolates tested were obtained from Australian pigs in diagnostic disease investigations and then submitted to the Microbiology Research Group, EcoSciences Precinct, Department of Agriculture Fisheries and Forestry (DAFF), Queensland, Australia for confirmatory identification and/or serotyping. A total of 71 *A. pleuropneumoniae*, 51 *P. multocida* and 18 *B. bronchiseptica* isolates collected between the years 2002 to 2013 were selected from the culture collection of the Microbiology Research Group. All isolates were diagnostic submissions from Australian pig herds. The *A. pleuropneumoniae* isolates represent 19% of the total available culture collection of the Microbiology Research Group and originated from New South Wales (8 isolates), Queensland (24 isolates), South Australia (8 isolates), Victoria (24 isolates) and Western Australia (7 isolates). The *P. multocida* isolates came from New South Wales (12 isolates), Queensland (22 isolates), South Australia (3 isolates), Victoria (1 isolate) and Western Australia (13 isolates). The *B. bronchiseptica* isolates came from New South Wales (4 isolates), Queensland (13 isolates) and South Australia (1 isolate). All isolates of *P. multocida* and *B. bronchiseptica* existing in the culture collection were included in this study. With the exception of *B. bronchiseptica*, all the isolates had been previously identified by a relevant species specific polymerase chain reaction (Gram and Ahrens, 1998; Townsend et al., 1998; Miflin and Blackall, 2001). The *B. bronchiseptica* isolates had been previously identified by sequencing of the 16S rDNA gene using a previously described method (Blackall et al., 2001).

Antimicrobial resistance was detected by determination of MIC in duplicate using CLSI standards and recommendations (CLSI, 2013). The media used were chocolate Mueller Hinton agar (BD) for *A. pleuropneumoniae*; and cation adjusted Mueller Hinton broth (BD) for *P. multocida* and *B. bronchiseptica.* The antimicrobials used were ampicillin, ceftiofur,

co-trimoxazole, florfenicol, erythromycin, penicillin, tetracycline, tilmicosin and tulathromycin. As per the CLSI (2013), the quality control strains used were *A. pleuropneumoniae* (ATCC 27090) and *S. aureus* (ATCC 29213).

The MIC was defined as the lowest antimicrobial concentration that inhibited bacterial growth. The interpretation of MIC of each antimicrobial agent against the three bacterial species was based on the breakpoints provided by the CLSI (2013), where available. As there are no CLSI interpretative breakpoints for penicillin, the one for ampicillin was used (CLSI, 2013). The breakpoints (shown in Table 1) for some antimicrobial agents were taken from other published studies and are detailed in the following text. For *A. pleuropneumoniae*, breakpoints for erythromycin and co-trimoxazole were the ones used by Archambault et al. (2012). For *P. multocida*, the breakpoints used were from the CLSI guidelines (CLSI, 2013) except for erythromycin (Tang et al., 2009) and co-trimoxazole (Archambault et al., 2012). The breakpoints used for *B. bronchiseptica* were the values provided by the CLSI guidelines (CLSI, 2013) where available while some were taken from the published literature - erythromycin (Tang et al., 2009) and co-trimoxazole (Archambault et al., 2012).

3. Results and Discussion

The MIC distribution of 71 *A. pleuropneumoniae*, 51 *P. multocida* and 18 *B. bronchiseptica* isolates, the percentage of resistance in each antimicrobial as well as the MIC_{50} and MIC_{90} are shown in Table 1.The MICs of the reference strains in each test run were within the CLSI acceptable quality control ranges. All *A. pleuropneumoniae* were susceptible to ceftiofur, co-trimoxazole, florfenicol and tulathromycin. Overall, 66 of 71 (93%) of the *A. pleuropneumoniae* isolates were resistant to one or more antimicrobials, showing seven antimicrobial resistance patterns. Resistance to ampicillin (8.5%), penicillin

(8.5%), tilmicosin (25%), tetracycline (75%) and erythromycin (89%) was detected. All *P. multocida* isolates were susceptible to ceftiofur, tilmicosin and tulathromycin. Twenty one (41%) of the isolates exhibited resistance, showing five antimicrobial resistance patterns in which 2% were resistant to co-trimoxazole, 2% to florfenicol 4% to ampicillin and penicillin, 14% to erythromycin and 28% to tetracycline. All *B. bronchiseptica* isolates were susceptible to co-trimoxazole and tulathromycin and resistant to all beta-lactams included in this study. The obtained MICs showed resistance to florfenicol (6%), tilmicosin (22%), tetracycline (39%) and erythromycin (94%). The antimicrobial resistance of *B. bronchiseptica* isolates demonstrated six patterns.

In examining the results of the current study, there are a number of issues that need to be considered. Firstly, it is important to understand that the study is based on a collection of isolates submitted for identification and/or serotyping from across Australia. The collection, however, cannot be regarded as being representative of the full diversity of these pathogens present in the Australian pig herd. A much larger study, seen for example in the recent North American study by Portis et al. (2013), would be required to gain insight into the national picture in Australia. Secondly, while there is no specific knowledge, it is highly likely that the isolates used in the current study would have come from pigs exposed to antimicrobial treatment. Indeed, the antimicrobial agents used in this study are all registered for use in Australian pigs (https://portal.apvma.gov.au/pubcris). The VetPath program in Europe (de Jong et al., 2012) is seeking to address this issue by examining isolates obtained prior to the commencement of any antimicrobial treatment program. Because of these issues (i.e. use of a collection based on diagnostic submissions with an unknown history of antimicrobial treatment), no direct comparisons of the levels of resistance found in the current study with those reported in other studies in other countries will be made.

130 There was a lower prevalence of antimicrobial resistance found in *P. multocida* isolates
131 compared with the detected resistance in *A. pleuropneumoniae* (41% showed resistance to at
132 least one antimicrobial agent compared to 93% of *A. pleuropneumoniae* isolates). One isolate
133 showed co-resistance to all antimicrobials except for ceftiofur, tilmicosin and tulathromycin
134 and gave a high MIC to florfenicol (≥128µg/ml). This particular *P. multocida* strain and one
135 *B. bronchiseptica* are the only isolates amongst all strains in this study to show florfenicol
136 resistance. Florfenicol resistance has previously been detected in *P. multocida* isolates in
137 Germany (Kehrenberg et al., 2005) and in the Czech Republic (Nedbalcová et al., 2013). The
138 *B. bronchiseptica* isolates showed resistance to all antimicrobial agents tested except for
139 tulathromycin and gave high MICs (64 to ≥128 µg/ml) to ceftiofur and penicillin. Resistance
140 to beta-lactam agents (ampicillin, ceftiofur and penicillin) matches previous reports (Kadlec
141 et al., 2004; Chander et al., 2011). The beta-lactam resistance of *B. bronchiseptica* has been
142 previously detected to be associated with the production of beta lactamase enzymes and
143 reduced membrane permeability to ceftiofur (Kadlec et al., 2007; Chander et al., 2011).
144 For the purpose of this study, multidrug-resistance (MDR) was defined as resistance to
145 three or more antimicrobial classes. The MDR patterns obtained from the tested isolates are
146 shown in Table 2. In this study, there were two MDR patterns detected in 9.1% (6/66) of the
147 antimicrobial resistant *A. pleuropneumoniae* isolates. Only one (1/21) *P. multocida* isolate
148 showed MDR, while five *B. bronchiseptica* isolates (5/18) showed MDR. Interestingly, the
149 MDR patterns detected differed between Australian bacterial species. The variation across
150 species might be associated with the resistance mechanisms distinctive to each species which
151 can be evaluated by genetic characterisation (Kehrenberg et al., 2005; San Millan et al., 2009;
152 Chander et al., 2011; Archambault et al., 2012). Further investigations are essential to explain
153 the mechanisms involved in the antimicrobial resistance of Australian species.

In summary, this study presented data on the antimicrobial resistance profiles of *A. pleuropneumoniae, P. multocida* and *B. bronchiseptica* isolated from Australian pigs. The study found that Australian strains showed resistance to older (ampicillin, co-trimoxazole, erythromycin, penicillin and tetracycline) and newer (ceftiofur, florfenicol, tilmicosin and tulathromycin) generation antimicrobial agents. Antimicrobial susceptibility monitoring programs of important veterinary pathogens are necessary to provide evidence based guidance for antimicrobial therapy of bacterial diseases.

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Table 1
Minimum inhibitory concentration distribution of 71 *A. pleuropneumoniae*, 51 *P. multocida* and 18 *B. bronchiseptica*.

Antimicrobial agents and bacterial species	Number of isolates with MIC (μg/ml) of ≤0.12	0.25	0.5	1	2	4	8	16	32	64	≥128	MIC_{50}	MIC_{90}	% R
Ampicillin														
A. pleuropneumoniae	38	27				2	4					≤ 0.12	0.25	8.5
P. multocida	38	11	0	0	0	0	1	1				≤ 0.12	0.25	4
B. bronchiseptica						5	8	3	2			9	16	100
Ceftiofur														
A. pleuropneumoniae	71											≤ 0.12	≤ 0.12	0
P. multocida	49	2										≤ 0.12	≤ 0.12	0
B. bronchiseptica										14	4	64	≥128	100
Co-trimoxazole														
A. pleuropneumoniae	70	1										≤ 0.12	≤ 0.12	0
P. multocida	49	1				1						≤ 0.12	≤ 0.12	2
B. bronchiseptica	9			9								≤ 0.12	1	0
Erythromycin														
A. pleuropneumoniae						8	38	25				8	16	89
P. multocida			3	3	20	18	7					2	8	14
B. bronchiseptica						1	15	1	1			8	8	94
Florfenicol														
A. pleuropneumoniae		3	40	28								0.25	0.5	0
P. multocida	1		35	13		1					1	0.5	1	2
B. bronchiseptica					4	13			1			4	4	6
Penicillin														
A. pleuropneumoniae	7	40	18			1	5					0.25	0.5	8.5
P. multocida	46	2		1			2					≤ 0.12	≤ 0.12	4
B. bronchiseptica										14	4	64	≥128	100
Tetracycline														

A. pleuropneumoniae			2	16	5	7	8	14	14	5		8	32	75
P. multocida		1	22	14	5	4	4		1			1	2	28
B. bronchiseptica			11		7							0.5	2	39
Tilmicosin														
A. pleuropneumoniae							7	46	16	2		16	32	25
P. multocida				2	16	17	13	3				4	8	0
B. bronchiseptica							2	12	3	1		16	32	22
Tulathromycin														
A. pleuropneumoniae								20	51			32	32	0
P. multocida	1	22	19	9								0.5	1	0
B. bronchiseptica			2	9	5	2						1	2	0

Vertical lines indicate breakpoints for resistance; % R means percentage of resistance

MIC_{50}, MIC_{90} - the lowest concentration of antimicrobial agent capable of inhibiting the growth of 50% and 90% of isolates, respectively

Co-trimoxazole- trimethoprim and sulfamethoxazole in the ratio 1:19; test ranges are expressed as the trimethoprim concentration.

242

Table 2
Antimicrobial resistant (AMR) patterns detected in *A. pleuropneumoniae, H. parasuis, P. multocida and B. bronchiseptica* cultured from Australian pigs

Species	AMR pattern	Number of isolates[a]
A. pleuropneumoniae	EryTet	31
	EryTetTil	13
	Ery	12
	[b]AmpEryPenTetTil	4
	Tet	3
	[b]AmpEryPenTet	2
	EryTil	1
P. multocida	Tet	13
	Ery	5
	EryTet	1
	AmpPen	1
	[b]AmpCotriEryFfcPenTet	1
B. bronchiseptica	AmpCefEryPen	9
	AmpCefEryPenTil	3
	[b]AmpCefEryPenTetTil	2
	[b]AmpCefEryTetPen	2
	[b]AmpCefEryFfcPenTetTil	1
	AmpCefPen	1

[a]Number of isolates showing respective AMR pattern
[b]Multidrug-resistant patterns exhibited by resistant isolates
Amp- ampicillin, Cef- ceftiofur, Cotri- cotrimoxazole, Ery- erythromycin, Ffc- florfenicol, Pen- penicillin, Til- tilmicosin and Tula- tulathromycin