Ribotype diversity of porcine *Pasteurella multocida* from Australia

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Objective To use the technique of ribotyping to investigate the genetic diversity of Australian isolates of *Pasteurella multocida* associated with outbreaks of clinical disease in Australian pigs.

Design One hundred and seven porcine *P multocida* isolates were analysed by ribotyping using the restriction enzymes *Hpa*I and *Hind*III. The genetic population structure of the Australian porcine *P multocida* isolates was determined through statistical analysis of the joint ribotype patterns, and this was then compared with biochemical and epidemiological data available for the population.

Results A total of 25 combined ribotypes were recognised, which were grouped into five ribotype clusters. Despite the deliberate selection of diverse isolates, the study revealed only a limited degree of genetic diversity. Fourteen of the ribotypes contained multiple isolates, and 12 of these ribotypes were present on more than one farm. Three of the seven biovars analysed in the study showed very limited diversity. All fifteen biovar 2 isolates (subsp *multocida*) were found in a single cluster (III), while all four biovar 8 isolates, which correspond to *P multocida* subsp *gallicida*, were allocated by themselves to a single cluster (IV). All nine of the biovar 12 isolates (lactose-positive subsp *multocida*) were assigned to a single cluster (I), together with the single biovar 14 isolate, which was the only other lactose-positive isolate in the population (ODC-negative).

Conclusion A limited number of ribotypes of *P multocida* are associated with Australian pigs. The majority of these ribotypes are widely distributed across multiple farms, and across multiple states. Individual farms can possess multiple ribotypes of *P multocida*. Some of the unusual biochemical variants of P multocida present in Australian pigs have a very limited genetic diversity. The nature of pig production in Australia, primarily involving continuous flow systems with few closed herds, has possibly contributed to the widespread distribution of a limited number ribotypes.

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DIG	Digoxigenin
ODC	Ornithine decarboxylase
PAR	Progressive atrophic rhinitis

Pasteurella multocida is a major pathogen of pigs and is involved in a number of different disease conditions. Pneumonic pasteurellosis, one of the most common and widespread diseases of intensively housed pigs[1] and PAR, an important disease of pigs in North America and Europe[2] are the two major disease conditions associated with *P multocida*.

Three subspecies within *P multocida* (*P multocida* subsp *multocida*, *P multocida* subsp *septica* and *P multocida* subsp *gallicida)* are now recognised.[3] We have recently completed a phenotypic characterisation of 150 indole-positive *Pasteurella*-like organisms isolated from diseased Australian pigs,[4] using a biotyping scheme we originally developed for avian *P multocida*.[5] In this recent study, we found 91% of the isolates were subsp *multocida* and the remaining 9% were subsp *gallicida.* Six biovars were recognised within the Australian subsp *multocida* isolates (including two that contain only lactose-positive isolates), and a single biovar was recognised within the Australian subsp *gallicida* isolates.[4]

Ribotyping appears to be a useful tool for investigating genetic relationships between members of the genus *Pasteurella*. Petersen et al[6] recently reported that for the classification of maltose-positive isolates of *Pasteurella*, results from ribotyping (using the *Hin*dIII and *Hpa*II enzymes) correlated closely with data generated from phenotypic and 16S rRNA sequencing studies. Ribotyping has also been used extensively to sub-type both porcine and avian isolates of *P multocida*. Examples of studies on porcine *P multocida* include the epidemiological studies of Gardner et al[7] and Zhao and colleagues.[8,9]

The information generated from studies of genetic diversity can have significant practical outcomes. A knowledge of the genetic structure of the *P multocida* population in Australian pig herds will help to assess the value of ribotyping as an epidemiological tool for porcine *P multocida*. A knowledge of the genetic diversity present in a collection of strains allows the rational selection of strains to evaluate new diagnostic tests or new vaccines. In the current study, we used ribotyping to examine and quantify the diversity present in a collection of Australian porcine isolates of *P multocida*. Isolates in this collection were deliberately selected to be as epidemiologically diverse as possible.

To our knowledge this is the first time that a study has attempted to quantify the genetic diversity present in a porcine *P multocida* population, although a similar study has been performed at this laboratory on avian *P multocida.*[10]

Materials and methods

Bacteria

A total of 107 isolates of *P multocida* were used in this study. All were obtained from Australian pigs and all had been phenotypically characterised.[4] These field isolates had each been assigned to one of 14 biovars and were found to represent two of the *P multocida* subspecies (subsp *multocida* and *gallicida*), on the basis of ODC activity and the ability to ferment seven carbohydrates : arabinose, dulcitol, maltose, sorbitol, galactose, trehalose and xylose.[4,5] The isolates were deliberately selected to be as diverse as possible. Isolates were obtained from New South Wales (19 isolates from 19 farms), Queensland (69 isolates from 39 farms) and Victoria (19 isolates from 19 farms). Isolates were generally limited to one per farm unless there was some evidence (such as biovar variation) which indicated that the

isolates may not have been clonal in nature. Table 1 shows the properties of the isolates used in this study.

Bacterial growth and DNA extraction

Isolates were grown overnight at 37°C in Brain Heart Infusion broth (BBL). Following incubation, samples were plated onto sheep blood agar to check for purity. The bacteria were harvested by centrifugation (15,000 *g*, 20 min, 4°C). The resultant pellet was washed in 5 mL of SE buffer (150 mM NaCl, 100 mM EDTA, pH 8) and duplicate pellets were retained and were held at -20°C until required for DNA extraction. DNA extraction was performed by a standard methodology as described previously.[11]

Ribotyping

Total DNA (5 μg) was digested with both *Hpa*II and *Hin*dIII (Boehringer Mannheim) in separate reactions. The digestions were performed in Tris/acetate buffer[12] using 6.25 U enzyme per 50 mL reaction. The digestion was performed at 37°C for 3 h and terminated by heating at 65°C for 20 min. DNA fragments were separated by horizontal electrophoresis through a 0.7% agarose gel at 25V for 16 h in Tris/Borate/EDTA electrophoresis buffer.[13] DIG-labelled DNA Molecular Weight Marker II (Boehringer Mannheim) was used as a marker. The DNA from the gel was transferred to a positively charged nylon membrane (Boehringer Mannheim) with the VacuGene XL Vacuum Blotting System (Pharmacia) according to the manufacturer's instructions.

The probe used in the ribotyping was a PCR-generated 16S rRNA gene fragment of the type strain of *P multocida* subsp *multocida* (NCTC 10322), prepared and used as described previously for *Haemophilus paragallinarum*.[14] Southern hybridisation and detection were performed using the Nucleic Acid Detection Kit (Boehringer Mannheim) at 68°C for both prehybridisation and hybridisation steps and posthybridisation washes.

Analysis of ribotyping patterns

Fragment patterns for each restriction enzyme were compared visually and distinct patterns were designated as ribotypes. All bands present in the population were identified and each ribotype assigned a band profile with a value of 1 or 0 corresponding to the presence or absence of each band in the population, respectively. Once the binary band profile for each enzyme was elucidated for each ribotype, combined ribotypes were created by bringing together the binary profiles for each individual restriction enzyme into a single extended profile. A matrix describing the designated band profiles for each ribotype was converted to ASCII file data and imported into the NTSYS computer program (Exeter Publishing, Setauket, NY, USA). A similarity matrix was constructed according to the Dice coefficient with the SIMQUAL function of the NTSYS program. A phenogram of genetic similarity was constructed according to the UPGMA clustering strategy using the SAHN clustering tree and TREE DISPLAY functions of NTSYS. The cophenetic correlation co-efficient was calculated using the MXCOMP function of NTSYS.

Results

Both restriction enzymes produced well-resolved patterns that contained five to seven bands and allowed easy visual distinctions to be made between the strains. The use of *Hin*dIII gave 13 unique ribotypes among the 107 isolates whereas *Hpa*II gave 12 unique ribotypes. The combination of the two enzyme patterns resulted in 25 unique ribotypes, numbered 1 to 25. The allocation of the isolates to the ribotypes is shown in Table 1. The details of the ribotype bands and their distribution within the combined ribotypes is outlined in Table 2.

A phenogram of genetic relatedness within the *P multocida* population, based on the combined ribotyping results, is presented in Figure 1. The cophenetic correlation coefficient for this phenogram was found to have a value of 0.82, indicating that it was a good reflection of the raw similarity data. Five phenotypically coherent major clusters, numbered I to V, are apparent at a genetic similarity of 0.7. The properties of these five clusters are shown in Table 3.

Of the 25 ribotypes recognised in this study, 14 contained more than one isolate. Within these 14 multi-isolate ribotypes, 12 consisted of isolates from more than one farm. Of these 12 multi-farm ribotypes, three consisted of isolates from farms in just one State, five consisted of isolates from farms in two States and four consisted of isolates from farms in three States. All five ribotype clusters were present on at least two farms, while three clusters (I, II and III) were present on farms in all three States represented in the study.

Of the 14 ribotypes that contained more than one isolate, six consisted of multiple biovars while eight consisted of only one biovar. All 14 multi-isolate ribotypes contained only a single subspecies. While three of the five clusters (I, II and III) contained multiple biovars, clusters IV and V contained only a single biovar, 8 and 3 respectively. Each of the five ribotype clusters contained only one subspecies.

Four unusual biovars were present in the isolates used in this study: biovar 8 (which corresponds to subsp *gallicida*), biovars 12 and 14 (which correspond to lactose-positive subsp *multocida* and lactose-positive, ODC-negative subsp *multocida*) and biovar 13 (which corresponds to ODC-negative subsp *multocida*).

All four biovar 8 isolates were assigned to a single ribotype cluster (IV), which contained only that biovar. This cluster was markedly distinct from the remainder of the population, and was most closely related to the two isolates that comprised ribotype cluster V.

All nine biovar 12 isolates and the sole biovar 14 isolate were assigned to ribotype cluster I. Seven of the nine biovar 12 isolates were assigned to a single ribotype (ribotype 1). These seven isolates were obtained from seven different farms, with these farms being located in all three States represented in the study.

A total of 16 farms were represented by more than one isolate, and half of these had more than one biovar present. Of the eight farms with more than one biovar all were shown to have isolates that belonged to different ribotypes. In addition, six of the eight had isolates from different ribotype clusters. Of the eight farms with multiple isolates of a single biovar, three were shown to have isolates that belonged to different ribotypes and one had isolates that belonged to different ribotype clusters.

Discussion

This study was undertaken to examine the diversity present in Australian isolates of porcine *P multocida*. For this reason, the collection of isolates examined was deliberately selected to be as diverse as possible. This was done by generally selecting only one isolate from a piggery. In some cases where a prior

Table 1. Properties of isolates used in this study.

Cluster	Joint ribotype	Study Code	Biovar	Farm code[a]	*Hind*III Ribotype	*Hpa*II Ribotype
I	1	PM47, 129	3	N1, Q1	1	1
		PM21, 110, 122, 127, 203, 262 351	12	N2, Q2, Q3, Q4, Q5, Q6, V1	1	1
		PM346	14	V2	1	1
I	2	PM23, 98, 101, 102, 106, 108, 109, 253, 267, 271, 272, 273, 281, 282, 287, 303, 306, 337, 340, 341, 418, 430	3	N3, N4, N5, N6, N7, N8, N9, Q7, Q8, Q9, Q9, Q10, Q11, Q11, Q9, Q12, Q12, V3, V4, V5, Q13, Q14	1	4
I	5	PM296	12	Q15	2	1
I	13	PM254, 427	3	Q7, Q16	6	1
I	14	PM104, 107, 289, 428, 431	3	N10, N11, Q14, Q17, Q14	6	4
I	9	PM347	12	V6	5	1
I	10	PM270, 286, 290		Q9, Q9, Q14	5	4
		PM 293	3	Q14		
I	11	PM309, 323, 342, 348, 349, 350, 352, 353, 354	3	Q18, Q19, V7, V8, V9, V10, V11, V12, V13	5	5
I	4	PM128, 338	1	Q20, V14	1	10
II	16	PM42, 99, 100, 105, 126, 247, 252, 256, 266, 294, 301, 356, 407, 408, 409, 411, 425, 426	3	N12, N13, N14, N15, Q21, Q22, Q23, Q24, Q25, Q26, Q18, V15, Q27, Q27, Q27 Q22, Q28, Q28	7	4
		PM 291	13	Q14		
II	20	PM237, 280	3	Q29, Q29	9	4
II	22	PM278, 279	3	Q30, Q30	10	4
II	24	PM113	3	N16	12	9
II	17	PM308	1	Q31	7	11
II	18	PM263	1	Q8	7	12
III	3	PM29	2	N17	1	6
III	12	PM103, 125, 265, 339, 355	2	N18, Q32, Q25, V16, V17	5	6
		PM307	3	Q33	5	6
III	25	PM111	3	N19	13	6
		PM429	2	Q19	13	6
III	15	PM244, 248, 250, 344, 345, 370, 410, 436	2	Q22, Q34, Q15, V18, V19, Q35, Q36, Q37	6	6
		PM264	3	Q8	6	
III	19	PM288	3	Q38	8	6
IV	6	PM295, 332	8	Q15, Q22	3	2
IV	7	PM236	8	Q29	3	3
IV	8	PM197	8	Q5	4	2
V	21	PM195	3	Q5	9	7
V	23	PM124	3	Q39	11	8

[a]N = New South Wales, Q = Queensland, V = Victoria (number indicates individual farms within each state).

biotyping study[4] had indicated diversity existed within isolates of *P multocida* from a single piggery, isolates representing some of the different biovars were included. A matching number of piggeries with multiple isolates that did not show biovar variation were also included. Hence, of the 77 farms represented in the study, only 16 farms had multiple isolates. Of these 16 farms with multiple isolates, eight had isolates that were known to vary in biovar (a total of 26 isolates) and eight had isolates of the same biovar (a total of 20 isolates).

Despite this strategy of deliberately selecting diverse isolates, the majority of the ribotypes recognised in this study (14 of 25) consisted of multiple isolates. Further, 12 of the 25 ribotypes consisted of isolates from multiple farms. These results suggest that a relatively limited number of types of *P multocida* are present in the Australian pig herd. In contrast, a recent study with restriction endonuclease analysis of 10 *P multocida* isolates from pneumonic lung lesions in Australian pigs[15] showed that there was considerable heterogeneity amongst these isolates.

Table 2. Band size and distribution among the 25 combined *Hin*dIII and *Hpa*II ribotypes of *P multocida* revealed in this study.

Combined ribotype bands[a]	Molecular weight	Combined ribotypes[b]																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
C1 (Hd1)	12451	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
C2 (Hd2)	9938	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
C3 (Hp1)	9118	0	1	1	1	0	0	0	1	0	1	1	1	0	1	1	1	1	1	1	1	0	1	0	1	1
C4 (Hd3)	8895	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
C5 (Hp2)	8252	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
C6 (Hp3)	8046	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C7 (Hd4)	7743	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	0	1
C8 (Hd5)	7544	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
C9 (Hd6)	7253	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1
C10 (Hp4)	7253	1	1	0	0	1	0	0	0	1	1	0	0	1	1	0	1	0	1	0	1	0	1	0	1	0
C11 (Hd7)	7063	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0
C12 (Hp5)	6875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
C13 (Hd8)	6690	1	1	1	1	1	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
C14 (Hp6)	6599	1	0	0	1	1	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0
C15 (Hd9)	6239	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	1
C16 (Hd10)	5802	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	1	1	1	1	0
C17 (Hp7)	5631	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
C18 (Hp8)	5378	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	1
C19 (Hd11)	4805	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
C20 (Hd12)	4487	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
C21 (Hp9)	2987	0	0	1	0	0	1	1	1	0	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0	1
C22 (Hp10)	958	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C23 (Hp11)	831	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C24 (Hp12)	578	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
C25 (Hp13)	391	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
C26 (Hp14)	<100	1	1	0	0	1	0	0	0	1	1	1	0	1	1	0	1	0	0	0	1	0	1	0	1	0

[a] Bands numbered in order of size, Hd = present in *Hin*dIII ribotype, Hp = present in *Hpa*II ribotype.
[b] 0 = Ribotype band absent, 1 = Ribotype band present.

However the small number of isolates used in this study and the fact that no quantitative estimate of diversity was performed, makes comparisons with the ribotyping data generated from the present study difficult.

While there is no specific knowledge regarding the management practices of all the farms included in the study, the majority of Australian pig farms are a continuous flow type operation and few herds are closed. It is likely that this style of management, combined with the movement of live pigs onto farms, has resulted in the wide distribution of ribotypes across multiple farms.

Three of the seven biovars represented in this study showed limited genetic diversity. The genetic diversity present in biovar 2 was very limited, with all fifteen isolates distributed among four of the five ribotypes present in cluster III. The majority of the biovar 2 isolates were found in only two of the ribotypes, with six placed in ribotype 12 and a further six placed in ribotype 15. All of the biovar 2 isolates originated from different farms, and the farms were located in Queensland, New South Wales and Victoria. These results suggest that biovar 2 isolates may form a distinct genetic group within *P multocida* subsp *multocida*. Interestingly, four biovar 3 isolates comprised the remainder of cluster III, and three of these isolates shared ribotypes with the biovar 2 isolates

The diversity present in biovar 12, containing lactose-positive variants of *P multocida* subsp *multocida*, was limited. Seven of the nine isolates were placed in a single ribotype (ribotype 1), with all nine isolates being placed in the same ribotype cluster (cluster I). Of the seven isolates placed in ribotype 1, all seven were from different farms, with the farms being located in Queensland, New South Wales and Victoria. There is no known common connection between all seven piggeries. Interestingly

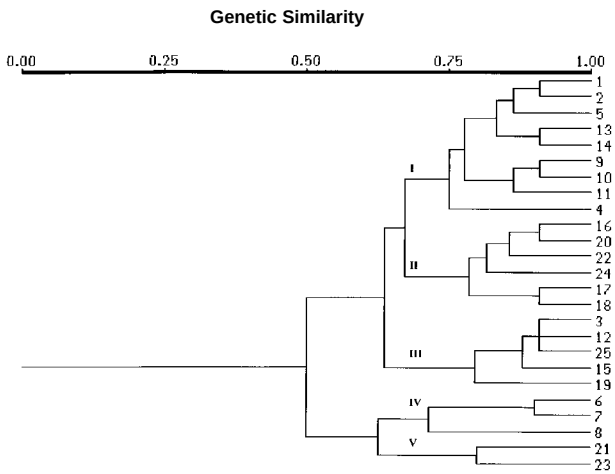

Figure 1. Phenogram of genetic distance amongst 25 ribotypes (numbered 1-25) of 107 porcine isolates of P multocida clustered by the UPGMA strategy. The five major ribotype clusteers (I to V) are marked. Full details are provided in Table 1.

the other lactose-positive biovar, biovar 14, which is also ODC-negative, was also placed in the same ribotype (ribotype 1) as the majority of the biovar 12 isolates. The limited diversity within the lactose-positive isolates of *P multocida* is in contrast to the diversity seen within the isolates previously characterised as maltose-positive *P multocida* examined by Petersen et al.[6] These maltose-positive isolates were shown to be very diverse, representing at least three separate species within the genus *Pasteurella*.[6]

Lactose-positive variants of *P multocida* subsp *multocida* have not been widely reported before. Indeed, the only reports of this

Table 3. Properties of the *Pasteurella multocida* isolates in the ribotype clusters.

Ribotype Cluster	No. of Isolates	Ribotypes	No. of Farms	States	Biovar[a]	Subspecies[a]
I	56	1, 2, 4, 5, 9, 10, 11, 13, 14	45	QLD, NSW, VIC	1 (3); 3 (43); 12 (9); 14 (1)	*multocida* (46) lactose$^+$ *multocida* (9) lactose$^+$ ODC$^-$ *multocida* (1)
II	26	16, 17, 18, 20, 22, 24	20	QLD, NSW, VIC	1 (2); 3 (23); 13 (1)	*multocida* (25) ODC$^-$ *multocida* (1)
III	19	3, 12, 15, 19, 25	19	QLD, NSW, VIC	2 (15); 3 (4)	*multocida* (19)
IV	4	6, 7, 8	4	QLD	8 (4)	*gallicida* (4)
V	2	21, 23	2	QLD	3 (2)	*multocida* (2)

[a]Number in brackets is the number of isolates of that biovar or subspecies.

variant in pigs have been from Australia and Vietnam, with the latter being a single strain only.[4,16,17] Bisgaard et al[18] have noted that some isolates of the subspecies *multocida* and *septica* obtained from calf lungs were lactose-positive. Given the rare reporting of lactose-positive isolates in pigs, our finding of nine lactose-positive biovar 12 isolates of very limited genetic diversity in Australian pigs is unusual. These isolates may be representative of a genotype that has found a common ecotypic niche in the environment, or alternatively may represent a genotype that has become widespread after originating from a point source.

The second biovar that showed very limited genetic diversity is biovar 8, *P multocida* subsp *gallicida*. The only four isolates of this biovar present in the study were found to represent three ribotypes (which possessed only minor pattern variations). These ribotypes were all placed in a single ribotype cluster (IV) that was genetically quite distinct from the majority of the rest of the *P multocida* study population (Clusters I to III). There is no known connection between the four Queensland piggeries that yielded these four isolates. Isolates of subsp *gallicida* are not common in pigs, with the case report of Cameron et al[16] and our previous phenotypic study[4] being the only reports of this subspecies in pigs. There have been few previous studies on the genetic diversity of isolates of subsp *gallicida*. A study of avian isolates of this subspecies found all five Australian isolates belonged to a single ribotype,[10] which supports the findings of the current study that isolates within subsp *gallicida* possess limited diversity. In contrast with our finding that the porcine *gallicida* isolates were quite distinct from the other *P multocida* isolates by ribotyping, the avian isolates were not markedly different from the other avian isolates by either ribotyping or multi-locus enzyme electrophoresis typing.[10] Overall, however, it is apparent that both avian and porcine Australian isolates of *P multocida* subsp *gallicida* show limited genetic diversity. One of the *gallicida* isolates included in the current study was isolated as the causative agent of a very severe outbreak of septicaemia that killed over 2000 pigs.[16] As this study has suggested that all available Australian isolates of *P multocida* subsp *gallicida* are genetically closely related, further studies investigating the pathogenic potential for this subspecies in pigs would appear worthwhile.

There were two ODC-negative isolates included in this study: one of biovar 14 (lactose-positive and ODC-negative) and one of biovar 13 (just ODC-negative). As the biovar 14 isolate was placed in the same ribotype as the majority of the lactose-positive biovar 12 isolates while the biovar 13 isolate was placed in a different ribotype cluster, the biovar 13 and 14 isolates are clearly not closely related. This ability to establish how closely isolates are related is an important advantage of the use of ribotype clustering.

Ribotyping appears to be a useful tool for investigating genetic relationships between members of the genus *Pasteurella*. Petersen et al[6] recently reported that, with maltose-positive isolates of *Pasteurella*, the results from ribotyping (using the *Hin*dIII and *Hpa*II enzymes) correlated closely with data generated from phenotypic and 16S rRNA gene sequencing studies. In our study, we have found some disagreement between classification into biovars and ribotypes within *P multocida* subsp *multocida*. Unlike our previous study on avian isolates,[10] we found that with the porcine isolates, almost half of the multi-isolate ribotypes (6 of 14) contained multiple biovars. However our current study has also found that of the eight farms that had multiple isolates of different biovars, ribotyping confirmed that multiple ribotypes were present on all eight farms. For the eight farms with multiple isolates of the same biovar, ribotyping identified multiple ribotypes in three. Based upon the methods described in this study, ribotyping is a better tool than biotyping for the purpose of establishing if different "types" of *P multocida* are present on a farm. Our results indicate that, in the absence of a capacity to perform ribotyping, biotyping may give some idea of the diversity of *P multocida* present in a pig herd, although the use of biotyping is likely to give an under-estimate of diversity.

In conclusion, this study has provided new knowledge on the diversity of Australian porcine isolates of *P multocida*. We were able to recognise only a relatively limited number of ribotypes, despite deliberately selecting a diverse collection of isolates. Most of the ribotypes consisted of isolates from multiple farms. These discoveries suggest that a limited number of ribotypes have been widely distributed across pig farms in Eastern Australia, and this may have been due to management practices that have permitted the movement of live pigs between herds.

We have established that some of the unusual biochemical variants of *P multocida* present in Australian pigs have a very limited genetic diversity.

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Reducing *Salmonella* in pigs

In many developed countries, salmonellosis has become one of the most important meat-transmitted zoonotic diseases. In some studies, over 10% of *Salmonella* outbreaks attributed to meat have been associated with pork. Infection may be introduced onto farms by contaminated feed, rodents, fomites and carrier pigs. Efforts to identify and eliminate the carrier pig population are hindered by, amongst other things, the lack of reliable practical means to control the carriage of *Salmonella* by subclinically infected pigs and limit spread to other pigs in contact with them. At the abattoir, the carrier pig is the initial source of contamination of the environment, other animals and, potentially, meat products. Whereas attention to good hygienic practice at the abattoir is important in avoiding or limiting contamination, removal of infection from herds has the potential to provide a longer term solution.

In an experiment, pigs were challenged with a virulent field isolate of *Salmonella* Typhimurium after receiving one of a range of treatments. Effect of treatment was assessed by comparing the number of isolations of the organism from various tissues and faeces at 14 days. *Salmonella* excretion was not reduced by acidification of water, use of specific immunoglobulins or vaccination with an endotoxin vaccine. Number of *Salmonella* in colonised mesenteric lymph nodes was reduced with the use of bambermycins and a live attenuated vaccine and shedding in faeces was reduced after supplementation with fructo-oligosaccharides in drinking water. Whereas the use of probiotics and prebiotics caused changes in faecal flora this was not associated with a reduction in shedding of *Salmonella*.

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