Australian Government
Department of Agriculture and Water Resources

AUSTRALIAN Pork

Surveillance for antimicrobial resistance in enteric commensals and pathogens in Australian pigs

Final Report
APL Project 2015/2213

August 2017

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Acknowledgements

This project is supported by funding from Australian Pork Limited and the Department of Agriculture and Water Resources.
The funding provided by the DAWR as well as the assistance of DAWR staff are gratefully acknowledged.

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1 Background to Research

Since 2013, the Australian Government has been actively progressing the development of a coordinated plan for the management of antimicrobial resistance (AMR) and antimicrobial use (AMU) in humans and animals. Broad support for the development of the "National Antimicrobial Resistance Strategy" (Australian Government Department of Health and Australian Government Department of Agriculture, 2015). was obtained from key stakeholders across the medical, health, veterinary, agricultural and pharmaceutical communities at the "Australian One Health Antimicrobial Resistance Colloquium" in 2013 (Australian Commission on Safety and Quality in Health Care, 2013). The Australian Government Department of Agriculture then sponsored a review of the national surveillance programs in place for monitoring AMR and AMU in animals around the world with a view to defining a program suitable for Australia and combined this with roundtable discussions with key stakeholders in the agriculture and veterinary sectors. The review "Surveillance and reporting of antimicrobial resistance and antibiotic usage in animals and agriculture in Australia" (Shaban et al., 2014) identified one of the major components of surveillance as the assessment of AMR in commensal bacteria and pathogens present in the gut of food animals at slaughter.

In March 2015, a one day meeting convened by the Department of Agriculture established the "Antimicrobial Resistance Surveillance Task Group". Present at the meeting were representatives from the Department of Agriculture, Animal Health Australia, scientists working in the area of AMR, most of the major Research and Development Corporations or industry bodies involved in animal production (MLA, APL, ACMF, Dairy Australia) and representatives from the Australian pharmaceutical industry. The Task Group reviewed the recommendations from the surveillance report and provided advice from technical and industry perspectives for developing an AMR surveillance component based on the collection of faecal samples from food animals at slaughter.

The Task Group proposed a surveillance model for use in the Australian pig industry that may also be applied to other major food animal industries in the future and to examine issues such as feasibility, cost, timing, methodology and logistics. This report presents the results of this proof of concept study and provides recommendations for future surveillance strategies.

2 Objectives of the Research Project

The objectives were to estimate the prevalence of resistance against specified antimicrobials amongst *E. coli, Salmonella* spp., *Enterococcus* spp., and *Campylobacter* spp. isolated from the gut of Australian finisher pigs at slaughter.

3 Research Methodology

The study design for this project 'Surveillance for antimicrobial resistance in enteric commensals and pathogens in Australian pigs – study design and implementation' is available via the link in Appendix A.

The number of caecal specimens collected from pigs was limited to 200 in total to be affordable, provide reasonable confidence limits, and to be consistent with the many international surveillance programs that evaluate AMR in commensal bacteria from food animals.

3.1 Collection, isolation, and identification of isolates

Commensal *E. coli* were isolated from diluted caecal material; and *Salmonella* spp., *Enterococcus* spp. and *Campylobacter* spp. from faecal samples collected at each abattoir by ACE Laboratories, Bendigo, as indicated in Appendices B1 and B2. Isolates were identified using standard procedures and confirmed using mass spectrometry (MALDI-TOF).

3.2 Susceptibility testing

Isolates were forwarded to either the Australian Centre for Antimicrobial Resistance Ecology (ACARE), the University of Adelaide (*E. coli* and *Salmonella* spp.) or the School of Veterinary and Life Science, Murdoch University, Perth (*Enterococcus* spp. and *Campylobacter* spp.) for antimicrobial susceptibility testing (AST).

AST was performed by broth microdilution using Veterinary Reference Card panels (Sensititre®, Trek Diagnostics, East Grinstead, UK). The CMV3AGNF format was used to test *E. coli* and *Salmonella* spp.; CMV3AGPF for *Enterococcus* spp., and CAMPY for *Campylobacter* spp. Inoculation and incubation were as by the manufacturers' guidelines. In addition, in-house broth microdilution panels made according to Clinical and Laboratory Standards Institute (CLSI) standards, were used to test *E. coli* and *Salmonella* spp. against colistin, florfenicol and kanamycin, and *Enterococcus* spp. against ampicillin, teicoplanin and virginiamycin (CLSI, 2015a). The antimicrobial concentration range for each agent is shown in Table 1.

Quality control strains *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and *C. jejuni* ATCC 33560 were used throughout the study period.

3.3 Interpretation

The minimum inhibitory concentrations (MIC) were interpreted according to CLSI VET01S (CLSI, 2015b) or the European Committee for Antimicrobial Susceptibility Testing (EUCAST) epidemiological cut-off values (ECOFFs) as indicated in Tables 1 to 3 (EUCAST, 2016). CLSI M100S breakpoints were used where animal species antimicrobial agent combinations were not available (CLSI, 2016). Interpretation of the MICs were based on Clinical and Laboratory Standards Institute (Wayne, PA) interpretive criteria when available; otherwise European Committee on Antimicrobial Susceptibility Testing (EUCAST; Basel, Switzerland). The dual EUCAST/CLSI system was used in order that the results were able to be completely internationally relevant i.e. there were two prevalence

estimates: 1) EUCAST ECOFF for the percent non-wild, and 2) CLSI intermediate break point for the percent non-susceptible.

Where no EUCAST or CLSI interpretative criteria were available, breakpoints were harmonised with those of the National Antimicrobial Resistance Monitoring System (NARMS), USA (CDC, 2013).

Table 1 Breakpoints used for Susceptibility Testing of Escherichia coli *and* Salmonella *species*

			ECOFF [a]		**CLSI [b] or NARMS [c]**		
Antimicrobial Class	**Antimicrobial Agent**	**Range (mg/L)**	***E. coli***	***Salmonella***	**S**	**I**	**R**
Aminoglycosides	Gentamicin	0.25 - 16	2	2	≤4	8	>8
	Kanamycin	2 - 256	8	-	≤16	32	>32
	Streptomycin	2 - 64	16	16	≤32	-	>32
β-lactam / β-lactam inhibitor combination	Amoxicillin-clavulanate (2:1 ratio)	1 - 32	- [d]	-	≤8	16	>16
Cephems	Cefoxitin	0.5 - 32	8	8	≤8	16	>16
	Ceftiofur	0.12 - 8	1	2	≤2 [e]	4	>4
	Ceftriaxone	0.25 - 64	0.12	-	≤1	2	>2
Fluoroquinolones	Ciprofloxacin (*E. coli*)	0.015 - 4	0.06	0.06	≤1	2	>2
	Ciprofloxacin (*Salmonella*)	0.015 - 4	0.06	0.06	≤0.06	0.12-0.5	>0.5
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole (1:19)	0.12 - 4	1	1	≤2	-	>2
Macrolides	Azithromycin (*Salmonella*)	0.12 - 16	-	-	≤16	-	>16
Penicillins	Ampicillin	1 - 32	8	8	≤8	16	>16
Phenicols	Chloramphenicol	2 - 32	16	16	≤8	16	>16
	Florfenicol	1 - 128	16	16	≤4 [f]	8	>8
Polymyxins	Colistin	0.12 - 8	2	-	-	-	-
Tetracyclines	Tetracycline	4 - 32	8	8	≤4	8	>8

[a] EUCAST epidemiological cut-off values (mg/L)
[b] CLSI VET01S (CLSI, 2015b) or M100S (CLSI, 2016) breakpoints (mg/L), S = sensitive; I = intermediate; R = resistant
[c] NARMS breakpoints (mg/L) (orange text) (CDC, 2013).
[d] Not defined
[e] *E. coli* only
[f] *Salmonella* Choleraesuis only

Table 2 Breakpoints used for Susceptibility Testing of Campylobacter *species*

					NARMS [a]	
Class	**Agent**	**Species**	**Range (mg/L)**	**ECOFF [b]**	**S**	**R**
Aminoglycosides	Gentamicin	All	0.12 - 32	2	≤2	>2
Ketolides	Telithromycin	*C. jejuni*	0.008 - 8	4	≤4	>4
Lincosamide	Clindamycin	*C. coli* [c]	0.03 - 16	1	≤1	>1
		C. jejuni	0.03 - 16	0.5	≤0.5	>0.5
Macrolides	Azithromycin	*C. coli*	0.015 - 64	0.5	≤0.5	>0.5
		C. jejuni	0.015 - 64	0.25	≤0.25	>0.25
	Erythromycin	*C. coli*	0.03 - 64	8	≤8	>8
		C. jejuni	0.03 - 64	4	≤4	>4
Phenicols	Florfenicol	All	0.03 - 64	4	≤4	>4
Quinolones	Ciprofloxacin	All	0.015 - 64	0.5	≤0.5	>0.5
	Nalidixic acid	All	4 - 64	16	≤16	>16
Tetracyclines	Tetracycline	*C. coli*	0.06 - 64	2	≤2	>2
		C. jejuni	0.06 - 64	1	≤1	>1

[a] NARMS breakpoints (mg/L), adapted from epidemiological cut-off values, , S = sensitive; R = resistant (CDC, 2013).
[b] EUCAST epidemiological cut-off values (mg/L)
[c] *C. coli* and species other than *C. jejuni*

Table 3 Breakpoints used for Susceptibility Testing of Enterococcus *species*

					CLSI [a] or NARMS [b]		
Class	**Agent**	**Species**	**Range (mg/L)**	**ECOFF [c]**	**S**	**I**	**R**
Aminoglycosides (high-level)	Gentamicin	All	128 - 1024	- [d]	≤512	-	>512
	Kanamycin [d]	All	128 - 1024	-	≤512	-	>512
	Streptomycin	All	512 - 2048	-	≤512	-	>512
Glycopeptides	Vancomycin	All	0.25 - 32	4	≤4	8-16	>16
	Teicoplanin	All	0.25 - 128	2	≤8	16	>16
Lincosamide	Lincomycin [d]	All	1 - 8	-	≤2	4	>4
Lipopeptides	Daptomycin	All	0.25 - 16	4	≤4	-	-
Macrolides	Erythromycin	*E. faecium*, *E. faecalis*	0.25 - 8	4	≤0.5	1-4	>4
		E. hirae	0.25 - 8	2	≤0.5	1-4	>4
Oxazolidinones	Linezolid	All	0.5 - 8	4	≤2	4	>4
Penicillins	Ampicillin	All	0.25 - 64	4	≤8	-	>8
	Benzylpenicillin	*E. faecium*, *E. faecalis*	0.25 - 16	16	≤8	-	>8
Phenicols	Chloramphenicol	*E. faecium*, *E. faecalis*	2 - 32	32	≤8	16	>16
		E. hirae	2 - 32	8	≤8	16	>16
Streptogramins	Quinupristin-dalfopristin	*E. faecium*	0.5 - 32	-	≤1	2	>2
	Virginiamycin	*E. faecium*	0.25 - 128	4	-	-	-
		E. faecalis	0.25 - 128	32	-	-	-
		E. hirae	0.25 - 128	-	-	-	-

Tetracyclines	Tetracycline	All	1 – 32	4	≤4	8	>8

[a] CLSI VET01S (CLSI, 2015b) or M100S (CLSI, 2016) breakpoints (mg/L), S = sensitive; I = intermediate; R = resistant
[b] NARMS breakpoints (mg/L) (orange text) (CDC, 2013).
[c] EUCAST epidemiological cut-off values (mg/L)
[d] Not defined

3.4 Resistance Profiles

Resistance profiles to the antimicrobial classes were generated to examine co-resistance for *E. coli*, *Salmonella* spp., *E. faecium* and *C. coli*. An isolate was considered non-susceptible to an antimicrobial agent when it tested resistant, intermediate or non-susceptible when using clinical breakpoints as interpretative criteria, and not ECOFFs, provided by EUCAST or CLSI. Only acquired (and not intrinsic) resistance was taken into consideration when defining an isolate as exhibiting multidrug resistance (MDR). MDR was defined as a profile comprising non-susceptibility to at least one agent in three or more associated antimicrobial classes[9] as listed in Table 4.

Table 4 Definitions of antimicrobial classes for determining multi-drug resistance by genus

Antimicrobial class	**Escherichia and Salmonella** [a]	**Campylobacter** [b]	**Enterococcus** [c]
Aminoglycosides	Gentamicin	Gentamicin	Gentamicin (high-level)
Streptomycin			Streptomycin (high-level)
β-lactam / β-lactam inhibitor combination	Amoxicillin-clavulanate		
Cephems (extended-spectrum cephalosporins)	Ceftriaxone		
Cephems (cephamycins)	Cefoxitin		
Fluoroquinolones	Ciprofloxacin		
Quinolone		Ciprofloxacin	
		Naladixic acid	
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole		
Glycopeptides			Vancomycin
			Teicoplanin
Ketolides		Telithromycin	
Lipopeptides			Daptomycin
Macrolides		Azithromycin	
Oxazolidinones			Linezolid
Penicillins	Ampicillin		Ampicillin
Phenicols	Chloramphenicol	Florfenicol	
Polymyxins	Colistin		
Tetracyclines	Tetracycline	Tetracycline	
Streptogramins			Quinupristin-dalfopristin [d]

[a] An isolate is considered multi-resistant if resistant to three or more of the ten antimicrobial classes
[b] An isolate is considered multi-resistant if resistant to three or more of the six antimicrobial classes
[c] An isolate is considered multi-resistant if resistant to three or more of the seven or six (*E. faecalis*, *E. gallinarum* or *E. casseflifavus*) antimicrobial classes
[d] Enterococcus species other than *E. faecalis*, *E. gallinarum*, or *E. casseliflavus*

3.5 Statistical Analysis

Confidence intervals of proportions were calculated where appropriate using GraphPad Prism version 7.01 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com.

4 Results

4.1 Isolates recovered

Caecal specimens were obtained at slaughter from pigs representing 31 farms throughout Australia. A total of 601 isolates were available for susceptibility testing as indicated in Table C1 in Appendix C. *E. coli* was isolated from samples from all farms. No *Salmonella* spp. were recovered from pigs originating from ten (32%) farms. Enterococci were isolated from pigs originating from all but one farm. Eight enterococcal species were recovered, three of which contributed to 93.8% of all species (*E. faecium,* 57.5%; *E. hirae,* 24.7%; *E. faecalis,* 11.6%). *Campylobacter* spp. was recovered from all farms; *C. coli* (91.8%) was the dominant species, followed by *C. hyointestinalis* (7.0%). One campylobacter isolate could only be identified to the genus level by MALDI-TOF (Table 5).

Table 5 Isolates recovered

Species	Number (% of genus)
Escherichia coli	200
Salmonella species	84
Campylobacter species	171
C. coli	157 (91.8)
C. hyointestinalis	12 (7.0)
C. jejuni	1
C. species	1
Enterococcus species	146
E. faecium	84 (57.5)
E. hirae	36 (24.7)
E. faecalis	17 (11.6)
E. durans	4
E. galinarum	2
E. hermanniensis	1
E. mundtii	1
E. avium	1
TOTAL	601

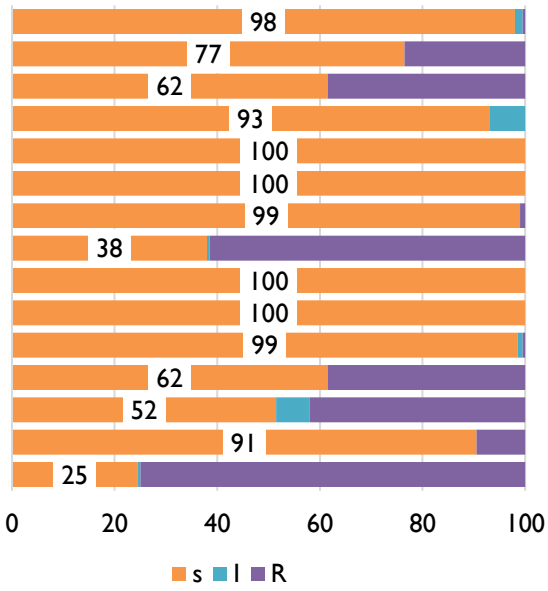
4.2 MIC Distributions

4.2.1 Escherichia coli *and* Salmonella *species*

Non-susceptibility (i.e. isolates classified as either intermediate or resistant according to clinical breakpoints) to tetracycline, ampicillin and streptomycin in both *E. coli* (Table D1, Appendix D). and *Salmonella* spp. (Table D2, Appendix D) was high (range 55–77%). None of the isolates showed non-susceptibility to ceftiofur and no isolate had an extended-spectrum β-lactamase (ESBL) phenotype (ceftriaxone MIC > 1 mg/L). Florfenicol and gentamicin non-susceptibility among *E. coli* and *Salmonella* spp. was less than 10% and 2%, respectively. Four (2.0%) *E. coli* and three (3.6%) *Salmonella* spp. isolates had ciprofloxacin non-wild type MICs, but none were regarded as clinically resistant (MIC > 1 mg/L for *E. coli* and > 0.5 mg/L for *Salmonella*). None of the isolates showed non-susceptibility to colistin.

The antimicrobial resistance patterns for *E. coli* and *Salmonella* spp., based clinical breakpoints, are summarised for Figure 1 and Figure 2 respectively. Resistance by species and agent is shown in Table 6.

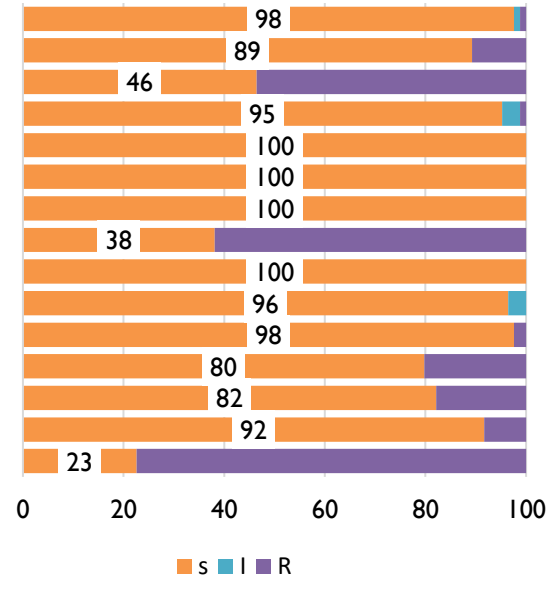
Rank*	Antimicrobial Class	Antimicrobial Agent	Susceptible, intermediate and resistant proportion
I	Aminoglycosides	Gentamicin	98
		Kanamycin	77
		Streptomycin	62
	B-lactam + inhibitor	Amoxicillin-clavulanate	93
	Cephems	Ceftiofur	100
		Ceftriaxone	100
	Macrolides	Azithromycin	99
	Penicillins	Ampicillin	38
	Polymyxins	Colistin	100
	Fluoroquinolone	Ciprofloxacin	100
II	Cephems	Cefoxitin	99
	Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	62
	Phenicols	Chloramphenicol	52
		Florfenicol	91
	Tetracyclines	Tetracycline	25



* Rank of antimicrobial agents based on World Health Organization's categorisations of critical importance in human medicine: Rank I, Critically important; Rank II, Highly important (Collignon et al., 2016).

Figure 1 Antimicrobial resistance pattern for Escherichia coli *(n=200), proportion susceptible, intermediate and resistant*

Rank*	Antimicrobial Class	Antimicrobial Agent	Susceptible, intermediate and resistant proportion
I	Aminoglycosides	Gentamicin	98
		Kanamycin	89
		Streptomycin	46
	B-lactam + inhibitor	Amoxicillin-clavulanate	95
	Cephems	Ceftiofur	100
		Ceftriaxone	100
	Macrolides	Azithromycin	100
	Penicillins	Ampicillin	38
	Polymyxins	Colistin	100
	Quinolones	Ciprofloxacin	96
II	Cephems	Cefoxitin	98
	Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	80
	Phenicols	Chloramphenicol	82
		Florfenicol	92
	Tetracyclines	Tetracycline	23

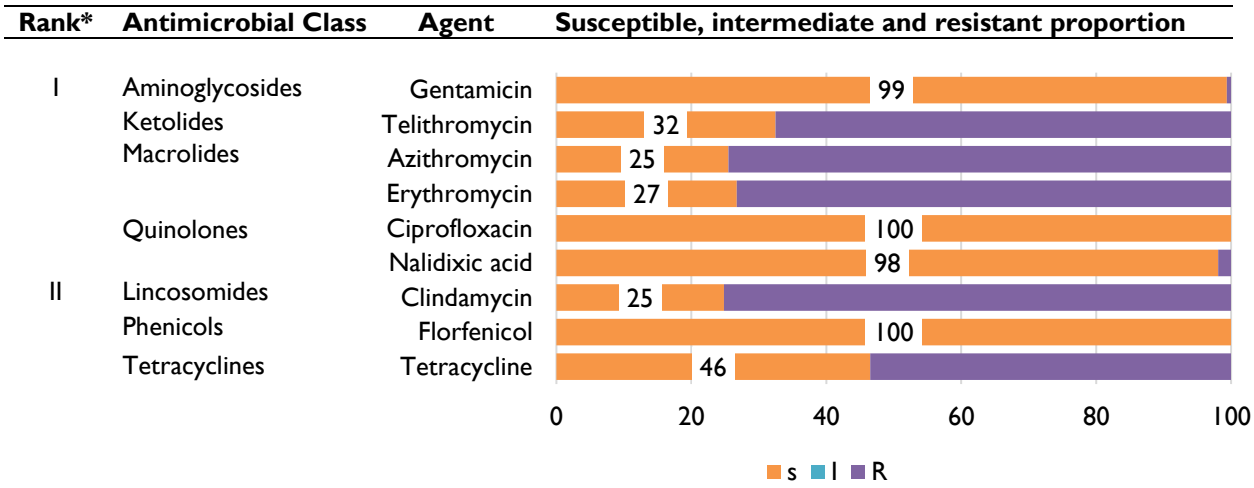


* Rank of antimicrobial agents based on World Health Organization's categorisations of critical importance in human medicine: Rank I, Critically important; Rank II, Highly important (Collignon et al., 2016).

Figure 2 Antimicrobial resistance pattern for Salmonella *species (n=84), proportion susceptible, intermediate and resistant*

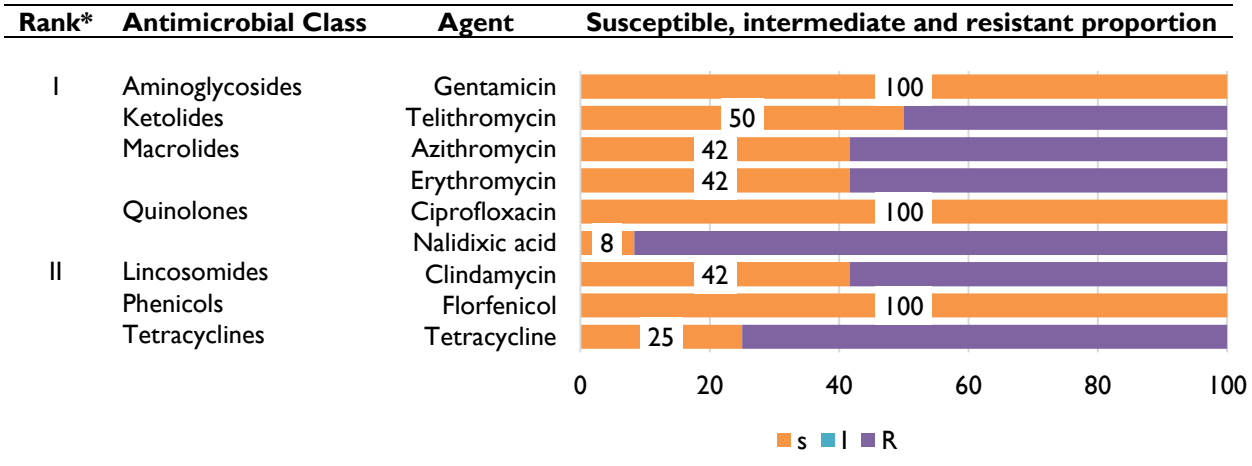
4.2.2 Campylobacter *species*

Campylobacter spp. are intrinsically resistant to lincosamides. Resistance in *C. coli* was high for macrolides (73.2–74.5%), ketolides (67.5%) and tetracyclines (53.5%) (Table D3, Appendix D). There was no resistance to ciprofloxacin or florfenicol, and only one isolate (0.6%) was gentamicin-resistant. The antimicrobial resistance patterns for *C. coli* and *C. hyoinstestinalis* are summarised in Figure 3 and Figure 4 respectively. Resistance by species, antimicrobial class and agent is shown in Table 7.



* Rank of antimicrobial agents based on World Health Organization's categorisations of critical importance in human medicine: Rank I, Critically important; Rank II, Highly important (Collignon et al., 2016).

Figure 3 Antimicrobial resistance pattern for Campylobacter coli *(n=157), proportion susceptible, intermediate and resistant*



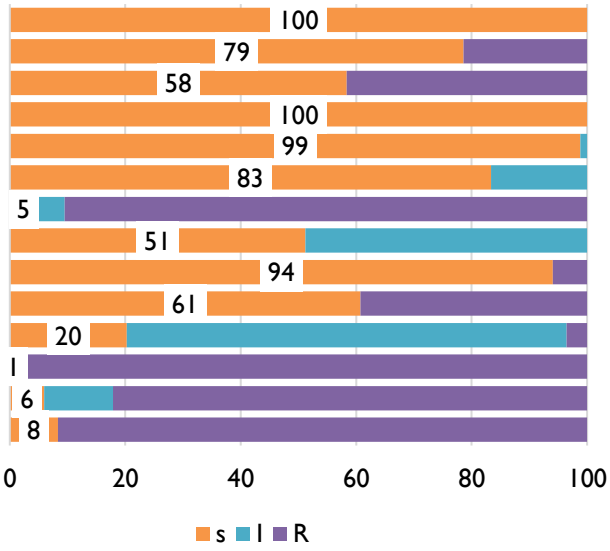
* Rank of antimicrobial agents based on World Health Organisation's categorizations of critical importance in human medicine: Rank I, Critically important; Rank II, Highly important (Collignon et al., 2016).

Figure 4 Antimicrobial resistance pattern for Campylobacter hyointestinalis *(n=12), proportion susceptible, intermediate and resistant*

4.2.3 Enterococcus *species*

Enterococcus spp. are considered intrinsically resistant to lincosamides and *E. faecalis* is intrinsically resistant to streptogramins, therefore *in vitro* susceptibility data for these agents should be reviewed with caution (Table D4, Appendix D. Only a single isolate was vancomycin non-susceptible (MIC = 8 mg/L). The resistance patterns for *E. faecium*, based on CLSI clinical breakpoints, are summarised in Figure 5. Quinupristin-dalfopristin resistance was recorded as high (82.1%) for this species. The resistance pattern was similar for *E. hirae* (Table D5, Appendix D and Figure 6). No resistance to penicillins, lipopeptides, and glycopeptides was seen in *E. faecalis* (Figure 7). Resistance by species, antimicrobial class and agent is shown in Table 8.

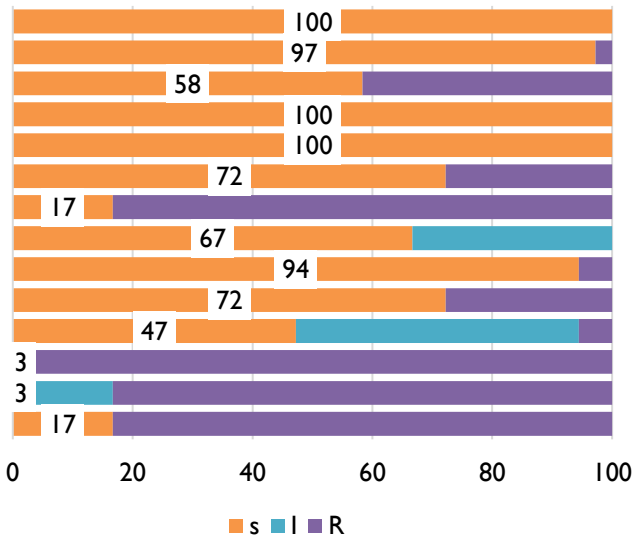
Rank*	Antimicrobial Class	Antimicrobial Agent	Susceptible, intermediate and resistant proportion
I	Aminoglycosides	Gentamicin (high-level)	100
		Kanamycin (high-level)	79
	Streptomycin	Streptomycin (high-level)	58
	Glycopeptides	Teicoplanin	100
		Vancomycin	99
	Lipopeptides	Daptomycin	83
	Macrolides	Erythromycin	5
	Oxazolidinones	Linezolid	51
	Penicillins	Ampicillin	94
		Benzylpenicillin	61
	Phenicols	Chloramphenicol	20
II	Lincosamides	Lincomycin	1
	Streptogramins	Quinupristin-dalfopristin	6
	Tetracyclines	Tetracycline	8



* Rank of antimicrobial agents based on World Health Organisation's categorizations of critical importance in human medicine: Rank I, Critically important; Rank II, Highly important (Collignon et al., 2016).

Figure 5 Antimicrobial resistance pattern for Enterococcus *faecium (n=84), proportion susceptible, intermediate and resistant*

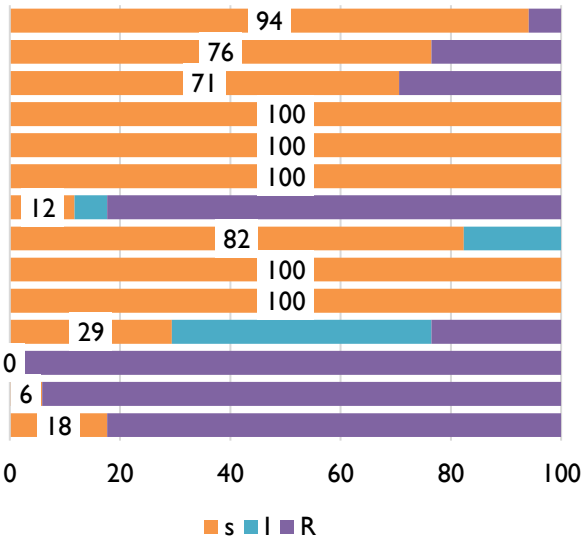
Rank*	Antimicrobial Class	Antimicrobial Agent	Susceptible, intermediate and resistant proportion
I	Aminoglycosides	Gentamicin (high-level)	
		Kanamycin (high-level)	
	Streptomycin	Streptomycin (high-level)	
	Glycopeptides	Teicoplanin	
		Vancomycin	
	Lipopeptides	Daptomycin	
	Macrolides	Erythromycin	
	Oxazolidinones	Linezolid	
	Penicillins	Ampicillin	
		Benzylpenicillin	
	Phenicols	Chloramphenicol	
II	Lincosamides	Lincomycin	
	Streptogramins	Quinupristin-dalfopristin	
	Tetracyclines	Tetracycline	



* Rank of antimicrobial agents based on World Health Organisation's categorizations of critical importance in human medicine: Rank I, Critically important; Rank II, Highly important (Collignon et al., 2016).

Figure 6 Antimicrobial resistance pattern for Enterococcus hirae *(n=36), proportion susceptible, intermediate and resistant*

Rank*	Antimicrobial Class	Antimicrobial Agent	Susceptible, intermediate and resistant proportion
I	Aminoglycosides	Gentamicin (high-level)	
		Kanamycin (high-level)	
	Streptomycin	Streptomycin (high-level)	
	Glycopeptides	Teicoplanin	
		Vancomycin	
	Lipopeptides	Daptomycin	
	Macrolides	Erythromycin	
	Oxazolidinones	Linezolid	
	Penicillins	Ampicillin	
		Benzylpenicillin	
	Phenicols	Chloramphenicol	
II	Lincosamides	Lincomycin	
	Streptogramins	Quinupristin-dalfopristin	
	Tetracyclines	Tetracycline	



* Rank of antimicrobial agents based on World Health Organisation's categorizations of critical importance in human medicine: Rank I, Critically important; Rank II, Highly important (Collignon et al., 2016).

Figure 7 Antimicrobial resistance pattern for Enteroccoccus faecalis *(n=17), proportion susceptible, intermediate and resistant*

Table 6 Antimicrobial susceptibility of Escherichia coli *and* Salmonella *species by antimicrobial agent*

		Number (and percentage) of isolates resistant by antimicrobial class and agent [a]													
Species		**GEN**	**KAN**	**STR**	**AMC**	**FUR**	**CTR**	**AMP**	**COL**	**CIP**	**CFT**	**SXT**	**CHL**	**FFN**	**TET**
Escherichia coli	200	1 (0.5)	47 (23.5)	77 (38.5)	0	0	0	123 61.5	–	0	1 (0.5	77 (38.5)	84 (42.0)	–	150 (75.0)
Salmonella	84	1 (1.2)	9 (10.7)	45 (53.6)	1 (1.2)	0	0	52 61.9	–	0	2 (2.4)	17 (20.2)	15 (17.9)	7 (8.3)	65 (77.4)

[a]GEN = gentamicin, KAN = kanamycin, STR = streptomycin, AMC = amoxicillin-clavulanate, FUR = ceftiofur, CTR = ceftriaxone, AMP = ampicillin, COL = colistin, CIP = ciprofloxacin, CFT = cefoxitin, SXT = trimethoprim-sulfamethoxazole, CHL = chloramphenicol, FFN = florfenicol, TET = tetracycline

Table 7 Antimicrobial susceptibility of Campylobacter *species[a] by antimicrobial agent*

Species	Number (%) of isolates	Number (and percentage) of isolates resistant by antimicrobial class and agent [b]								
		Aminoglycosides	Ketolides	Lincosamides	Macrolides		Phenicols	Quinolones		Tetracyclines
		GEN	TEL	CLN	AZI	ERY	FFN	CIP	NAL	TET
C. coli	157 (91.8)	1 (0.6)	106 (67.5)	118 (75.2)	117 (74.5)	115 (73.2)	0	0	3 (1.9)	84 (53.5)
C. hyointestinalis	12 (7.0)	0 (0.0)	6 (50.0)	7 (58.3)	7 (58.3)	7 (58.3)	0	0	11 (91.7)	9 (75.0)
Other species [a]	2 (1.2)	0	1	1	2	1	0	0	1	1
Total	171	1 (0.6)	113 (66.1)	126 (73.7)	126 (73.7)	123 (71.9)	0 (0.0)	0 (0.0)	15 (8.8)	94 (55.0)

[a] *C. jejuni* (n=1); *Campylobacter* not speciated (n=1)

[b] GEN = gentamicin, TEL = telithromycin, CLN = clindamycin, AZI = azithromycin, ERY = erythromycin, FFN = florfenicol, CIP = ciprofloxacin, NAL = nalidixic acid, TET = tetracycline

Table 8 Antimicrobial susceptibility of Enterococcus *species[a] by antimicrobial class and agent*

Species	Number (%) of isolates	Number (and percentage) of isolates resistant by antimicrobial class and agent [b]									
		Glycopeptides		Lipopeptides	Macrolides	Oxazolidinones	Penicillins		Phenicols	Streptogramins	Tetracyclines
		VAN	TEI	DAP	ERY	LNZ	AMP	PEN	CHL	QD	TET
E. faecium	84 (57.5)	0	0	14 (16.7)	76 (90.5)	0	5 (6.0)	12 (14.3)	3 (3.6)	69 (82.1)	77 (91.7)
E. hirae	36 (24.7)	0	0	10 (27.8)	30 (83.3)	0	2 (5.6)	4 (11.1)	2 (5.6)	30 (83.3)	30 (83.3)
E. faecalis	17 (11.6)	0	0	0	14 (82.4)	0	0	0	4 (23.5)	16 (94.1)	14 (82.4)
Other species[a]	9 (6.2)	0	0	2	7	0	0	0	1	4	7
Total	146	0 (0.0)	0 (0.0)	26 (17.8)	127 (87.0)	0 (0.0)	7 (4.8)	16 (11.0)	10 (6.8)	119 (81.5)	128 (87.7)

[a] *E. durans* (n=4); *E. gallinarum* (n=2); *E. mundtii* (n=1); *E. avium* (n=1); *E. hermanniensis* (n=1)

[b] VAN = vancomycin, TEI = teicoplanin, DAP = daptomycin, ERY = erythromycin, LNZ = linezolid, AMP = ampicillin, PEN = penicillin, CHL = chloramphenicol, QD = Quinupristin-dalfopristin, TET = tetracycline

4.3 Non-susceptible profiles

A total of 71 non-susceptible profiles were found among the 200 *E. coli* isolates. The top 24 profiles shown in Table 9 account for 71.5% of all isolates. Susceptibility to all 14 agents tested, representing nine antimicrobial classes, was observed in 8.5% of isolates.

Table 9 Non-susceptible profiles with the highest frequency in Escherichia coli *isolates (n=200)*

Resistance profile *				
Gen	Kan	Str	Amc	Fur	Ctr	Amp	Col	Cip	Cft	SXT	Chl	Ffn	Tet	**N (%) †**
														17 (8.5)
						Amp							Tet	16 (8.0)
													Tet	15 (7.5)
						Amp					Chl		Tet	10 (5.0)
		Str				Amp							Tet	7
						Amp					Chl			7
	Kan	Str				Amp				SXT	Chl		Tet	6
	Kan	Str								SXT	Chl		Tet	5
		Str				Amp				SXT			Tet	5
						Amp				SXT	Chl		Tet	5
		Str											Tet	5
		Str				Amp				SXT	Chl		Tet	5
	Kan					Amp				SXT			Tet	5
											Chl		Tet	4
	Kan					Amp				SXT	Chl		Tet	4
						Amp				SXT			Tet	3
						Amp				SXT	Chl	Ffn	Tet	3
		Str									Chl		Tet	3
		Str								SXT	Chl		Tet	3
		Str				Amp				SXT				3
		Str				Amp				SXT	Chl	Ffn	Tet	3
	Kan					Amp								3
	Kan					Amp							Tet	3
	Kan	Str				Amp							Tet	3
Other profiles (n=47)														57 (28.5)

* Gen = gentamicin, Kan = kanamycin, Str = streptomycin, Amc = amoxicillin-clavulanate, Fur = Ceftiofur, Ctr = ceftriaxone, Amp = ampicillin, Col = colistin, Cip = ciprofloxacin, Cft = cefoxitin, SXT = trimethoprim/sulfamethoxazole, Chl = chloramphenicol, Ffn = florfenicol, Tet = tetracycline

† Percentage of isolates shown n > 10

There were 18 non-susceptible profiles among the *Salmonella* spp. isolates, with 89.3% of strains found in the top nine profiles (Table 10). The top two profiles were non-susceptibility to ampicillin, streptomycin and tetracycline (34.5%); and susceptible to all 14 agents tested (15.5%).

Table 10 Non-susceptible profiles with the highest frequency in Salmonella *spp. Isolates (n=84)*

Resistance profile *														N (%) †
Gen	Kan	Str	Amc	Fur	Ctr	Amp	Col	Cip	Cft	SXT	Chl	Ffn	Tet	
		Str				Amp							Tet	29 (34.5)
														13 (15.5)
						Amp							Tet	11 (13.1)
	Kan	Str								SXT	Chl		Tet	7
													Tet	6
		Str	Amc			Amp							Tet	3
						Amp								2
		Str								SXT	Chl	Ffn	Tet	2
						Amp		Cip		SXT	Chl	Ffn	Tet	2
Other profiles (n = 9)														9 (10.7)

* Gen = gentamicin, Kan = kanamycin, Str = streptomycin, Amc = amoxicillin-clavulanate, Fur = Ceftiofur, Ctr = ceftriaxone, Amp = ampicillin, Col = colistin, Cip = ciprofloxacin, Cft = cefoxitin, SXT = trimethoprim/sulfamethoxazole, Chl = chloramphenicol, Ffn = florfenicol, Tet = tetracycline
† Percentage of isolates shown n > 10

Clinical breakpoints have only been defined by CLSI for *C. jejuni* and *C. coli* against ciprofloxacin, erythromycin and tetracycline. For this report, breakpoints harmonized by NARMS have been used throughout. There were 11 non-susceptible profiles generated for the eight agents tested against *C. coli* (Table 11). The top three profiles (77.7% of all strains) were resistance to ketolides and macrolides alone (29.9%), or in combination with tetracyclines (35.0%); and no resistance to any of the eight antimicrobial agents tested (12.7%).

Table 11 Non-susceptible profiles with the highest frequency in Campylobacter coli *isolates (n=157)*

Resistance profile *							N (%) †
Gen	Tel	Azi	Ery	Cip	Nal	Ffn	Tet	
	Tel	Azi	Ery				Tet	55 (35.0)
	Tel	Azi	Ery					47 (29.9)
								20 (12.7)
							Tet	19 (12.1)
		Azi	Ery				Tet	6
		Azi	Ery					4
	Tel	Azi	Ery		Nal		Tet	2
					Nal		Tet	1
		Azi						1
	Tel	Azi					Tet	1
Gen	Tel	Azi	Ery					1

* Gen = gentamicin, Tel = telithromycin, Azi = azithromycin, Ery = erythromycin, Cip = ciprofloxacin, Nal = nalidixic acid, Ffn = florfenicol, Tet = tetracycline
† Percentage of isolates shown n > 10

As *Enterococcus* species are considered intrinsically resistant to lincomycin, and *E. faecalis* to streptogramins; these antimicrobials were not included when determining non-susceptibility profiles. Almost all *E. faecium* were non-susceptible to quinupristin-dalfopristin, a result which required further investigation (Australian Government Department of Health and Australian Government Department of Agriculture, 2015) (Table 12). Although chloramphenicol non-susceptibility was 80%, only 3.6% were resistant. Similarly, for linezolid non-susceptibility was common (48.8%), but no isolate was classified as resistant.

Resistance profile *														**N (%) †**
HLg	HLk	HLS	Tei	Van	Dap	Lnz	Amp	Pen	Chl	Q	D	Ery	Tet	
								Pen	Chl	Q	D	Ery	Tet	10 (11.9)
						Lnz		Pen	Chl	Q	D	Ery	Tet	8
									Chl	Q	D	Ery	Tet	6
						Lnz			Chl	Q	D	Ery	Tet	6
		HLS				Lnz		Pen	Chl	Q	D	Ery	Tet	5
		HLS				Lnz			Chl	Q	D	Ery	Tet	4
		HLS							Chl	Q	D	Ery	Tet	3
	HLK	HLS			Dap	Lnz			Chl	Q	D	Ery	Tet	3
						Lnz				Q	D			2
		HLS								Q	D	Ery	Tet	2
		HLS			Dap					Q	D	Ery	Tet	2
		HLS			Dap	Lnz			Chl	Q	D	Ery	Tet	2
Other profiles (n =27)														27 (32.1)

* HLg = gentamicin (high-level), HLk = kanamycin (high-level), HLS = streptomycin (high-level), Tei = teicoplanin, Van = vancomycin, Dap = daptomycin, Lnz = linezolid, Amp = ampicillin, Pen = penicillin, Chl = chloramphenicol, QD = quinupristin-dalfopristin, Ery = erythromycin, Tet = tetracycline

† Percentage of isolates shown n > 10

4.4 Multidrug resistance

The proportion of *E. coli* and *Salmonella* spp. that were resistant or showed reduced susceptibility to nine different antimicrobial classes is shown in Figure 8. Forty-six percent of *E. coli* and 23% of *Salmonella* spp. were classified as multidrug-resistant (exhibiting non-susceptibility to at least one agent in ≥ 3 antimicrobial classes). The antimicrobial agents tested against *Campylobacter* spp. represent six different classes. Forty percent of *C. coli* and 33.3% *C. hyointestinalis* were multidrug-resistant (Figure 9). MDR among *Enterococcus* spp. (nine different classes) ranged from 94.0% in *E. faecium*, and 88.9% in *E. hirae* to 29.4% among *E. faecalis* (Figure 10).

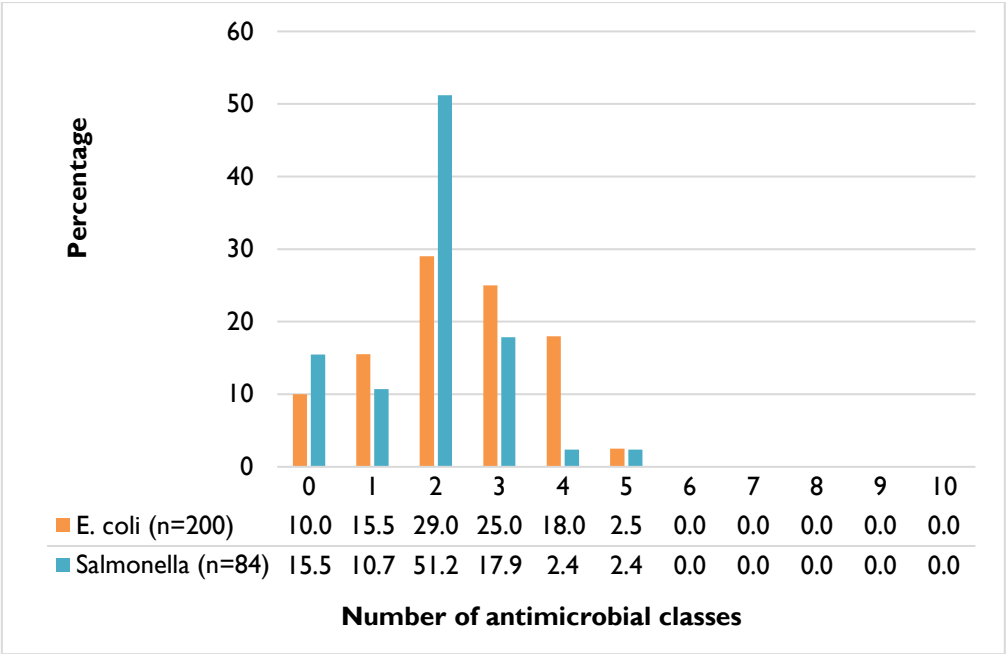


Figure 8 Percentage of Escherichia coli *and* Salmonella *spp. isolates exhibiting non-susceptibility to multiple antimicrobial classes*

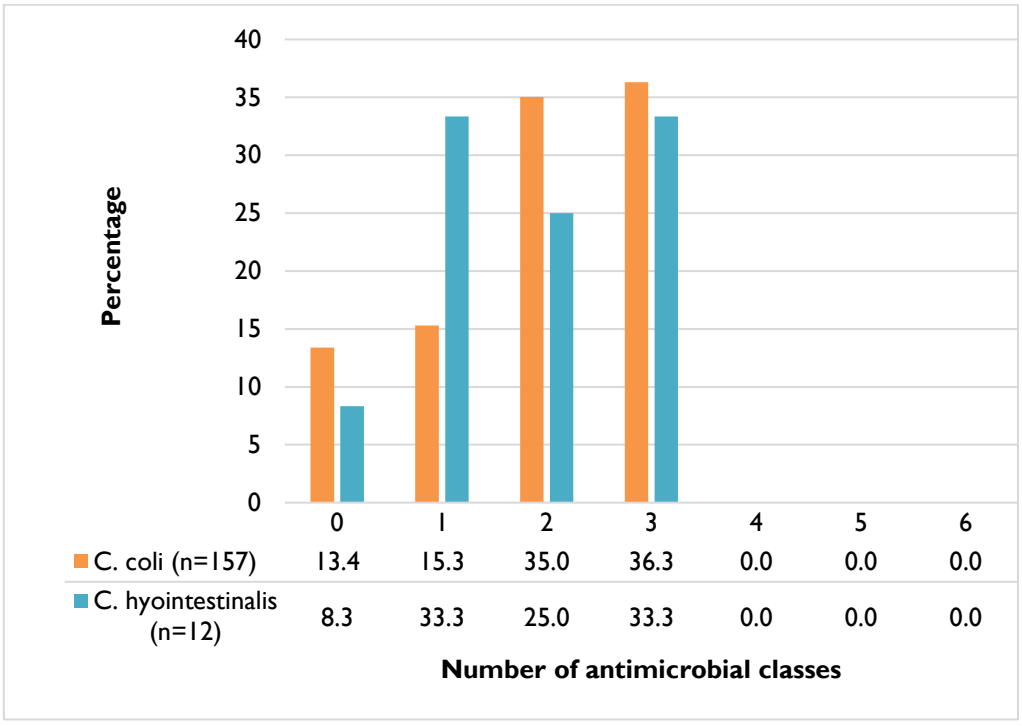
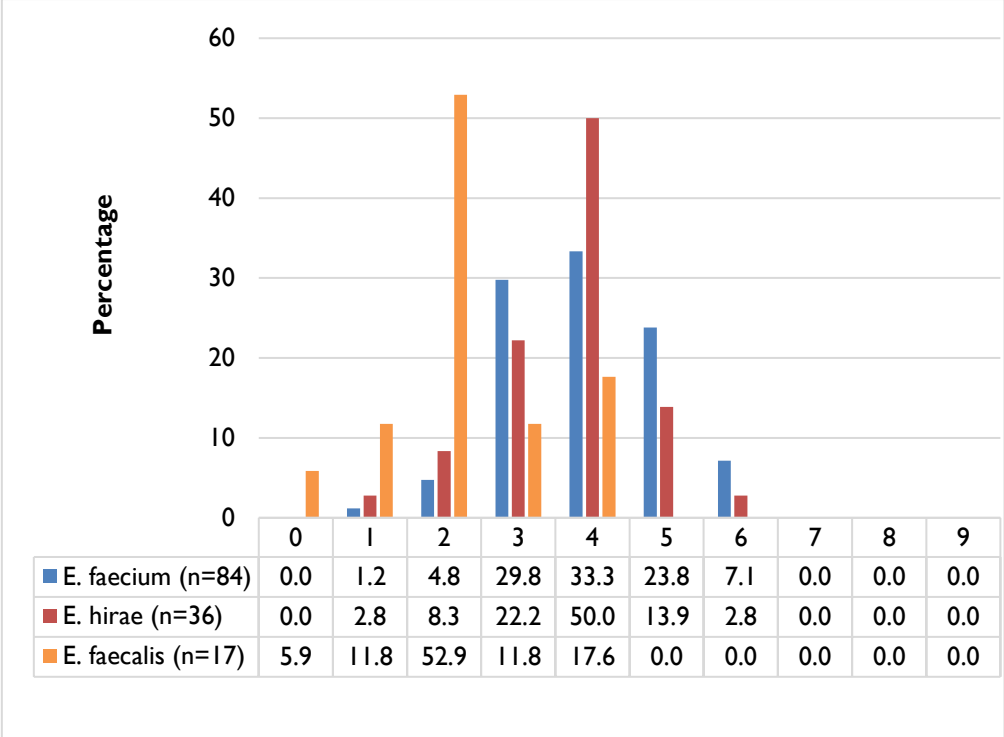


Figure 9 Percentage of Campylobacter *species exhibiting non-susceptibility to multiple antimicrobial classes*



	0	1	2	3	4	5	6	7	8	9
E. faecium (n=84)	0.0	1.2	4.8	29.8	33.3	23.8	7.1	0.0	0.0	0.0
E. hirae (n=36)	0.0	2.8	8.3	22.2	50.0	13.9	2.8	0.0	0.0	0.0
E. faecalis (n=17)	5.9	11.8	52.9	11.8	17.6	0.0	0.0	0.0	0.0	0.0

Figure 10 Percentage of Enterococcus *species exhibiting non-susceptibility to multiple antimicrobial classes*

5 Discussion

For international benchmarking, the most recent annual reports from DANMAP (DANMAP, 2014), CIPARS (Government of Canada, 2015), NARMS (CDC, 2013), and SVARM (SVARM, 2015) were assessed for comparison with the baseline established in the current proof of concept Australia study. All studies had a similar sampling protocol from caecal material collected at slaughter and used similar ECOFF values and clinical breakpoints. The exception to this was Wasyl et. al. who monitored indicator *E. coli* from swine slaughtered in Poland, using material collected on rectal swabs, in 2013.

It should be noted, however, that all studies presented in the table were conducted from one to three years earlier than the Australian proof of concept study, and several countries have had active policies in place to reduce antimicrobial usage in livestock for a number of years (e.g. Denmark and Sweden).

Relatively high rates of resistance were observed in Australian pig commensal *E. coli* isolates to antimicrobials with a lower importance rating (i.e. tetracycline, ampicillin, streptomycin and chloramphenicol) compared to some of the comparator countries (Table 13). Overall rates of resistance were more similar to those observed in North American AMR surveillance programs rather than European programs. However, the proportion of isolates showing non-susceptibility to critically important agents such as fluoroquinolones, 3rd generation cephalosporins and gentamicin was very similar across the six studies. No resistance to colistin was observed in line with absence of any colistin containing products currently registered by the Australian Pesticides and Veterinary Medicines Authority (APVMA) for use in pigs and anecdotal reports that this agent has not been used in pigs in Australia for over thirty years.

Table 13 Comparative frequency of resistance or reduced susceptibility to 14 antimicrobial agents among Escherichia coli *isolates from pigs at slaughter obtained in current AMR surveillance studies*

	Country (year)					
Antimicrobial agent	**Australia (2016)**	**Sweden[1] (2015)**	**Denmark[2] (2014)**	**Canada[3] (2013)**	**USA[4a] (2013)**	**Poland[5b] (2012)**
Amoxicillin-clavulanate (2:1 ratio)	7.0 [c]	— [d]	—	1.8 [c]	0.8 [c]	—
Ampicillin	62.0	40	32.5	38.6	21.2	42.3
Cefoxitin	1.0	—	—	1.8	0.8	—
Ceftiofur	0.0	—	—	1.2	4.2	—
Ceftriaxone	0.0 [e]	—	—	1.2 [e]	4.2[e]	—
Colistin	0.0	0.0	0.0	—	—	—
Chloramphenicol	48.0	3.0	1.9	16.4	5.1	10.1
Ciprofloxacin	0.0	2.5	0.5	0.0	1.7	39.0
Florfenicol	9.5	—	—	—	—	3.8
Gentamicin	2.0	0.0	1.0	2.9	1.7	5.4
Kanamycin	23.0	—	—	17.5	—	7.8
Streptomycin	38.0	26	—	40.4[f]	3.4	34.4
Tetracycline	75.0	18	36.8	74.3	89.0	43.3
Trimethoprim-sulfamethoxazole	38.0	39	—	11.7	3.4	—
Number of isolates	200	84	209	171	118	3430

[a] Market hogs; [b] Rectal swabs EUCAST epidemiological values; [c] CLSI non-susceptible; [d] not tested; [e] MIC > 0.25 mg/L, due to concentration range limitation; [f] not able to interpret due to concentration range

[1]SVARM. Swedres-Svarm 2015. Consumption of antibiotics and occurrence of antibiotic resistance in Sweden: Table 5.1 Distribution of MICs and resistance (%) in Escherichia coliform pigs 2015; page 85.

[2]DANMAP. Web Annex DANMAP 2014 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Table A7.4-Distribution of MICs and resistance (%) in Escherichia coli from broilers (n=191), cattle (n=136) and pigs (n=209)

[3]CIPARS 2013 Annual Report - Chapter 2. Antimicrobial Resistance. Table 40. Distribution of minimum inhibitory concentrations among *Escherichia coli* isolates from pigs-page 77.

[4]2014 NARMS Integrated Report Data Tables-Antimicrobial Resistance among *E. coli* Isolates, 2003-2014 (Tables 63a-63d)

[5]Wasyl D, Hoszowski A, Zajac M et al. Antimicrobial resistance in commensal Escherichia coli isolated from animals at slaughter. *Front Microbiol* 2013; 4: 221; Table 2 Minimal Inhibitory concentration distribution of *E. coli* isolates.

Although reduced susceptibility to azithromycin, ceftiofur and ceftriaxone among *Salmonella* spp. isolated from healthy pigs at slaughter was not observed in the Australian proof of concept study, it was reported at low levels in other countries (Table 14). Ciprofloxacin reduced susceptibility (i.e. non-wild type) was observed in a small number of *Salmonella* isolates, both in the NARMS survey and the Australian proof of concept study, however none of the Australian isolates could be regarded as resistant according to CLSI clinical breakpoints.

Table 14 Comparative frequency of reduced susceptibility to 14 antimicrobial agents among Salmonella *isolates from pigs at slaughter obtained in current AMR surveillance studies*

Antimicrobial agent	Country (year)			
	Australia (2016)	**Canada[1] (2013)**	**Denmark[2] (2014)**	**USA[3a] (2013)**
Amoxicillin-clavulanate (2:1 ratio)	4.8 [b]	12.2 [b]	— [c]	1.9 [b]
Ampicillin	61.9	22.1	33.5	9.6
Azithromycin [d]	0.0	0.6	1.2	0.4
Cefoxitin	2.4	3.4	—	1.9
Ceftiofur	0.0	2.8	—	2.3
Ceftriaxone	0.0 [b]	2.8 [b]	—	2.3[b]
Colistin [e]	0.0	—	1.7	—
Chloramphenicol	17.9	15.5	4.6	3.5
Ciprofloxacin	3.6	0.0	0.0	0.4
Florfenicol	8.3	—	—	—
Gentamicin	2.4	1.7	0.6	3.1
Kanamycin	10.7 [b]	7.2 [b]	—	2.4 [b]
Streptomycin	54.8	— [f]	—	18.1
Tetracycline	77.4	48.6	49.1	31.2
Trimethoprim-sulfamethoxazole	20.2	6.6	-	0.8
Number of isolates	84	181	173	279

[a] Market hogs; [b] CLSI non-susceptible; [c] not tested; [d] azithromycin MIC > 16 mg/L, [e] colistin MIC > 2 mg/L; [f] not able to interpret due to concentration range

[1]CIPARS 2013 Annual Report - Chapter 2. Antimicrobial Resistance. Table 39. Distribution of minimum inhibitory concentrations among *Salmonella* isolates from pigs-page 77

[2]DANMAP. Web Annex DANMAP 2014 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Table A6.1 Distribution of MICs and resistance (%) in *Salmonella* (all serovars) from pigs, Denmark

[3]2014 NARMS Integrated Report Data Tables-Antimicrobial Resistance among all Non-Typhoidal *Salmonella* Isolates, 2003-2014 **(**Tables 10a-10e)

No fluoroquinolone resistance was observed among *Campylobacter* species (Table 7). However, high rates of macrolide, lincosamide, ketolide (telithromycin) and tetracycline resistance were observed. This is likely due to use of first line antimicrobials (Veterinary use of antibiotics highly important to human health-Australian Veterinary Association, April 2017) i.e. those with a lower importance rating, to treat and control respiratory and enteric infections among pigs in Australia (Appendix E).The multidrug-resistant *Campylobacter* species isolated in the Australian study require molecular characterization to further elucidate their public health significance, but it is hypothesized to be low based on the *Campylobacter* species identified.

None of the enterococci isolates were resistant to vancomycin and linezolid. The observed resistance to quinupristin-dalfopristin is unexpected; virginiamycin use in pigs was banned in Australia over 13 years ago. It is still registered for use in other animal species, namely horses and feedlot cattle, however, label recommendations prohibit its use in pigs and the compound also has a Schedule 4 rating (i.e the compound can only be dispensed on prescription by a registered veterinarian): https://apvma.gov.au/sites/default/files/publication/14231-virginiamycin-final-review-report.pdf.
Surveillance data from other countries have documented *Enterococcus* isolates returning to full susceptibility to quinupristin-dalfopristin quite rapidly following removal of virginiamycin. We suspected there was an issue with the elevated resistance to quinupristin-dalfopristin since the percentage of non-wild type for virginiamycin was low (28.6%; Appendix D). Repeat quinupristin-

dalfopristin MIC testing on a subset of isolates using broth microdilution confirmed the original result, indicating a possible the break-point issue for the classification of non-susceptible phenotypes. In order to resolve this we investigated the *Enterococcus* isolates by whole genome sequencing to evaluate if the quinupristin-dalfopristin-resistant isolates carry genetic determinants encoding this resistance (Appendix F). No vancomycin resistance was detected among the enterococci isolates. The genome sequencing revealed that only one isolate carried both previously reported streptogramin A and B resistance gene. The genotypic results shows that the elevated resistance (82%) observed for quinupirstin-dalforpristin among *E. faecium* is not due to the carriage of any known resistance gene. This warrant further investigations.

The elevated resistance to quinpristin-dalfopristin enterococcus may be due to the following reasons.

1. Inappropriate break point for both clinical and epidemiological breakpoint.
2. Presence of new resistance mechanism.

6 Implications & Recommendations

This proof of concept study has provided a baseline for the Australian Pig Industry and a benchmark for the other livestock industries in Australia to establish further animal-specific proof of concept surveys, as the basis for an ongoing integrated livestock AMR surveillance program. It is recommended that the generated data are integrated into current antimicrobial stewardship programs being developed by the intensive livestock industries.

No resistance to critically important drugs including colistin, fluoroquinolones and third-generation cephalosporins, was identified in either *E. coli* or *Salmonella* isolates, and only a small number of isolates showed reduced susceptibility to fluoroquinolones. No resistance to vancomycin and linezolid was identified in *Enterococcus* isolates. All *Campylobacter* isolates were susceptible to fluoroquinolones. Nevertheless, it is recommended that the *Salmonella* and *E. coli* isolates showing reduced susceptibility to fluoroquinolones, the multidrug-resistant enterococci and a selection of multidrug-resistant *Campylobacter* and enterococci isolates are subjected to whole genome sequence analysis to further elucidate their epidemiology, likely origins and public health significance.

This proof of concept study successfully integrated industry-facilitated collection of samples from abattoir specimens, primary culture of commensal and pathogenic bacterial species at a NATA accredited laboratory selected by industry and antimicrobial susceptibility testing at specialist reference laboratories currently undertaking AMR surveillance of human and veterinary pathogens. This will be a successful model to follow for further industry proof of concept studies.

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8 Appendices

8.1 Appendix A – Study Protocol

8.1.1 Introduction

Since 2013, the Commonwealth Government has been actively progressing the development of a coordinated plan for the management of antimicrobial resistance (AMR) and antimicrobial use (AU) in humans and animals. Broad support for the development of the "National Antimicrobial Resistance Strategy" was obtained from key stakeholders across the medical, health, veterinary, agricultural and pharmaceutical communities at the "Australian One Health Antimicrobial Resistance Colloquium" in 2013. The Department of Agriculture and Water Resources (DAWR) then sponsored a review of the national surveillance programs in place for monitoring AMR and AU in animals around the world with a view to defining a program suitable for Australia and combined this with roundtable discussions with key stakeholders in the agriculture and veterinary sectors. The review "Surveillance and reporting of antimicrobial resistance and antibiotic usage in animals and agriculture in Australia" identified one of the major components of surveillance being the assessment of AMR in commensal bacteria and pathogens present in the gut of food animals at slaughter.

In March 2015, a one day meeting convened by the DAWR established the "Antimicrobial Resistance Surveillance Task Group". Present at the meeting were representatives from the DAWR, Animal Health Australia, scientists working in the area of AMR, most of the major Research and Development Corporations or industry bodies involved in animal production (MLA, APL, ACMF, Dairy Australia) and representatives from the Australian pharmaceutical industry. The Task Group reviewed the recommendations from the surveillance report and provided advice from technical and industry perspectives for developing an AMR surveillance component based on the collection of faecal samples from food animals at slaughter. This plan is the result of that meeting. It defines a surveillance model for use in the Australian pig industry that may also be applied to other major food animal industries in future.

At the moment smaller farms are regarded as out of scope of this study because they are from a "minor" production system. It is recognised that there are significant number of non-commercial farms – this is dealt with in the AMRIA report. Nonetheless it is a matter of prioritising to keep within budget and resources. Hence within the AMRIA it has been recommended that the surveillance proceeds on a "risk" basis and a major component of risk is the volume of product/extent of human exposure. Another method of dealing the tail-end of the production curve would be potentially revisit some of them in time where problems are detected.

A number of people involved in the Technical Group and the Antimicrobial Resistance Surveillance Task Group, have given freely of their time and expertise to assist this collaboration between the pig industry and the Federal DAWR, and their contributions are gratefully acknowledged. The outcomes of this project will assist the DAWR in their negotiations with our trading partners on behalf of Australia's livestock industries.

8.1.2 Objectives

The primary aim of the work was to estimate the prevalence of resistance against specified antimicrobials amongst *E. coli, Salmonella* spp., *Enterococcus* spp. and *Campylobacter* spp. isolated from the gut of Australian finisher pigs at slaughter.

8.1.3 Functions and roles

Successful completion of this work required collaboration amongst a number of individuals and institutions.

Table A1 Roles, role descriptions, and responsibilities of the individuals and institutions involved.

Roles	Role Description	Responsibilities
Project coordinator Dr Patricia Mitchell, Australian Pork Limited (APL)	Required an individual with project management skills and technical background in microbiology or epidemiology, although assistance from the Technical Group will be available to support most functions.	Overall coordination of the project and first contact point for major stakeholders and on administration issues. Liaised with laboratories to develop protocols. Liaised with establishments on collection of caecal samples. Provided protocols to laboratories. Collated protocols and data for inclusion in report. Primary authorship of the report. Various other tasks as defined in this report.
Primary laboratories: Ace Laboratory Services 12 Gildea Lane, Bendigo East, Vic 3550 Medical and Molecular Sciences School of Veterinary and Life Sciences Murdoch University PERTH WA	One or more laboratories with NATA accreditation, general expertise in veterinary microbiology and having capacity and infrastructure for collation of caecal samples, isolation and identification of target organisms, storage of isolates and collation of data.	A nominated officer within the lab was responsible for all aspects and acted as the contact person for communication with the Project Coordinator. Roles for the lab included provision of collection material to processing establishments, receipt and processing of samples. The nominated officer provided error-free data in an electronic format to AMR testing laboratories and the project coordinator on request.
AMR testing laboratories Medical and Molecular Sciences School of Veterinary and Life Sciences Murdoch University (MU) PERTH WA Australian Centre for Antimicrobial Resistance Ecology North Adelaide Campus The University of Adelaide (UA) ADELAIDE SA	One or more laboratories with specialist ability at performing phenotypic AMR testing on bacterial isolates using the Sensititre technique.	A nominated officer within the lab was responsible for all aspects and acted as the contact person for communication with the Project Coordinator.
Collection technician ACE Laboratory Services and Murdoch University Technicians Pig Production Veterinary Consultant	Trained Pig Health Monitoring Scheme (PHMS) inspectors and Quality Assurance staff at participating establishments	Coordinated and supervised the collection of caecal samples and their dispatch according to protocols. Ensured supporting data was provided to the Project Coordinator. Collected caecal samples, recorded required data and dispatched samples to primary testing laboratories
Technical group Dr Pat Mitchell Prof. Darren Trott (UA) Dr Jan Bell (UA) Ms Amanda Kidsley (UA) Dr Sam Abraham (MU) Dr Mark O'Dea (MU) Dr Skye Badger (MU) Ms Aileen Vanderfeen (ACE) Dr David Jordan (NSW DPI)	Comprised of the Project Coordinator and microbiologists in charge of testing at the AMR testing laboratories and personnel appointed by the DAWR.	Provided scientific and technical advice to the project as requested and assist the Project Coordinator in analysis and interpretation of results and compilation of the report.

8.1.4 Sampling of caecal contents from animals at abattoirs for AMR surveillance

Animal population under study

The work focused on antimicrobial resistance in bacteria of finisher pigs at slaughter in Australian export abattoirs. Eligible establishments include those processing finishing pigs where a DAWR on-plant veterinarian is present. Approximately 85% of pigs in Australia are slaughtered in eligible establishments.

Number of samples

This is a trial project and the number of caecal specimens collected from pigs was limited to 200 in total to be affordable, provide reasonable confidence limits, and to be approximately the same as many international surveillance programs that evaluate AMR in commensal bacteria from food animals. The numbers of samples (200 for a single major production system on the grounds of "international comparability") is also going to give reasonable statistical accuracy. It must be remembered that this plan has a number of objectives and limited resources. The AMRIA report formed the agreed basis of the direction of this project and was referred back to, to ensure the project remained focused and avoided cost and time overruns.

Discussions with the Export Meat Program (Dr. Clare Jones) and the National Residue Survey (Mr Travis Tobin) indicated that while both areas are very supportive of this work, alternative arrangements for sample collection and transport logistics respectively were likely to be more appropriate for a 'proof of concept' project. To this end, the Project Coordinator engaged the ACE Laboratories, Bendigo Victoria to co-ordinate a number of activities including;

- the making-up of sample collection kits
- collection of samples
- primary isolation from all samples taken and
- submission of isolates to secondary laboratories for AMR testing.

To reduce the chance for bias in results it was imperative to avoid sampling on the basis of convenience, for example, all at once or close together on the production chain. Thus, a systematic-random method of sampling was used, as follows:

- The target number of specimens are collected at regular intervals along the chain during the day.
- The set number of carcasses used as a sampling interval, was consistently applied within that plant. This sampling interval was reported to the Project Coordinator.
- Allowance was made for time to dispatch samples and the submission of collection advice to the Project Coordinator at the end of the day.

Data obtained at specimen collection

Data obtained and recorded at the time of sample collection included: date and time of collection, establishment name, animal species and age (finisher pig in this study), the name of the specimen collector, the within-establishment sample number (a unique number within each establishment written on the label identifying each specimen), and a farm identification code. This data was forwarded to the Project Coordinator at the same time as samples were dispatched to the laboratory. Data accompanying samples to the lab included: the date and time of collection, establishment name, animal species and age, the name of the specimen collector and the within establishment sample

numbers present in the consignment. Note: Farm data is not provided to laboratories and is only given to the Project Coordinator.

Act of specimen collection
Sampling at pig abattoirs was carried out by persons suitably trained and who are well-experienced with specimen collection at slaughter.

Transportation of specimens to primary laboratories
Once collected, specimens were stored at 2-4^{0}C before being packed and shipped to the primary laboratories along with accompanying documents. The chilled specimens were prepared for dispatch according to instructions and materials provided by the primary laboratory, and by using the usual means of transporting specimens indicated by the laboratories. Samples were shipped on the same day they were collected and were required to arrive at the laboratories within 24 hours of collection.

Isolation and confirmation of target organisms (to species level) in primary laboratories
Two primary laboratories were appointed by Project Coordinator in consultation with DAWR. These laboratories had the responsibility of coordinating the despatch of sampling equipment and disposables to the collection points, to receive faecal samples, to extract from samples the isolates of interest, to store the isolates of interest in duplicate, to forward one copy of the isolates to the AMR testing laboratory for susceptibility testing and to manage the data and information around this process. The Project Coordinator ensured that these laboratories are contractually constrained from using the data acquired, specimens, any living organisms or DNA or other biological material derived from the specimens, for any other purpose than for the completion of the surveillance tasks assigned to them unless written approval is provided by DA and industry.

Prior to the commencement of work, the Project Coordinator liaised with the abs to define the details of the above protocols for isolation, identification and AMR assays. Protocols were documented by labs and are attached as an appendix to the Final Report.

The isolate collections submitted to specialist AMR testing laboratories were accompanied by electronic files in a standard format that included the sample collection information from the abattoir, any sample identifying numbers assigned at primary laboratories (e.g. accession numbers etc.) and unique numbers that identified each isolate. Formats for the storage of data at laboratories were standardized and discussed with the Project Coordinator prior to commencement.

Interpretation of resistance was performed with reference to break points published by CLSI and EUCAST after cross-checking with Australian experts on this topic. Data collection and storage in specialist AMR labs was rigorous and performed to an agreed standard. Completed data sets were forwarded to the Project Coordinator on completion.

8.1.5 Collation, analysis and reporting of data

The following data sets were collated:

- Primary laboratory data: defining the information submitted with each specimen, the data on isolates extracted from each specimen

- Specialist AMR data sets included the AMR assay results, as well as bacterial identification results for each of the following organisms:
    - *E. coli*
    - *Enterococcus*
    - *Salmonella*
    - *Campylobacter*

Analysis of data

Data was analysed to produce findings tabulated in similar fashion to all major international surveillance programs for AMR in animals (e.g. DANMAP, CIPARS, SVARM etc.). This consisted of findings aggregated at the national level, showing for each class of organism:

- The number of isolates tested.
- The drugs used in sensitivity testing.
- The percentage of isolates resistant to each drug at the nominated break points.
- The distribution of minimal inhibitory concentrations for each drug.
- Genus and species of isolates where that is relevant.

For the purpose of aiding design of future work, data on the recovery of isolates from samples and issues experienced in the processing and identification of isolates was collated.

The minimum inhibitory concentrations (MIC) were interpreted according to CLSI VET01S (CLSI, 2015b) or the European Committee for Antimicrobial Susceptibility Testing (EUCAST, 2016). epidemiological cut-off values (ECOFFs) as indicated in Tables 1 to 3. (CLSI, 2016) breakpoints were used where animal species antimicrobial agent combinations were not available. Interpretation of the MICs were based on Clinical and Laboratory Standards Institute (Wayne, PA) interpretive criteria when available; otherwise European Committee on Antimicrobial Susceptibility Testing (EUCAST; Basel, Switzerland). The dual EUCAST/CLSI system was used in order that the results were able to be completely internationally relevant i.e. there were two prevalence estimates: 1) EUCAST ECOFF for the percent non-wild, and 2) CLSI intermediate break point for the percent non-susceptible.

Where no EUCAST or CLSI interpretative criteria were available, breakpoints were harmonised with those of the National Antimicrobial Resistance Monitoring System (NARMS), USA (CDC, 2013).

Data was not analysed for reporting AMR findings at the level of herds or processing plants.

Compilation of report drafts

The first draft of the report was produced for the purpose of review by the technical contributors to the work (microbiologists and epidemiologists directly involved in the work) and by the DAWR. The purpose of this report was to identify all technical issues with the data.

The final report focuses on the methods and the results. There is not expansive discussion on the interpretation of the information. Interpretation that is offered focuses on issues to do with study validity, impact of issues encountered during implementation etc. Interpretation with respect to the ramifications for any of the stakeholders is not within the scope of the report.

8.1.6 Consultation and communication

Stage 1: The Project Coordinator liaised closely with DAWR for the duration of the work. APL representatives saw the first draft of the report once scientific and technical details were finalised.

Stage 2. The AMR task group and the DAWR reviewed the work and commented, as appropriate.

Stage 3. The DAWR will table the final report to the ASTAG AMR meeting. Simultaneously APL will be free to distribute the work to the pork industry.

8.2 Appendix B1 – Sampling Protocol

8.2.1 Sampling activities

As directed, the protocol '*Surveillance of AMR in enteric commensals and pathogens in Australian pigs – study design and implementation'* was followed. In addition, the United States Department of Agriculture, Food Safety and Inspection Service directive '*FSIS sampling for the national AMR monitoring system (NARMS)*' was used as a visual guide for appropriate sample collection.

It was decided that better information would be gained if samples were collected samples from at least 10 animals per property and the properties were be selected in such a way as to capture as many representatives as possible of the various production systems

As per the surveillance study design and implementation protocol provided, the interval between the collection of individual samples was calculated as a function of chain speed, daily throughput and shift length. For example, in Western Australia this equated to 1 sample every 30 minutes until 10 samples were collected each day.

Data collected at time of sampling was as per the sampling protocol and labelled on each specimen and provided to the project coordinator.

8.2.2 Sample collection

Sample collection was performed as per the provided protocol and aseptic techniques were used to prevent cross contamination of samples.

Note – it was not possible to use a standard 18 gauge needle and 50ml syringe to suck up caecal contents as directed in the protocol due to frequent blockages. To expedite the process of sample collection the NARMS protocol demonstrated an effective method to collect caecal contents which was subsequently followed. This involved making an incision in the apex of the caecum and catching the contents of the caecum into a sterile pot and since the abattoir condemned the offal from the sampled animals, post-collection contamination from caecal contents was not an issue.

Additionally, a 3x3cm (approximately) tissue sample from the caecal lumen of each individual pig was collected using sterile surgical scissors. The tissue was placed in a sterile jar in no medium as advised by project co-ordinator.

While rectal samples were not included in the sample collection protocol, the decision was made to also collect rectal contents as well and store for later investigation if required. Collection of rectal contents was made via an incision made in the rectal wall of each individual and the faeces subsequently collected into a 70ml sterile pot. (Not all animals sampled had sufficient rectal contents for collection).

After collection, samples were immediately stored in an ice-chest and transported to the primary laboratory each day. The samples were placed in large chilled fridges until processed by the laboratory.

8.3 Appendix B2 – Isolation Methods

8.3.1 *Isolation of* Enterococci, Campylobacter, Salmonella, and E. coli

Initial sample preparation

1) Weigh out 10g of faecal sample into a sterile sample collection jar.
2) Add 7ml of 0.1% Buffered Peptone Water into each jar.
3) Mix well and allow to settle.
4) Extract 1ml of faecal mixture into 1.5ml microfuge tube for *E. coli* and enterococci isolation. The tube was centrifuged for 5000rpm for 5mins and used for *E. coli* and enterococci isolation.
5) Extract 1ml of faecal mixture into 9ml Preston *Campylobacter* broth for *Campylobacter* isolation.
6) Retain remaining faecal mixture for *Salmonella* isolation.

E. coli Isolation

1) A sterile cotton tip applicator is inserted in to the Microfuge tube and plated on to MacConkey agar and streaked using a sterile loop.
2) Incubate the plate at 37°C for 24hrs.
3) Select 3 large isolated pink colonies and sub-culture on to individual sheep blood agar plates.
4) Incubate the plates at 37°C for 24hrs.
5) Typical Isolates are subjected to MALDI or other biochemical identification.
6) One confirmed *E. coli* isolate is frozen down and entered in to the survey
7) Collect a loop-full of colonies using a sterile loop and inoculate 1ml Tryptone Soya Broth w/ 20% glycerol in a freeze down tube.
8) Store at -80°C.
9) Using Amies Charcoal swab send isolate to secondary lab for MIC.

Enterococcus Isolation

1) A sterile cotton tip applicator is inserted in to the microfuge tube and plated on to Slanetz & Bartley Agar Plate.
2) Slanetz & Bartley Agar Plate is incubated at 42°C for 48hrs.
3) Select 3 well isolated colonies that resemble enterococcus colonies and plate on Sheep Blood Agar for identifying the bacteria.
4) Incubate the plates at 37°C for 24hrs.
5) Isolates that were α- haemolytic are subjected to MALDI or other biochemical identification.
6) One enterococcus isolate is frozen down and entered in to the survey
7) Collect a loop-full of colonies using a sterile loop and inoculate 1ml Tryptone Soya Broth w/ 20% glycerol in a freeze down tube.
8) Store at -80°C.
9) Using Amies Charcoal swab send isolate to secondary lab for MIC.

Campylobacter Isolation

1) Incubate Preston Campylobacter Broth at 42°C for 48hrs.
2) Using a sterile cotton tip applicator, inoculate a plate of Campy agar and streak using a sterile loop.
3) Incubate at 42°C for 24hours.
4) Select 3 well isolated colonies using a sterile loop and sub-culture on individual sheep blood agar. (If unable to get isolation, subculture on sheep blood agar or on MCCDA).
5) Incubate the plates at 42°C for 24 hrs.

6) Typical isolates are subjected to MALDI or other biochemical identification.
7) One *Campylobacter* isolate is frozen down and entered in to the survey
8) Collect a loop-full of colonies using a sterile loop and inoculate 1ml Tryptone Soya Broth w/ 20% glycerol in a freeze down tube.
9) Store at -80°C.
10) Using Amies Charcoal swab send isolate to secondary lab for MIC.

Salmonella Isolation

1) Incubate remaining faecal sample in BPW at 37°C for 24hrs.
2) Transfer 0.1ml of incubated peptone into 10ml of Rappaport-Vassiliadis (RV) Broth
3) Incubate 42°C for 18hrs.
4) Using a sterile cotton tip applicator, inoculate a plate of *Salmonella* Brilliance agar and XLD agar followed by streaking using a sterile loop.
5) Incubate the plates at 37°C for 24hrs.
6) Select 3 well isolated colonies and sub-culture on sheep blood agar.
7) Incubate at 37°C for 24hrs.
8) Typical isolates are subjected to MALDI or other biochemical identification.
9) One *Salmonella* isolate is frozen down and entered in to the survey
10) Collect a loop-full of colonies using a sterile loop and inoculate 1ml Tryptone Soya Broth w/ 20% glycerol in a freeze down tube.
11) Store at -80°C.
12) Using Amies Charcoal swab send isolate to secondary lab for MIC.

Media

Table B2 – Media for the isolation of samples.

0.1% Buffered peptone water	Prepared in house (Thermo Fisher)
Sheep Blood Agar	Thermo Fisher
MacConkey Agar	Thermo Fisher
Preston Campylobacter Broth	Prepared in house (Thermo Fisher)
Campylobacter Agar (PP2005)	Thermo Fisher
Sheep Blood Agar	Thermo Fisher
RV Broth	Micromedia (Edwards)
XLD Agar	Micromedia (Edwards)
Tryptone Soya Broth w/ 20% glycerol	Prepared in house (Thermo Fisher)
Slanetz & Bartley Agar Plate	Thermo Fisher
Salmonella Brilliance agar	Thermo Fisher

8.4 Appendix C – Isolates Recovered

Table C1 Isolates recovered by species

			Campylobacter †					Enterococcus ‡									
	Escherichia	***Salmonella***	**CCOL**	**CHYO**	**CJEJ**	**CAM**	**All**	**EFAC**	**EHIR**	**EFAE**	**EDUR**	**EGAL**	**EHER**	**EMUN**	**EAVI**	**All**	**Total**
	10	*10*	10				10	8	2	1						6	40
	10	*1*	10				10	3	2	1						6	27
	10	8	10				10	3	2	1						6	34
	10	4	10				10	9							1	10	34
	10	4	5				5	3	4	2						9	28
	10	8	5				5	8	2							10	33
	10		10				10	1	2							3	23
	10	4	6	3			9	6	1	2						9	32
	10	2	9				9	7						1		8	29
	10	1	5	1	1		7		1	1						2	20
	10	9	6	3			9	4	6							10	38
	10	8	5	3			8	5	4		1					10	36
	10	1	3	1			4	6	3		1					10	25
	10	6	10				10	6		1	1	2				10	36
	10	1	10				10	1	2	1						4	25
	10		7			1	8	1								1	19
	10	10	7	1			8	3	4	3						10	38
	10	2	10				10	1								1	23
	4		4				4	1		2						3	11
	1		1				1			1						1	3
	2		2				2			2						2	6
	2		1				1	1			1					2	5
	1		1				1	1								1	3
	1		1				1	1								1	3
	1	1	1				1	1								1	4
	2	1	2				2	2								2	7
	2		2				2										4
	1		1				1		1							1	3
	1	1	1				1		1							1	4
	1	1	1				1	1								1	4
	1	1	1				1	1								1	4
Total (%)	200	84	157 (91.8)	12 (7.0)	1	1	171	84 (57.5)	36 (24.7)	17 (11.6)	4	2	1	1	1	146	601

†CCOL = *C. coli*; CHYO = *C. hyointestinalis*; CJEJ = *C. jejuni*; CAM = *Campylobacter* species‡ EFAC = *E. faecium*; EFAE = *E. faecalis*; EDUR = *E. durans*; EGAL = *E. gallinarum*; EHER = *E. hermanniensis*; EMUN = *E. mundtii*; EAVI = *E. avium*

8.5 *Appendix D – Minimum Inhibitory Concentration (MIC) Distributions*

Table D1 Minimum inhibitory concentration (MIC) distribution of Escherichia coli *isolates (n=200)*

	Number and percentage of isolates with MICs (mg/L) at: [a]																		
Antimicrobial agent	**0.008**	**0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**>256**	**%NWT** [b] **[95% CI]**	**%NS** [c]
Amoxicillin-clavulanate								1 0.5	18 9.0	75 37.5	92 46.0	14 7.0	0 - [d]					— [e]	7.0
Ampicillin								8 4.0	33 16.5	33 16.5	2 1.0	1 0.5	0 -	123 61.5				62.0 [55.1 - 68.4]	62.0
Azithromycin					0 -	0 -	0 -	2 1.0	69 34.5	116 58.0	11 5.5	0 -	2 1.0					—	1.0
Cefoxitin							0 -	1 0.5	16 8.0	132 66.0	48 24.0	2 1.0	1 0.5					1.5 [0.4 - 4.3]	1.5
Ceftiofur					0 -	60 30.0	132 66.0	8 4.0	0 -	0 -	0 -							0.0 [0.0 – 1.9]	0.0
Ceftriaxone						198 99.0	2 1.0	0 -	0 -	0 -	0 -	0 -	0 -	0 -				1.0 [f] [0.2 - 3.6]	0.0
Chloramphenicol									2 1.0	29 14.5	72 36.0	13 6.5	37 18.5	47 23.5				42.0 [35.4 - 48.9]	48.5
Ciprofloxacin		174 87.0	22 11.0	0 -	0 -	3 1.5	1 0.5	0 -	0 -	0 -								2.0 [0.8 - 5.0]	0.0
Colistin					26 13.0	168 84.0	6 3.0	0 -	0 -	0 -	0 -							0.0 [0.0 – 1.9]	—
Florfenicol								0 -	1 0.5	9 4.5	90 45.0	81 40.5	12 6.0	0 -	7 3.5			9.5 [6.2 - 14.4]	—
Gentamicin						3 1.5	81 40.5	102 51.0	9 4.5	1 0.5	3 1.5	0 -	1 0.5					2.5 [1.1 - 5.7]	2.0
Kanamycin									142 71.0	10 5.0	1 0.5	0 -	0 -	2 1.0	10 5.0	2 1.0	33 16.5	23.5 [18.2 - 29.8]	23.5
Streptomycin									1 0.5	14 7.0	53 26.5	21 10.5	34 17.0	25 12.5	52 26.0			55.5 [48.6 - 62.2]	38.5
Tetracycline										49 24.5	1 0.5	0 -	9 4.5	141 70.5				75.0 [68.6 - 80.5]	75.5
Trimethoprim/ sulfamethoxazole					82 41.0	29 14.5	8 4.0	3 1.5	1 0.5	1 0.5	76 38.0							39.0 [32.5 - 45.9]	38.5

[a] Unshaded areas indicate MIC range for each agent available on the Sensititre CMV3AGNF card. MICs > than highest concentration available are indicated in the shaded region

Vertical green lines indicate EUCAST epidemiological cut-off (ECOFF) values; CLSI susceptible (blue) and resistant (red) breakpoints; and NARMS breakpoints (red dashes

[b] Percentage non-wild-type (EUCAST); [c] Percentage non-susceptible, CLSI or NARMS (orange)

[d] not applicable; [e] Not defined; [f] Ceftriaxone MIC > 0.25 mg/L used; as unable to accurately determine non-wild type population

Table D2 Minimum inhibitory concentration (MIC) distribution of Salmonella spp. *Isolates (n=84)*

Antimicrobial agent	Number and percentage of isolates with MICs (mg/L) at: [a]																	%NWT [b] [95% CI]	%NS [c]
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256		
Amoxicillin-clavulanate								29 34.5	1 1.2	11 13.1	39 46.4	3 3.6	0 - [d]	1 1.2				— [e]	4.8
Ampicillin								29 34.5	1 1.2	2 2.4	0 -	0 -	0 -	52 61.9				61.9 [51.2 – 71.6]	61.9
Azithromycin					0 -	0 -	0 -	0 -	15 17.9	60 71.4	9 10.7	0 -						—	0.0
Cefoxitin							0 -	0 -	52 61.9	25 29.8	5 6.0	0 -	0 -	2 2.4				2.4 [0.4 – 8.3]	2.4
Ceftiofur					0 -	0 -	19 22.6	60 71.4	5 6.0	0 -	0 -							0.0 [0.0 – 4.4]	—
Ceftriaxone						83 98.8	1 1.2	0 -	0 -	0 -	0 -	0 -	0 -	0 -				—	0.0
Chloramphenicol									1 1.2	17 20.2	51 60.7	0 -	0 -	15 17.9				17.9 [11.1 – 27.4]	17.9
Ciprofloxacin		36 42.9	44 52.4	1 1.2	1 1.2	2 2.4	0 -	0 -	0 -	0 -								3.6 [1.0 – 10.0]	3.6
Colistin					0 -	53 63.1	28 33.3	3 3.6	0 -	0 -								—	0.0
Florfenicol								0 -	0 -	37 44.0	34 40.5	6 7.1	1 1.2	1 1.2	0 -	5 6.0		8.3 [4.1 – 16.2]	8.3
Gentamicin						67 79.8	15 17.9	0 -	0 -	0 -	1 1.2	0	1 1.2					2.4 [0.4 – 8.3]	2.4
Kanamycin									74	1	0	0	0	3	3	1	2		

					88.1	1.2	-	-	-	3.6	3.6	1.2	2.4	—	10.7
Streptomycin					1	14	12	11	1	7	38				
					1.2	16.7	14.3	13.1	1.2	8.3	45.2			54.8 [44.1 – 65.0]	53.6
Tetracycline						19	0	0	0	65					
						22.6	-	-	-	77.4				77.4 [67.4 – 85.0]	77.4
Trimethoprim/sulfamethoxazole	66	0	1	0	0	0	17								
	78.6	-	1.2	-	-	-	20.2							20.2 [13.0 – 30.0]	20.2

[a] Unshaded areas indicate MIC range for each agent available on the Sensititre CMV3AGNF card. MICs > than highest concentration available are indicated in the shaded region
Vertical green lines indicate EUCAST epidemiological cut-off (ECOFF) values; CLSI VET01S susceptible (blue) and resistant (red) breakpoints; NARMS breakpoint (red dashes)

[b] Percentage non-wild-type (EUCAST)

[c] Percentage non-susceptible, CLSI or NARMS (orange)

[d] Not applicable

[e] Not defined

Table D3 Minimum inhibitory concentration (MIC) distribution of Campylobacter coli *isolates (n=157)*

Antimicrobial agent	Number and percentage of isolates with MICs (mg/L) at: [a]															%NWT [b] [95% CI]	%NS [c]
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64		
Azithromycin		0	3	21	14	2	0	0	0	0	0	0	0	0	117		
		- [d]	1.9	13.4	8.9	1.3	-	-	-	-	-	-	-	-	74.5	74.5 [67.2 – 80.7]	74.5
Ciprofloxacin		0	7	41	90	15	4	0	0	0	0	0	0	0			
		-	4.5	26.1	57.3	9.6	2.5	-	-	-	-	-	-	-		0.0 [0.0 – 2.4]	0.0
Clindamycin			0	0	6	16	16	1	9	20	72	15	2				
			-	-	3.8	10.2	10.2	0.6	5.7	12.7	45.9	9.6	1.3			75.2 [67.9 – 81.3]	75.2
Erythromycin			0	0	0	3	16	19	2	0	2	0	2	2	111		
			-	-	-	1.9	10.2	12.1	1.3	-	1.3	-	1.3	1.3	70.7	73.2 [65.8 – 79.6]	73.2
Florfenicol			0	0	2	9	69	69	8	0	0	0	0	0			
			-	-	1.3	5.7	43.9	43.9	5.1	-	-	-	-	-		0.0 0.0 [0.0 – 2.4]	0.0
Gentamicin					0	6	59	91	0	1	0	0	0				
					-	3.8	37.6	58.0	-	0.6	-	-	-			0.6 [0.03 – 3.5]	0.6
Telithromycin		0	0	0	0	1	4	22	16	8	30	76					
		-	-	-	-	0.6	2.5	14.0	10.2	5.1	19.1	48.4				67.5 [59.8 – 74.3]	67.5
Tetracycline				3	13	28	17	11	1	0	7	14	11	28	24		
				1.9	8.3	17.8	10.8	7.0	0.6	-	4.5	8.9	7.0	17.8	15.3	53.5 [45.7 – 61.1]	53.5
Nalidixic acid										65	81	8	0	0	3		
										41.4	51.6	5.1	-	-	1.9	1.9 [0.5 – 5.5]	1.9

[a] Unshaded areas indicate MIC range for each agent available on the Sensititre CAMPY card. MICs > than highest concentration available are indicated in the shaded region
Vertical green lines indicate EUCAST epidemiological cut-off (ECOFF) values; CLSI susceptible (blue) and resistant (red) breakpoints; NARMS breakpoints (dashes)

[b] Percentage non-wild-type (EUCAST)

[c] Percentage non-susceptible, NARMS (orange)

[d] not applicable

Table D4 Minimum inhibitory concentration (MIC) distribution of Enterococcus faecium *isolates (n=84)*

	Number and percentage of isolates with MICs (mg/L) at: [a]																	
Antimicrobial agent	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**>2048**	**%NWT [b] [95% CI]**	**%NS [c]**
Ampicillin		4 4.8	4 4.8	10 11.9	20 23.8	17 20.2	24 28.6	4 4.8	1 1.2	0 - [d]							34.5 [25.2 – 45.2]	6.0
Benzylpenicillin		2 2.4	2 2.4	5 6.0	5 6.0	27 32.1	10 11.9	21 25.0	12 14.3								14.3 [8.4 – 23.3]	39.3
Chloramphenicol					1 1.2	1 1.2	15 17.9	64 76.2	3 3.6								0.0 [0.0 – 4.4]	79.8
Daptomycin		5 6.0	6 7.1	12 14.3	14 16.7	33 39.3	13 15.5	1 1.2									16.7 [10.0 – 26.1]	16.7
Erythromycin		2 2.4	2 2.4	0 -	1 1.2	3 3.6	1 1.2	75 89.3									90.5 [82.3 – 95.1]	95.2
Gentamicin (high-level)											83 98.8	1 1.2	0 -	0 -			—[e]	0.0
Kanamycin (high-level)											29 34.5	30 35.7	7 8.3	6 7.1	12 14.3		—	21.4
Lincomycin				1 1.2	0 -	1 1.2	3 3.6	79 94.0									—	97.6
Linezolid			0 -	0 -	43 51.2	41 48.8	0 -										0.0 0.0 [0.0 – 4.4]	48.8
Quinuprisrin-dalfopristin			2 2.4	3 3.6	10 11.9	33 39.3	24 28.6	5 6.0	6 7.1	1 1.2							—	94.0
Streptomycin (high-level)													41 48.8	8 9.5	10 11.9	25 29.8	—	51.2
Teicoplanin		65 77.4	8 9.5	10 11.9	1 1.2	0 -	0 -	0 -	0 -	0 -	0 -						0.0 0.0 [0.0 – 4.4]	0.0
Tetracycline				7 8.3	0 -	0 -	0 -	4 4.8	2 2.4	71 84.5							91.7 [83.8 – 95.9]	91.7
Vancomycin		4 4.8	67 79.8	7 8.3	4 4.8	1 1.2	1 1.2	0 -	0 -								1.2 [0.01 – 6.4]	1.2
Virginiamycin		6 7.1	6 7.1	9 10.7	16 19.0	23 27.4	13 15.5	8 9.5	3 3.6	0 -	0 -						28.6 [20.0 – 39.0]	—

[a] Unshaded areas indicate MIC range for each agent available on the Sensititre CMV3AGPF card. MICs > than highest concentration available are indicated in the shaded region
Vertical green lines indicate EUCAST epidemiological cut-off (ECOFF) values; CLSI susceptible (blue) and resistant (red) breakpoints; NARMS breakpoints (dashes)

[b] Percentage non-wild-type (EUCAST)
[c] Percentage non-susceptible, CLSI or NARMS (orange)
[d] not applicable
[e] Not defined

Table D5 Minimum inhibitory concentration (MIC) distribution of Enterococcus hirae *isolates (n=36)*

	Number and percentage of isolates with MICs (mg/L) at: [a]																	
Antimicrobial agent	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**>2048**	**%NWT [b] [95% CI]**	**%NS [c]**
Ampicillin		12 33.3	1 2.8	5 13.9	7 19.4	5 13.9	4 11.1	2 5.6	0 - [d]	0 -							16.7 [7.9 – 31.9]	5.6
Benzylpenicillin		9 25.0	1 2.8	5 13.9	6 16.7	0 -	5 13.9	6 16.7	4 11.1								11.1 [4.4 – 25.3]	27.8
Chloramphenicol					0 -	0 -	17 47.2	17 47.2	2 5.6								0.0 0.0 [0.0 – 9.6]	52.8
Daptomycin		0 -	1 2.8	2 5.6	7 19.4	16 44.4	9 25.0	1 2.8									27.8 [15.8 – 44.0]	27.8
Erythromycin		6 16.7	0 -	0 -	0 -	0 -	1 2.8	29 80.6									83.3 [68.1 – 92.1]	83.3
Gentamicin (high-level)											36 100	0 -	0 -	0 -			—	0.0
Kanamycin (high-level)											35 97.2	0 -	0 -	0 -	1 2.8		—	2.8
Lincomycin				0 -	1 2.8	0 -	0 -	35 97.2									—	97.2
Linezolid			0 -	0 -	24 66.7	12 33.3	0 -										0.0 0.0 [0.0 – 9.6]	33.3
Quinuprisrin-dalfopristin			1 2.8	0 -	5 13.9	19 52.8	10 27.8	1 2.8	0 -								—	97.2
Streptomycin (high-level)													18 50.0	3 8.3	2 5.6	13 36.1	—	50.0
Teicoplanin		36 100	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -						0.0 0.0 [0.0 – 9.6]	0.0
Tetracycline				6 16.7	0 -	0 -	0 -	0 -	1 2.8	29 80.6							83.3 [68.1 – 92.1]	83.3
Vancomycin		0 -	26 72.2	10 27.8	0 -	0 -	0 -	0 -	0 -								0.0 [0.0 – 9.6]	0.0
Virginiamycin		3 8.3	5 13.9	8 22.2	14 38.9	5 13.9	1 2.8	0 -	0 -	0 -	0 -						2.8 [0.1 – 14.2]	—

[a] Unshaded areas indicate MIC range for each agent available on the Sensititre CMV3AGPF card. MICs > than highest concentration available are indicated in the shaded region
Vertical green lines indicate EUCAST epidemiological cut-off (ECOFF) values; CLSI susceptible (blue) and resistant (red) breakpoints; NARMS breakpoints (dashes)

[b] Percentage non-wild-type (EUCAST)
[c] Percentage non-susceptible, CLSI or NARMS (orange)
[d] not applicable
[e] Not defined

8.6 Appendix E – Veterinary use of antibiotics in pigs

Table E1 Veterinary use of antimicrobials in pigs

Veterinary use in pigs [a]			
First line	**Second line**	**Third line**	**Prohibited**
Amoxicillin Erythromycin Chlortetracycline Oxytetracycline Sulphonamides Kitasamycin Tilmicosin Tylosin Penicillin Florfenicol Neomycin	Amoxicillin-clavulanate Apramycin Lincomycin Trimethoprim-Sulphonamides Tiamulin Tulathromycin Spectinomycin	Ceftiofur	Fluoroquinolones Gentamicin Chloramphenicol Nitrofurans

Source: Australian Veterinary Association, 2014

Following diagnosis, consider using the first line antimicrobials along with alternative treatment approaches.
Second line use should be limited where possible to when susceptibility testing or clinical results have proven that first line antibiotics are not effective.
Third line antimicrobials are for use as a last resort. They should be used only when other options are unavailable and wherever possible only after susceptibility testing has been completed.

8.7 Appendix F – Genomic characterisation of E. faecium *among Australian pigs*

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8.7.1 Introduction

During surveillance for antimicrobial resistance in enteric commensals and pathogens in Australian pigs, several resistance phenotypes to clinically important antimicrobials such as quinupristin-dalfopristin were observed in *E. faecium*. Further investigations were carried out to determine the genetic origin of these resistances and to confirm the presence of elevated antimicrobial resistance in these isolates.

The AMR pilot survey among Australian pigs report noted a high prevalence (82%) of resistance to the streptogramin A and B combination antimicrobial, quinupirstin-dalforpristin, in *E. faecium*. While each by itself is only a bacteriostatic agent, the combination of the pair produces a synergistic bactericidal effect. The binding of dalfopristin to the 23s portion of the 50s ribosomal unit results in a conformational change to the ribosome which increases the affinity of quinupristin binding by 100-fold. Resistance to quinupristin-dalfopristin can be conferred by the presence of one or more streptogramin A resistance genes (Hershberger et al., 2004). Genetically, resistance to streptogramin A antimicrobials including dalfopristin can be conferred by the *vatD,* (*satA*) or *vatE* (*satG*) genes while resistance to streptogramin B antimicrobials including virginiamycin and quinupristin can be conferred by the *vgbA*, *msrC* or *ermB* genes. Besides quinupristin-dalfopristin resistance, a single isolate of *E. faecium* was also identified to be vancomycin resistant. Resistance to vancomycin is conferred by acquisition of the van gene cluster for which there are several types. Clinically important van genes such as *vanA*, *vanB* and *vanM* can be carried on plasmids and therefore can be shared among bacteria through horizontal gene transfer (Cetinkaya, Falk, and Mayhall, 2000).

8.7.2 Methods

Whole Genome sequencing
The isolates were cultured on blood agar from -80˚C storage and further sub-cultured before testing. DNA extraction was performed using the MagMax DNA extraction kit (Thermofisher Scientific, USA). A total of 84 *E. faecium* was isolated during the surveillance project, however, only 81 of these isolates were successfully recovered after cryopreservation at -80˚C. DNA libraries were prepared using the Illumina Nextera Xt library preparation kit (Illumina, USA) and sequenced on the Illumina Nextseq platform (Illumina, USA). Raw sequence data was parsed through the Nullarbor pipeline which assembles and annotates the sequences before identifying resistance genes and multi-locus sequence types MLST) (Seeman et al.). The assembled sequences were aligned using snippy which also identifies single nucleotide polymorphisms before adjusting for recombination using Gubbins and generating the phylogenetic tree (Croucher et al., 2014).

Identification of local Epidemiological cut-off values (ECOFFs)
The distribution of the MIC's for each of quinupristin-dalfopristin, virginiamycin and erythromycin for 84 *E. faecium* isolates from pigs were input into the ECOFFinder software.

8.7.3 Results and discussion

Whole Genome sequencing

Using the Illumina NextSeq platform, 81 *E. faecium* isolates identified during the APL project were sequenced. Genome sequencing returned sequence data for 9 isolates that was unsuitable for further analysis. This was due to the genomes sequenced not matching with *E. faecium* (likely due to maldi-tof identification error from the previous study or mixed cultures in freezedown). Therefore, the final number of isolates to be reported was reduced to 72.

A total of 23 unique MLSTs were identified and 29 isolates had unknown STs. The most common ST was ST5 with 9 isolates. 26 isolates had STs that were associated with clonal complex 17 which associated with hospital acquired *E. faecium* infections (See Table F1).

Table F1 MLST and profile of resistance genes. aadD, aadE, aph(3')-III, spc, str confers aminoglycoside resistance. Cat confers chloramphenicol resistance. ermA, ermB, ermT confers macrolid, lincosamide and streptogramin B (MLSB) resistance. lnuB confers lincosamide resistance. msrC confers erythromycin, macrolides and streptogramin B resistance. tetL, tetM, tetS confers tetracycline resistance. vatE confers streptogramin A resistance.

Number of isolates	MLST	Number of resistance genes	Resistance Profile
1	5	3	*ermB|msrC|tetM*
1	5	4	*ermT|msrC|tetL|tetM*
1	5	4	*ermB|msrC|tetM|tetS*
1	5	4	*aadE|ermB|msrC|tetM*
1	5	4	*ermB|msrC|tetL|tetM*
1	5	5	*ermA|ermB|msrC|spc|tetM*
1	5	6	*ermA|ermB|msrC|spc|tetM|tetS*
1	5	5	*ermB|msrC|str|tetL|tetM*
1	5	6	*ermA|ermB|msrC|spc|tetM|tetS*
2	6	5	*ermB|msrC|str|tetL|tetM*
1	22	6	*cat|ermB|lnuB|msrC|tetL|tetM*
1	27	5	*aadE|ermB|lnuB|msrC|tetL*
1	32	6	*aadE|ermB|lnuB|msrC|tetL|tetM*
1	32	5	*aph(3')-III|ermB|lnuB|msrC|tetL*
1	94	1	*msrC*
1	108	1	*msrC*
1	116	5	*aadE|aph(3')-III|msrC|tetL|tetM*
1	133	5	*ermB|msrC|str|tetL|tetM*
1	133	5	*ermB|lnuB|msrC|tetL|tetM*
1	133	7	*aadE|ermB|lnuB|msrC|str|tetL|tetM*
1	133	5	*aadE|ermB|msrC|str|tetL*
1	137	3	*ermB|msrC|tetM*

1	137	4	*aadE*|*ermB*|*msrC*|*tetM*
1	140	3	*ermB*|*msrC*|*tetM*
1	178	4	*ermB*|*msrC*|*tetL*|*tetS*
1	185	3	*ermB*|*msrC*|*tetM*
1	185	5	*ermB*|*msrC*|*str*|*tetL*|*tetM*
1	185	5	*aadE*| *aph(3')-III*|*ermB*|*msrC*|*tetM*
2	185	4	*aadE*|*ermB*|*msrC*|*tetL*
1	240	1	*msrC*
1	291	5	*aph(3')-III*|*ermB*|*msrC*|*tetL*|*tetM*
1	509	3	*ermB*|*msrC*|*tetM*
1	529	7	*aadD*|*aadE*|*ermB*|*lnuB*|*msrC*|*str*|*tetL*
1	621	5	*aph(3')-III*|*ermB*|*msrC*|*tetL*|*tetM*
1	822	7	*aadD*| *aadE*|*ermB*|*lnuB*|*msrC*|*tetL*|*tetM*
2	1006	6	*aadE*|*ermB*|*lnuB*|*msrC*|*tetL*|*tetM*
1	1103	3	*ermB*|*msrC*|*tetS*
1	1176	6	*aadE*|*ermB*|*lnuB*|*msrC*|*tetL*|*tetM*
1	1258	2	*msrC*|*tetM*
1	1258	5	*aadE*|*ermB*|*msrC*|*tetL*|*tetS*
1	-	1	*msrC*
1	-	3	*ermB*|*msrC*|*tetS*
2	-	4	*ermT*|*msrC*|*tetL*|*tetM*
1	-	4	*aadE*|*msrC*|*tetL*|*tetM*
2	-	4	*ermB*|*msrC*|*tetL*|*tetM*
1	-	5	*ermB*|*ermT*|*msrC*|*tetL*|*tetM*
1	-	5	*ermB*|*msrC*|*tetL*|*tetM*|*tetS*
1	-	2	*msrC*|*vatE*
1	-	5	*aadE*|*ermB*|*msrC*|*tetL*|*tetS*
1	-	5	*aadE*|*ermB*|*lnuB*|*msrC*|*tetL*
2	-	6	*aadE*|*aph(3')-III*|*ermB*|*msrC*|*tetL*|*tetM*
1	-	5	*ermB*|*lnuB*|*msrC*|*tetL*|*tetM*
1	-	2	*ermB*|*msrC*
1	-	6	*aadE*|*ermB*|*lnuB*|*msrC*|*tetL*|*tetM*
1	-	6	*aadE*|*ermB*|*lnuB*|*msrC*|*tetL*|*tetS*
1	-	6	*ermA*|*lnuB*|*msrC*|*spc*|*tetL*|*tetM*
1	-	6	*aadE*|*ermB*|*lnuB*|*msrC*|*tetL*|*tetS*
1	-	6	*aadE*|*ermB*|*lnuB*|*msrC*|*tetL*|*tetM*
1	-	7	*aadD*|*aadE*|*ermB*|*lnuB*|*msrC*|*tetL*|*tetM*
1	-	6	*aadE*|*ermB*|*lnuB*|*msrC*|*tetL*|*tetM*
1	-	5	*aph(3')-III*|*ermB*|*msrC*|*tetL*|*tetS*
1	-	5	*aph(3')-III*|*ermB*|*msrC*|*tetL*|*tetM*
1	-	6	*aadD*|*aadE*|*ermB*|*msrC*|*tetL*|*tetM*

1	-	6	*aph(3')-III|ermB|lnuB|msrC|tetL|tetM*
1	-	5	*aadE|ermB|lnuB|msrC|tetL*
1	-	8	*aph(3')-III|ermA|ermB|lnuB|msrC|spc|tetL|tetM*

A total of 17 resistance genes were identified in the *E. faecium* sequenced. The most common resistance gene, *msrC*, which encodes for a chromosomal-encoded ABC-efflux pump that confers resistance to erythromycin, other macrolides and streptogramin B antimicrobials, was found in 100% of isolates. The second most common resistance gene, *ermB*, which confers the macrolide-lincosamide-streptogramin B (MLSb) resistance phenotype was identified in 90.2% of isolates. Although the *ermA* and *ermT* genes were also identified, they were less prevalent at 6.6% and 4.9% respectively. Overall, only three isolates did not possess any of the genes from the *erm* family. The *vatE* gene (conferring streptogramin A resistance) was only identified in one isolate.

Other notable resistance genes identified were tetracycline resistance genes *tetL*, *tetM* and *tetS* (77%, 82% and 18% respectively) and *cat* gene encoding chloramphenicol resistance (1 isolate). In conclusion, although a high prevalence of resistance to streptogramin B was identified in the isolates tested, most isolates did not possess any known resistance genes to streptogramin A.

ECOFF analysis

Distribution of MIC for quinupristin-dafloprístin (QID), erythromycin (ERY) and virginiamycin (VIR) are shown in Figure F1. Tentative local Epidemiological cut-off values (mg/L) for assessing sensitivity of *E. faecium* isolates from pigs (n=84) to quinupristin-dalfopristin, virginiamycin and erythromycin and corresponding current ECOFF values used for interpretation in this report are shown in Table F2.

The total number of observations in each case numbered 84 and because of this limitation in sample size it was only appropriate to calculate "tentative ECOFF" values as described by EUCAST. Other limitations in the data include the lack of clearly defined susceptible and resistance subpopulations in the data which would greatly enhance the accuracy of estimates. Therefore, the current "tentative" estimates are not suitable for re-interpretation of results but do indicate the need for generating more robust ECOFF values for these drugs.

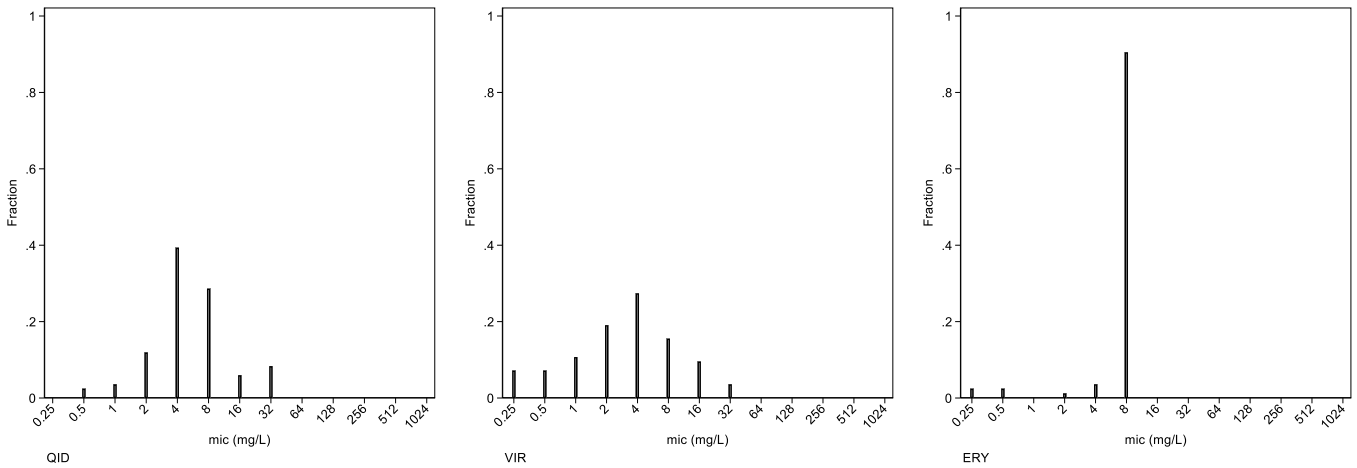

Figure F1 Distribution of minimal inhibitory concentrations (MIC – mg/L) used for assessing susceptibility of Enterococcus faecium isolates from pigs in this report (n=84) to quinupristin-dafloprístin (QID), erythromycin (ERY) and virginiamycin (VIR).

Table F2 Tentative ecological cut-off values (mg/L) for assessing sensitivity of Enterococcus faecium isolates from pigs (n=84) to quinupristin-dalfopristin, virginiamycin and erythromycin and corresponding current ECOFF values** used for interpretation in this report.*

	ECOFF mg/L	
Antimicrobial	**Tentative *** **	**Current**
Quinupristin-dalfopristin	16	Not available
Virginiamycin	32	4
Erythromycin	Not available****	4

*Tentative ECOFF values obtained from ECOFFinder software.
** Current ECOFF values as for Table D4, Appendix D in this report and derived from EUCAST.
*** Caution in interpretation is required due to the limitations in sample size
**** Tentative ECOFF could not be determined due to data limitations.

8.7.4 Conclusion

No vancomycin resistance was detected among the enterococci isolates. The genome sequencing revealed that only one isolate carried both previously reported streptogramin A and B resistance gene. The genotypic results shows that the elevated resistance (82%) observed for quinupirstin-dalforpristin among *E. faecium* is not due to the carriage of any known resistance gene. This warrant further investigations.

The elevated resistance to quinpristin-dalfopristin enterococcus may be due to the following reasons and require further investigation.

1. Inappropriate break point for both clinical and epidemiological breakpoint
2. Presence of new or yet to be identified resistance mechanism.

8.7.5 References

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