

Investigation into *Salmonella* Carriage and Antimicrobial Resistance in Australian Weaner Pigs

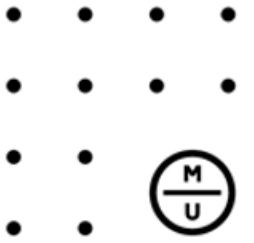
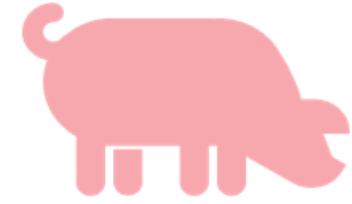
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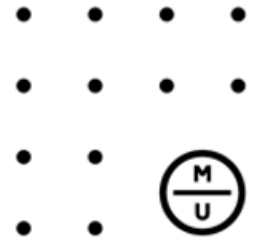
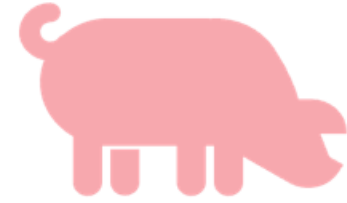
Overview



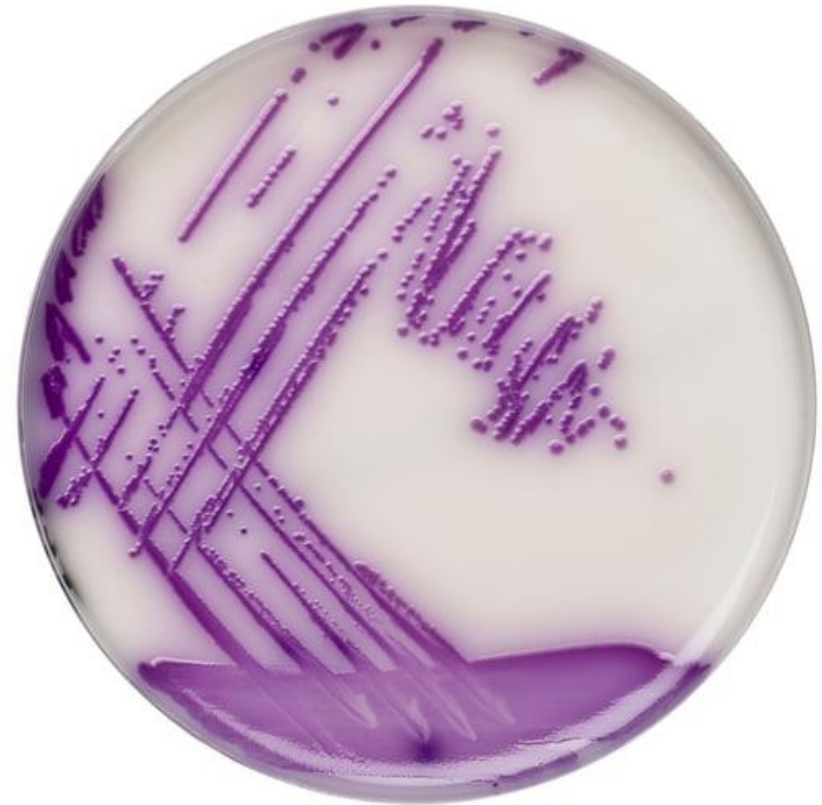
- Background
- Aims and Hypothesis
- Methods
- Results
- Limitation and future directions
- Conclusion



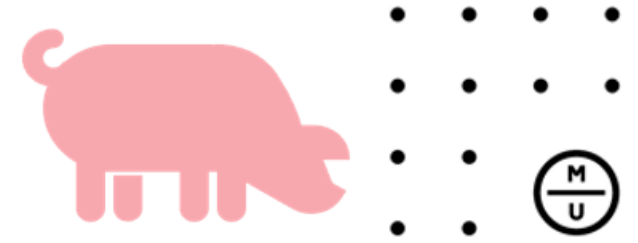
Salmonella enterica



- Gram Negative and zoonotic bacteria
- Salmonellosis – enteritis, diarrhea, septicaemia, death
- Non-typhoidal *Salmonella* - top foodborne pathogen worldwide that causes human disease
- Persistent pathogen (many serovars)



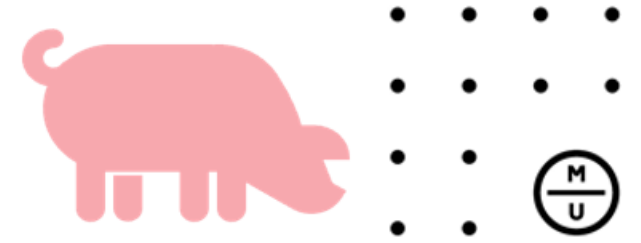
A Cause for Concern : Human and Animal Health



- Zoonosis means both animals and humans are affected
- Negative impact on livestock industry
- Young, old, immunocompromised – more susceptible
- May lead to decrease in production yield / premature death



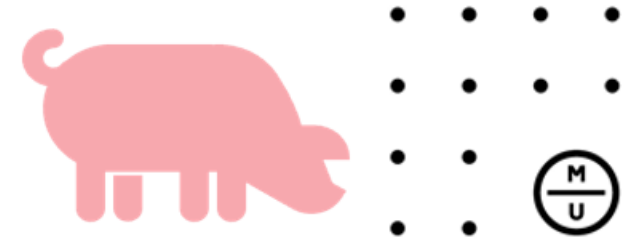
The Disease in Pigs



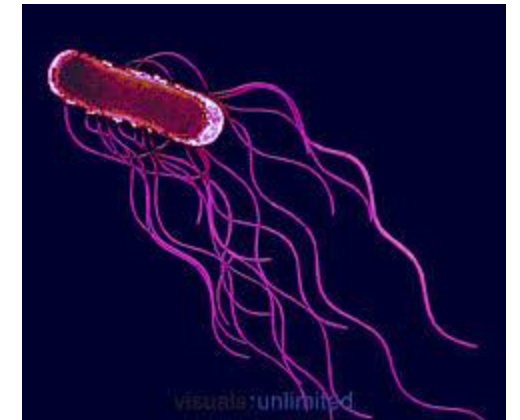
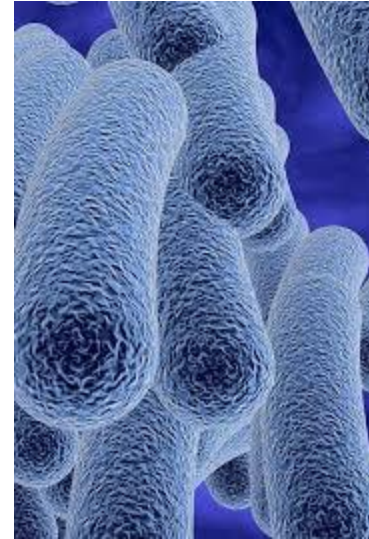
- Often asymptomatic
- If any manifestations: diarrhea, loss of appetite, gastroenteritis, sepsis
- Often prophylactically treated with antimicrobials in food and drinking water
- Gives rise to antimicrobial resistance



Previous Work in Pigs



- Disease often systemic
- Multidrug resistance detected in many countries such as Australia, Thailand and Croatia
- Monophasic Variant of *Salmonella enterica* serovar Typhimurium found in increasing numbers in Australia and around the world

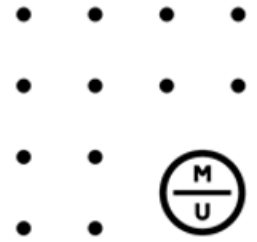
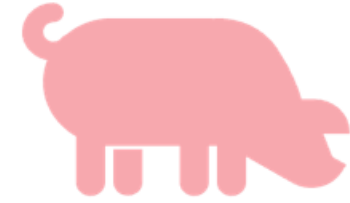


Weaver T. Monophasic Salmonella Typhimurium in Australian pigs. minerva-access.unimelb.edu.au [Internet]. 2017; Available from: <https://minerva-access.unimelb.edu.au/items/9a0b2006-7cb7-53d0-b93b-8ba433846a63>

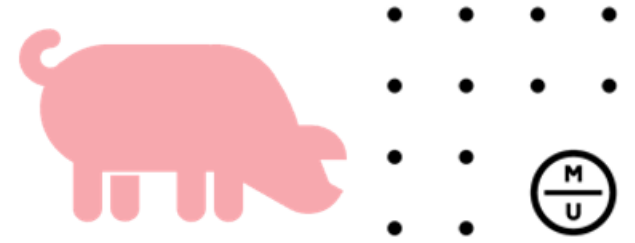
Kidsley AK, Abraham S, Bell JM, O'Dea M, Laird TJ, Jordan D, et al. Antimicrobial Susceptibility of Escherichia coli and Salmonella spp. Isolates From Healthy Pigs in Australia: Results of a Pilot National Survey. Frontiers in Microbiology. 2018 Jul 9;9

The Study

- Investigate *Salmonella* carriage in young pigs in Australian piggeries
- Weaner pigs (4-6 weeks)
- *Salmonella* prevalence, Antimicrobial Resistance profiles and Serovar diversity

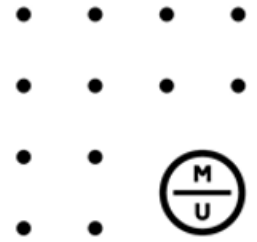
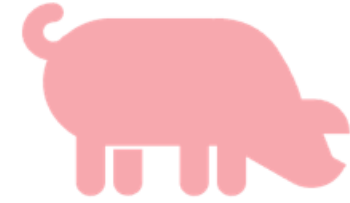


Hypothesis



- We expected to find *Salmonella* at moderate prevalence among weaner pigs in Australian Piggeries
- Expected to detect antimicrobial Resistance (AMR) against some broad-spectrum antimicrobials, but not against Critically Important Antimicrobials (CIAs) in human treatment
- Expected to find a wide range of serovars

Methods and Materials - Overview



Sample collection, isolation and identification of presumptive colonies

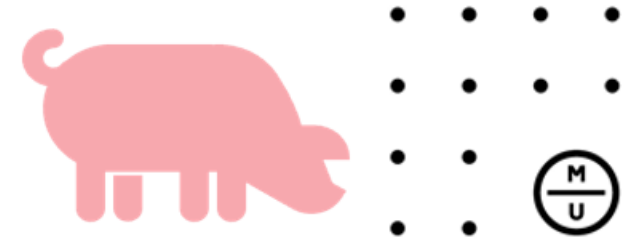
Antimicrobial Susceptibility Testing (AST)

Whole Genome Sequencing (WGS)

Robotic Antimicrobial Susceptibility Platform (RASP)



Methods and Materials



Sample receipt (n=1020, 34 Farms, 30/Farm),
isolation and identification

DNA Extraction
(n=109, at least 5/Farm)

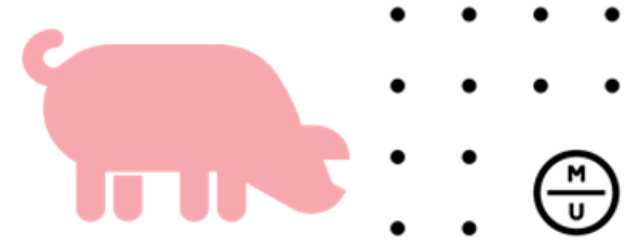
Whole Genome
Sequencing (WGS)

Serovars

AMR Genes

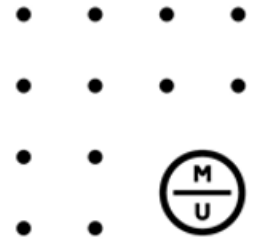
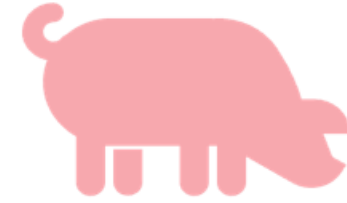
Antimicrobial
Susceptibility Testing
(n=235)

AMR Phenotypes

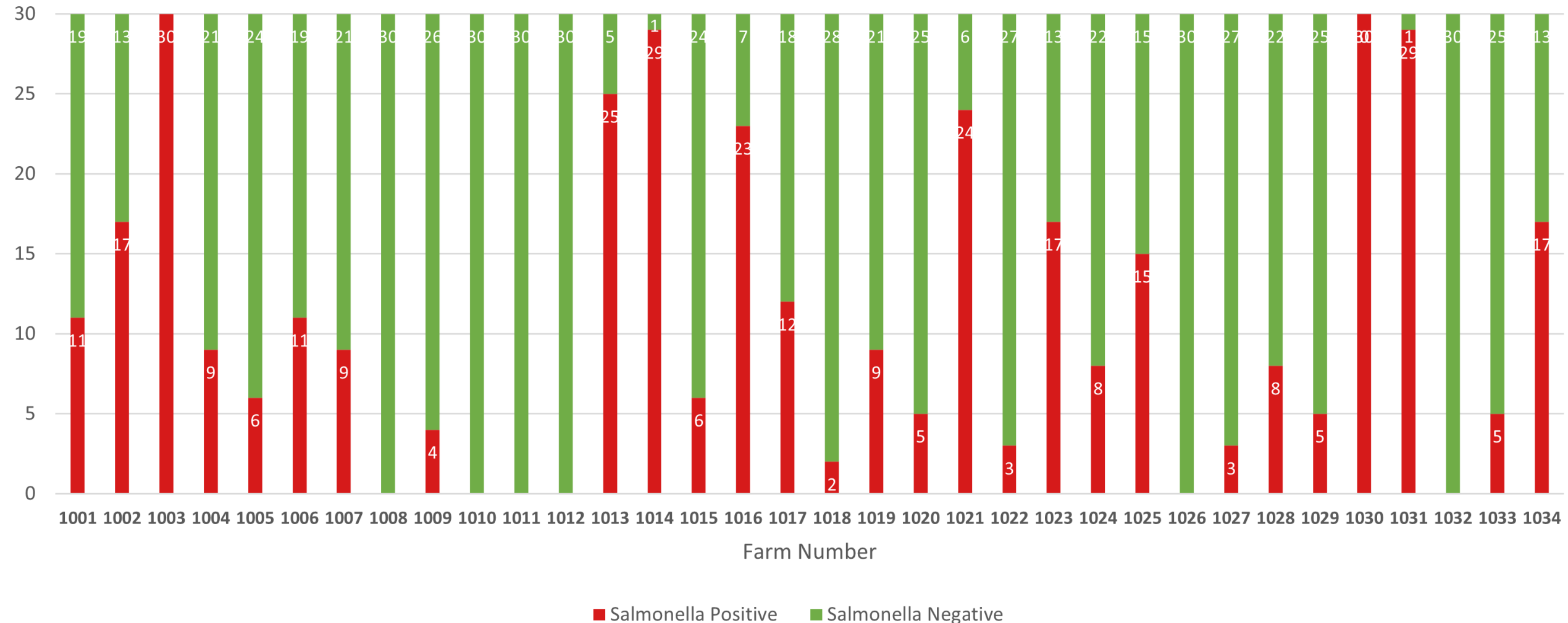


Results

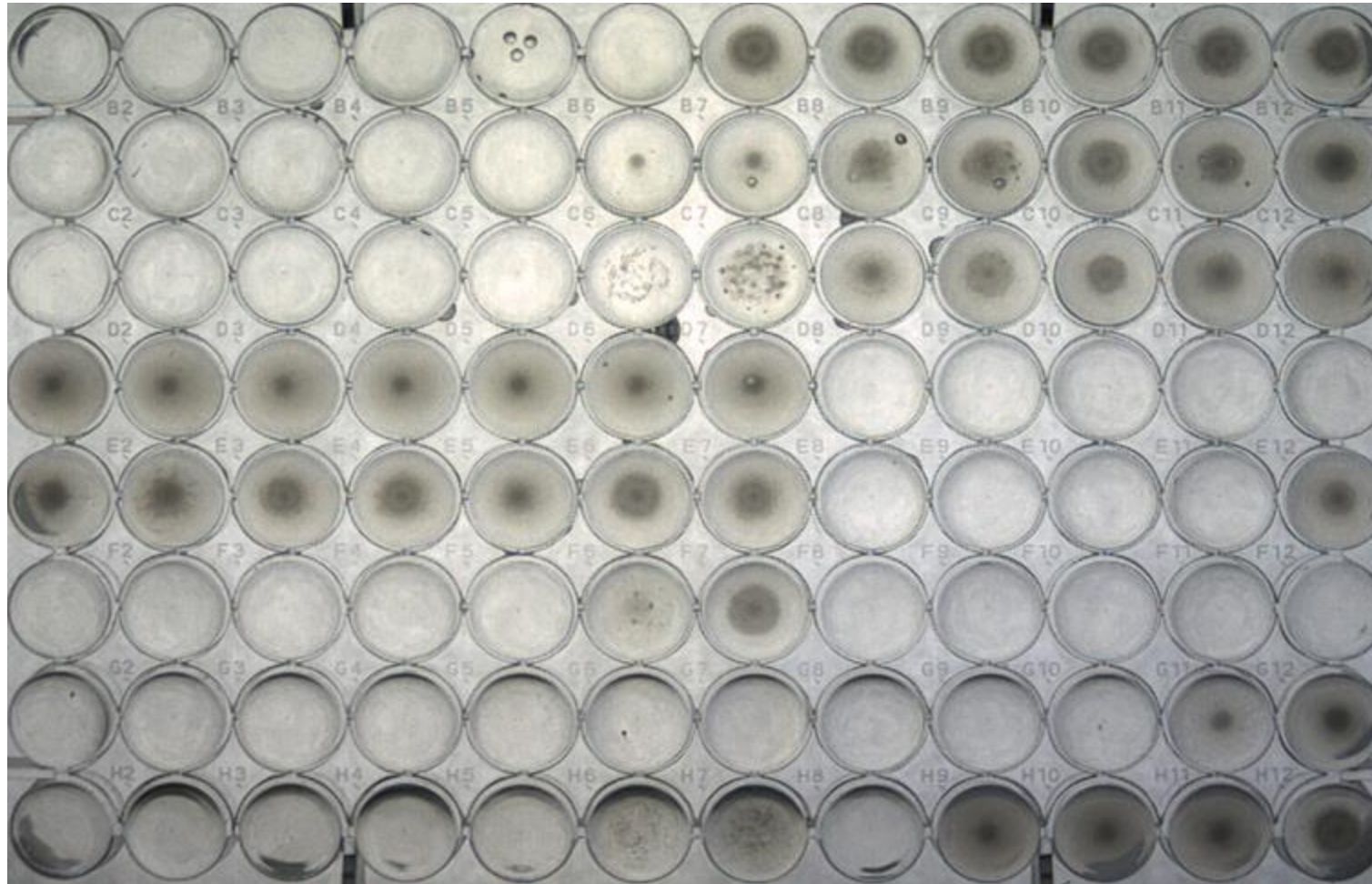
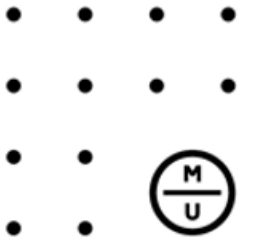
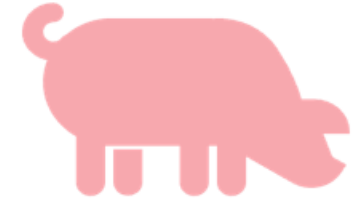
Farm based *Salmonella* carriage



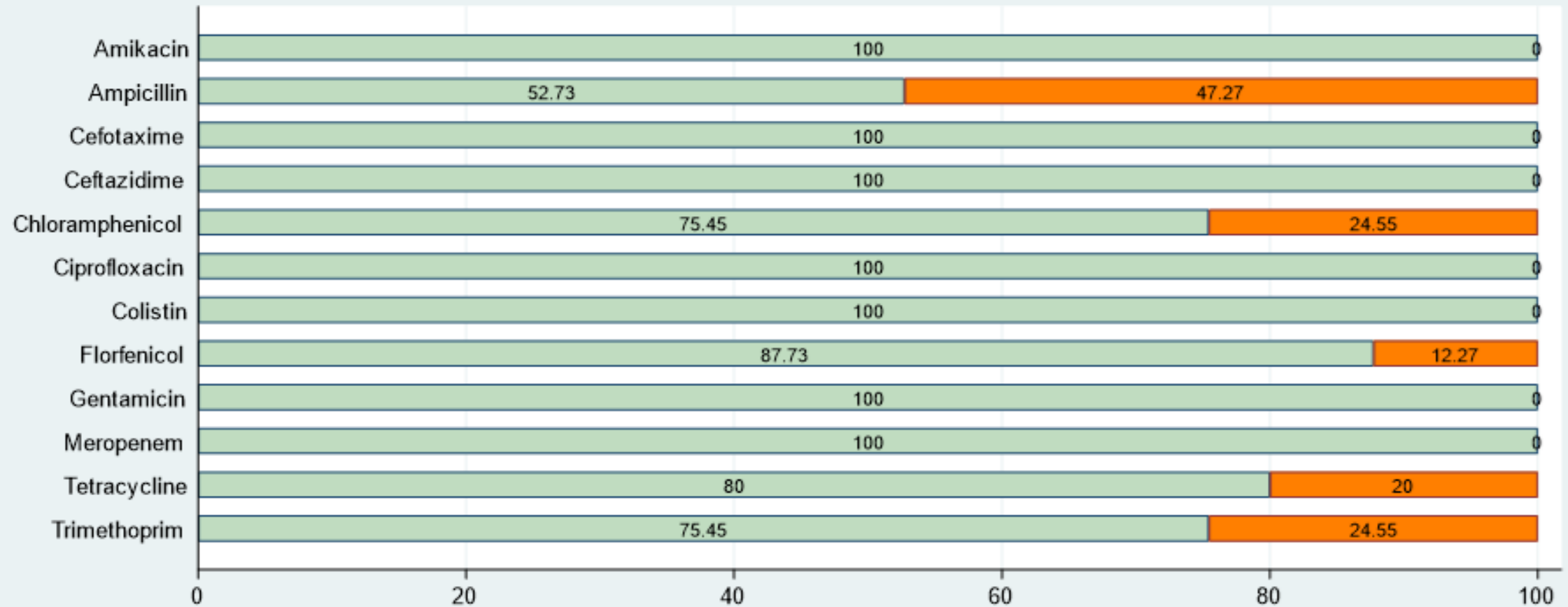
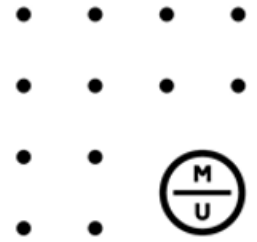
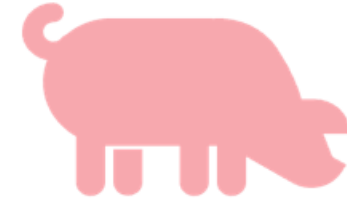
Prevalence of Salmonella in each farm (n=30)



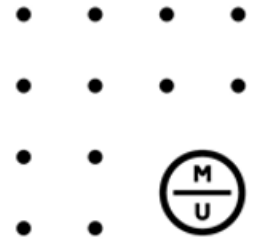
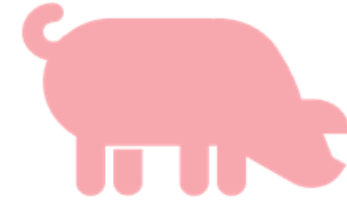
Antimicrobial Susceptibility Testing



AMR – *Salmonella enterica* (n=235)



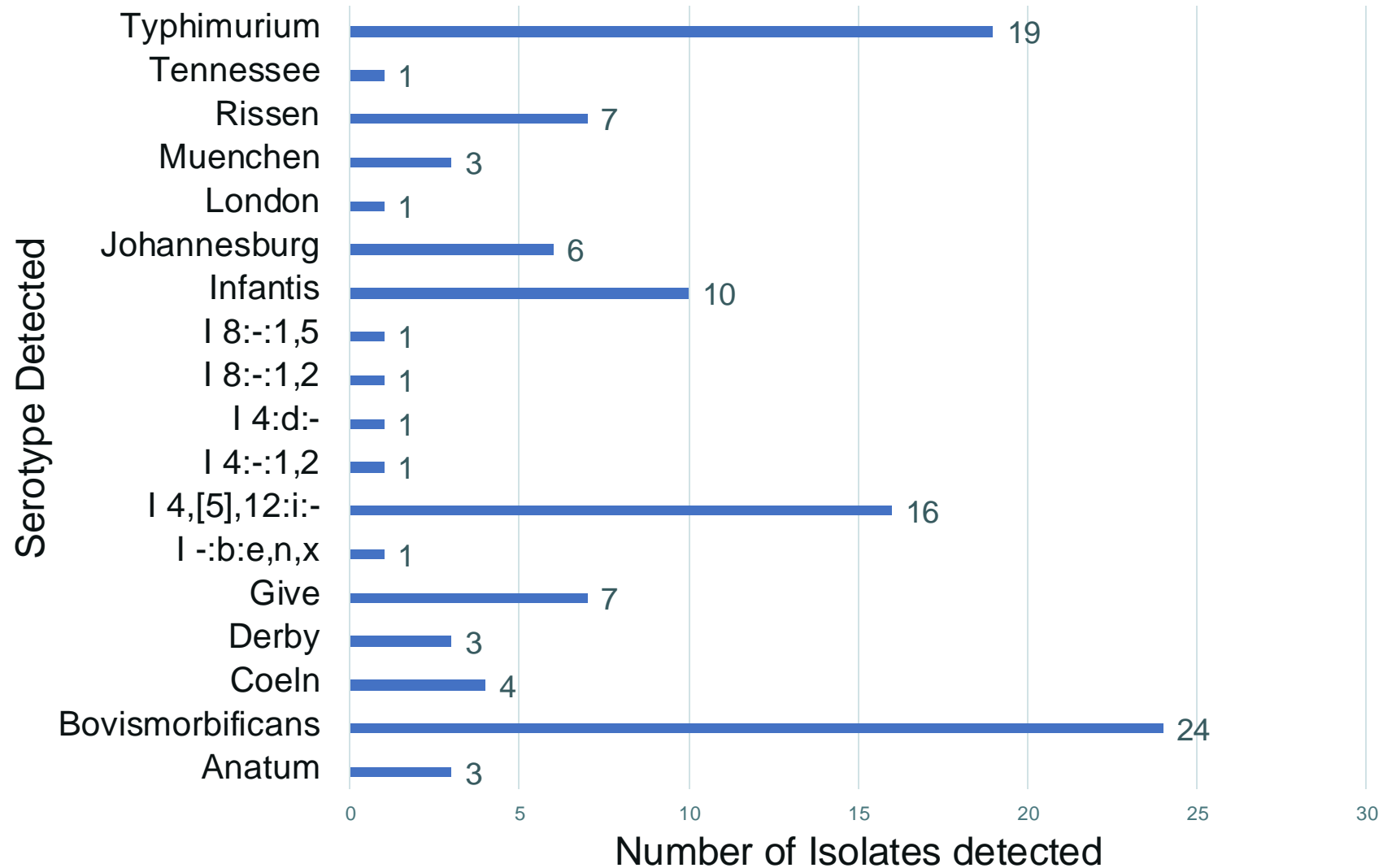
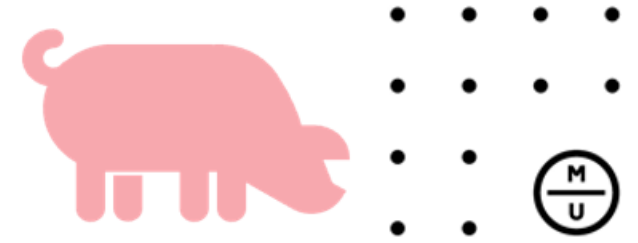
Resistance Phenotypes



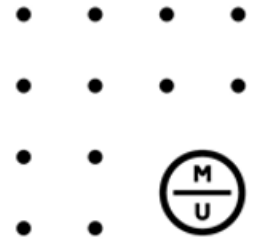
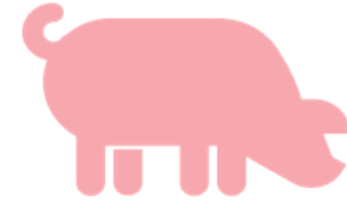
Phenotype	<i>Salmonella</i> n=235	
	n	%
nil	116	52.73
bla	33	15.00
bla_tet	17	7.73
bla_fpi_phe	27	12.27
bla_fpi_phe_tet	27	12.27

AMR profiles based on phenotypic analysis : bla – beta-lactams, tet – tetracyclines, fpi – folate pathway inhibitors, phe – phenicols

Serovar Diversity (n=109)



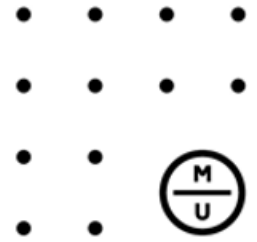
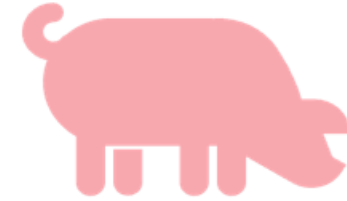
Serovar Distribution Per Farm



- Up to 4 different serovars in a single farm

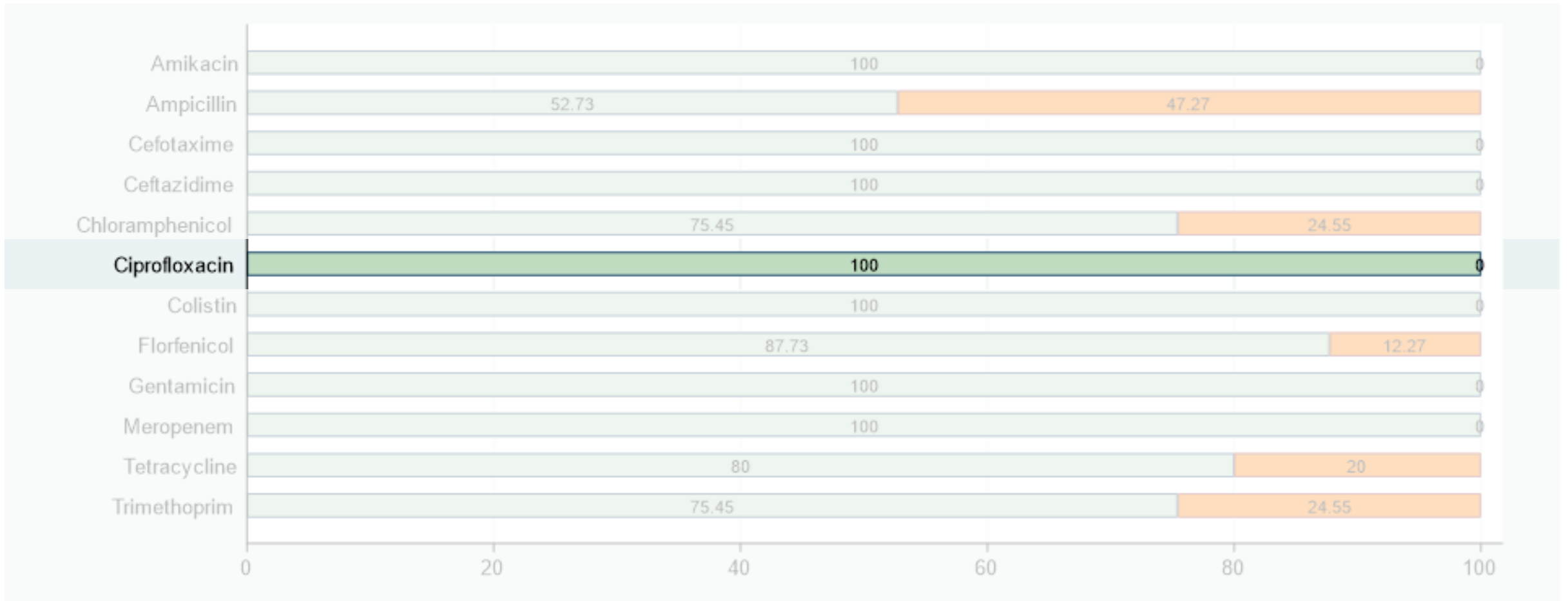
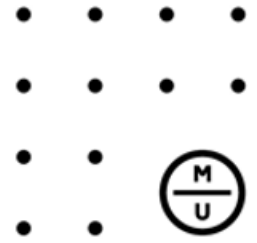
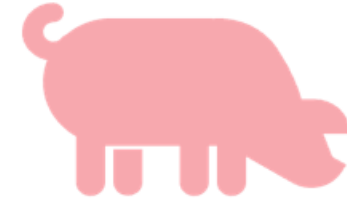
Farm Number	Serotypes detected	Number			
1001	Bovismorbificans	2	1013	Bovismorbificans	10
	I 4,[5],12:i:-	4		I 8:-:1,5	1
1002	Bovismorbificans	3	1014	Bovismorbificans	2
	Give	1		Coeln	2
	Muenchen	1		I 4:-:1,2	1
	Tennessee	1		Typhimurium	4
1003	Bovismorbificans	6	1015	I 8:-:1,2	1
	Give	2		Muenchen	2
1004	I 4,[5],12:i:-	1		Rissen	2
	Rissen	4	1016	Coeln	1
1005	Bovismorbificans	1		Typhimurium	11
	Give	3	1017	I 4,[5],12:i:-	1
	I 4:d:-	1		Rissen	1
1006	Coeln	1	1018	Derby	1
	Infantis	5		Infantis	1
	Typhimurium	3	1019	I 4,[5],12:i:-	3
1007	Give	1		Johannesburg	2
	I 4,[5],12:i:-	1	1020	Give	2
	Infantis	4		I 4,[5],12:i:-	2
1009	I 4,[5],12:i:-	4		London	2
			1021	I -:b:e,n,x	1
				Johannesburg	4
			1022	Anatum	3

Results and Discussion – AMR Genes

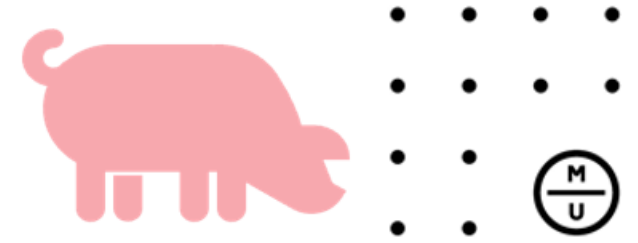


Antimicrobial Class	Salmonella n=109	
	AMR genes	Frequency (%)
AMINOGLYCOSIDE	<i>aadA1</i>	18.35
	<i>aadA2</i>	15.60
	<i>aph(3'')-Ib</i>	11.93
	<i>aph(6)-Id</i>	11.93
BETA-LACTAM	<i>blaTEM-1</i>	31.19
EFFLUX	<i>mdsA</i>	79.82
	<i>mdsB</i>	79.82
FOSFOMYCIN	<i>fosA7.3</i>	1.83
PHENICOL	<i>cmlA1</i>	15.60
	<i>floR</i>	7.34
SULFONAMIDE	<i>sul2</i>	19.27
	<i>sul3</i>	17.43
TETRACYCLINE	<i>tet(A)</i>	8.26
	<i>tet(B)</i>	8.26
	<i>tet(M)</i>	2.75
TRIMETHOPRIM	<i>dfrA12</i>	15.60
QUINOLONE	<i>qnrS1</i>	8.26

AMR - *Salmonella enterica* (n=235)

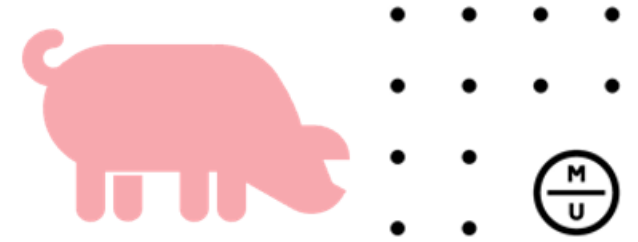


Conclusions

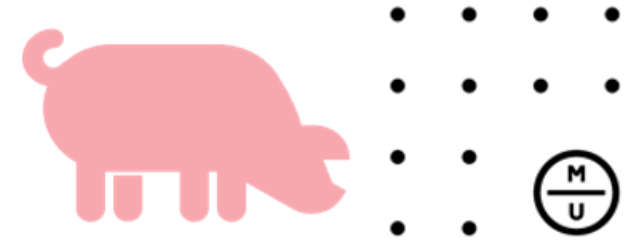


- High levels of *Salmonella* carriage in weaner pigs
- Low levels of antimicrobial resistance (no AMR detected in more than 50% of samples)
- No CIA resistance detected – positive outcome
- Resistance results are consistent with other *Salmonella* work in cattle and horses
- Wide variety of serovars detected

Limitations and Future Direction



- Enrichment steps may have generated bias for certain serovars
- Single colony testing eliminated any potential for multiple strains to be found in any single fecal sample
- Only 5-15 isolates were selected for WGS per farm
- Not all districts across Australia were sampled – potentially not an accurate representation of *Salmonella* in weaners across the country
- Include as many as possible in all stages of testing (Prevalence, AST, WGS)



Thank you