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Introduction

- BRD is a major disease in dairy cattle, causing illness, death, and economic loss.^{1,2,3}
- Triggered by bacteria, viruses, and stress-induced weakened immunity^{1,2,3}.
- Antimicrobials are commonly used to manage BRD, raising concerns about antimicrobial resistance (AMR).¹
- Ongoing surveillance and advanced lab techniques are crucial for tracking AMR trends in BRD-associated bacteria.^{1,2,3}

Objectives

- To identify the phenotypic and genotypic antimicrobial resistance profiles of the isolates
- To evaluate the relationship between observed resistance and the presence of AMR genes

Material & Methods

1. Isolation & Identification

- Nasal swabs collected from healthy dairy calves
- Cultured and incubated to separate pure colonies
- Identified using MALDI-TOF mass spectrometry

2. Phenotypic Antimicrobial Resistance Testing

- 51 isolates tested: 41 *Pasteurella multocida*, 9 *Mannheimia haemolytica*, 1 *Histophilus somni*
- Minimum Inhibitory Concentration (MIC) testing performed with Sensititre Aris HiQ using BOPO7F plates (Thermo Fisher)
- Bacterial suspensions standardized to 0.5 McFarland
- Plates incubated
- Results interpreted using CLSI guidelines

3. Genomic Analysis

- 46 isolates sequenced: 37 *Pasteurella multocida*, 8 *Mannheimia haemolytica*, 1 *Histophilus somni*
- DNA extracted and sequenced on Oxford Nanopore Mk1B
- Genome assembly and AMR gene detection completed using the EPI2ME bioinformatics platform.
- CARD and ResFinder databases used for AMR gene identification

Results

Table 1. Summary of Antimicrobial Resistance (AMR) Genes Detected in *Mannheimia haemolytica* Isolates

Isolate	AMR Gene	AMR Class	Database	Accession No.	% Identity	% Coverage
<i>Mannheimia haemolytica</i> #4	aph(6)-Id	Aminoglycoside	CARD, ResFinder	AB109805	99.9	100
	aph(3')-Ia	Aminoglycoside	CARD, ResFinder	X62115	99.9	100
	aph(3'')-Ib	Aminoglycoside	CARD, ResFinder	AF024602	99.8	100
	sul2	Sulfonamide	CARD, ResFinder	AY034138	100	100
	tet(H)	Tetracycline	CARD, ResFinder	Y15510	100	100
<i>Mannheimia haemolytica</i> #18	blaROB-1	β-lactam	CARD, ResFinder	AF022114	99.9	100
<i>Mannheimia haemolytica</i> #19	msr(E)	Macrolide, Streptogramin	CARD, ResFinder	FR751518	99.9	100
	mph(E)	Macrolide	CARD, ResFinder	DQ839391	99.8	100
	floR	Phenicol	CARD, ResFinder	AF118107	98.1	99.9
	aph(3')-Ia	Aminoglycoside	CARD, ResFinder	X62115	100	100
	aadA14	Aminoglycoside	CARD, ResFinder	AJ884726	81.1	99.9
	tet(H)	Tetracycline	CARD, ResFinder	Y15510	99.75	100

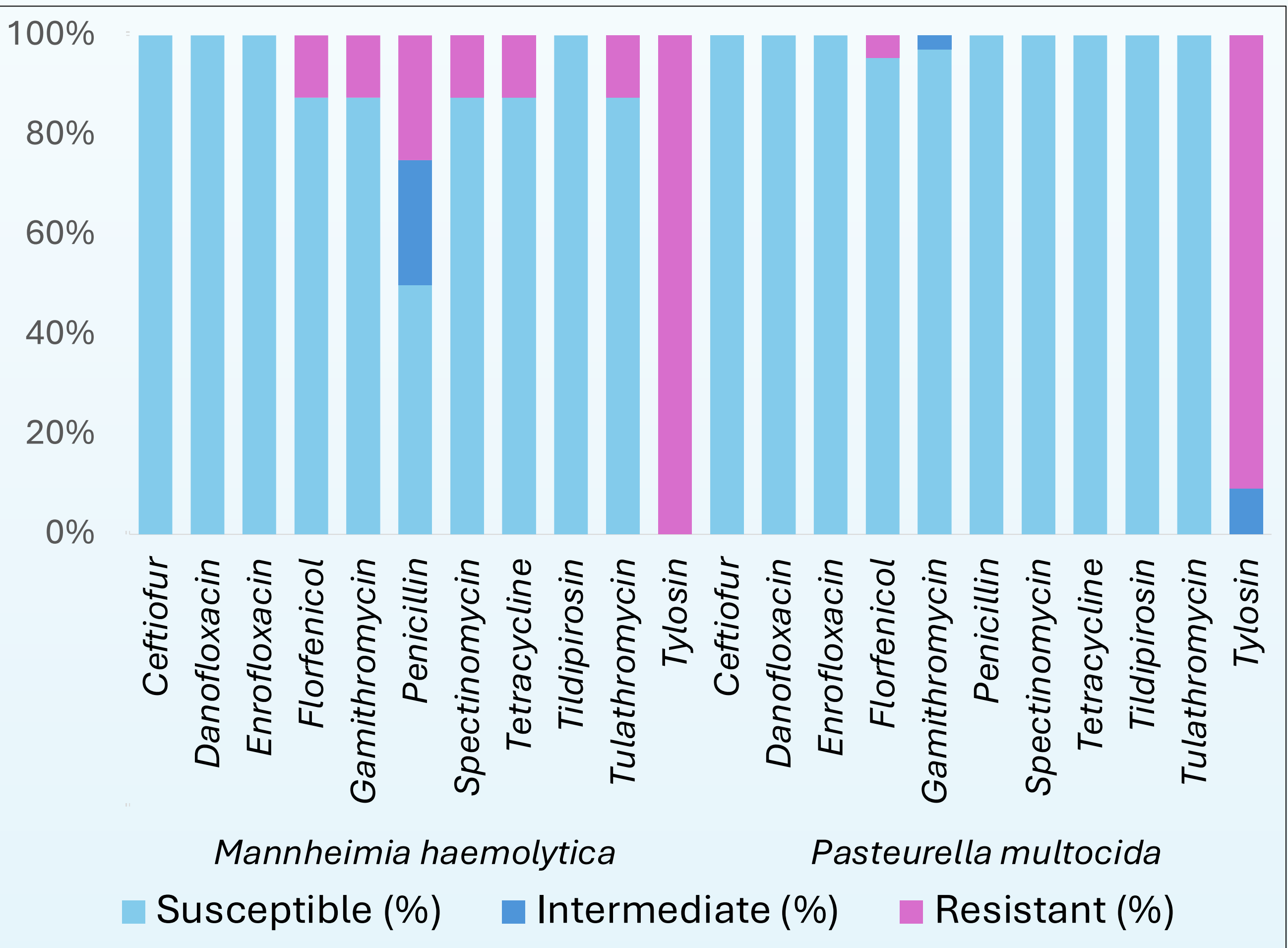


Figure 1. Proportion of the MIC test results (susceptible, intermediate, resistant) in 11 antimicrobials in *Pasteurella multocida* and *Mannheimia haemolytica* isolates

Table 2. Herd-level Distribution of *Histophilus somni*, *Mannheimia haemolytica*, and *Pasteurella multocida* Isolates

Herd	<i>Histophilus somni</i>	<i>Mannheimia haemolytica</i>	<i>Pasteurella multocida</i>	Total
1	0	0	7	7
101	0	0	6	6
201	0	0	3	3
301	0	1	4	5
401	0	0	1	1
501	0	0	1	1
601	0	1	5	6
701	1	4	5	10
801	0	1	6	7
901	0	1	3	4
902	0	1	0	1
Total	1	9	41	51

Conclusion

Table 1 and Figure 1:

- One *Histophilus somni* isolate was susceptible to all antimicrobials tested, and no AMR genes were detected
- Two *Mannheimia haemolytica* isolates carried multiple resistance genes, while one isolate had a single AMR gene, which matched with MIC test results
- Most *Pasteurella multocida* isolates showed phenotypic resistance to tylosin, but no AMR genes were detected, suggesting either a limitation in current databases or the presence of unknown resistance mechanisms

Table 2:

- Differences in herd management, environmental conditions, and sampling variation likely contributed to the diverse rate of bacterial detection across herds

Future Directions

Future directions

- Expand sampling to include healthy and BRD-affected calves for broader resistance data
- Use whole genome analysis to find more AMR genes and strengthen databases
- Assess how farm antimicrobial use may drive resistance in BRD bacteria
- Screen for virulence genes to better classify pathogens and guide control efforts

Acknowledgements and References

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