

Rapid tetracycline resistance detection in bovine Pasteurella multocida isolates by MALDI Biotyper antibiotic susceptibility test rapid assay (MBT-ASTRA)

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Pasteurella multocida





 Infectious bronchopneumonia: leading cause antimicrobial use in calves



 Pressure intensive antimicrobial consumption food producing animals





 Reduction and rational antimicrobial use
TOP PRIORITY!



- Formularies
 - 1st choice products
 - Certain classes: identification and susceptibility test requested







Keuze

antibioticum/chemotherapeuticum

Bovine Respiratory disease (indeling niet voor Mycoplasma spp.)

Eerste keuze(s) Florfenicol

Procaïne benzylpenicilline^A

Trimethoprim + sulfonamiden^A

Tweede keuze(s) Amoxicilline

Procaïne benzylpenicilline + AmoxicIIIne + clavulaanzuur® Procaïne benzylpenicilline Ampicilline*

Doxycycline[®] Gamithromycine®

Lincomycine + spectinomycine[®].

Oxytetracycline[®].

Derde keuze(s) Cefquinomec





dihydrostreptomycine⁸

+ neomycine[®]

Difloxacine® Verduidelijking:

AR 2: Matige prevalentie van resistentie

AR 3: Hoge prevalentie van resistentie

Door de betere orale beschikbaarheid van doxycycline, geniet deze molecule de voorkeur op de andere molecules van de tetracycline-groep bij orale toediening





GHENT UNIVERSITY

=>minimum 2 days!

- MBT-ASTRA: Maldi Biotyper-antibiotic susceptibility test rapid assay
 - => semi-quantitative MALDI-TOF MS to analyse susceptibility/ resistance status of bacteria in few hours







 Design and validate MBT-ASTRA for *Pasteurella multocida* (ubiquitous respiratory pathogen in cattle) and tetracycline (frequently used antimicrobial)

 Determine diagnostic accuracy of MBT-ASTRA and disk diffusion with MIC-gradient strip test





Conditions to determine:

- 1. Growth medium
- 2. Starting concentration of P. multocida
- 3. Concentration of tetracycline
- 4. Incubation time





Determination of standard testing conditions

Growth medium
BHIB and CAMHB



 Starting concentration of *P. multocida* 10⁶-10⁷-10⁸ CFU/mL







Incubation time (hours)

Determination of standard testing conditions

Antibiotic concentration
0-2-4-8 µg/mL



Incubation time
0-3-4-5-6 hours





Determination of standard testing conditions





Determination of standard testing conditions



DIAGNOSTIC ACCURACY MBT-ASTRA

 Database 100 recent clinical strains of *P. multocida* with MIC- values for tetracycline by MIC-gradient strip test





DIAGNOSTIC ACCURACY MBT-ASTRA



DIAGNOSTIC ACCURACY MBT-ASTRA AND

DISK DIFFUSION

Test		Reference test (MIC-gradient strip test)	
		Resistant	Susceptible
MBT-ASTRA	Resistant	95.7% (44/46)	0% (0/54)
	Susceptible	4.3% (2/46)	100% (54/54)
	Total	100% (46/46)	100% (54/54)
Disk diffusion	Resistant	91.3% (42/46)*	7.4% (/54)
	Susceptible	8.7% (4/46)	92.6% (50/54)
	Total	100% (46/46)	100% (54/54)

*Intermediate results were classified as resistant for disk diffusion

DIAGNOSTIC ACCURACY MBT-ASTRA AND

DISK DIFFUSION

	Disk diffusion	MBT-ASTRA
Essential agreement	91%	98%
Very major error	2%	2%
Major error	6%	0%
Minor error	1%	0%
Sensitivity	91.3% (83.2%, 99.4%)	95.7% (89.8%, 101.5%)
Specificity	92.6% (85.6%, 99.6%)	100% (100.0%, 100.0%)
RPV	91.3% (83.2%, 99.4%)	100% (100.0%, 100.0%)

92.6%	96.4%
(85.6%, 99.6%)	(91.6%, 101.3%)

SPV

CONCLUSION

- Fast: Susceptibility testing result after 3 hours of incubation: identification and susceptibility testing possible within the same day!
- Reliable: sensitivity 95,7%, specificity 100%









