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

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INTRODUCTION

*Pasteurella multocida*
INTRODUCTION

• Infectious bronchopneumonia: leading cause antimicrobial use in calves

• 🚿 Pressure intensive antimicrobial consumption food producing animals
INTRODUCTION

• Reduction and rational antimicrobial use
  => TOP PRIORITY!

• Formularies
  • 1\textsuperscript{st} choice products
  • Certain classes: identification and susceptibility test requested
INTRODUCTION

Keuze antibioticum/chemotherapeuticum

**Bovine Respiratory disease** *(Indeling niet voor Mycoplasma spp.)*

**Eerste keuze(s)**
- Florfenicol
- Procaine benzylpenicilline
- Trimethoprim + sulfonamiden

**Tweede keuze(s)**
- Amoxicilline
- Amoxicilline + clavulaanuur
- Ampicilline
- Doxycycline
- Garamthromycine
- Uncomycine + spectinomycine
- Oxytetracycline

**Derde keuze(s)**
- Cefquinome
- Cefiturf
- Danofloxacine
- Difloxacin

**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.
INTRODUCTION

Broncho-alveolar lavage

=>minimum 2 days!

Identification

Disk diffusion
MBT-ASTRA: Maldi Biotyper-antibiotic susceptibility test rapid assay

=> semi-quantitative MALDI-TOF MS to analyse susceptibility/ resistance status of bacteria in few hours

Sample 1: Control
Sample 2

Incubation
Protein extraction + Internal standard

Susceptible: Peptides=\/

Resistant: Peptides \/

GHENT UNIVERSITY
OBJECTIVE

• 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
DESIGN MBT-ASTRA

Conditions to determine:

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

Determination of standard testing conditions

• Growth medium
  BHIB and CAMHB

• Starting concentration of *P. multocida*
  $10^6$-$10^7$-$10^8$ CFU/mL
AUC: area under the curve

CAMHB

BHB
DESIGN MBT-ASTRA

Determination of standard testing conditions

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

• Incubation time
  0-3-4-5-6 hours
DESIGN MBT-ASTRA

Determination of standard testing conditions
DESIGN MBT-ASTRA

Determination of standard testing conditions

$$\text{RG: } \frac{\text{AUC} + \text{Ab}}{\text{AUC} - \text{Ab}}$$

4µg/mL of tetracycline
3 hours of incubation
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

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference test (MIC-gradient strip test)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Resistant</strong></td>
<td><strong>Susceptible</strong></td>
</tr>
<tr>
<td>MBT-Astra</td>
<td><strong>Resistant</strong></td>
<td>95.7% (44/46)</td>
<td>0% (0/54)</td>
</tr>
<tr>
<td></td>
<td><strong>Susceptible</strong></td>
<td>4.3% (2/46)</td>
<td>100% (54/54)</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>100% (46/46)</td>
<td>100% (54/54)</td>
</tr>
<tr>
<td>Disk diffusion</td>
<td><strong>Resistant</strong></td>
<td>91.3% (42/46)*</td>
<td>7.4% (4/54)</td>
</tr>
<tr>
<td></td>
<td><strong>Susceptible</strong></td>
<td>8.7% (4/46)</td>
<td>92.6% (50/54)</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>100% (46/46)</td>
<td>100% (54/54)</td>
</tr>
</tbody>
</table>

*Intermediate results were classified as resistant for disk diffusion
## Table 2: Diagnostic accuracy of disk diffusion and MBT-ASTRA for tetracycline susceptibility testing in 100 clinical *P. multocida* isolates from cattle compared to the MIC-gradient strip test

<table>
<thead>
<tr>
<th></th>
<th>Disk diffusion</th>
<th>MBT-ASTRA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Essential agreement</strong></td>
<td>91%</td>
<td>98%</td>
</tr>
<tr>
<td><strong>Very major error</strong></td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Major error</strong></td>
<td>6%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Minor error</strong></td>
<td>1%</td>
<td>0%</td>
</tr>
</tbody>
</table>

- **Sensitivity (95% confidence interval):**
  - Disk diffusion: 91.3% (83.2%, 99.4%)
  - MBT-ASTRA: 95.7% (89.8%, 101.5%)

- **Specificity (95% confidence interval):**
  - Disk diffusion: 92.6% (85.6%, 99.6%)
  - MBT-ASTRA: 100% (100.0%, 100.0%)

- **RPV (95% confidence interval):**
  - Disk diffusion: 91.3% (83.2%, 99.4%)
  - MBT-ASTRA: 100% (100.0%, 100.0%)

- **SPV (95% confidence interval):**
  - Disk diffusion: 92.6% (85.6%, 99.6%)
  - MBT-ASTRA: 96.4% (91.6%, 101.3%)
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%