Antibiotics resistance and mastitis

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Outline

- Issues about antibiotic resistance and mastitis
- Methods
- Preliminary results
- Summary/Future work

Antimicrobial resistance and mastitis

**Mastitis:** concern to human society because
- Antimicrobial residues
- Antimicrobial resistance
- Animal welfare
- Milk quality
- *Staph. aureus:* the most common cause of mastitis
- Frequency of *S. aureus* resistance to penicillin
  47% Italy (Moreni et al., 2006), 40% Argentina (Gentile et al., 2000), 47% Uruguay (Gianneccini et al., 2002) e 52% Finland (Pikalá et al., 2004)

Antimicrobial resistance and mastitis

- Mastitis
  - Single most common cause of antibiotic use in dairy cows
  - Main cause of antibiotic residue violation
  - Antibiotics in dairy cows
  - Incriminated as a potential cause for resistance in bacteria isolated from treated cows and milk

Key issues of antimicrobial resistance and mastitis

- Does scientific data demonstrate emerging antimicrobial resistance of mastitis pathogens?
- How about Brazilian dairy situation?

*no apparent progression* of antimicrobial resistance in mastitis pathogens after four decades of antimicrobial use in dairy cows” IDF Factsheet, Feb/2012

Key issues of antimicrobial resistance and mastitis

- Is there evidence that drug resistance in mastitis pathogens is an concern for human public health?
- Great concern: *S. aureus* methicillin resistant (MRSA) - 1980
- Very low prevalence of MRSA in dairy cows
- Is bacterial resistance impacting outcomes for mastitis therapy?
Center for Monitoring antimicrobial resistance of mastitis pathogens in Brazil

- 7 Brazilian research institutions
- Research proposal in 2009; approved in 2010
- Funded by National Council of Technological and Scientific Development (CNPq-Brazil)

Main research questions

- Lack of information about antimicrobial resistance in a national basis
- Comparison should not be made between studies from different time periods
- Previous studies used different AR techniques
- Sample collection, culture methods, failure to speciate the microorganisms
- Need for consistency laboratory techniques

2. Objectives

- To establish a collaboration center for monitoring antimicrobial resistance of mastitis pathogen in 6 different Brazilian regions: use of standardized methods
- To evaluate the in vitro antimicrobial sensitivity of bovine mastitis pathogens (disk diffusion test)
- To collect herd information about risk factors for antibiotic resistance and mastitis control
- To quantify the occurrence of methicillin resistant S. aureus (MRSA) - mecA gene

3. Methods

3.1 Experimental design

- Characterization of dairy herds in the Pirassununga-SP region
- Determination of number of herds to be sampled
- Average milk production, number of cows/herd
- Experimental unit: bacteria isolate

<table>
<thead>
<tr>
<th>Cow/herd</th>
<th>Number of herds</th>
<th>Cows sampled</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-19</td>
<td>6</td>
<td>all</td>
<td>72</td>
</tr>
<tr>
<td>20-29</td>
<td>3</td>
<td>all</td>
<td>73</td>
</tr>
<tr>
<td>30-39</td>
<td>2</td>
<td>all or 30 if &gt; 30</td>
<td>60</td>
</tr>
<tr>
<td>40-49</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>50-60</td>
<td>1</td>
<td>30 (random)</td>
<td>30</td>
</tr>
<tr>
<td>&gt;60</td>
<td>1</td>
<td>30 (random)</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>------</td>
<td>265</td>
</tr>
</tbody>
</table>

Selection of 13 dairy herds

- Pirassununga-SP
- Desalvado-SP
- Leme-SP
- Santa Cruz Conceição-SP
- Santa Rita Passa Quatro-SP
- São João da Boa Vista-SP

1179 samples
3. Methods
3.2 Sampling

Four sampling periods during 2 years
Composite samples (no clinical cases)

3. Methods
3.3 Microbiological culture (NMC, 2004)

Gram +
Gram -

Colony appearance
TSA

Acetoin
Coagulase

Staphylococcus spp.

Hippurate
CAMP

Streptococcus spp.
Catalase

3. Methods
3.4 Disk diffusion test - (CLSI, 2008)

Culture in BHI

Vortex

McFarland scale (1 to 4 X10^8 UFC/ml)

Mutiipication com Swab

Inoculation of bacteria

1. Ampicillin 10µg
2. Clindamycin 2µg
3. Penicillin GP 1µg
4. Cefotiofor 30µg
5. Gentamicin 10µg
6. Sulfametropin 25µg
7. Enrofloxacina 6µg
8. Sulfonamide 300µg
9. Tetracyclina 30µg
10. Oxacilina 1µg
11. Cefotaxima 30µg
12. Erythromicina 5µg

Incubation 37°C 18 hrs
Inhibition zone reading

3.5 meca gene identification - PCR

DNA extraction
DNA quantification

Agarose gel electrophoresis
DNA amplification

4. Preliminary results

Frequency of mastitis pathogens in composite milk samples of 13 herds

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Isolates</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>213</td>
<td>28</td>
</tr>
<tr>
<td>Staphylococcus spp. coag neg</td>
<td>238</td>
<td>31</td>
</tr>
<tr>
<td>Staphylococcus spp. coag pos</td>
<td>93</td>
<td>12.2</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>91</td>
<td>12.0</td>
</tr>
<tr>
<td>Streptococcus dysgalactiae</td>
<td>17</td>
<td>2.2</td>
</tr>
<tr>
<td>Streptococcus esculine pos</td>
<td>23</td>
<td>3.0</td>
</tr>
<tr>
<td>Enterococcus sp.</td>
<td>01</td>
<td>0.13</td>
</tr>
<tr>
<td>Corynebacterium sp.²</td>
<td>80</td>
<td>10.5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>00</td>
<td>0.0</td>
</tr>
<tr>
<td>Levedura</td>
<td>02</td>
<td>0.26</td>
</tr>
<tr>
<td>Total</td>
<td>758</td>
<td>100</td>
</tr>
</tbody>
</table>

FMVZ-USP
4. Preliminary results

- Table 3. Number (%) of susceptibility of Staph. aureus isolates from mastitis (disk diffusion test)

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Staphylococcus aureus (n= 213)</th>
<th>CNS (n = 230)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>73 (34%)</td>
<td>92 (38%)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>52 (24%)</td>
<td>21 (13%)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>41 (19%)</td>
<td>77 (33%)</td>
</tr>
<tr>
<td>Cefalotin</td>
<td>69 (4%)</td>
<td>76 (3.7%)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>99 (3.7%)</td>
<td>99 (3.7%)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>37 (17%)</td>
<td>33 (13.8%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>40 (1.9%)</td>
<td>95 (2.5%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>11 (5%)</td>
<td>29 (12%)</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>104 (6%)</td>
<td>52 (23.6%)</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>63 (29%)</td>
<td>70 (33.6%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>48 (22%)</td>
<td>50 (21.7%)</td>
</tr>
</tbody>
</table>

5. Conclusions/future research

- Coagulase-negative Staphylococci, S. aureus, Strep. agalactiae were the most common mastitis pathogens.
- Frequency of methicillin resistant Staph. aureus + CNS is low (only 3 isolates out of 74 - partial)

Future research

- Use quantitative susceptibility test
- Broth dilution susceptibility test (MIC)
- Study genetic diversity of MRSA (pulsed field gel electrophoresis (PFGE) and spa typing)
- Survey the antibiotic use for mastitis therapy in dairy herds