

Figure 3. Individual test variation reported as percent of isolates positive for the specific test at either 4 or 24 hours.

| | VP | HIP | ESC | PYRA | AGAL | BGUR | BGAL | PAL | LAP | ADH* | RIB | ARA | MAN* | SOR | LAC | TRE* | INU | RAF | AMD* | GLYG | HEM |
|-------------|------|-----|------|------|------|------|------|-----|-----|------|------|-----|------|-----|-----|------|-----|-----|------|------|-----|
| 4 hr | 100% | 4% | 100% | 4% | 0% | 4% | 88% | 0% | 96% | 21% | 100% | 0% | 4% | 0% | 75% | 21% | 0% | 0% | 4% | 0% | 0% |
| 24 hr | 100% | 4% | 100% | 4% | 0% | 4% | 88% | 0% | 96% | 88% | 100% | 21% | 42% | 13% | 96% | 92% | 13% | 25% | 88% | 13% | 0% |
| API library | 90% | 40% | 99% | 35% | 3% | 0% | 35% | 30% | 96% | 95% | 95% | 15% | 45% | 1% | 72% | 87% | 4% | 5% | 90% | 3% | 1% |

* Denotes tests where % positive study samples more closely resembles API library for *Lactococcus lactis* ssp. *lactis* at 24 hours versus 4 hours

Figure 4. Characteristics of farms representing *Lactococcus lactis* subspecies *lactis* isolates from Part 1.

| | Farm | | | | |
|------------------------------|------|------|------|------|------|
| | A | B | C | D | E |
| Herd Size(milking cows) | 870 | 1491 | 213 | 2464 | 328 |
| Avg Milk- Milking cows | 78 | 92 | 86 | 87 | 77 |
| Bulk Tank Somatic Cell Count | 306 | 97 | 435 | 253 | 174 |
| New Infection Rate | 11% | 7% | 10% | 10% | 12% |
| Chronic Infection Rate | 23% | 8% | 27% | 14% | 16% |
| % <i>Strep</i> species | 22% | 16% | 9% | 8% | 23% |
| % <i>Lactococcus</i> samples | 42% | 29% | 17% | 8% | 4% |
| Bedding Type | Sand | Sand | Sand | Sand | Sand |

New and Chronic Infection Rates are calculated based on the 12 months leading up to study enrollment.

% *Strep* Species refers to percent of total farm milk cultures identified as non-hemolytic, esculin-positive *Streptococcus* species in the 12 months leading up to study enrollment.

% *Lactococcus* samples refers to percent of *Lactococcus lactis* ssp. *lactis* isolates from part 1 attributable to each farm.

Figure 5. MIC evaluation for *Lactococcus lactis* ssp. *lactis* isolated from milk samples

| Drug | Sensitive | Intermediate | Resistant | % Isolates Sensitive |
|------------------------|------------------|---------------------|------------------|-----------------------------|
| Ampicillin | 23 | 1 | 0 | 96% |
| Penicillin | 5 | 19 | 0 | 21% |
| Erythromycin | 22 | 0 | 2 | 92% |
| Pirlimycin | 21 | 0 | 3 | 88% |
| Penicillin/ Novobiocin | 23 | 1 | 0 | 96% |
| Tetracycline | 15 | 0 | 9 | 63% |
| Cephalothin | 24 | 0 | 0 | 100% |
| Oxacillin* | n/a | n/a | n/a | n/a |
| Ceftiofur* | n/a | n/a | n/a | n/a |
| Sulphadimethoxine* | n/a | n/a | n/a | n/a |

•No cutoffs provided for Oxacillin, Ceftiofur, or Sulphadimethoxine, so not analyzed at this time.

Figure 6. MIC evaluation for *Lactococcus lactis* ssp. *lactis* isolated from sand bedding

| Drug | Sensitive | Intermediate | Resistant | % Isolates Sensitive |
|-----------------------|------------------|---------------------|------------------|-----------------------------|
| Ampicillin | 8 | 0 | 0 | 100% |
| Penicillin | 1 | 7 | 0 | 12.5% |
| Erythromycin | 8 | 0 | 0 | 100% |
| Pirlimycin | 8 | 0 | 0 | 100% |
| Penicillin/Novobiocin | 8 | 0 | 0 | 100% |
| Tetracycline | 3 | 0 | 5 | 37.5% |
| Cephalothin | 8 | 0 | 0 | 100% |
| Oxacillin* | n/a | n/a | n/a | n/a |
| Ceftiofur* | n/a | n/a | n/a | n/a |
| Sulphadimethoxine* | n/a | n/a | n/a | n/a |

•No cutoffs provided for Oxacillin, Ceftiofur, or Sulphadimethoxine, so not analyzed at this time.

•8 total isolates evaluated[Farm A (n=3), Farm B (n=1), Farm C(n=1), Farm D (n=2), Farm E (n=1)]

Figure 7. Percent of *Lactococcus* milk isolates matching MIC of environmental *Lactococcus* isolates by farm.

| | Ampicillin | Penicillin | Erythromycin | Pirlimycin | Penicillin/Novobiocin | Tetracycline | Cephalothin | Oxacillin | Ceftiofur | Sulphadimethoxine |
|--------|------------|------------|--------------|------------|-----------------------|--------------|-------------|-----------|-----------|-------------------|
| Farm A | 70% | 80% | 80% | 100% | 80% | 90% | 30% | 80% | 80% | 20% |
| Farm B | 0% | 0% | 100% | 86% | 86% | 100% | 57% | 86% | 100% | 57% |
| Farm C | 75% | 50% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| Farm D | 75% | 50% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 0% |
| Farm E | 100% | 0% | 100% | 0% | 100% | 100% | 0% | 100% | 100% | 100% |