Proficiency Testing as a tool to point out criticalities in the strategy for control of antibiotic residues in milk: the Italian experience

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Introduction

As starting point, screening methods are a critical point of the entire antibiotic residue control. Various commercial screening methods are available for this.

- **Screening**
  - positive
  - negative

- **Confirmation**
  - positive and >MRL*
  - negative or <MRL

- **Sample processing**

* Maximum Residue Limits

The proficiency test for the screening of antibiotic residues in milk completed in 2013 by the Italian National Reference Laboratory (NRL) for antimicrobial residues in food (ISS) was finalized to:

1. assess the competence of official control laboratories with this regard;
2. get an insight of the routine screening methods applied in Italy;
3. point out criticalities, if any;
4. evaluate the effect of the milk fat content level (1.8% and 3.5%) on the identification of equal concentrations of the same antibiotics in milk samples.

Organization

Thirty-eight laboratories received 2 series (respectively at 1.8 % and 3.5 % of fat content) of 9 randomly encoded lyophilized milk samples, spiked with:

- β-lactam: clindamycin (CLX), benzyl penicillin (PEN)
- β-lactam: oxytetracycline (OXY)
- sulfonamides: sulfadiazine (SDZ)

at concentrations of 1x and 2x MRLs.

A blank sample was also enclosed in each series. Laboratories were invited to perform the analyses following their routine procedures.

A reserved web-area was created so that each participant could fill in line forms for the direct transmission of data in a protected mode (HTTPS). Among them, pre-arranged forms were available with the corresponding samples codes.

<table>
<thead>
<tr>
<th>Molecules</th>
<th>CLX</th>
<th>CLX</th>
<th>PEN</th>
<th>PEN</th>
<th>OXY</th>
<th>OXY</th>
<th>SDZ</th>
<th>SDZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations (µg/kg)</td>
<td>30</td>
<td>60</td>
<td>4</td>
<td>8</td>
<td>100</td>
<td>200</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>false compliant results (n°)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>71</td>
<td>56</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>false compliant results (%)</td>
<td>1,3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>93,4</td>
<td>73,7</td>
<td>1,3</td>
<td>0</td>
</tr>
</tbody>
</table>

1. Results

2. Routine screening methods applied

- Delvo test SP-NT
- Copan test
- Snap Beta-Lactam ST

3. Criticalities

- **Interpretation of results**
  - Intermediate color changes recorded as "°"
  - (even when the presence of traces was signaled)

- **Choice of the methods**
  - Only 2 methods applied (Delvo test SP-NT and Copan test)
  - (similar spectrum of sensitivities)

- **General uniformity of results.**
  - Corrective action undertaken: finalized to the improvement of the reading and registration of results.

- **Only one lab used two methods in parallel: Snap Beta-Lactam ST although the contemporary use of Delvo SP-NT**

4. Effect of milk fat content

No significant differences were observed for results from samples at 2 fat content levels.

Conclusions

The NRL provided guidance to Italian participating laboratories in order to ensure the detection of a wider spectrum of antibiotic families implementing different screening methods complementing each other. Following the issuing of the report, the laboratories involved undertook corrective actions. The effectiveness of these actions will be evaluated with a new PT round currently planned.