ANTIBIOTICS

Vs.

BACTERIA
Antibiotic monitoring results and the effect of reduced antibiotic use on resistance data

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- The Netherlands Food and Consumer Product Safety Authority (NVWA)
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The task and activities of the NVWA:
To protect human and animal health by monitoring food and consumer products.

The Authority controls the whole production chain, from raw materials and processing aids to end products and consumption.

From 1 June 2019, Laboratory for Feed and Food Safety of the Dutch Food and Consumer Product Safety Authority (NVWA) and RIKILT Wageningen University & Research will form a new institute. The new organization is called Wageningen Food Safety Research (WFSR) and is part of Wageningen University & Research.
Microbial screening for antibiotics within the framework of the Dutch national residue monitoring plan

**Nouws Antibiotic Test** (NAT)

- **NAT-screening**: the test comprises five test plates enabling group specific identification (tetracyclines, beta-lactam, macrolides, quinolones, sulfonamides and aminoglycosides). The NAT-screening uses paper disks impregnated with renal pelvis fluid from kidney.

- **NAT meat/kidney post-screening**: method based on analysis of kidney and/or meat fluid. Post screening is only performed on the type of plate showing a positive NAT screening result.

- **Chemical conformation of suspected samples from the NAT meat and/or kidney post-screening.**
NAT-screening

- Five paper disks impregnated with renal pelvis fluid from kidney are applied into punch holes in each of the five test plates and supplemented with a synergistic buffer. After overnight incubation, inhibition zones are registered.
# NAT-screening

<table>
<thead>
<tr>
<th>Antibiotic group</th>
<th>Bacteria</th>
<th>Agar</th>
<th>Incubation temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrolides&amp;β-lactam</td>
<td><em>K. rhizophila</em></td>
<td>Iso-sensitest pH 8</td>
<td>37°C</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td><em>B. cereus</em></td>
<td>Iso-sensitest pH 6 + CAP</td>
<td>30°C</td>
</tr>
<tr>
<td>Quinolones</td>
<td><em>Y. ruckeri</em></td>
<td>PCA pH 6.5</td>
<td>30°C</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td><em>B. subtilis</em></td>
<td>PCA pH 8.0</td>
<td>37°C</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td><em>B. pumilus</em></td>
<td>DST pH 7 + TMP</td>
<td>37°C</td>
</tr>
</tbody>
</table>
NAT meat/kidney post-screening

- Post screening is only performed on the type of plate showing a positive NAT screening result. Fluid samples are prepared by homogenizing the sample using a household chopper. 20 g of homogenized material is heated (10 min 80°C) and centrifuged. For the analysis of aminoglycosides in meat additional sample preparation with Bromelain (protease) is required. The supernatant is applied into the punch hole of the plate and supplemented with a synergistic buffer. After overnight incubation the inhibition zone is registered.
Correlation between the inhibition zone (kidney fluid) NAT kidney post screening B.cereus plate and OTC residue concentration in meat
Flow scheme of the Nouws Antibiotic Test

Dutch National Residue Monitoring Plan (6000 samples / year)

NAT-screening
renal pelvis fluid paperdisk

No inhibition: Compliant

Inhibition zone: suspect

NAT-meat and/or NAT-kidney

No inhibition: Compliant

Inhibition zone: suspect

Chemical confirmation

< MRL: Compliant

> MRL: Not Compliant
Screening of antibiotic residues in chicken feather

**Introduction**

The use of antibiotics in poultry is controlled by monitoring programs, focused on the analysis of residues mainly in meats. The ACCEPT network (European network on antibiotic residues in chicken) is currently collecting data on residues in chicken, with a special emphasis on the monitoring of the antibiotic group. This study aims to evaluate a rapid, simple, and cost-effective method for screening of antibiotics on chicken feathers that can be used for monitoring correct registration of antibiotic use.

**Materials and methods**

The method consists of cutting 1 g of feathers, extracting with chloroform, spotting the extract onto paper, and incubating the paper with TCA (trichloroacetic acid). The results are compared to a standard curve.

**Results Validation**

The results are shown in the table below:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Residue 1</th>
<th>Residue 2</th>
<th>Residue 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>0.05 mg/L</td>
<td>0.06 mg/L</td>
<td>0.04 mg/L</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.03 mg/L</td>
<td>0.04 mg/L</td>
<td>0.02 mg/L</td>
</tr>
</tbody>
</table>

**Conclusion**

The developed method is simple, rapid, and sensitive for the detection of antibiotic residues in chicken feathers.
Antimicrobial veterinary medicinal product sales 1999-2016 in kg (thousands)
Rules on antibiotics in livestock farming

The livestock industry must comply with strict rules on antibiotic use:
- Antibiotics may only be prescribed by a veterinarian.
- Vets must inspect and assess a farm before prescribing antibiotics to sick animals. Farmers may only administer antibiotics themselves under strict conditions.
- Livestock farmers must register all the antibiotics they use, to show how much each animal receives. The Netherlands Veterinary Medicines Authority (SDA), which sets rules for responsible antibiotic use, collects this information.
- ‘Last-resort’ antibiotics for humans may only be administered to sick livestock under strict conditions.
- Farmers may not deliver animals for slaughter that contain antibiotic residues.

The Food and Consumer Product Safety Authority (NVWA) monitors the registration and use of antibiotics by farmers.
Results Antibiotic screening Dutch National Residue Monitoring Plan

NAT-screening suspected /MRL not compliant

- 2009: 0.2% > MRL, 0.1% NAT screening verdacht
- 2010: 0.2% > MRL, 0.15% NAT screening verdacht
- 2011: 4.5% > MRL, 4.5% NAT screening verdacht
- 2012: 4.0% > MRL, 4.0% NAT screening verdacht
- 2013: 3.5% > MRL, 3.5% NAT screening verdacht
- 2014: 3.0% > MRL, 3.0% NAT screening verdacht
- 2015: 2.5% > MRL, 2.5% NAT screening verdacht
- 2016: 2.0% > MRL, 2.0% NAT screening verdacht
- 2017: 1.5% > MRL, 1.5% NAT screening verdacht
- 2018: 1.0% > MRL, 1.0% NAT screening verdacht

Chart showing trends from 2009 to 2018.
Results Antibiotic screening Dutch National Residue Monitoring Plan

Suspected samples Antibiotics

[Bar chart showing the percentage of suspected samples for different antibiotics (Beta-lactam/Macroliden, Tetracyclines, Quinolones, Aminoglycosides, Sulfonamides) from 2009 to 2018.]
Antimicrobial susceptibility testing 2018

**Strains:**
- *Salmonella*
- *E. coli*
- Enterococci
- *Campylobacter*
- ESBL

**Panels Sensititre:**
- EUVSEC
- EUVSEC
- EUVENC
- EUCAMP2
- EUVSEC2

**Samples 2018 (n):**
- 320
- 300
- 170
- 200
- 300

**Equipment:**

![Equipment Images]
Antimicrobial susceptibility testing NVWA

The results are annually reported to the **EFSA** and published in the **MARAN** (Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands)

**MARAN** published by based on:

- Total sales data and animal specific usage of antimicrobial agents in animal husbandry.
- Development of antimicrobial resistance in bacteria of animal origin.
- Relevance to public health.

In collaboration with:

- [NVZA](#)
- [NVMM](#)
- [SDa](#)
- [SWAB](#)
- [Wageningen University & Research](#)
- [Rijksinstituut voor Volksgezondheid en Milieu](#)
- [Nederlandse Voedsel- en Warenautoriteit](#)
“The decrease in the use of antibiotics in animals goes hand in hand with the decrease in resistance in animals and is most noticeable in poultry” (Results MARAN 2018)

Resistance (%) to 0-9 antimicrobial classes among *E. coli* strains from broilers in the Netherlands from 1998-2016
Thank you for your attention