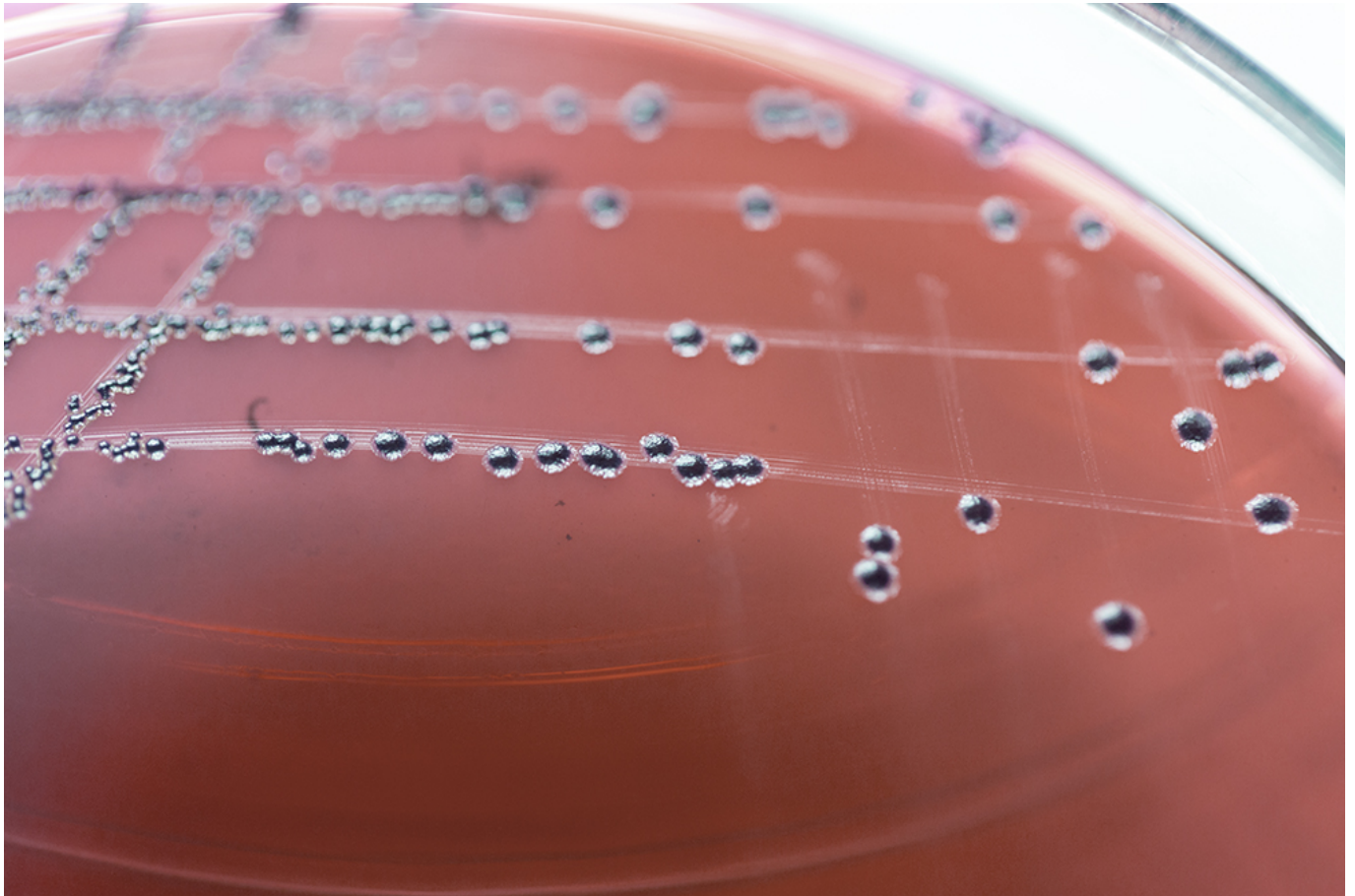
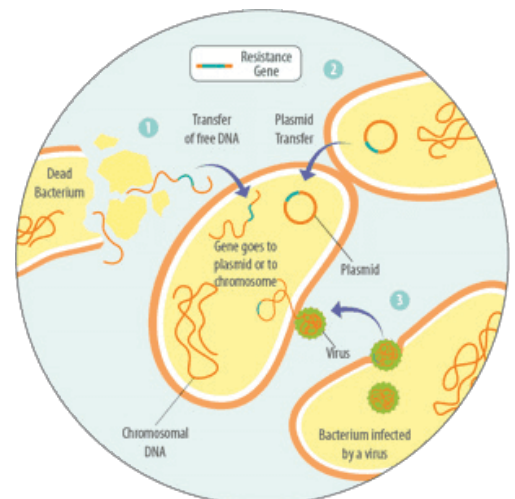


# Secondary Plant Compounds (SPC's) to reduce the use of antibiotics?



Initial in vitro trials give reason for hope

Antibiotic Resistance



Some bacteria, due to mutations, are less sensitive to certain antibiotics than others. This means that if

certain antibiotics are used, the insensitive ones survive. Because their competitors have been eliminated, they are able to reproduce better. This resistance can be transferred to daughter cells by means of „resistance genes“. Other possibilities are the intake of free DNA and therefore these resistance genes from dead bacteria 1, through a transfer of these resistance genes by viruses 2 or from other bacteria by means of horizontal gene transfer 3 (see figure 1). Every application of antibiotics causes a selection of resistant bacteria. A short-term use or an application at a low dosage will give the bacteria a better chance to adapt, promoting the generation of resistance (Levy, 1998).

### Antibiotics are promoting the development of resistance:

- Pathogenic bacteria possessing resistance genes are conserved and competitors that do not possess these genes are killed
- Useful bacteria possessing the resistance genes are conserved and serve as a gene pool of antibiotic resistance for others
- Useful bacteria without resistance, which probably could keep the pathogens under control, are killed

### Reducing the use of antibiotics

Ingredients from herbs and spices have been used for centuries in human medicine and are now also used in modern animal husbandry. Many SPC's have antimicrobial characteristics, e.g. Carvacrol and Cinnamon aldehyde. They effectively act against *Salmonella*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, Enter- and *Staphylococcus*, and *Candida albicans*. Some compounds influence digestion, others act as antioxidants. Comprehensive knowledge about the single ingredients, their possible negative but also positive interaction (synergies) is essential for developing solutions. Granulated or microencapsulated products are suitable for addition to feed, liquid products would be more appropriate for an immediate application in the waterline in acute situations.

| Central Poultry Diagnostic Laboratory, Kondapur, Hyderabad (India) | (Activo* Liquid) |    |    |
|--|------------------|----|----|
|  | 10%              | 2% | 1% |
| <i>E. coli</i> (reference strains)                                 | ++               | +  | +  |
| <i>Proteus vulgaris</i> (reference strains)                        | +                | +  | +  |
| <i>Pseudomonas fluorescens</i>                                     | ++               | +  | -  |
| <i>Salmonella pulmorum</i>   | ++               | ++ | +  |
| <i>Salmonella gallinarum</i>                                       | ++               | ++ | +  |
| <i>Staphylococcus aureus</i> (reference strains)                   | +++              | ++ | ++ |

|     |                                     |
|-----|-------------------------------------|
| +++ | 22 – 29 mm ZOI (zone of inhibition) |
| ++  | 15 – 21 mm ZOI                      |
| +   | 10 – 14 mm ZOI                      |
| -   | < 10 mm ZOI                         |

### SPC's (Activo Liquid) against livestock pathogens in vitro

In "agar diffusion tests", the sensitivity of different strains of farm-specific pathogens was evaluated with different concentrations of Activo Liquid. The effectiveness was determined by the extent to which they prevented the development of bacterial overgrowth. The larger the bacteria-free zone, the higher the antimicrobial effect.

In this trial, Activo Liquid showed an antimicrobial effect on all bacteria tested. The degree of growth inhibition positively correlated with its concentration.

Table 1: Inhibition of field isolated standard pathogens by different concentrations of Activo Liquid

## Activo Liquid against antibiotic resistant field pathogens in vitro

It cannot be excluded that resistant pathogens not only acquired effective weapons to render antibiotics harmless to them but also developed general mechanisms to rid themselves of otherwise harmful substances. In a follow-up laboratory trial, we evaluated whether the Activo Liquid composition is as effective against ESBL producing *E. coli* and Methicillin resistant *S. aureus* (MRSA) as to non-resistant members of the same species.

| Laboratory: Vaxxinova,<br>Muenster, Germany | SPC's (Activo® Liquid) |      |      |    |
|---|------------------------|------|------|----|
|   | 0.1%                   | 0.2% | 0.4% | 1% |
| <i>E.coli</i> reference<br>ATCC25922        | +                      | ++   | ++   | ++ |
| ESBL 1 (Pig)                                | -                      | ++   | ++   | ++ |
| ESBL 2 (Pig)                                | +                      | ++   | ++   | ++ |
| ESBL 3 (Poultry)                            | +                      | ++   | ++   | ++ |
| ESBL 4 (Poultry)                            | -                      | ++   | ++   | ++ |
| <i>S. aureus</i> reference<br>ATCC29213     | -                      | +    | +    | ++ |
| MRSA 1 (Pig)                                | -                      | +    | +    | ++ |
| MRSA 2 (Pig)                                | -                      | +    | +    | ++ |

- no effect  
+ growth inhibiting  
++ bactericide

Trial Design: Farm isolates of four ESBL producing *E. coli* and two MRSA strains were compared to nonresistant reference strains of the same species with respect to their sensitivity against Activo Liquid. In a Minimal Inhibitory Concentration Assay (MIC) under approved experimental conditions (Vaxxinova Diagnostic, Muenster, Germany) the antimicrobial efficacy of Activo Liquid in different concentrations was evaluated.

The efficacy of SPC's (Activo Liquid) against the tested strains could be demonstrated in a concentration-dependent manner with antimicrobial impact at higher concentrations and bacteriostatic efficacy in dilutions up to 0,1% (ESBL) and 0,2% (MRSA)(table 2).

### Conclusion:

To contain the emergence and spread of newly formed resistance mechanisms it is of vital importance to reduce the use of antibiotics. SPC's are a possibility to [decrease antibiotic use](#) especially in pro- and metaphylaxis, as [they show good efficacy against the common pathogens](#) found in poultry, even against resistant ones.



# Necrotic enteritis in poultry



Enteric diseases cause significant economic losses due to decreased weight gain, higher mortality, higher feed conversion, higher veterinary costs and medicine and a higher risk of contamination by poultry products in food production. The losses due to necrotic enteritis mainly occurring in broilers and fattening turkeys in intensive floor or free-range management are put at 2 billion US\$ per year.

After the ban of antibiotic growth promoters, the relevance of this formerly well controllable disease reappeared and increased.

## **Necrotic enteritis is a disease of the gut**

It is caused by specific gram-positive, anaerobic bacteria – *Clostridium perfringens*, mostly Type A. Clostridia are found in litter, faeces, soil, dust and in healthy animals' guts. These spore forming bacteria are extremely resistant against environmental influences and can survive in soil, feed, and litter for several years and even reproduce.

*Clostridium perfringens* is a component of the normal gut flora. It occurs in a mixture of diverse strains in a concentration of up to  $10^5$  CFU / g intestinal content. In animals suffering from necrotic enteritis particularly one strain of *Clostridium perfringens* is found in a much more higher concentration of  $10^6$ - $10^8$  CFU / g.

Necrotic enteritis affects chickens and turkeys at the age of 2-16 weeks, proliferating at the age of 3-6 weeks. There is an acute clinical, and a subclinical form.

Birds suffering from the clinical form clearly show symptoms like a poor general state of health and diarrhoea. Mortality rates up to 50 % can occur. Subclinical necrotic enteritis cannot be diagnosed easily, as there are no clear symptoms. This form, however, stays within the flock and causes losses due to decreased growth.

## **Factors promoting an infection with necrotic enteritis should be avoided!**

In general, factors have to be cited that create an intestinal environment favourable for the facultative anaerobic *Clostridium perfringens* or weaken the immune status of the host:

1. **Feed:**

Here **NSP's** have to be mentioned. Undigested NSP's serve as substrate and some of them cause higher production of mucus also serving as substrate and providing ideal anaerobic conditions. Undigested **proteins** due to high contents in the diet also serve as substrates. **Animal protein** and **fat** are worse than vegetable variants and a homogeneous size of particles in the diet is better than an **inhomogeneous mixture**.

2. **Stress**

Stresses such as feed change or high stocking density favour NE

3. **Diseases**

Immunosuppressive diseases such as infective chicken anaemia, Gumboro or Marek's decrease resistance against intestinal infections and facilitate their colonisation. Some pathogens exert pressure on the gut and prepare the way for clostridia. Here Cryptosporidia and salmonella have to be mentioned.

### **New approaches**

[Secondary plant compounds](#) show good results against the two microorganisms just mentioned. In a trial conducted with free range broilers in France, a combination of a vaccination against coccidia and a mixture of secondary plant compounds (Activo liquid) resulted in a reduced occurrence of necrotic enteritis in the trial group compared to the control. Additionally due to an improved feed conversion, the margin per animal in the trial group was 5 Cent higher than in the control (1,44 € vs 1,39 €).

In an *in vitro* test, [Activo liquid](#) also showed bactericidal efficacy against field isolated *Salmonella pulmorum* and *Salmonella gallinarum* at a 2 % concentration.

The trials show that combined with a good feeding and stress management, secondary plant compounds, could be a [good tool to eliminate predisposing factors for necrotic enteritis](#) and could therefore help control this economically important disease.

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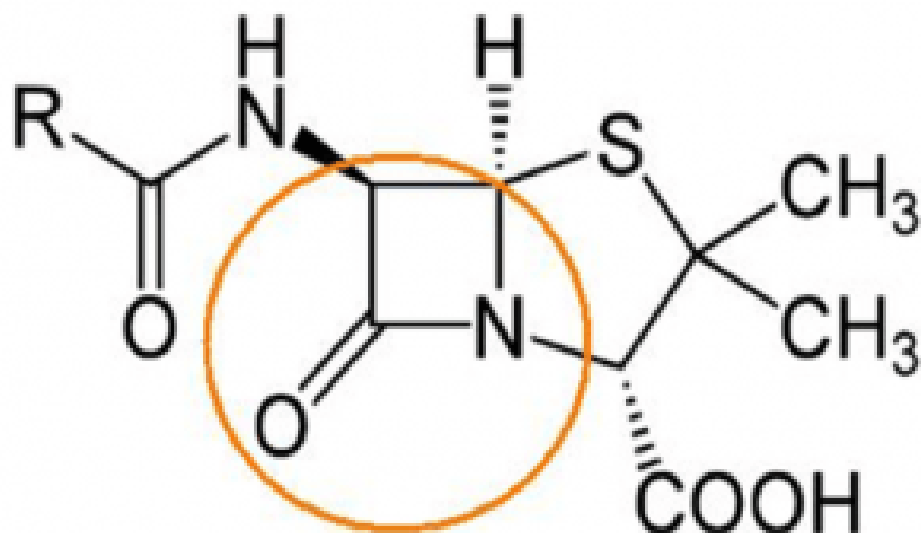
# **Secondary plant compounds against antibiotic-resistant E. coli**



Due to incorrect therapeutic or preventive use of antibiotics in animal production as well as in human medicine, occurrence of antibiotic resistant pathogens has become a widespread problem. Enterobacteria in particular (e.g. *Salmonella*, *Klebsiella*, *E. coli*) possess a special mechanism of resistance. By producing special enzymes ( $\beta$ -lactamases), they are able to withstand the attack of so-called  $\beta$ -lactam antibiotics. The genes for this ability (resistance genes) can also be transferred to other bacteria resulting in a continuously increasing problem. Divers point mutations within the  $\beta$ -lactamase genes lead to the occurrence of „Extended-Spectrum-Beta-Lactamases“ (ESBL), which are able to hydrolyse most of the  $\beta$ -Lactam-antibiotics. AmpC Beta-Lactamases (AmpC) are enzymes, which express a resistance against penicillins, cephalosporins of the second and third generation as well as cephamycins.

#### **What are $\beta$ -lactam antibiotics?**

The group of  $\beta$ -lactam antibiotics consists of penicillins, cephalosporins, monobactams and carbapenems. A characteristic of these antibiotics is the lactam ring (marked in orange):

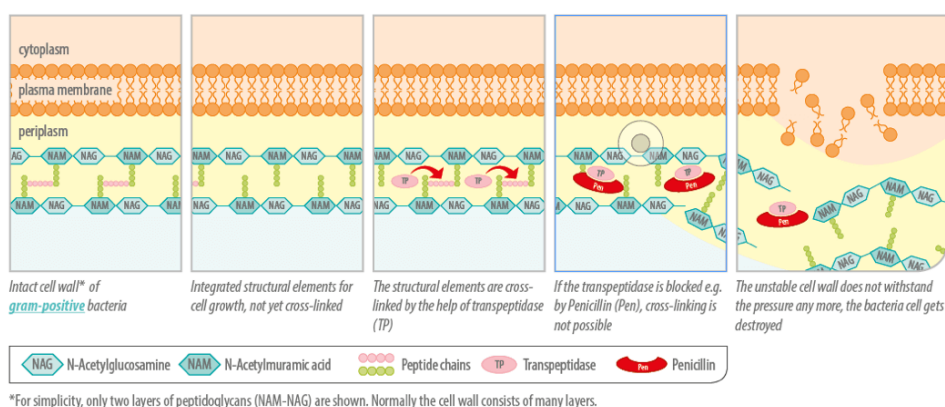


### Mode of action of $\beta$ -lactam antibiotic

If a bacterial cell is growing, the cell wall also has to grow. For this purpose, existing conjunctions are cracked and new components are inserted.  $\beta$ -lactam-antibiotics disturb the process of cell wall construction by blocking an enzyme needed, the transpeptidase. If crosslinks necessary for the stability of the cell wall cannot be created, the bacteria cannot survive. Resistant bacteria, which are able to produce  $\beta$ -lactamases, destroy the  $\beta$ -lactam antibiotics and prevent their own destruction.

### Secondary plant compounds

[Secondary plant compounds and their components](#) are able to prevent or slow down the growth of moulds, yeasts, viruses and bacteria. They attack at various sites, particularly the membrane and the cytoplasm. Sometimes they change the whole morphology of the cell. In the case of gram-negative bacteria, secondary plant compounds (hydrophobic) have to be mixed with an emulsifier so that they can pass the cell wall which is open only for small hydrophilic solutes. [The modes of action of secondary plant compounds](#) depend on their chemical composition. It also depends on whether single substances or blends (with possible positive or negative synergies) are used. It has been observed that extracts of spices have a lower antimicrobial efficacy than the entire spice.



The best explained mode of action is the one of thymol and carvacrol, the major components of the oils of thyme and oregano. They are able to incorporate into the bacterial membrane and to disrupt its integrity. This increases the permeability of the cell membrane for ions and other small molecules such as ATP leading to the decrease of the electrochemical gradient above the cell membrane and to the loss of energy equivalents of the cell.

### Trial (Scotland)

Design



Two strains of ESBL-producing and AmpC respectively, isolated from the field, a non-resistant strain of *E. coli* as control. Suspensions of the strains with  $1 \times 10^4$  KBE/ml were incubated for 6-7 h at 37°C together with different concentrations of [Activo Liquid](#) or with cefotaxime, a cephalosporin. The suspensions were then put on LB-Agar plates and bacteria colonies were counted after a further 18-22h incubation at 37°C. Evaluation of the effects of Activo Liquid on ESBL-producing as well as on *E. coli* resistant for aminopenicillin and cephalosporin (AmpC)

### *Results*

The antimicrobial efficacy of the blend of secondary plant compounds depended on concentration with bactericidal effect at higher concentrations and bacteriostatic at dilutions up to 0,1%. It is also possible that bacteria could develop a resistance to secondary plant compounds; the probability is however relatively low, due to the fact that essential oils contain hundreds of chemical components (more than antibiotics) making it difficult for bacteria to adapt.