Recent challenges in veterinary pharmaco therapy

Medicinal plants as an alternative to antibiotics?

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- Reduction of antibiotics
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Introduction

- Maria Groot
- Veterinarian, researcher
- Wageningen Food Safety Research, part of Wageningen University & Research
- Works on herbal medicine since 1999
- Chair of the study group “Animals and herbs” from the Netherlands Phytotherapy Association
- Works together with Foundation Natural Livestock Farming

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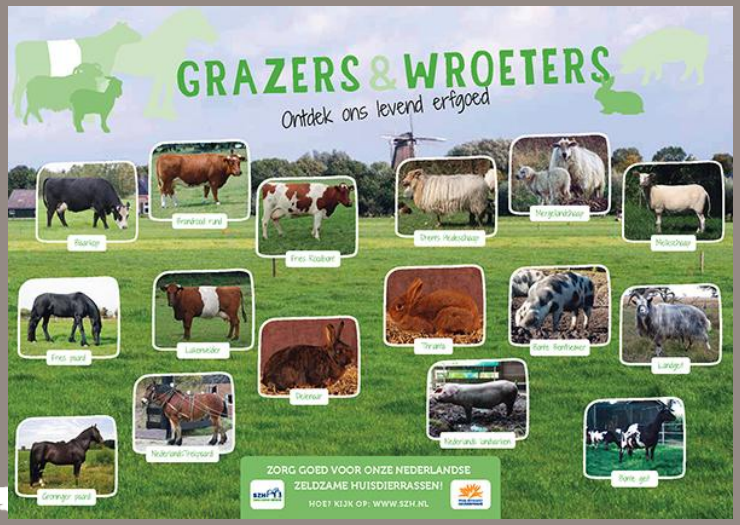
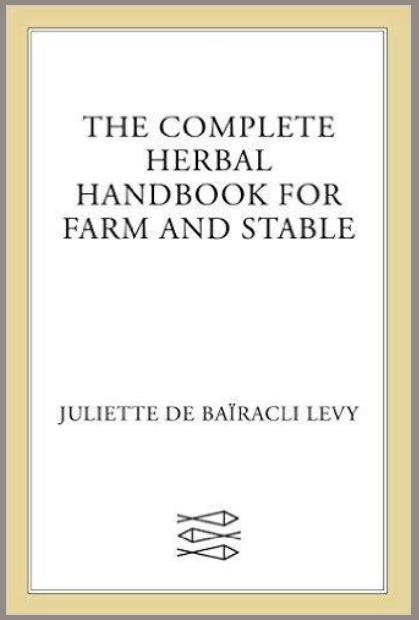
Nederlandse Vereniging voor Fytotherapie

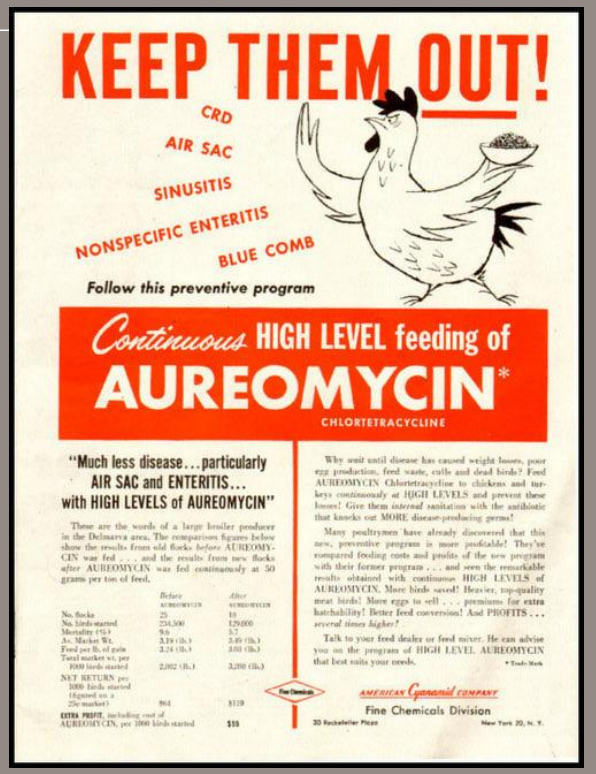
Past

- Pasture with biodiversity, > 20 different grasses and herbs
- Animal biodiversity, breeds per region
- Less production (grass and milk/meat)
- Herbal medicine only available remedy





Developments



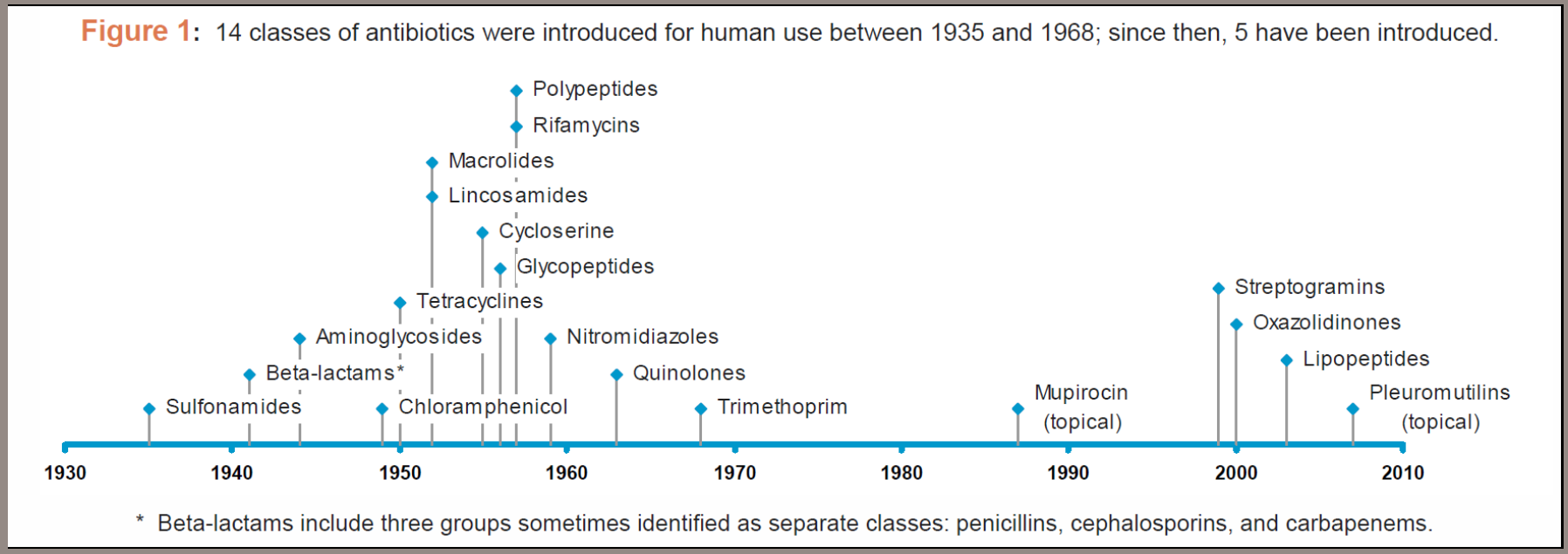
- Intensification
- More animals/farm
- Specialisation
- Less breeds/farm
- Genetic improvement for production
- Mechanisation
- Use of fertilizers, antibiotics for growth promotion, prevention of disease

Present

- High production breeds/ crosses
- Production pasture
- Feed for production
- Intensive farming
- **Antibiotic resistance**

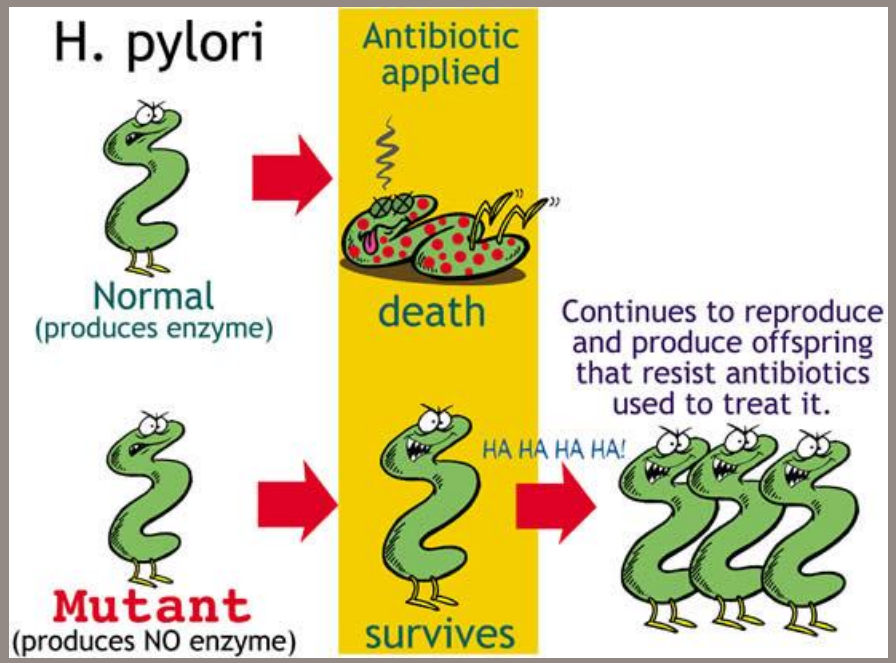
Development of antibiotics

- Most between 1940-1970
- Last years no new abs



Antibiotic resistance

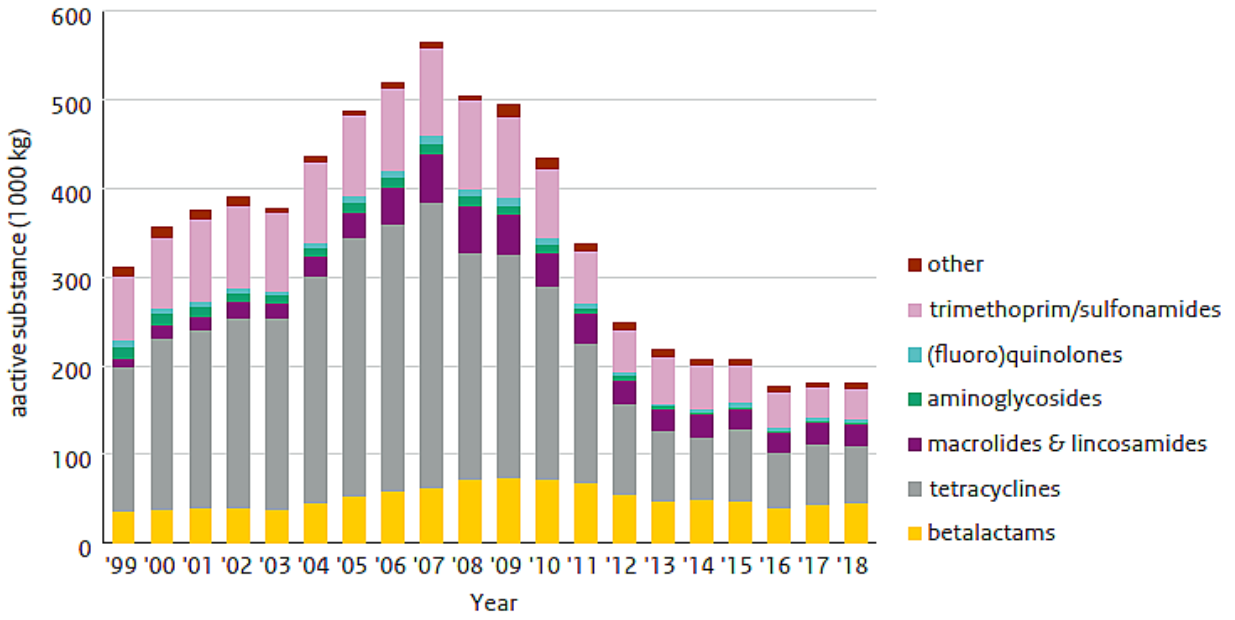
- spontaneous or induced genetic mutation in bacteria
- genes that confer resistance transferred between bacteria by conjugation, transduction or transformation.
- Exposure to antibiotics selects for the antibiotic resistant trait



Reduction of antibiotic use in the Netherlands

- Aim: 50 % reduction from 2009-2013
- 70 % reduction in 2015

Figure ABuse01 Antimicrobial veterinary medicinal product sales 1999-2018 in kg (thousands).



Reduction of veterinary antibiotic use in the Netherlands

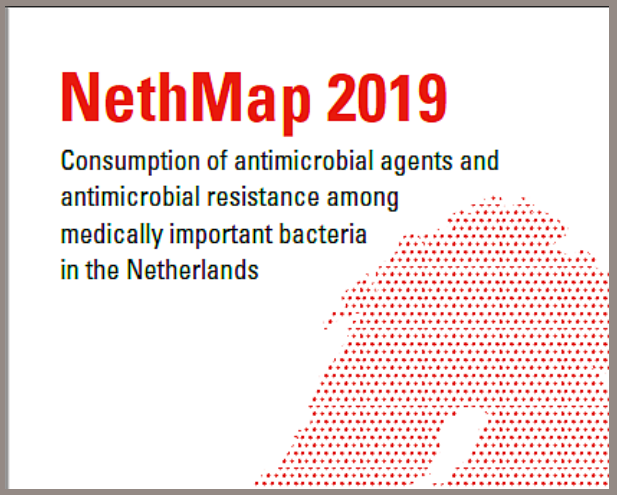
- ~ 63% reduction (2009-2018)
- Fluoroquinolones and 3rd/4th-gen cephalosporines usage reduced to a minimum
- Registration of all antibiotics used by veterinarians
- Netherlands Veterinary Medicines Authority (SDa) controls
- Defined Daily Dose animal levels for each animal production group
- When higher usage intervention occurs

Formularia for antibiotics in animals

- Guidelines to improve efficacy and tot reduce antibiotic resistance
- First, second and third choice of antibiotics
- Third choice only after susceptibility testing
- Goal is to reduce the induction of resistance
- And optimal efficacy of the antibiotics used

Monitoring antibiotics in animals

- Maran reports
- Use of antibiotics in different animal species and humans
- Occurrence of resistance



Some results on resistance

Table S04 Resistance (%) of S. Typhimurium (N tested) isolated from humans, cattle, pigs and other sources in 2018.

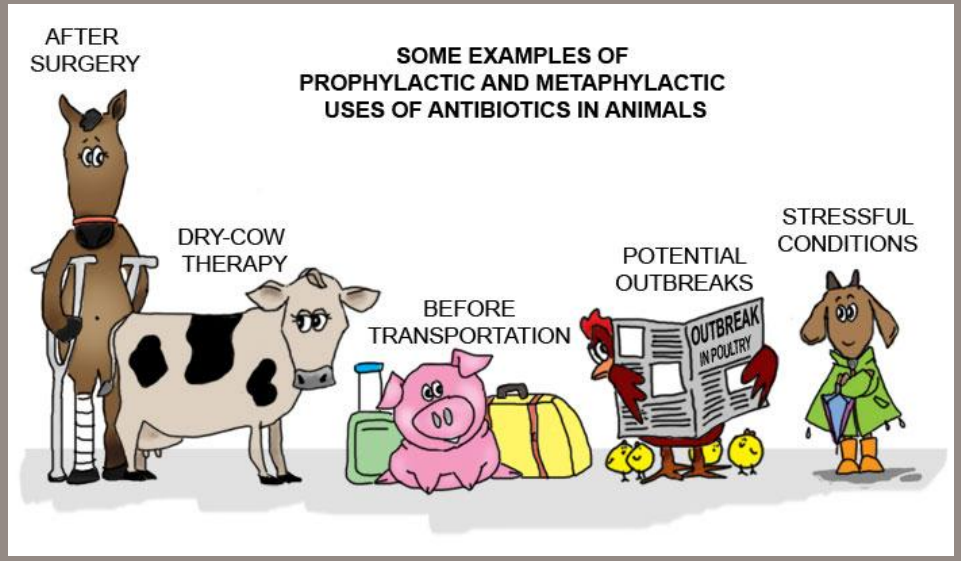
	S. Typhimurium (302)[a]			
	Humans (230)	Cattle (24)	Pigs (28)	Other sources (20)[b]
Ampicillin	47.0	54.2	64.3	35.0
Cefotaxime	1.7	0.0	0.0	0.0
Ceftazidime	0.9	0.0	0.0	0.0
Gentamicin	2.2	20.8	0.0	0.0
Tetracycline	32.6	58.3	50.0	35.0
Sulfamethoxazole	31.7	58.3	50.0	25.0
Trimethoprim	13.5	8.3	35.7	15.0
Ciprofloxacin	14.8	8.3	3.6	10.0
Nalidixic acid	10.4	8.3	3.6	10.0
Chloramphenicol	17.8	25.0	21.4	10.0
Azithromycin	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0
Tigecycline	1.3	0.0	0.0	0.0

a Monophasic variants (1,4,[5],12:i:-) are excluded.

b Other sources include broilers, layers, goats, horses, food and feed products.

Antibiotic resistance

- Need for reduction of the use of antibiotics in production animals
- Keeping animals healthy
- Prevention in stead of curing



Guides for farmers in the Netherlands

- Books for farmers, veterinarians and feed consultants
- Information on diseases, animal management, natural products, research and documentation
- Books for poultry, veal calves, pigs, dairy cattle, turkeys, rabbits, sheep and dairy goats
- Books in English, for organic farming (www.fyto-v.nl, pigs, poultry and dairy cows)

Course for veterinarians

- On behalf of Foundation Natural Livestock Farming with support from the province of Overijssel
- 4 days, two days background, 2 days dairy cows
- In 2018 a pilot with 14 vets
- New course from September

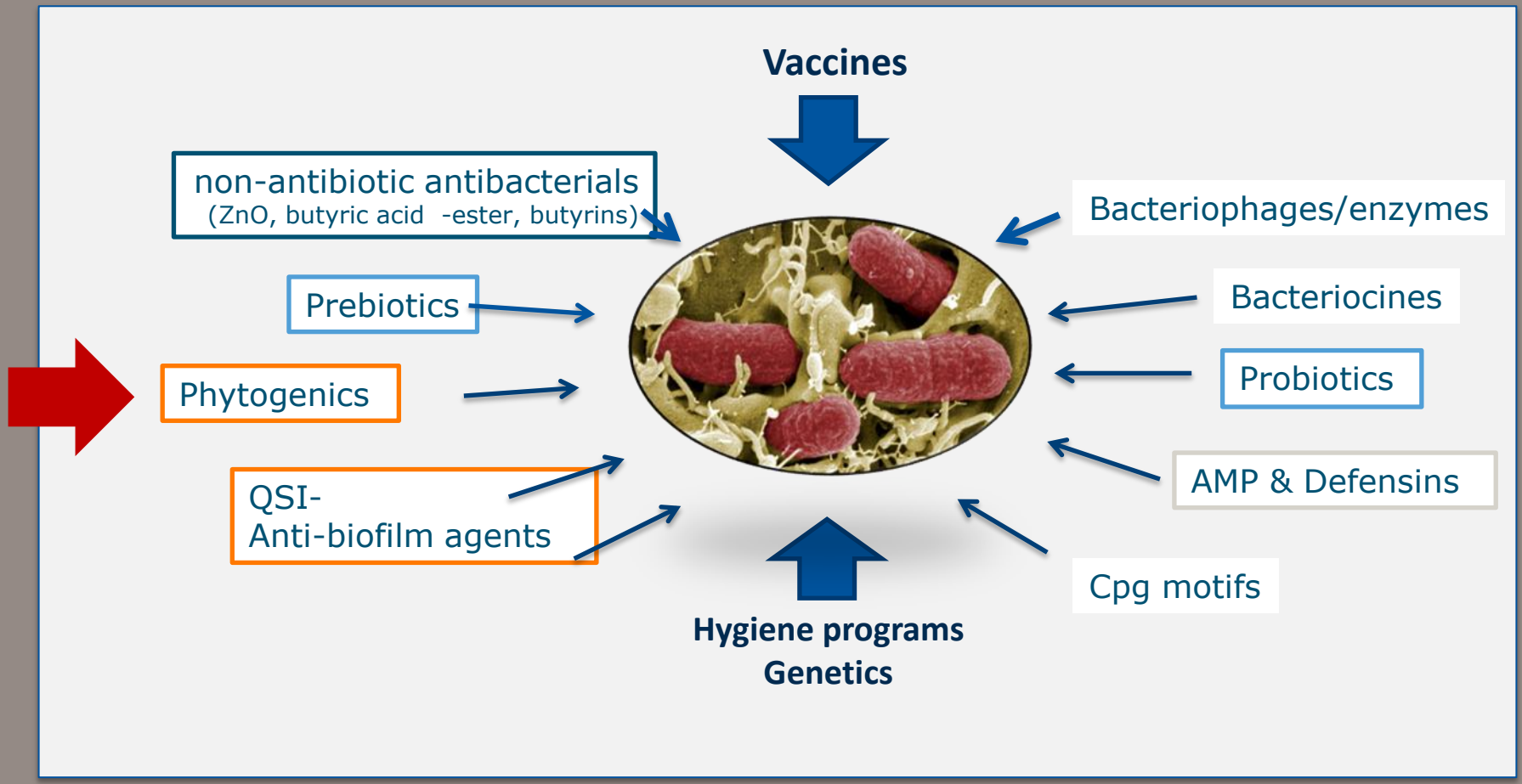
Herbs for animals

- As animal feed, feed ingredient, as feed additive or complementary feed
- As veterinary medicine
- As herbal medicine (phyto-therapeuticals, homeopathic medicine)
- Zoöpharmacognosy: self medication of animals with herbs, soils or insects to treat or prevent disease

Indications for use (production animals)

- Improving appetite and taste
- Stabilizing gut flora
- Improving resistance to stress
- Anti-oxidant activity
- Anti-microbial activity
- Stimulating digestion (enzymes, glandular secretions)
- Modulating immune system
- Animal welfare

EU strategy:
Reduce the need for antibiotics in animals



Animal husbandry: feed additives with positive effects on health and welfare

Antimicrobial effects essential oils

Table 2
Rankings of *in vitro* antimicrobial capacity of some essential oil components.

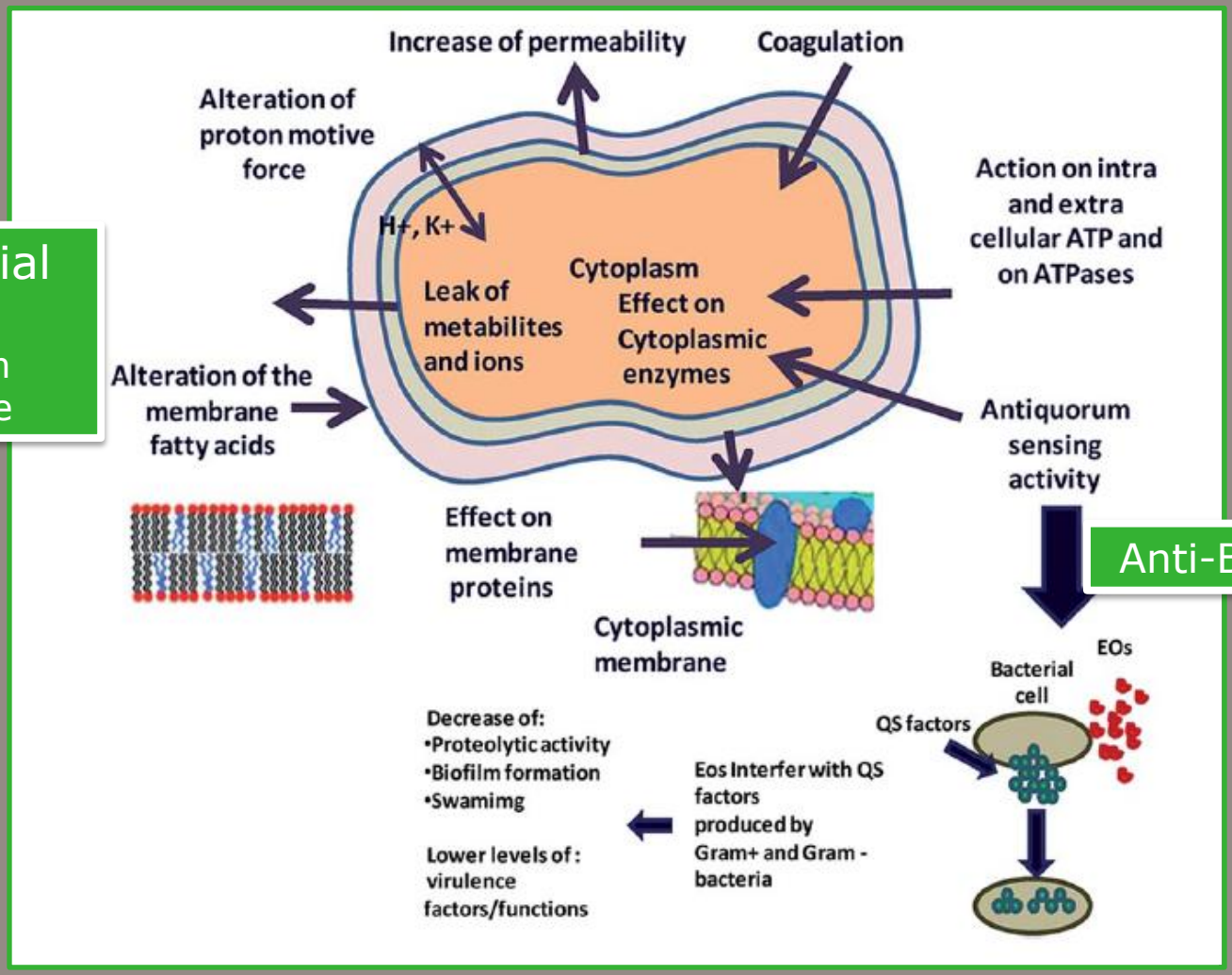
Reference	Test methods	Pathogens	Rankings
Kim et al., 1995 [1]	Disk diffusion method	*E. coli*	Citronellal > perillaldehyde > citral > geraniol > linalool > eugenol > terpineol > carvacrol
Kim et al., 1995 [1]	Disk diffusion method	*S. typhimurium*	Citronellal > citral > geraniol > perillaldehyde > linalool > eugenol > terpineol > carvacrol
Ait-Ouazzou et al., 2011	Disk diffusion method	*S. enteritidis*	Carvacrol > terpineol > linalool
Ait-Ouazzou et al., 2011	Disk diffusion method	*E. coli* O157:H7	Carvacrol > terpineol > linalool
Frideman et al., 2002	Microdilution + agar culture	*E. coli*	Carvacrol, cinnamaldehyde > thymol > eugenol > geraniol
Frideman et al., 2002	Microdilution + agar culture	*S. enterica*	Cinnamaldehyde > thymol > carvacrol > eugenol > geraniol
Frideman et al., 2002	Microdilution + agar culture	*C. jejuni*	Cinnamaldehyde > carvacrol > eugenol > thymol > geraniol
Si et al., 2006 [2]	Microdilution + optical density	*E. coli* K88	Thymol, carvacrol > cinnamon oil > clove oil > eugenol
Si et al., 2006 [2]	Microdilution + optical density	*E. coli* O157:H7	Cinnamon oil > thymol > geraniol, clove oil, carvacrol > eugenol
Si et al., 2006 [2]	Microdilution + optical density	*S. typhimurium* DT 104	Cinnamon oil > carvacrol > thymol > clove oil
Van Zyl., 2006 [3]	Microdilution + *p*-iodonitrotetrazolium violet	*S. aureus* ATCC 25923	Carvacrol > geraniol > linalool > citronellal > eugenol
Van Zyl., 2006 [3]	Microdilution + *p*-iodonitrotetrazolium violet	*B. cereus* ATCC 11778	Eugenol > carvacrol > geraniol > linalool > citronellal
Van Zyl., 2006 [3]	Microdilution + *p*-iodonitrotetrazolium violet	*E. coli* ATCC 11775	Eugenol > carvacrol > geraniol > linalool > citronellal
Michiels et al., 2009 [4]	Simulated stomach	Total anaerobic bacteria	Carvacrol > thymol > eugenol > trans-cinnamaldehyde
Michiels et al., 2009	Simulated jejunum	Coliform bacteria	Trans-cinnamaldehyde > carvacrol > thymol > eugenol
Michiels et al., 2009	Simulated jejunum	*E. coli*	Trans-cinnamaldehyde > carvacrol > thymol > eugenol

[1] The ranking was based on 5% concentration.
[2] The ranking was based on minimum bactericidal concentrations.
[3] The ranking was based on the concentration that resulted in complete growth inhibition of 10^7 cfu/mL.
[4] The ranking was based on the concentration that gives a reduction of 0.5 $\log_{10}$ cfu/mL compared to control.

Zhai, H., Liu, H., Wang, S., Wu, J., Kluenter, A.-M. 2018. Potential of essential oils for poultry and pigs. Animal Nutrition 4(2), pp. 179-186

Working mechanism of plant metabolites on bacteria (essential oils)

Antibacterial effects;
Anti-adhesion
Anti-virulence



Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., De Feo, V. 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals 6(12), pp. 1451-1474

Quorum sensing

Quorum Sensing in a Nut Shell

Production and release of signalling molecule (Auto inducer).

↓

Diffusion of Auto inducer (**AI**) molecule at low cell density leaving the QS circuit in-active

↓

Activation of QS circuit by **AI** at higher concentration due to Higher cell density.

↓

Transcriptional activation of down regulating genes by **AI** receptor complex or other activated signalling molecule.

↓

Activation of virulence, biofilm formation, sporulation, competence and antibiotic production.

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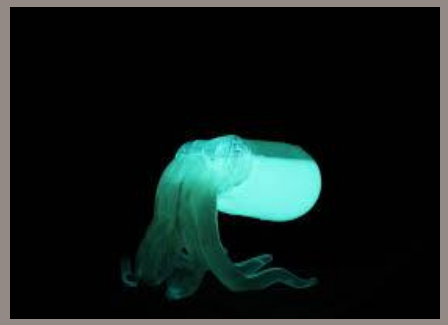

Quorum sensing

QS IN GRAM NEGATIVE BACTERIA

- The autoinducers are typically molecules called **acyl-homoserine lactones** (AHLs).
- AHLs diffuses extracellular and intracellular via passive transport.

QS IN GRAM POSITIVE BACTERIA

- The autoinducers are **oligopeptides**, short peptides typically 8-10 amino acids long.
- Oligopeptides excretes in extracellular environment via active transport.

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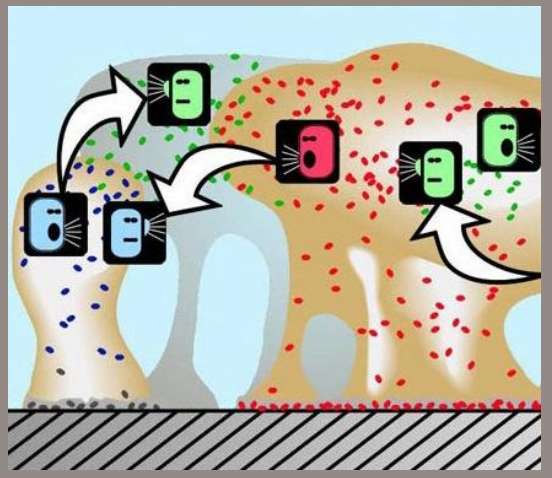


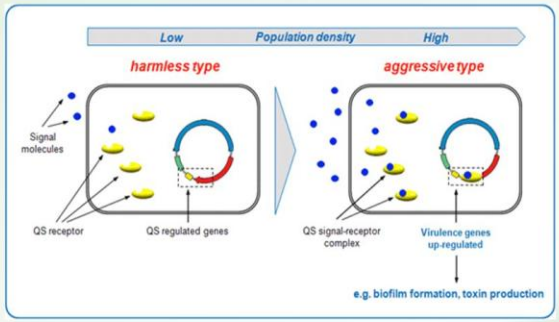
Plant metabolites effects

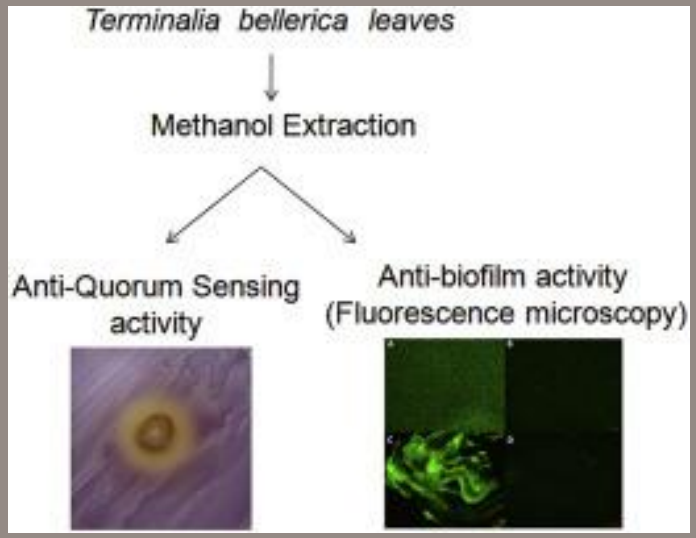
Inhibition of Quorum Sensing

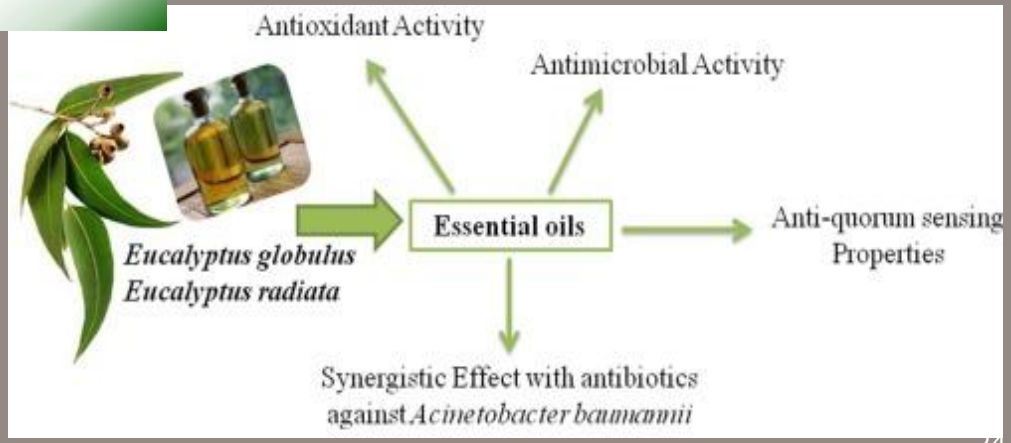
Cell signaling by organisms using auto inducers called Quorum Sensing determines gene expression, virulence, resistance, and the development of biofilms.

Botanicals which are known to inhibit Quorum Sensing:

- **Garlic**
- **Oregano**
- **Bilberry**
- **Bladderwrack**







Quorum sensing

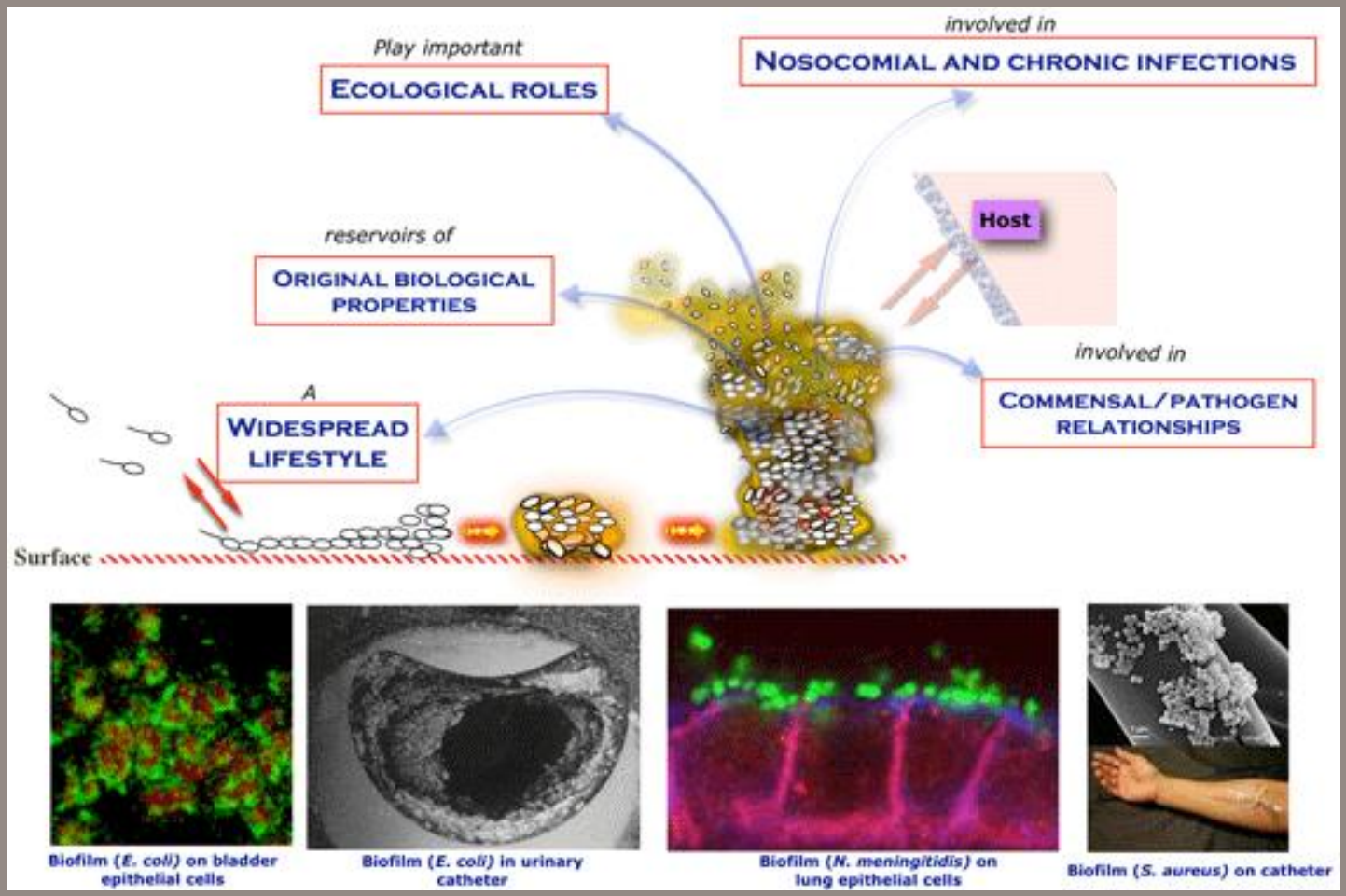
- Scopus essential oil quorum sensing: 102 hits
- Recent research (Vasconcelos et al., 2018): Cinnamon extracts, essential oils, and their compounds have been reported to inhibit bacteria by damaging cell membrane; altering the lipid profile; inhibiting ATP-ases, cell division, membrane porins, motility, and biofilm formation; and via anti-quorum sensing effects.

Vasconcelos, N.G., Croda, J., Simionatto, S. 2018. Antibacterial mechanisms of cinnamon and its constituents: A review Microbial Pathogenesis 120, 198-203

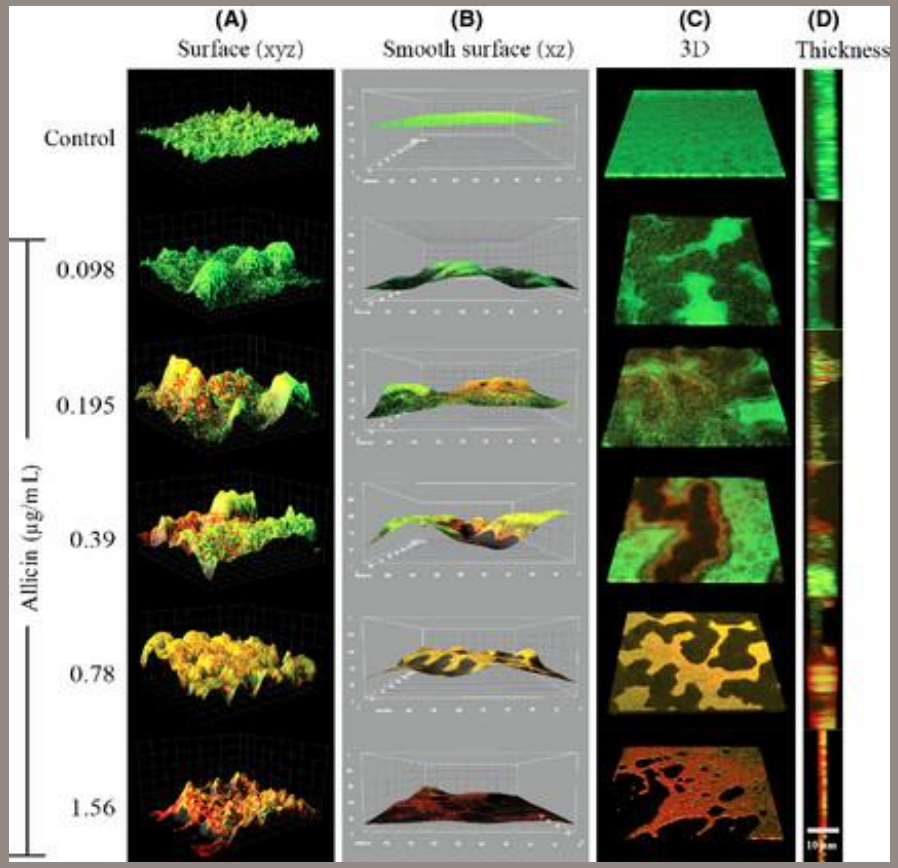
Nazzaro, F., Fratianni, F., Coppola, R. 2013. Quorum sensing and phytochemicals. International Journal of Molecular Sciences 14(6), pp. 12607-12619

Biofilms

Almost all pathogens can form biofilms



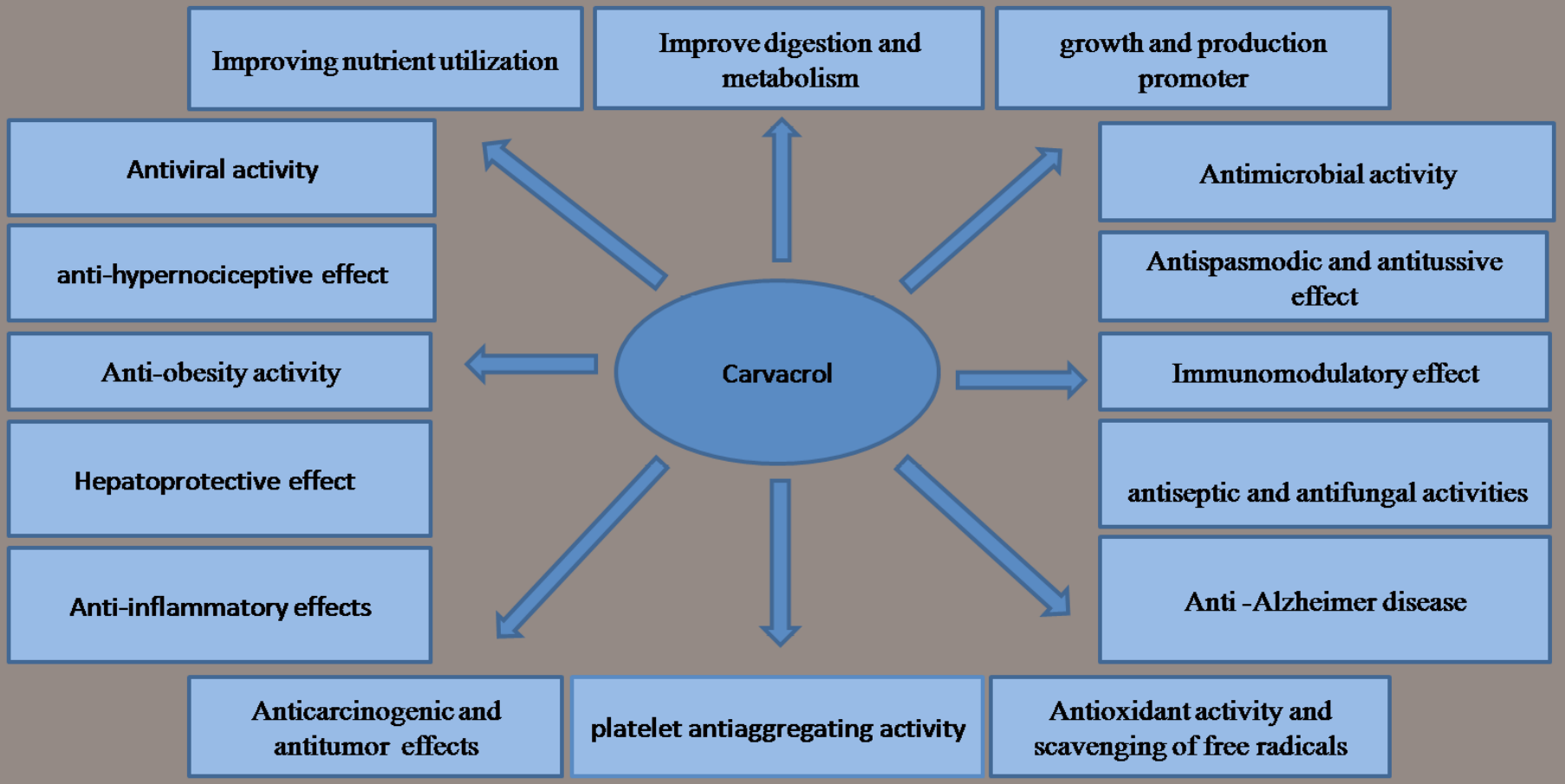
Anti-biofilm effect



Staphylococcus epidermidis (ATCC 35984) biofilm architecture after exposure to increasing concentrations of allicin. CSLM images of S. epidermidis biofilm surface and smooth surface from (xz) (A, B); biofilm 3D and thickness images (C, D) after exposure to different allicin concentrations (0.098–1.56 µg/mL). The biofilm thickness decreased from 10.7 (controls) concentration-dependently to 8.6, 7.7, 8.0, 7.9, and 4.7 µm (1.56 µg/mL allicin) with increasing allicin concentrations. Cells were distinguished by staining total bacterial cells with SYTO® green (green) and nonviable bacterial cells with propidium iodide (red).

Wu, X., Santos, R.R., Fink-Gremmels, J. 2015. Analyzing the antibacterial effects of food ingredients: Model experiments with allicin and garlic extracts on biofilm formation and viability of staphylococcus epidermidis. Food Science and Nutrition. 3(2), pp. 158-168

Other effects of herbal extracts



Diversity food

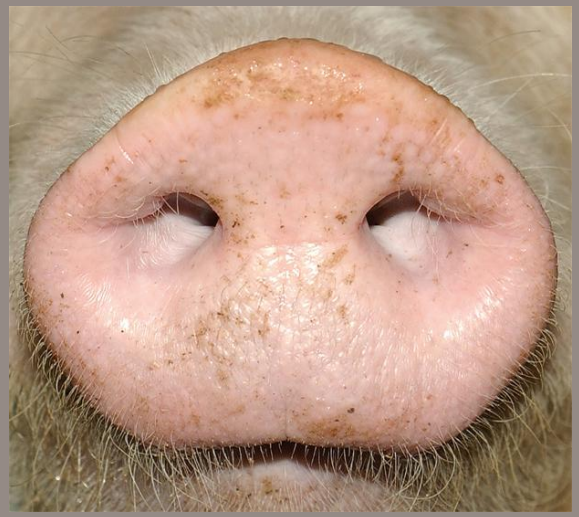
- Recent research with humans
- Diet with many different plant species lead to increased biodiversity of the microbiome
- Reduction of the expression of some antibiotic resistance genes
- More than 30 different plant species a week led to significant less antibiotic resistance of the microbiome

McDonald *et al*. American gut: an open platform for citizen science microbiome research. mSystems 2018;3(3): pii:e00031-18. doi: 10.1128/mSystems.00031-18.

Need for information

- Farmers, veterinarians, governments, feed industry, consumers
- Goal: **safe food from healthy animals with minimal use of antibiotics**
- Guides for different countries?
- Check the guides for what is important for the specific situation
- Involve stakeholders for commitment
- Monitor the use of antibiotics

Knowledge bank for herbs for animals



Planning

- Website
- Databases
- Literature, publications
- Forum for farmers and veterinarians
- Study groups for farmers and veterinarians
- Workshops
- Education for veterinarians

Conclusion

- Herbs have a broad spectrum of activity
- Contribute to the biodiversity of the feed
- Have several different systemic effects
- Concerning gut heath anti-QS, anti-biofilm and prebiotic effects important
- Can help stabilise the gut flora
- More resilient animals
- Contribute to health and animal welfare

Conclusions

- Need to reduce antibiotics
- Holistic approach needed: management, breeding, feed, herbal products
- Education of farmers and veterinarians
- Dissemination of knowledge
- Joining forces

Questions?

Thanks for your attention