

# Sophisticated plant extract inhibits *in vitro* the growth and biofilm formation of *Staphylococcus pseudintermedius* isolated from canine skin and ear infections

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## Introduction

Canine superficial pyoderma and otitis externa are very common clinical problems which are often associated with *Staphylococcus pseudintermedius* (Sp) a gram-positive coccoid bacteria. Sp can be an opportunistic pathogen in dogs with underlying conditions that compromise the skin barrier or immune system function. Treatment of such infections may necessitates the use of systemic antimicrobial drugs in combination with topical antibacterials. However. the emergence of multidrug-resistant Sp led to increased interest in novel topical therapeutic approaches.

## Objectives

- Study the **susceptibility of Sp** isolated from canine skin and ear infections to **classical antibiotics**.
- Evaluate the **antibacterial and antibiofilm activities** of a **Water Extracts of Complex Mixes of Edible Plants (WECMEPs)** against Sp strains.
- Demonstrate that plant extract preparations using **plants from different sources generate reproducible results**.

## Methods

- Forty-three Sp** were isolated from canine skin (23) and ear (20) infections.
- Susceptibility of Sp to **17 classical antibiotics** was evaluated using agar-disk diffusion assay.
- The **WECMEP 16P576** was generated using **artificial intelligence** algorithms to reach maximal synergistic activities.
- The **WECMEP 16P576 activity** was measured on planktonic agar growth and biofilm formation using optical density measurement. agar-well diffusion assay and crystal violet staining.

## Results



### Antibiogram of Sp strains and their susceptibility to 16P576 16P576 WECMEP

Staphylococcus pseudintermedius were isolated from skin or ear infections and 16P576 16P576 WECMEP activity was measured on planktonic, agar growth and biofilm formation using optical density measurement, agar-well diffusion assay and crystal violet staining.

|                                                                         |                                                                      |           | Sp STRAINS from SKIN INFECTIONS |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     | Sp STRAINS from EAR INFECTIONS |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|-------------------------------------------------------------------------|----------------------------------------------------------------------|-----------|---------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|-----|------|------|------|------|-----|-----|-----|-----|--------------------------------|------|------|------|-----|------|------|------|------|------|------|------|-----|-----|------|-----|-----|-----|------|----|--|
| SENSITIVE                                                               | RESISTANT                                                            | INTERMED. | 1                               | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15  | 16   | 17   | 18   | 19   | 20  | 21  | 22  | 23  | 24                             | 25   | 26   | 27   | 28  | 29   | 30   | 31   | 32   | 33   | 34   | 35   | 36  | 37  | 38   | 39  | 40  | 41  | 42   | 43 |  |
| BETA-LACTAMS                                                            | Penicillin                                                           |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Amoxicillin                                                          |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Cefalexin                                                            |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Oxacillin                                                            |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Cefovecin                                                            |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
| AMINOGLYCOSIDES                                                         | Streptomycin                                                         |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Neomycin                                                             |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Gentamicin                                                           |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
| MACROLIDES/<br>LINCOSAMIDES                                             | Clindamycin                                                          |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Erythromycine                                                        |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
| FLUOROQUINOLONES                                                        | Enrofloxacin                                                         |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Marbofloxacin                                                        |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
| SULFONAMIDES                                                            | Trimethop./SMX                                                       | nd        |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
| OTHER FAMILIES                                                          | Fusidic acid                                                         |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Chloramphenicol                                                      |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Florfenicol                                                          |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
|                                                                         | Doxycycline                                                          |           |                                 |      |      |      |      |      |      |      |      |      |      |      |      |      |     |      |      |      |      |     |     |     |     |                                |      |      |      |     |      |      |      |      |      |      |      |     |     |      |     |     |     |      |    |  |
| GLOBAL STATUS TO ANTIBIOTICS<br>(Sensitive. Resistant. Multi-Resistant) |                                                                      |           | S                               | S    | R    | R    | MR   | MR   | R    | R    | MR   | MR   | S    | S    | MR   | MR   | R   | MR   | R    | S    | MR   | R   | MR  | R   | R   | R                              | MR   | S    | R    | S   | MR   | MR   | R    | MR   | S    | R    | R    | S   | R   | MR   | R   | R   | R   | MR   | S  |  |
| 16P576 16P576<br>WECMEP<br>ANTIBACTERIAL<br>ACTIVITIES                  | Minimum Inhibitory<br>Concentration (g/l of<br>extract)              |           | 0.25                            | 0.25 | 0.05 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0.5 | 0.5 | 0.5 | 0.25                           | 0.25 | 0.25 | 0.25 | 2   | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 2   | 0.5 | 0.25 | 0.5 | 0.5 | 0.5 | 0.25 | 1  |  |
|                                                                         | BIOFILM INHIBITION at<br>WECMEP MIC<br>concentration (%)             |           | 95                              | 97   | 94   | 87   | 95   | 91   | 93   | 96   | 89   | 91   | 97   | 100  | 92   | 91   | 92  | 95   | 95   | 86   | 81   | 90  | 98  | 90  | 87  | 88                             | 95   | 96   | 91   | 86  | 94   | 92   | 93   | 73   | 97   | 79   | 74   | 92  | 90  | 97   | 88  | 90  | 93  | 91   | 88 |  |
|                                                                         | AGAR PLATE<br>INHIBITION DIAMETER<br>with 0.8 mg WECMEP<br>load (cm) |           | 1.7                             | 2.4  | 1.7  | 1.6  | 1.9  | 1.5  | 1.8  | 1.8  | 1.8  | 1.8  | 1.8  | 1.6  | 1.6  | 1.7  | 1.7 | 1.8  | 1.8  | 1.6  | 1.6  | 1.5 | 1.5 | 1.4 | 1.8 | 1.8                            | 1.7  | 1.7  | 1.1  | 1.8 | 1.5  | 1.6  | 1.6  | 1.7  | 1.6  | 1.7  | 1.0  | 1.5 | 1.6 | 1.6  | 1.5 | 1.4 | 1.7 | 1.4  |    |  |

### Study of plant variability impact on 16P576 WECMEP activities

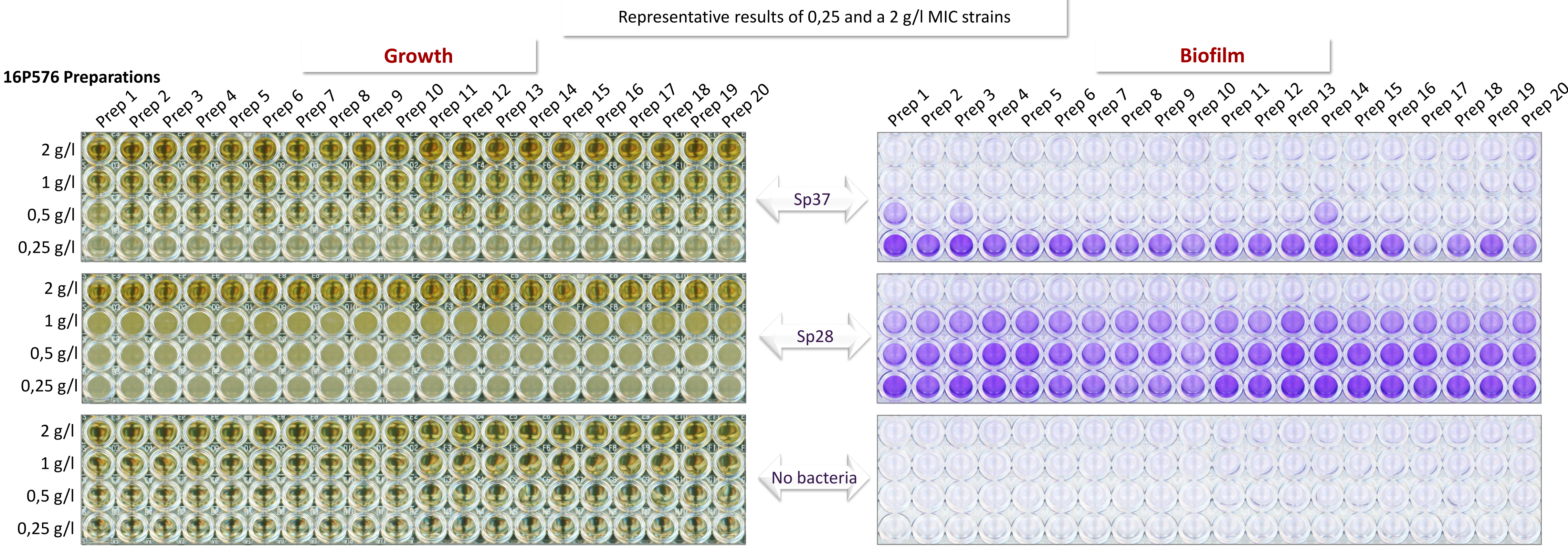
Twenty 16P576 WECMEPs were prepared using different plant batches and their activities against 20 Sp strains were measured on planktonic growth and biofilm formation using optical density measurement and crystal violet staining.

#### Plant extracts:

20 different 16P576 preparations (16 plants) with a choice among 3 different batches for each plant (producer, year, conventional or organic...). **Plant list:** *Arbutus unedo*, *Arctostaphylos uva-ursi*, *Camellia sinensis*, *Combretum micranthum*, *Eugenia caryophyllus*, *Foeniculum vulgare*, *Fucus vesiculosus*, *Juniperus communis*, *Lippia citriodora*, *Rheum palmatum*, *Rosmarinus officinalis*, *Satureja montana*, *Spiraea ulmaria*, *Tanacetum vulgare*, *Valeriana officinalis*, *Vitis vinifera* – Patent WO 2018/083115.

#### Bacterial strains:

20 clinical Sp: 10 from skin infections and 10 from ear infections (Sp2, 3, 4, 6, 9, 12, 14, 21, 25, 26, 28, 29, 30, 33, 37, 38, 39, 42).



## Conclusion

- Among the 43 strains isolated from dogs infections, **18 were resistant and 15 multi-resistant** to classical antibiotics.
- WECMEP 16P576** presented a MIC of 0.5 g/l for 40/43 Sp and induced more than 90% biofilm inhibition at this concentration.
- No differences** in the efficacy were observed **depending on the resistance status** to classical antibiotics nor the sample collection site.
- Results were **reproducible** for several WECMEPs 16P576 prepared with plants from **different sources**.

➡ **The WECMEP 16P576 appears as a candidate for a novel topical solution against Sp involved in superficial pyoderma and otitis externa, including antibiotic-resistant strains.**

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#### Conflicts of interest:

LL, CM, MF, LF and PM are employees of  
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