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# EFFICACY OF AKACID AS DISINFECTANT

## Introduction

In the last decade the development of new antibiotic substances has become more and more urgent, due to ever growing microbial resistance rates. Disinfection is a central measure in the area of prophylactic hygiene. By acting microbicidally on bacteria, fungi and virus and thereby leading to a quick killling; they prevent the emergence and spread of resistance.

The aim of the present study was to evaluate the antimicrobial activity of *Akacid*, a member of a new class of disinfectants. The mimimal inhibitory concentrations (MIC) of over 350 microorganisms (both clinical isolates and ATCC strains as reference) were determined. Furthermore, temperature-dependency and microbicidal activity of *Akacid* were evaluated.
The recent bioterrorist incidents in the USA have shown that the inhalative form of anthrax remains a distinct threat in the current world situation. Effective measures for decontaminating an environment contaminated with anthrax spores are urgently needed now. Since these spores are highly resistant to many physical and chemical agents, new substances should be looked for. One of the most promising compounds is akacid, a polymer originally used in the oil industry, with antimicrobial properties. The present study also examines the efficacy of akacid against anthrax spores.

## Materials and Methods

### Microorganisms

In order to attain a representative survey of the activity of *Akacid*, a wide range of clinically relevant microorganisms were used for the experiments. Overall 330 bacteria of different species, 10 yeasts, 2 moulds, 1 dermatophyte and spores of both Bacillus anthracis and Bacillus subtilis were tested. All

isolates were derived from the respiratory tract, the urogenital tract, blood or other sites. After isolation, strains were characterized using standard laboratory methods.

**Test substance**

The acitive substance of the tested ”Triple A Spray, Geopharma” is *Akacid*, batch no. 44/170502.
As stock solution *Akacid*, a poly [9C2-(2-ethoxy)-ethoxyethyl)-guanidium-chloride] 25 % aqueous solution was used. The stock solution was diluted with hard water to the desired concentration. The test substance was stored at room temperature.

**Determination of minimal inhibitory concentrations (MIC)**

Determination of MICs was performed by using the microdilution method with Mueller-Hinton broth, according to NCCLS guidelines. The microbial inoculum was at least $5x10^5$ CFU/ml; the incubation lasted for 16 – 20 hours at 36°/ambient air. *Akacid* was used in concentrations from 1000 μg/ml to 0.001 μg/ml.
The lowest concentration of test substance where there was no visible bacterial growth was defined as MIC. As quality control ATCC strains with known MICs were included in each run.

**Temperature Dependency**

The temperature dependency of the active substance was determined using Pseudomonas aeruginosa ATCC15442 and Staphylococcus aureus ATCC6538 at temperatures of –196, -20, 0, 20°C and after sterilisation for 15 minutes at 121°C.

**Development of resistance**

Based on the results of the MIC testing, 30 bacterial strains were chosen for these experiments:

| | |
|---|---|
| **Gram-positive strains (n=12)** | MSSA (n=1) |
| | MRSA (n=2) |
| | MRSE (n=4) |
| | VRE (n=5) |
| **Gram-negative strains (n=18)** | multiresistant E. coli (n=4) |
| | K. pneumoniae (n=1) |
| | K. oxytoca (n=1) |
| | P. aeruginosa (n=4) |
| | multiresistant P. aeruginosa (n=4) |
| | Acinetobacter sp. (n=2) |
| | E.coli ATCC 35218 and 25922 |

All strains were incubated with the test substance in concentrations ranging from 1000 μg/ml to 0.001 μg/ml for 24 hours. Test tubes, where bacterial growth was recorded after the first passage, were used as inoculum for the second passage. After each passage the MIC was determined and compared to the start value. Overall thirty passages were performed.

**Determination of Quantitative Bactericidal and Fungicidal Activity**

These tests were performed using the membrane filtration method, according to ÖNORM 1276 (3% albumine, 5 minutes incubation period).

**Determination of Sporocidal Activity**

These tests were performed using spores of Bacillus subtilis ATCC 6633 and Bacillus anthracis CH10 (anthrax spores reg.no. G112 WET/ACT 36/47) and Envirocheck Rodac GKZ agar plates with inhibitors. This method is in accordance with Draft European Standard CEN/TC 243/WG 2 (prEN 1632-3: 1994). The contact plates for evaluation of mivrobial contamination of surfaces contain the following inhibitors: Tween 80 (polysorbate), sodiumthiosulfate, lecithin, histidine and a combination of histidine and lecithine. The spore suspension ($10^7$ KBE) was distributed on the test surface. Drying time was two hours. After drying test surfaces were sprayed with saline solution as growth control. The other test surfaces were sprayed with 0.5 % Akacid and the time was noted. Test samples were taken after five minutes, 30 minutes, one hour and 24 hours in the following way (five plates per time point, three repetitions):

1. Opening of the lid and pressing the contact plate with even pressure to the contaminated surface for at least ten seconds.
2. Closing the lid and labelling of the plate.
3. Incubation for 68 hours at 37°C, afterwards for 10 days at ambient air.

## Results

**Minimal inhibitory concentrations (MIC)**

Results of MIC testing are summarized in Tables 1 to 5.

*Akacid* showed good activity against S. aureus and S. epidermidis, regardless of their resistance profile, with MICs from 4 to 32 μg/ml. Even multiresistant strains of S. aureus (MRSA) had similar values (Table 1).

Enterococcal MICs ranged from 16 to 32 μg/ml, where the MICs of multiresistant and vancomycin-resistant strains did not differ from the MICs of sensitive strains (Table 2).

Good results could also be obtained when testing the Enterobacteriacae: MICs of E. coli, Klebsiella sp., Enterobacter sp. and P. mirabilis ranged from 4 to 32 μg/ml. This also holds true for the tested Salmonella sp., Shigella sp and Yersinia enterocolitica (Table 3).

The non-fermenters, P. aeruginosa and Acinetobacter sp., proved to be sensitive to *Akacid*, with MICs of 4 to 32 μg/ml (Table 3). Also the five strains of Pseudomonas sp., which were resistant to all clinically relevant antibiotics, were suscepible to *Akacid*.

*Akacid* showed also good results against Mycobacterium tuberculosis, M. avium complex, M. kansasii and M. gordonae, with MICs from 16 to 32 μg/ml (Table 4).

The testing of clinically relevant fungi like C. albicans, C. tropicalis, C. parapsilosis, A. fumigatus and Trichophyton mentagrophytes proved the good antimycotic activity of *Akacid* with MICs of 8 to 32 μg/ml (Table 5).

**Temperature Dependency**

At all temperatures the MIC for S. aureus was 16 µg/ml, and for P.aeruginosa 32 µg/ml.

**Determination of Quantitative Bactericidal and Fungicidal Activity**

Tested strains:

S. aureus ATCC 6538, E. coli ATCC 10536, P. aeruginosa ATCC 15442 and C. albicans ATCC 10231. The demanded activity of a 5 log reduction after an incubation of five minutes against the tested strains E. coli, S. aureus and P. aeruginosa could be shown for concentrations of 0.5 to 3.75 %. For C. albicans concentrations ranged between 0.5 and 1%.

**Determination of Sporocidal Activity**

These tests were performed using spores of Bacillus subtilis ATCC 6633 and Bacillus anthracis CH10 (anthrax spores reg.no. G112 WET/ACT 36/47). Results are summarized in Tables 6 and 7.

**Development of resistance**

For this test a total of 30 strains (gram-positive agents like S. aureus and S. epidermidis and gram-negative agents like E. coli, Klebsiella sp., P. aeruginosa and Acinetobacter sp) was used. Tested were not only sensitive ATCC strains, but also multi-resistant clinical isolates. In both groups no development of resistance was observed after 30 passages, that is there was no rise in MIC values.

## Conclusion

Disinfection can be defined as the targeted killing of microorganisms, aiming at the prevention of the spreading of these organisms, regardless of their state of function (therefore also in the stationary phase). In order to guarantee this, a desinfectant must be able to kill microbes at the recommended concentration, it must be microbicidal. Another important aspect is the spectrum which is covered by the disinfectant.

Furthermore these result show that Akacid has safe efficacy in five minutes against the tested strains of E. coli, S. aureus and P. aeruginosa at concentrations of 0.5 to 5 % in the quantitative suspensions test. Against fungi, activity sets in after 20 to 30 minutes.
**In the real-life test it could also be shown that Akacid spray at 0.5 % posesses sporocidal activity after five minutes.**

The present study demonstrates without doubt that Akacid meets the demand for both microbicidal properties and broad spectrum to the fullest extent. From the microbiological point of view the use of Akacid as a desinfectant can be recommended wihout restriction.

**Table 1. Efficacy of *Akacid* against gram-positive bacteria**

| species | number of strains n (%) with a MiC (in mg/l) of | | | |
|---|---|---|---|---|
| | **4** | **8** | **16** | **32** |
| **MSSA** | | | | |
| **(n = 35)** | 0 (0) | 6 (17,1) | 20 (57,1) | 9 (25,8) |
| **MRSA** | | | | |
| **(n = 60)** | 3 (5) | 10 (16,7) | 38 (63,3) | 9 (15) |
| **Enterococcus faecalis** | | | | |
| **(n = 32)** | 0 (0) | 0 (0) | 16 (50) | 16 (50) |

**Table 2. Efficacy of *Akacid* against Enterobacteriacae**

| Species | number of strains n (%) with a MiC (in mg/l) of | | | |
|---|---|---|---|---|
| | **4** | **8** | **16** | **32** |
| **Klebsiella spp.** | | | | |
| **(n = 43)** | 0 (0) | 5 (11,6) | 18 (41,9) | 20 (46,5) |
| **Escherichia coli** | | | | |
| **(n = 59)** | 2 (3,4) | 17 (28,8) | 39 (66,1) | 1 (1,7) |
| **Proteus spp.** | | | | |
| **(n = 7)** | 0 (0) | 0 (0) | 3 (42,9) | 4 (57,1) |

**Table 3. Efficacy of *Akacid* against gram-negative bacteria**

| species | number of strains n (%) with a MiC (in mg/l) of | | | |
|---|---|---|---|---|
| | **4** | **8** | **16** | **32** |
| **Pseudomonas spp..** | | | | |

| | | | | |
|---|---|---|---|---|
| **(n = 55)** | 1 (1,8) | 5 (9,1) | 32 (58,2) | 17 (30,9) |
| **Salmonella spp.** | | | | |
| **(n = 6)** | 0 (na) | 2 (na) | 4 (na) | 0 (na) |
| **Shigella spp.** | | | | |
| **(n = 2)** | 0 (na) | 0 (na) | 1 (na) | 1 (na) |
| **Yersinia enterocolitica** | | | | |
| **(n = 1)** | 0 (na) | 0 (na) | 0 (na) | 1 (na) |

**Table 4. Efficacy of *Akacid* against Mycobacterium spp.**

| **species** | **number of strains n (%) with a MiC (in mg/l) of** | | | |
|---|---|---|---|---|
| | **4** | **8** | **16** | **32** |
| **Mycobakterium spp.** | | | | |
| **(n = 6)** | 0 (na) | 0 (na) | 5 (na) | 1 (na) |

**Table 5. Efficacy of *Akacid* against Candida spp.**

| **species** | **number of strains n (%) with a MiC (in mg/l) of** | | | |
|---|---|---|---|---|
| | **4** | **8** | **16** | **32** |
| **Candida spp.** | | | | |
| **(n = 10)** | 0 (na) | 1 (na) | 3 (na) | 6 (na) |

**Table 6.** Activity of Akacid spray against spores of Bacillus anthracis and Bacillus subtilis (0.5 %)

| **Time** | **Experiment no** | | | | | | |
|---|---|---|---|---|---|---|---|
| **(Min)** | **Spores of B. anthracis** | | | **Spores of B. subtilis** | | | **Control** |
| | ***1*** | ***2*** | ***3*** | ***1*** | ***2*** | ***3*** | |
| 5 min | 0 | 0 | 0 | 0 | 0 | 0 | L |
| 30 min | 0 | 0 | 0 | 0 | 0 | 0 | L |
| 1 hour | 0 | 0 | 0 | 0 | 0 | 0 | L |
| 24 hours | 0 | 0 | 0 | 0 | 0 | 0 | L |

L Lawn, (more than 10.000 CFU, according to European Standard CEN/TC 243/WG 1 (prEN 1632-3: 1994).

**Table 7.** Activity of Akacid spray against spores of Bacillus anthracis and Bacillus subtilis (0.1 %)

| **time** | **Experiment no** | | | | | | |
|---|---|---|---|---|---|---|---|
| **(Min)** | **Spores of B. anthracis** | | | **Spores of B. subtilis** | | | **Control** |
| | ***1*** | ***2*** | ***3*** | ***1*** | ***2*** | ***3*** | |
| 5 min | > 300 | > 300 | > 300 | > 300 | > 300 | > 300 | L |
| 30 min | 30 | 4 | 0 | 20 | 8 | 10 | L |
| 1 hour | 1 | 0 | 0 | 3 | 0 | 0 | L |
| 24 hours | 1 | 0 | 0 | 1 | 0 | 0 | L |

L Lawn, (more than 10.000 CFU, according to European Standard CEN/TC 243/WG 1 (prEN 1632-3: 1994).