Antimicrobial activities of essential oil and methanol extract of Boswellia sacra Flueck. and Boswellia papyrifera (Del.) Hochst from Djibouti

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ABSTRACT

*Boswellia sacra* and *Boswellia papyrifera* belong to the family of Burseraceae. The daily and ritual use of *Boswellia* is characteristic cultural of the horn of Africa which transcends the ethical and religious memberships. Plants samples of *Boswellia sacra* and *Boswellia papyrifera* were collected in Djibouti. The essential oil and methanol extracts of each plant were collected. They were evaluated for its antimicrobial activity using disc diffusion and microdilution methods. The essential oil and methanol extract of *Boswellia* showed activity against bacterial species than against yeast.

Key words: *Boswellia sacra*, *Boswellia papyrifera*, Burseraceae and antimicrobial activity.

INTRODUCTION

The genus *Boswellia* (family Burseraceae) consists of many species widespread throughout the world. It includes approximately 23 species of small trees that grow mainly in Arabia, on eastern coast of Africa and in India. Olibanum is a natural oleo-gum-resin that exudes from tappings in the bark of *Boswellia* trees (Hamm \textit{et al.}, 2005). The genus is represented in Soqotra Island by 8 species. The oleogum resin is used traditionally by the native inhabitants for relieving the pain in cavities, sweetening the breath, or to soothe a disturbed stomach (Miller and Morris, 2004). *Boswellia sacra* and *Boswellia papyrifera* belong to the family of Burseraceae. These high trees of ten meters to the maximum, attend arid regions more particularly the horn of Africa where they form more or less dense settlements (Chikamai \textit{et al.}, 2000). The bark contains schizogenous olea-gum-resin pockets (Verghese, 1988). Leaves are large, compound, arranged on long stalks with 11 to 29 leaflets which are narrowly ovate to oblong, waved or toothed along the margin. *Boswellia sp.* possesses sweet scented flowers which are
white to pink, arranged on long red flower stalks, in loose panicles at the end of branches. Fruits are obtetrahedral, which are red capsules about 2 cm long, usually containing three tapered seeds (Vollesen, 1989).

In the Arabic Peninsula as in Somalia, settlements concentrated of *Boswellia* on the piemonts with a few kilometers of the dimension where they are sprinkled by monsoons. The resins known under the name of myrrh and of incense do not come from a species but several groups of species. The flowering of these trees is generally discrete and fugacious. Their foliage often falls very quickly under the effect of the dryness (Monod, 1979). Incense *Boswellia* or tree is regarded as a gift of nature and a compensation with the lack of resource of the area.

The daily and ritual use of *Boswellia* is characteristic cultural of the horn of Africa which transcends the ethical and religious memberships. The dromedaries nourish sheets and fruits of these trees (Groom, 1981). The men chew the resin of *Boswellia*. *Boswellia* is aromatic and releases by combustion a pleasant and strong odor. It is originating in the septentrional Somali dimension and southernmost Arabia. It is cultivated in India. All the species of this kind contain resins which are extracted by incision from the bark. After being transformed into essential oils, absolute and resinoid, these gum-oleo-resins are used in perfumery and cosmetic. They also enter certain pharmacopeias in particular the Chinese pharmacopeia.

*Boswellia papyrifera and Boswellia sacra* are two of the most important multipurpose tree species in Central and Eastern Africa. It is a drought-resistant species that continues to grow in marginal lands, produce incense, flower and grow leaves even in harsh and unpredictable biophysical conditions. Finally, issues and concerns related to the population decline of *B. papyrifera* are highlighted. So these two species of those possess multiple economic and ecological benefits in Africa. It is found in Ethiopia, Nigeria, Cameroon, Central African Republic, Chad, Sudan, Uganda and Eritrea (Vollesen, 1989). The species are widely known for its frankincense. Despite its multiple benefits, *Boswellia* is nowadays reported to be in plight conditions and needs priority in conservation. Its population is degrading due to extensive farming, overgrazing, fire, poor incense harvesting practices, shifting cultivation, termite and other insect infestations (Oqbazghi, 2001). Considerable work on the composition of the essential oils from different species of *Boswellia* is reported in literature. Monoterpenoids seem to be the dominant class of compounds found in the oils. The main compounds of *B. dalzielii* essential oil from Nigeria were α-pinene (45.7 %), α -terpinene (11.5 %) and trans-sabinene hydrate (Kubmarawa et al., 2006).

The essential oil of *B. frereana* from Somalia was dominated by α-thujene (10.10 %) and p-cymene (4.3 %) (Chiavari et al., 1991). The essential oil of *B. serrata* from India was found to contain mainly α- thujene (61.0 %), α-pinene (7.7 %) and sabinene (5.1 %) (Kasali et al., 2002). Since the chemical composition of essential oils depends on various environmental factors, three studies on the essential oils composition of *B. carteri* from Somalia revealed this composition variety: α-thujene (19.2 %), sabinene (9.4 %), limonene (7.8 %) and α-pinene (7.2 %) (Chiavari et al., 1991); octyl acetate (60.0 %), octanol (12.7 %) and p-cymene (8.7 %) (Wang et al., 1993); α-pinene (41.0 %), limonene (12.8 %) (Abdulwahab et al., 1987). Several studies investigated the anti-inflammatory, immunomodulatory, anti-leukotriene, antiacetylcholinesterase, and anticancer
activity of the resin and especially its major components, boswellic acid derivatives (Ammon et al., 1991; Akihisa et al., 2006). In addition, the essential oil showed antibacterial, antifungal and immunostimulating activity (Gangwal and Vardhan, 1995; Mikhail et al., 2003; Ota and Houghton, 2005).

The aim of this study is the analysis of antimicrobial activities of two species of *Boswellia* (*Boswellia papyrifera* and *Boswellia sacra*) belonging in Arta (Djibouti), in the object to optimize medicinal exploitation.

**MATERIALS AND METHODS**

**Isolation of essential oil**
The plant samples of *Boswellia sacra* and *Boswellia papyrifera* were collected in Arta in June 2010. Identification of the species was carried out at the Centre d’Études et de Recherches de Djibouti, where a voucher specimen is kept. Fractions of 500 g of air-dried plant material (branch), previously cut into small pieces, were submitted, separately, to hydrodistillation for 4 h using a Clevenger-type apparatus. The resulting essential oils collected by decantation were dried over anhydrous sodium sulfate, and kept in a sealed flask at 10°C until antimicrobial testing. The yields are expressed in percent, w/w of the dry vegetable material.

**Preparation of the methanol extract**
The dried and powdered branch (500 g) were extracted with 1 L of methanol using a Soxhlet extractor for 7 h at a temperature (64°C) not exceeding the boiling point of the solvent (Lin et al., 1999). The extract was filtrated using Whatman filter paper (no. 1) and then concentrated in vacuum at 40°C using a rotary evaporator. The residues obtained were stored in freezer at -80°C until further tests.

**Microbial strains**

**Microbiological methods**

**Antimicrobial screening**
The agar disk diffusion method was employed for the screening of antimicrobial activities of the essential oils and methanol extract (NCCLS, 1999). The dried plant extracts were dissolved in methanol to final concentration of 30 mg/ml and sterilized by filtration through 0.45 µm Millipore filters (Schleicher and Schuell, Microscience, Dassel, Germany). Antimicrobial tests were then carried out by disk diffusion (Murray et al., 1995) using 100 µl of suspension containing 10<sup>8</sup> colony forming units (CFU)/ml of bacteria, 10<sup>4</sup> spore/ml of fungi spread on nutrient agar (NA) and potato dextrose agar (PDA) medium, respectively. The disk impregnated with 10 µl of essential oil or 10 µl of the methanol solution of the dried plant extracts (300 µg/disk) were placed on the inoculated agar. Negative controls were prepared with the same solvent used to dissolve the plant extracts.
Tetracyclin (30 UI) and ticarcillin (75 μg) were used as standard antibiotics (BIO-RAD Marnes-la coquette-France). Benomyl (100 μg) and griseofulvin (100 μg) were used as standard antifungics (BIO-RAD Marnes–la coquette-France). Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay was repeated twice (Edris and Farrag, 2003; Kordali et al., 2005).

The positive controls were used to determine the sensitivity of one strain/isolated in each microbial species tested. The inoculated plates were incubated aerobically at 30°C (Gram-negative) or 37°C (Gram-positive) according to strain for 24 h and 72 h for fungi isolated. Plants-associated microorganisms were incubated at 27 °C.

Antimicrobial activity

The MIC values were determined for the bacterial strains that were sensitive to the essential oil in the disk diffusion assay. The inocula of the bacterial strains were prepared from 10 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils and extracts of *Boswellia sacra* and *Boswellia papyrifera*, dissolved in 10 % dimethyl sulfoxide (DMSO), were first diluted to the highest concentration (500 μg/ml) to be tested and then serial fold dilutions were made to obtain a concentration range from 7.8 to 500 μg/ml in 10 ml sterile test tubes containing nutrient broth. The MIC values of *Boswellia sacra* and *Boswellia papyrifera* extracts against bacterial strains and fungal isolates were determined on the basis of a microwell dilution method with some modification (Obame et al., 2008a and b). The 96-well plate transparent microwells were prepared by dispensing 95 μl of nutrient broth and 5 μl of the inoculums into each well. One hundred microliters from the stock solutions of *Boswellia sacra* and *Boswellia papyrifera* essential oil prepared at the 500 μg/ml concentration was added into the first wells. Then, 100 μl from the serial dilutions was transferred into the six consecutive wells. The last well containing 195 μl of nutrient broth without compound and 5 μl of the inoculums on each strip was used as a negative control. The final volume in each well was 200 μl. Maxipime at a concentration range of 7.8 - 500 μg/ml was prepared in nutrient broth and used as a standard drug for positive control. The plate was covered with a sterile plate shaker at 300 rpm for 20 s and then incubated at appropriated temperatures for 24 h.

Microbial growth in each well determined by reading the respective absorbance (Abs) at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, VT) and confirmed by plating 5 μl samples from clear wells on nutrient agar medium. The oil tested in this study was screened twice against each organism.

MIC Agar dilution assay.

The agar dilution method was used to determine the MIC values of the fungus isolates. The essential oil of *Boswellia sacra* and *Boswellia papyrifera* were added aseptically to sterile molten PDA medium, containing tween 20 (Sigma 0.5 % (v/v), at appropriate volume to produce the concentration range of 7.8–500 μg/ml. The resulting PDA solutions were immediately poured into Petri plates after vortexing. The plates were spot incubated with 5 μl (104 spores/ml) of each fungal isolate. In addition, PDA plates treated with benomyl (12.0 mg/Petri dishes) and griseofulvin (100 μg) were used as positive controls (BIO-RAD Marnes-la coquette-France). The inoculated plates were incubated at 27°C and 37°C for 72 h for plant and clinical fungus isolates, respectively. At the end of the incubation period, the plates were evaluated for the presence or absence of growth.
MIC values were determined as the lowest concentration of the essential oil at which the absence of growth was recorded. Each assay was repeated at least twice.

To determine MBCs, 10 µl suspension were taken from each well and inoculated in Mueller–Hinton Agar (Becton Dickinson, USA) for 24 h at 30 or 37 °C. The MBC is defined as the lowest concentration of the essential oil (Michel-Briand, 1986).

RESULTS AND DISCUSSION

The yield of essential oils of branch of *Boswellia sacra* and *Boswellia papyrifera* were respectively 0.37 % (w/w) and 0.14 % (w/w). The yield of methanol extracts of branch of *B. sacra* and *B. papyrifera* were respectively 3.45 % (v/v) and 3.51 % (v/v).

### Antimicrobial screening

The test of sensitivity of the extracts of *Boswellia sacra* and *Boswellia papyrifera* to the various bacterial strains shows that the methanol extracts and essential oils have an inhibiting potential. The methanol extracts and essential oils are active on bacteria Gram + and Gram – (Table 1). They gave a great antimicrobial activity and an inhibition with the diameters from 11 to 40 mm for oil essential and from 6 to 34 mm for the methanol extract. Essential oils are more active than the methanol extract.

The best zone of inhibition of essential oil of *Boswellia sacra* for bacteria was obtained for *Salmonella enterica* CIP 105150 (34 mm), *Listeria innocua* LMG 1135668 (30 mm). The other strains had sensitivities between 19-29 mm.

The best zone of inhibition of essential oil of *Boswellia papyrifera* for bacteria was obtained for *Salmonella enterica* CIP 105150 (40 mm), *Bacillus cereus* LMG 13569 (39 mm), *Enterococcus faecalis* CIP 103907 (39 mm), *Shigella dysenteria* CIP 5451 (31 mm), *Staphylococcus camorum* LMG 13567 (30 mm). The other strains had sensitivities between 15-28 mm.

The best zone of inhibition of methanol extract of *Boswellia papyrifera* were obtained for *Enterococcus faecalis* CIP 103907 (30 mm), *Bacillus cereus* LMG 13569 (27 mm). The other strains had sensitivities between 6-24 mm.

Essential oils of *Boswellia sacra* and *Boswellia papyrifera* present an antimicrobial activity stronger than the tetracycline. However, the methanol extracts of *Boswellia sacra* and *Boswellia papyrifera* have an antimicrobial activity weaker than the tetracycline for *Escherichia coli* CIP 105182, *Shigella dysenteria* CIP 5451, *Staphylococcus camorum* LMG 13567, *Proteus mirabilis* CIP 104588 and *Pseudomonas aeruginosa*. *Proteus mirabilis* CIP 104588 is resistant to the methanol extracts of *Boswellia sacra* and *Boswellia papyrifera* while *Shigella dysenteria* CIP 5451 is resistant to the methanol extract of *Boswellia sacra*. Essential oils of *Boswellia sacra* and *Boswellia papyrifera* present an antimicrobial activity stronger than the ticarcycline for *Enterococcus faecalis* CIP 103907, *Escherichia coli* CIP 105182, *Shigella dysenteria* CIP 5451, *Staphylococcus camorum* LMG 13567, *Pseudomonas aeruginosa* and
Proteus mirabilis CIP 104588 (only for essential oil of Boswellia sacra).
The methanol extracts of Boswellia sacra and Boswellia papyrifera have an antimicrobial activity weaker than the ticaracycline except for Escherichia coli CIP 105182.
The essential oil and methanol extract of Boswellia sacra and Boswellia papyrifera were tested against Candida albicans and Aspergillus as pathogenic fungal species in human body and compared with benomyl and griseofulvin.
The result showed that the growth of fungal species was significantly inhibited by the essential oil and methanol extract (Table 1).
Clinical origin C. albicans was less sensitive to the essential oil (15 mm for essential oil of Boswellia sacra and 19 mm for essential oil of Boswellia papyrifera) than reference C. albicans strain (20 mm). Oil and methanol extract of Boswellia sacra and Boswellia papyrifera were also interesting to find that the inhibition effect against C. albicans were higher than that of benomyl (C. albicans, 13 to 19 mm) and griseofulvin (C. albicans, 15 to 19 mm). Clinical origin C. albicans was less sensitive to the methanol extract of Boswellia sacra (13 mm) than that of griseofulvin. C. albicans was more sensitive to the methanol extract (17 mm) than Aspergillus sp and Aspergillus niger (11 mm and 14 mm for methanol extract of Boswellia sacra and 15 mm for methanol extract of Boswellia papyrifera).
The values of the MIC and MBC of essential oil and methanol extract of Boswellia sacra and Boswellia papyrifera with the bacterial strains are consigned (Table 2). The methanol extract of Boswellia sacra contains bactericidal properties with MIC and MBC equal to 100 µg/ml on Staphylococcus aureus ATCC 9244 and Staphylococcus aureus. The extract is bactericidal on the other strains with MIC and MBC equal to 200 µg/ml. The essential oil of Boswellia sacra showed the strongest bactericidal activities on Salmonella enterica CIP 105150 (1 µg/ml), Enterococcus faecalis CIP 103907 (2 µg/ml), Staphylococcus aureus ATCC 9244 (2 µg/ml), Staphylococcus aureus (2 µg/ml).
It is bacteriostatic action on Staphylococcus camorum LMG 13567.
The methanol extract of Boswellia papyrifera contains bactericidal properties with MIC and MBC equal to 50 µg/ml on Salmonella enterica CIP 105150 and Staphylococcus camorum LMG 13567.
The essential oil of Boswellia papyrifera showed the strongest bactericidal activities on Enterococcus faecalis CIP 103907 (2 µg/ml) and Staphylococcus aureus (2 µg/ml). The weakest bactericidal activity was observed on Pseudomonas aeruginosa (32 µg/ml).
Essential oil of Boswellia papyrifera was fungicidal activity for Candida albicans, Candida albicans ATCC 10231 and Aspergillus sp. Essential oil of Boswellia sacra and methanol extract of Boswellia papyrifera were microbicidal action for Candida albicans, Candida albicans ATCC 10231, Aspergillus sp. and Aspergillus niger. Methanol extract of Boswellia sacra was fungicidal activity for Candida albicans ATCC 10231.
The antimicrobial activity was expressed in two different ways. In term of many inhibited micro-organisms (MIC), essential oils are more effective than the methanol extracts. The essential oil of Boswellia papyrifera is more active than Boswellia sacra. In term of the smallest value of MBC, essential oils are powerful that the methanol extracts. The essential oil of Boswellia sacra is more powerful than Boswellia papyrifera.

CONCLUSION
The study has shown that essential oil and methanol extract of Boswellia sacra and Boswellia papyrifera have in vitro antimicrobial activities which could support
the use of the plant by traditional healers to treat various infective diseases. Further studies could lead to the most active essential oil and could lead to a new antimicrobial agent.

REFERENCES


Activity of Frankincence Oil. Z. Naturforsch. 58c, 230-238.


Table 1: Antimicrobial activity (mm) from essential oil and methanol extract of *Boswellia sacra* and *Boswellia papyrifera* from Djibouti

Each value represents mean of three different observations.

<table>
<thead>
<tr>
<th>Origin</th>
<th><em>Boswellia sacra</em></th>
<th><em>Boswellia papyrifera</em></th>
<th>Te&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Ti&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMG</td>
<td>Oil</td>
<td>MeOH</td>
<td>Oil</td>
<td>MeOH</td>
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<tr>
<td>Reference strains</td>
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<td></td>
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<tr>
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<td>27</td>
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<tr>
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<td>CIP 37</td>
<td>24</td>
<td>39</td>
<td>30</td>
</tr>
<tr>
<td><em>Escherichia coli</em> CIP 105182</td>
<td>CIP 27</td>
<td>11</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td><em>Listeria innocua</em> LMG 1135668</td>
<td>LMG 34</td>
<td>30</td>
<td>28</td>
<td>21</td>
</tr>
<tr>
<td><em>Salmonella enterica</em> CIP 105150</td>
<td>CIP 35</td>
<td>34</td>
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</tr>
<tr>
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<td>CIP 37</td>
<td>9</td>
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<td>15</td>
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<tr>
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<td>21</td>
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<tr>
<td><em>Staphyloccocus camorum</em> LMG 13567</td>
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<tr>
<td><em>Proteus mirabilis</em> CIP 104588</td>
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<td>14</td>
<td>21</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Clinical</td>
<td>27</td>
<td>25</td>
<td>21</td>
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<td><em>Staphyloccocus aureus</em></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Fungal strains</td>
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<td>17</td>
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<td>17</td>
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<td>13</td>
<td>19</td>
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<tr>
<td><em>Candida albicans</em></td>
<td>Clinical</td>
<td>15</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Clinical</td>
<td>16</td>
<td>11</td>
<td>17</td>
</tr>
</tbody>
</table>

Te: tetracycline, Ti: ticarcycline, ND: not determined, MeOH: methanol extract, Griseo: Griseofulvin
**Table 2:** MIC and MBC (µg/ml) from essential oil and methanol extract of *Boswellia sacra* and *Boswellia papyrifera* from Djibouti

Each value represents mean of three different observations.

<table>
<thead>
<tr>
<th>Reference strains</th>
<th>Origin</th>
<th>MIC</th>
<th>MBC</th>
<th>MIC</th>
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<tr>
<td><em>Enterococcus faecalis</em> CIP 103907</td>
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<td>2</td>
<td>2</td>
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<td>2</td>
<td>2</td>
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<td>32</td>
<td>&gt;32</td>
<td>200</td>
<td>200</td>
<td>4</td>
<td>4</td>
<td>&gt;200</td>
<td>ND</td>
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<tr>
<td><em>Listeria innocua</em> LMG 1135668</td>
<td>LMG</td>
<td>4</td>
<td>4</td>
<td>200</td>
<td>200</td>
<td>4</td>
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<td>&gt;200</td>
<td>ND</td>
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<tr>
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<td>CIP</td>
<td>32</td>
<td>&gt;32</td>
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<td>200</td>
<td>16</td>
<td>&gt;16</td>
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<td>200</td>
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<td>16</td>
<td>&gt;32</td>
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<td>8</td>
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<td>2</td>
<td>2</td>
<td>100</td>
<td>100</td>
<td>4</td>
<td>4</td>
<td>&gt;200</td>
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<tr>
<td><em>Staphylococcus camorum</em> LMG 13567</td>
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<td>8</td>
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<td>200</td>
<td>4</td>
<td>4</td>
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</table>

**Hospital strains:**

| *Pseudomonas aeruginosa* | Clinical | >32 | ND | 200 | 200 | 32 | >32 | >200 | ND |
| *Staphylococcus aureus* | Clinical | 2 | 2 | 100 | 100 | 2 | 2 | 200 | 200 |

**Fungal strains**

| *Candida albicans* ATCC 10231 | ATCC | 0.25 | 0.25 | 50 | 50 | 0.25 | 0.25 | 50 | 50 |
| *Candida albicans* | Clinical | 1 | 1 | >200 | >200 | 0.25 | 0.25 | 50 | 50 |
| *Aspergillus niger* | Clinical | 1 | 1 | 100 | >200 | 4 | >4 | 100 | 100 |
| *Aspergillus sp* | Clinical | 0.5 | 0.5 | >200 | >200 | 0.5 | 0.5 | 100 | 100 |

ND: not determined