In vitro susceptibility of *Bacillus anthracis* to various antibacterial agents and their time–kill activity

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**Objectives:** To investigate the in vitro acquisition of resistance to antibiotics by *Bacillus anthracis*.

**Methods:** The in vitro activities of 18 antibacterial agents against two strains of *B. anthracis*, the Sterne strain and the Russian anthrax vaccine strain ST-1, were tested by determining the MICs and by measuring the rates of antibiotic kill at 5× and 10× MIC.

**Results:** The fluoroquinolones ciprofloxacin, ofloxacin, levofloxacin and moxifloxacin, the β-lactams penicillin G and amoxicillin, the macrolide clarithromycin, the ketolide telithromycin, as well as clindamycin, rifampicin and quinupristin/dalfopristin had MICs in the range of 0.03–0.25 mg/L. Minocycline had an MIC of 0.03 mg/L, as did penicillin, against the ST-1 strain. Ciprofloxacin had an MIC of 0.03 mg/L against both strains. Erythromycin, vancomycin and the oxazolidinone linezolid were less active (MIC 0.5–2.5 mg/L). Ceftriaxone was the least active, having an MIC of 8.0 mg/L. Chloramphenicol was inactive (MIC > 256 mg/L). Quinupristin/dalfopristin, rifampicin and moxifloxacin showed the most rapid bacterial killing, achieving a complete eradication of detectable organisms (2 log\(_{10}\) reduction within 0.5–3 h and 4 log\(_{10}\) reduction within 0.5–4 h for both strains at concentrations of 5× and 10× the MIC). The β-lactams and vancomycin demonstrated a 2–4 log\(_{10}\) reduction within 5–15 h. Ceftriaxone had a similar effect to penicillin and amoxicillin against the ST-1 strain, but a slower effect than these two β-lactams against the Sterne strain. The macrolides, tetracyclines and linezolid demonstrated a lower kill rate, while chloramphenicol did not kill at all.

**Conclusions:** These data expand on the spectrum of agents recommended for the treatment of anthrax (ciprofloxacin, penicillin G and tetracyclines) and add new options, such as other fluoroquinolones, amoxicillin, rifampicin and quinupristin/dalfopristin, as potential therapeutic agents.

Keywords: anthrax, fluoroquinolones, macrolides, β-lactams

**Introduction**

Anthrax has been the recent focus of attention as a potential biological warfare agent. It has been estimated that 50 kg of *Bacillus anthracis* spores released upwind over a population centre of 500,000 would result in up to 95,000 fatalities, with an additional 123,000 persons incapacitated from inhalational anthrax.\(^1\)

Methods of prevention of inhalational anthrax include: protective masks capable of filtering 1–5 μm particles, appropriate sheltering, use of pre- and post-exposure vaccination, and preventive and therapeutic antibiotic regimens. In unvaccinated individuals, antibiotics are the single most effective mode of management of this situation. Recently in the USA, antibiotic prophylaxis has been administered to ~32,000 individuals suspected to have been exposed to anthrax.\(^2\) Up until the recent bioterror attack in the USA, the recommended antibiotics had not been tested in cases of human anthrax, but only in a monkey model with doses of ciprofloxacin, doxycycline and penicillin mimicking human pharmacokinetics.\(^1\) This publication did not study the pharmacodynamics of these agents, as in 1993 pharmacodynamics were at an early stage of development. The possible development of resistance was also not addressed in this study.

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The importance of antibiotic prophylaxis received special inter-
est, mainly because of the current shortage of vaccine and lack of
toxin neutralizing agents (antitoxins). Although the in vitro suscep-
tibility of representative B. anthracis strains has been tested using a
wide variety of antibiotics, little is known about the rate of bacterial
killing by these agents; only a single paper has described the kill
activity of five antibiotics against seven B. anthracis clinical iso-
lates.3 It is accepted that the likelihood of emergence of resistant
mutants may depend on the rate of bacterial killing; the more rapid the
rate the less likely the chance for the emergence of resistant strains.4,5
Moreover, rapid killing diminishes bacterial toxin formation, and
thus may reduce resulting tissue damage.

In the present study we determined the susceptibility and the rate of
to Kill of two B. anthracis strains by 18 antibacterial agents belonging
to different antibiotic classes.

Materials and methods

Antibacterial agents

The antibiotics tested in this study were: ofloxacin and levofloxacin (gifts
from Aventis, Netanya, Israel and Aventis, Paris, France, respectively),
ciprofloxacin and moxifloxacin (a gift from Bayer, Leverkusen,
Germany), minocycline (obtained from Dексон, Caesarea Or-Aqvia,
Israel), tetracycline (obtained from Sigma, Rehovot, Israel), penicillin G
(obtained from Rafa Laboratories, Jerusalem, Israel), amoxicillin
(obtained from GlaxoSmithKline, Petach-Tiqva, Israel), ceftriaxone
(obtained from Roche, Tel-Aviv, Israel), vancomycin (obtained from
Eli Lilly, Indianapolis, IN, USA), erythromycin (purchased from Sigma,
Rehovot, Israel), clarithromycin [obtained from Abbott (Promedico,
Petach-Tiqva, Israel)], telithromycin and quinupristin/dalfopristin (a gift
from Aventis, Paris, France), clindamycin and linezolid [a gift from
Pharmacia (Agis, Bnei-Braq, Israel)], rifampicin (purchased from Sigma,
Rehovot, Israel) and chloramphenicol (obtained from Teva, Jerusalem,
Israel).

Penicillin G, minocycline, vancomycin, erythromycin, rifampicin, clindamycin, linezolid, ceftriaxone and quinupristin/dalfopristin were
each received as a dry laboratory powder and were dissolved in phos-
phate-buffered saline (PBS) (pH 7.2). Amoxicillin was dissolved in
distilled water. Clarithromycin was dissolved in analytical acetone.

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Antibacterial activity against *B. anthracis*

Table 1. MICs of 18 antibiotics against the *B. anthracis* strains ST-1 and the Sterne

<table>
<thead>
<tr>
<th>Antibacterial agent</th>
<th><em>B. anthracis</em> (ST-1)</th>
<th><em>B. anthracis</em> (Sterne)</th>
<th>NCCLS breakpoint (susceptibility) for <em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg/L)</td>
<td>MIC (mg/L)</td>
<td>≤8</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>0.25</td>
<td>0.25</td>
<td>≤8</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.03</td>
<td>0.03</td>
<td>≤4</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.125</td>
<td>0.06</td>
<td>–</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.125</td>
<td>0.125</td>
<td>≤8</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.06</td>
<td>0.125</td>
<td>≤8</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>0.03</td>
<td>0.25</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>8.0</td>
<td>8.0</td>
<td>≤64</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1.25</td>
<td>2.5</td>
<td>≤32</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.125</td>
<td>0.125</td>
<td>≤16</td>
</tr>
<tr>
<td>Minocycline</td>
<td>0.03</td>
<td>0.03</td>
<td>≤16</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1.0</td>
<td>0.5</td>
<td>≤8</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.125</td>
<td>0.125</td>
<td>≤8</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>0.125</td>
<td>0.125</td>
<td>–</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.25</td>
<td>0.125</td>
<td>≤4</td>
</tr>
<tr>
<td>Quinupristin/dalfopristin</td>
<td>0.06</td>
<td>0.125</td>
<td>≤4</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.25</td>
<td>0.125</td>
<td>≤4</td>
</tr>
<tr>
<td>Linezolid</td>
<td>2.0</td>
<td>2.0</td>
<td>≤4</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>512</td>
<td>256</td>
<td>≤32</td>
</tr>
</tbody>
</table>

whereas erythromycin and telithromycin were poorly bactericidal (Table 2).

Tetracycline and minocycline exhibited a similar pattern of killing. No reduction in the bacterial viability was observed within the first 15 h of exposure, while at 24 h these agents caused complete eradication (Table 2).

Quinupristin/dalfopristin was found to be the most potent agent, showing complete kill within 30 min with the ST-1 strain and within 4 h with the Sterne strain (Table 2).

Rifampicin also exhibited a rapid killing activity, with 100% of the bacteria killed within 1–3 h (Table 2).

Chloramphenicol caused no killing effect within 12 h, and only a 2–3 log₁₀ reduction in bacterial count after 22 h (Table 2).

**Discussion**

*In vitro* development of resistance to some antimicrobial agents following serial passages has been reported for *B. anthracis.* In addition, penicillin-resistant *B. anthracis* clinical isolates have also been described. Therefore, additional agents were evaluated to expand the therapeutic options for treatment of resistant *B. anthracis.*

The *B. anthracis* strains used in the present study were highly susceptible to fluoroquinolones, penicillin G and amoxicillin, to the macrolides (except erythromycin), tetracyclines, quinupristin/dalfopristin and rifampicin, with MICs ranging between 0.03 and 0.25 mg/L. Similar results were obtained in other studies using different strains; the slight differences (e.g. one doubling dilution) are probably due to differences in strains tested, growth medium used and methods of MIC determination, and differences in cell cycle stage tested (spores versus vegetative cells).

Vancomycin, erythromycin and linezolid showed an intermediate activity (MIC range 0.5–2 mg/L), as was reported in previous studies.

Ceftriaxone possessed low activity (MIC 8 mg/L). It has been shown previously by Mohammed *et al.* and Bryskier, and others, that *B. anthracis* may be resistant to penicillins and cephalosporins, either by the presence of a β-lactamase or by a different mechanism, and that resistance may even develop during therapy. Thus ceftriaxone and other cephalosporins should not be indicated as therapeutic options for anthrax. Chloramphenicol was inactive (>256 mg/L), consistent with previous observations and is therefore recommended neither for therapy nor as prophylaxis.

The fluoroquinolones showed a rapid killing effect, demonstrating a 3–4 log₁₀ decrease within 4–6 h, with relatively low MICs. Among the fluoroquinolones, moxifloxacin demonstrated the most rapid effect, achieving a complete kill within 2 h. Drago *et al.* reported a similar killing effect of levofloxacin and gatifloxacin at similar concentrations to those used in the present study. Levo- floxacin, on the other hand, needed longer to achieve a 4 log₁₀ kill, and was less potent than ofloxacin. This latter phenomenon may be related to the biphasic response seen with fluoroquinolones rather than a real difference between levofloxacin and ofloxacin.

For rifampicin, a killing effect similar to the effect induced by the fluoroquinolones was observed. The β-lactams and quinupristin/dalfopristin were found to possess the highest bacterial killing effect, achieving a 100% kill of the ST-1 strain within 30 min and a 100% kill of the Sterne strain within 2 h. The killing effects of rifampicin and quinupristin/dalfopristin have not yet been described.

The macrolides and tetracyclines exerted a slow killing effect, with a reduction of only 3 log₁₀ kill, and was less potent than ofloxacin. This latter phenomenon may be related to the biphasic response seen with fluoroquinolones rather than a real difference between levofloxacin and ofloxacin.
B. anthracis addition, rapid kill may diminish the host damage induced by the effect of those agents on that particular microorganism. 15,16 In gatifloxacin has been suggested to be due in part to the rapid killing reduced incidence of be associated with a decreased emergence of resistant strains, e.g. the rate of killing of the test strains, and draws attention to the need for individual study of antibiotic action against each strain. A rapid killing effect is believed to be associated with a decreased emergence of resistant strains, e.g. the reduced incidence of S. pneumoniae resistance to moxifloxacin and gatifloxacin has been suggested to be due in part to the rapid killing effect of those agents on that particular microorganism.15,16 In addition, rapid kill may diminish the host damage induced by the B. anthracis toxins released, such as the lethal factor, oedema factor and protective antigen, as has been shown with the action of ciprofloxacin on several exotoxins of Pseudomonas aeruginosa.17 On the other hand, rapid killing may release larger amounts of toxins and thus increase the damage, as has been suggested for some β-lactams and the release of endotoxin from some Gram-negative bacteria.18 Reduction in bacterial toxin production and release is considered important as clindamycin, which has only a limited killing activity on B. anthracis, has been introduced into various therapeutic schemes in anthrax patients. To our knowledge, no previous studies have compared MICs with the rate of killing of B. anthracis. A rapid killing effect is believed to be associated with a decreased emergence of resistant strains, e.g. the reduced incidence of S. pneumoniae resistance to moxifloxacin and gatifloxacin has been suggested to be due in part to the rapid killing effect of those agents on that particular microorganism.15,16 In addition, rapid kill may diminish the host damage induced by the B. anthracis toxins released, such as the lethal factor, oedema factor and protective antigen, as has been shown with the action of ciprofloxacin on several exotoxins of Pseudomonas aeruginosa.17 On the other hand, rapid killing may release larger amounts of toxins and thus increase the damage, as has been suggested for some β-lactams and the release of endotoxin from some Gram-negative bacteria.18 Reduction in bacterial toxin production and release is considered important as clindamycin, which has only a limited killing activity on B. anthracis, has been introduced into various therapeutic schemes in anthrax patients. To further studies in B. anthracis need to establish the correlation between rapid killing and reduced resistance development. In summary, our results point to additional antibiotics that might be tested as therapeutic agents against anthrax. The rapidity of bacterial kill should also be entertained as a therapeutic advantage in the treatment of clinical cases of anthrax.

### Acknowledgements

This study was supported by a grant from Aventis France and Bayer AG, and by partial support from the Israeli Ministry of Science and Culture.

### References


### Table 2. Rate of killing of B. anthracis strains ST-1 and Sterne

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>5 × MIC 2 log_{10}</th>
<th>10 × MIC 2 log_{10}</th>
<th>5 × MIC 4 log_{10}</th>
<th>10 × MIC 4 log_{10}</th>
<th>5 × MIC 2 log_{10}</th>
<th>10 × MIC 2 log_{10}</th>
<th>5 × MIC 4 log_{10}</th>
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<td>Moxifloxacin</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1.5</td>
<td>1.5</td>
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<td>2</td>
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<td>Ciprofloxacin</td>
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<td>10</td>
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<td>–</td>
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<td>2.5</td>
<td>2.5</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

*The time (h) required to decrease the viability of the bacteria 2 logs by treatment with 5 × MIC of various antibiotics.
*The time (h) required to decrease the viability of the bacteria 2 logs by treatment with 10 × MIC of various antibiotics.
*The time (h) required to decrease the viability of the bacteria 4 logs by treatment with 5 × MIC of various antibiotics.
*The time (h) required to decrease the viability of the bacteria 4 logs by treatment with 10 × MIC of various antibiotics.
*Level of kill not reached at 24 h.
Antibacterial activity against \textit{B. anthracis}


