Susceptibility of *Yersinia pestis* to novel and conventional antimicrobial agents

John Frean¹, Keith P. Klugman²,³*, Lorraine Arntzen¹ and Stanley Bukofzer⁴

¹National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg; ²MRC/University of the Witwatersrand/NHLS Respiratory and Meningeal Pathogens Research Unit, Johannesburg, South Africa; ³Department of International Health, Emory University, Atlanta, GA; ⁴Abbott Laboratories, Abbott Park, Chicago, IL, USA

Received 3 April 2003; returned 28 April 2003; revised 4 June 2003; accepted 10 June 2003

**Objectives:** To determine the susceptibility of southern African strains of *Yersinia pestis* to novel as well as conventional antimicrobial agents.

**Materials and methods:** The MICs of 28 strains of *Yersinia pestis* from a southern African plague focus were determined by agar dilution.

**Results:** The most active agents were cefditoren and the fluoroquinolones, both conventional and novel. The in vitro activity of macrolides was poor against this member of the Enterobacteriaceae.

**Conclusion:** Further investigation of the novel quinolones olamufloxacin (HSR 903) and ABT 492 in animal models of plague would seem to be justified.

Keywords: plague, antibiotics, fluoroquinolones, macrolides, ketolides

**Introduction**

*Yersinia pestis*, the agent of plague, is one of a limited number of organisms that are regarded as serious potential bioterrorism or biowarfare threats.¹* Y. pestis* is a zoonotic pathogen which currently causes naturally-acquired plague in a number of countries around the world. The reservoir is principally rodents of various species, and transmission is usually flea-borne. It is amenable to mass production and aerosol dispersion (thus bypassing the need for flea vectors) and is highly infectious in this form. It is well known that several nations actively pursued research into the development of bioweapons utilizing *Y. pestis* in recent decades.¹,² Untreated primary pneumonic plague has a high mortality, and it has been estimated that 50 kg of aerosolized *Y. pestis*, released over a city of 5 million, would cause up to 150,000 cases of pneumonic plague, of whom 36,000 might be expected to die.³ Apart from the actual deaths which would occur in this scenario, it is likely that there would be widespread civil disruption and general panic, as occurred during the natural outbreak of urban plague in Surat, India, in 1994.⁴ For many decades, reliance has been placed on a limited number of antibiotics for treatment and prophylaxis of plague. Only streptomycin and tetracycline or doxycycline are approved for this purpose by the Food and Drug Administration (FDA) of the United States; however, due to limited availability of streptomycin, gentamicin is often successfully substituted.¹ Chloramphenicol has been advocated for treatment of plague meningitis because of good meningeal penetration, but this has not been tested in human clinical trials. Sulphonamides and trimethoprim–sulamethoxazole are usually regarded as second-line choices for management of plague.¹ There is in vitro and animal experiment evidence for efficacy of fluoroquinolones,⁵,⁶ but clinical trials have not been carried out. The United States Working Group on Civilian Biodefense recommended streptomycin or gentamicin as the preferred choice in a contained casualty setting (i.e. modest numbers of patients requiring treatment), with doxycycline, ciprofloxacin, or chloramphenicol as alternative choices. For a mass casualty situation, the US Working Group recommended oral therapy with doxycycline or tetracycline, or ciprofloxacin.¹ Novel ketolide and penem antimicrobials have previously shown potentially useful in vitro activities,⁵ and we now report on further susceptibility studies in clinical isolates of *Y. pestis*.

**Materials and methods**

*Yersinia pestis* isolates and control organisms

The 28 strains of *Y. pestis* were human blood culture isolates from a plague focus in northern Namibia, isolated between 1982 and 1991. Isolates had been stored in semi-solid agar; purity was checked by plating.

*Corresponding author. Tel: +1-404-712-9001; Fax: +1-404-727-4590; E-mail: kklugma@sph.emory.edu*
Susceptibility of *Y. pestis* to antimicrobial agents

### Table 1. Antimicrobial susceptibility of 28 strains of *Y. pestis*

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (mg/L)</th>
<th>Controls</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>4–32</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16</td>
<td>32</td>
<td>16–32</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25–0.5</td>
<td></td>
</tr>
<tr>
<td>Cefdinir</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.125</td>
<td>0.125</td>
<td>0.063–0.125</td>
<td></td>
</tr>
<tr>
<td>Cefditoren</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.063</td>
<td>0.063</td>
<td>0.031–0.063</td>
<td></td>
</tr>
<tr>
<td>Cethromycin (ABT 773)</td>
<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>4</td>
<td>2–4</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.016</td>
<td>0.031</td>
<td>0.016–0.031</td>
<td></td>
</tr>
<tr>
<td>Temofoxacin</td>
<td>0.125&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.031</td>
<td>0.031</td>
<td>0.016–0.031</td>
<td></td>
</tr>
<tr>
<td>Olamufloxacin (HSR 903)</td>
<td>0.031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.016</td>
<td>0.016</td>
<td>0.008–0.016</td>
<td></td>
</tr>
<tr>
<td>ABT 492</td>
<td>0.031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.016</td>
<td>0.016</td>
<td>0.008–0.016</td>
<td></td>
</tr>
<tr>
<td>Tosufloxacin</td>
<td>0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.004</td>
<td>0.008</td>
<td>0.004–0.008</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>E. coli 25922.  
<sup>b</sup>S. aureus 29213.

onto horse blood agar. The identity of the strains was confirmed by bacteriophage susceptibility testing and key carbohydrate fermentation reactions.

The control organisms used were *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213.

Antibiotics tested

Clarithromycin, erythromycin, cefdinir, cefditoren, ciprofloxacin, tefamoxacin, tosufloxacin and the experimental agents cethromycin (ABT 773, a ketolide), olamufloxacin (HSR 903) and ABT 492 (fluoroquinolones), were all obtained from Abbott Laboratories, Abbott Park, IL, USA. Doxycycline was supplied by Sigma (S. Africa), Midrand, South Africa.

### Determination of antimicrobial susceptibility

All work was done in a class 2 biosafety cabinet in the biosafety level 3 laboratory of the National Health Laboratory Service, Johannesburg, South Africa. We used the agar dilution method to determine MICs, according to the method of the National Committee for Clinical Laboratory Standards (NCCLS), with the following minor modifications: for safety reasons all culturing of *Y. pestis* was done at 28°C, as its Fraction 1 capsular virulence factor is not produced at this temperature, and growth was assessed at 18–20 h (controls) and at 48 h (*Y. pestis*). Incuba of *Y. pestis* were prepared by suspending 18- to 20-h colonies in trypticase soy broth, and adjusting the turbidity to the equivalent of a 0.5 McFarland standard. The MICs at which 50% and 90% of isolates were inhibited (MIC<sub>50</sub> and MIC<sub>90</sub>, respectively) were determined.

### Results and discussion

Results of antimicrobial testing are shown in Table 1. The MICs for the control organisms were within the expected ranges for previously characterized antibiotics. Cefditoren and the fluoroquinolones, both novel and conventional, showed excellent activity against the *Y. pestis* strains tested. Likewise, in our previous study which tested a larger number of isolates (n = 100), the most active agents were the extended spectrum cephalosporin cefotaxime, and the fluoroquinolones levofloxacin and ofloxacin. Although extended-spectrum cephalosporins showed good efficacy in experimental murine septicemic plague, a mouse pneumonic plague model suggested that β-lactam antibiotics are not sufficiently active for treatment purposes, and none are currently recommended for human plague management. The efficacy of fluoroquinolones has been demonstrated in a mouse model of plague and our data support further investigation of fluoroquinolones in animal experiments. Not surprisingly, the *in vitro* activity of macrolides was poor against this member of the Enterobacteriaceae. The ketolide cethromycin (ABT 773) showed enhanced activity compared to the macrolides, but was less active than telithromycin. Antibiotic resistance has not proved to be a major problem in naturally occurring plague, but as in the case of anthrax and other bacterial biowarfare threats, there is the potential for production of resistant strains. Isolates of *Y. pestis* showing tetracycline resistance or reduced susceptibility to streptomycin have been described in some African countries and rare plasmid-mediated multidrug-resistant isolates have been reported from Madagascar. Experimental induction of fluoroquinolone resistance in *Y. pestis* by exposure to nalidixic acid has been described. Both the present and our previous studies have utilized isolates of *Y. pestis* of Southern African origin. These isolates are all the same biotype (orientalis), and ribotyping of five strains revealed two different ribotypes, one of which is typical of most third pandemic strains. A multicentre study of plague antimicrobials, utilizing a range of geographically and genetically diverse pathogen strains, is clearly
required. In the present climate of incipient bioterrorism, it would seem prudent to proactively investigate potential additions to the anti-plague armamentarium.

Acknowledgements

We thank Dr E. Carniel, Institut Pasteur, Paris, for the ribotyping data on our strains. This study was financially supported by Abbott Laboratories, Abbott Park, IL, USA. Some of these data were presented at the 42nd Interscience Conference of Antimicrobial Agents and Chemotherapy, San Diego, CA, USA, September 2002.

References


