Lack of Antimicrobial Resistance in *Yersinia pestis* Isolates from 17 Countries in the Americas, Africa, and Asia

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*Yersinia pestis* is the causative agent of plague, a fulminant disease that is often fatal without antimicrobial treatment. Plasmid (IncA/C)-mediated multidrug resistance in *Y. pestis* was reported in 1995 in Madagascar and has generated considerable public health concern, most recently because of the identification of IncA/C multidrug-resistant plasmids in other zoonotic pathogens. Here, we demonstrate no resistance in 392 *Y. pestis* isolates from 17 countries to eight antimicrobials used for treatment or prophylaxis of plague.

*Yersinia pestis*, the causative agent of plague, is a zoonotic pathogen endemic in rodent populations throughout the Americas, Asia, and Africa (5, 20). Humans contract plague from the bite of infected rodent fleas, contact with infected animals, or inhalation of respiratory aerosols from infected people or animals. In the absence of treatment, plague is a severe and often fatal disease (30 to 100% mortality) (20). Antimicrobial therapy is effective in alleviating illness, particularly when administered early after disease onset (4, 15). Traditional antimicrobials used for treatment and/or prophylaxis of plague patients include aminoglycosides (streptomycin and gentamicin), tetracyclines (doxycycline and tetracycline), chloramphenicol, and trimethoprim-sulfamethoxazole (15). Fluoroquinolones show efficacy in animals and nonhuman primates and have been proposed as newer broad-spectrum antibiotics for the treatment of human plague patients (18, 19, 21).

Naturally occurring plasmid-mediated resistance to antimicrobial agents used for the treatment of plague is a public health concern (4, 11). Plasmid-mediated single- and multiple-drug resistance (MDR) was documented in two *Y. pestis* isolates recovered from separate patients in Madagascar in 1995 (10, 12). The MDR *Y. pestis* plasmid pIP1202, isolated from one of these cases, confers high-level resistance to eight antimicrobial agents (streptomycin, chloramphenicol, tetracycline, sulfonamides, ampicillin, kanamycin, spectinomycin, and minocycline) and is a member of the IncA/C plasmid family (23). IncA/C MDR plasmids have been found in Enterobacteriaceae (Salmonella, Escherichia, and Klebsiella) isolated from agricultural sources, Vibrio cholerae, two fish pathogens (Photobacterium damselae and Aeromonas hydrophila), and the soil bacterium Yersinia ruckeri (1, 9). Phylogenetic analysis of 91 genes conserved among 8 IncA/C plasmids indicates that the pLP1202 plasmid from *Y. pestis* is most closely related to pP99018 and pP91278 from *Photobacterium damselae* (1).

Under laboratory conditions, conjugative transfer of a streptomycin resistance plasmid from *E. coli* (transformed with the pIP1203 plasmid, isolated from the single-drug-resistant *Y. pestis* strain from Madagascar) to *Y. pestis* in the midgut of Xenopsylla cheopis fleas has been documented, generating concern that this scenario could also occur in nature (14). The potential for plasmid exchange in nature between MDR enteric pathogens and *Y. pestis* has also been suggested based on identification of IncA/C MDR plasmids in Enterobacteriaceae isolated from retail meats (poultry and cattle) in the United States (from 2002 onward) and the geographic overlap of MDR *Salmonella* and *Y. pestis* in the southwestern United States (23).

Here, antimicrobial susceptibility was determined for 392 *Y. pestis* isolates (292 of the 392 isolates tested were isolated from 1995 to 2009) from 17 countries in North America, South America, Asia, and Africa and 16 states within the United States (Table 1). Isolates tested included those recovered from recent human cases of plague (1999 to 2009) in the United States (n = 47; all human cases where an isolate was recovered during this time period), Madagascar (n = 34), and Uganda (n = 57). *Y. pestis* isolates recovered from animals as well as fleas (n = 137) in the United States, including all isolates recovered from 2002 to 2009 (n = 55), were also tested. Susceptibility testing was performed using standard CLSI methods and interpretative and quality control criteria for *Y. pestis* (2, 3). Custom broth microdilution plates were prepared by Trek Diagnostic Systems (Cleveland, OH) and contained cation-adjusted Mueller-Hinton broth, pH 7.3 ± 0.1, growth and negative-control wells, and eight antimicrobial agents with doubling dilutions in their therapeutic ranges (0.03 to 64 μg/ml for gentamicin, streptomycin, tetracycline, doxycycline, ciprofloxacin, levofloxacin, and chloramphenicol and 0.03/0.6 to 16/304 μg/ml for trimethoprim-sulfamethoxazole). Plates arrived frozen and were stored at −70°C until use. Isolates were suspended in Mueller-Hinton broth (BD Diagnostic Systems, Franklin Lakes, NJ), with a final inoculum in each well of ~5 × 10⁸ CFU/ml, as determined by colony counts. Plates were incubated in ambient air at 35°C and results read at 24 or 48 h. All work with *Y. pestis* cultures was performed in a biosafety level 3 (BSL-3) laboratory using BSL-3 safety precautions. Quality control strains (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 0066-4804/12/$12.00 Antimicrobial Agents and Chemotherapy p. 555–558 aac.asm.org

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Yersinia pestis, isolated from retail meats (poultry and cattle) in the United States (from 2002 onward) and the geographic overlap of MDR *Salmonella* and *Y. pestis* in the southwestern United States (23).

Here, we demonstrate no resistance in 392 *Y. pestis* isolates from 17 countries to eight antimicrobials used for treatment or prophylaxis of plague.
27853) were tested with every batch of \textit{Y. pestis} isolates to verify that results fell within the acceptable limits.

The distribution of the MICs for the 392 \textit{Y. pestis} isolates with the eight antimicrobials is listed in Table 2. The MIC\textsubscript{50} and MIC\textsubscript{90} are shown in Table 3. For the eight antimicrobial agents tested, the MICs for 390 strains fell within the susceptible range for \textit{Y. pestis}. The two remaining isolates, one from Peru and one from India, each had a reproducible (\textit{Y. pestis}). The two remaining isolates, one from Peru and one from India, each had a reproducible (\textit{Y. pestis}). The two remaining isolates, one from Peru and one from India, each had a reproducible (\textit{Y. pestis}).

**TABLE 1** Geographic and temporal origins of \textit{Y. pestis} isolates tested in this study

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of isolates</th>
<th>Year(s) represented\textsuperscript{a}</th>
<th>No. from source\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolivia</td>
<td>3</td>
<td>1965, 1969, 1990</td>
<td>Animal: 0 Human: 3 Flea: 0</td>
</tr>
<tr>
<td>Brazil</td>
<td>6</td>
<td>1966</td>
<td>Animal: 3 Human: 3 Flea: 0</td>
</tr>
<tr>
<td>China</td>
<td>4</td>
<td>1940, 1958, 1983</td>
<td>Animal: 2 Human: 0 Flea: 2</td>
</tr>
<tr>
<td>Democratic Republic of Congo</td>
<td>6</td>
<td>2006</td>
<td>Animal: 0 Human: 6 Flea: 0</td>
</tr>
<tr>
<td>Ecuador</td>
<td>3</td>
<td>2005</td>
<td>Animal: 3 Human: 0 Flea: 0</td>
</tr>
<tr>
<td>India</td>
<td>4</td>
<td>1955, 1994</td>
<td>Animal: 0 Human: 3 Flea: 1</td>
</tr>
<tr>
<td>Indonesia</td>
<td>2</td>
<td>1983, 1998</td>
<td>Animal: 1 Human: 0 Flea: 1</td>
</tr>
<tr>
<td>Iran</td>
<td>1</td>
<td>1961</td>
<td>Animal: 0 Human: 1 Flea: 0</td>
</tr>
<tr>
<td>Kazakhstan</td>
<td>8</td>
<td>1997-1999</td>
<td>Animal: 4 Human: 2 Flea: 2</td>
</tr>
<tr>
<td>Nepal</td>
<td>3</td>
<td>1969</td>
<td>Animal: 0 Human: 3 Flea: 0</td>
</tr>
<tr>
<td>Peru</td>
<td>5</td>
<td>1994</td>
<td>Animal: 2 Human: 3 Flea: 0</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>5</td>
<td>1984</td>
<td>Animal: 2 Human: 3 Flea: 0</td>
</tr>
<tr>
<td>United States\textsuperscript{c}</td>
<td>214</td>
<td>1971-2009</td>
<td>Animal: 76 Human: 77 Flea: 61</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>4</td>
<td>1976, 1994</td>
<td>Animal: 0 Human: 4 Flea: 0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The avirulent lab strain A1122, originally isolated in 1939, was included.

\textsuperscript{b} The source of two isolates from China was unknown.

\textsuperscript{c} States from which isolates originated included Arizona, California, Colorado, Idaho, Kansas, Montana, Nebraska, Nevada, New Mexico, North Dakota, Oregon, South Dakota, Texas, Utah, Washington, and Wyoming.

h. Antimicrobial-resistant \textit{Y. pestis} isolates could not be included in this study, as the distribution of the single- and multiple-drug-resistant \textit{Y. pestis} isolates from Madagascar is restricted.

Of the patients from the United States, Madagascar, and Uganda from whom isolates were obtained, 32 died of plague, and the available data indicated that 10 of the 32 had been treated either singly or in combination with antimicrobials tested in this study: gentamicin, tetracycline, chloramphenicol, doxycycline, or streptomycin. Isolates recovered from these 10 patients showed no resistance to the antimicrobials used for treatment, providing evidence that treatment failure was not due to infection with an antibiotic-resistant strain of \textit{Y. pestis} or in vivo development of resistance in \textit{Y. pestis}.

Our data indicate no evidence of single-drug resistance or MDR in \textit{Y. pestis} in animals and fleas. This is consistent with the idea that opportunities for plasmid exchange are extremely rare in animals, given that \textit{Y. pestis} infects sterile sites (14). The most likely place for MDR plasmid exchange to occur between MDR pathogens and \textit{Y. pestis} is within the flea gut (14). However, for this to arise and be maintained naturally, the following must occur: (i) an animal must be septicemic with an MDR pathogen, in order for a flea to take up the MDR pathogen into the gut via feeding (i.e., ingestion of a blood meal); (ii) a flea infected with an MDR pathogen must feed on an animal septicemic with \textit{Y. pestis} in order for \textit{Y. pestis} to be transmitted to the bacterium. A number of factors contribute to the low likelihood of this scenario occurring in nature, including but not limited to the following: (i) vector efficiency of flea species for \textit{Y. pestis} is generally quite low; (ii) host specificity of fleas transmitting \textit{Y. pestis} is generally high, although host shifts may occur during plague epizootics; and (iii) host bacteremia levels of >10\textsuperscript{7} CFU/ml for \textit{Y. pestis} are required for infection of fleas, which...
TABLE 3

| Antibiotic                | Range (μg/ml) for:
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All strains (392)</td>
</tr>
<tr>
<td></td>
<td>U.S. strains (214)</td>
</tr>
<tr>
<td></td>
<td>Madagascar strains (53)</td>
</tr>
<tr>
<td></td>
<td>Uganda strains (63)</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.03/0.6–0.25/4.75</td>
</tr>
<tr>
<td></td>
<td>0.06/1.19</td>
</tr>
<tr>
<td></td>
<td>0.12/2.38</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.03/0.6–0.12/2.38</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.03–0.12</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.03–0.06</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>0.25–1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0.5–8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.12–1</td>
</tr>
</tbody>
</table>

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REFERENCES


