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Susceptibilities of *Yersinia pestis* Strains to 12 Antimicrobial Agents

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Ninety-two strains of *Yersinia pestis* recovered over a 21-year period were evaluated for susceptibility to traditional and newer antimicrobial agents. In vitro resistance was noted only against rifampin and imipenem (~20% of strains). The most active compounds (MIC at which 90% of the isolates tested are inhibited) against *Y. pestis* were cefixime, ceftriaxone, trimethoprim-sulfamethoxazole, and trovafloxacin.

Yersinia pestis is the causative agent of plague, and at least three major pandemics have been described in historical records. The second pandemic occurred between the 8th and 14th centuries, where an estimated 17 to 28 million Europeans died from the "Black Death" (6). Today, most episodes of plague are sporadic in nature and are thought to be exquisitely susceptible to commonly administered antimicrobial agents, although few recent studies have been published on the subject.

Several developments have generated a need for reassessment of chemotherapeutic regimens useful in treating *Y. pestis* infections. In 1994, a major outbreak of bubonic and pneumonic plague occurred in India (3). This event sparked international concern regarding the potential transmission of pneumonic plague globally via travel. In 1995, a multidrug-resistant strain of *Y. pestis* was isolated in Madagascar from a 16-year-old boy (5) that was resistant to ampicillin, chloramphenicol, kanamycin, streptomycin, sulfonamides, tetracycline, and minocycline; resistance determinants appeared to be located on a transferable plasmid. Of even more concern is the fact that *Y. pestis* is one of several agents likely to be used as a biological weapon in a bioterrorism event (1). Thus, the importance of defining the range of susceptibility of *Y. pestis* strains from diverse sources to key antimicrobial agents has taken on paramount importance with respect to the treatment and control of incidents due to the factors described above.

Few studies have looked at the in vitro susceptibility of *Y. pestis* strains to antimicrobial agents. Smith et al. (7) studied the susceptibility of 78 Vietnamese strains to 14 antimicrobial agents. The most active agents in vitro were ceftriaxone, ciprofloxacin, ofloxacin, and ampicillin. In 1996, Frean and colleagues (4) studied the in vitro susceptibility of 100 South African isolates to newer antimicrobial agents. Among oral antimicrobial agents, two quinolones (levofloxacin and ofloxacin) were found to be extremely active in vitro against *Y. pestis*, while cefotaxime was found to be the most active nonparenteral agent tested. However, these studies have focused on the analysis of strains from a limited number of sources. Because of concerns regarding bioterrorism (1) and recently described drug resistance in this species (5), we have investigated the susceptibility patterns of a large collection of *Y. pestis* strains to 12 antimicrobial agents.

Ninety-two strains of *Y. pestis* were tested in the present investigation. These strains were isolated by the Microbial Diseases Laboratory over a 21-year period (1977 to 1998). The sources of these 92 strains are as follows: animal carcasses (squirrels and chipmunks), $n = 39$; humans, $n = 26$; fleas, $n = 20$; cats, $n = 4$; and miscellaneous, $n = 3$. All strains were confirmed as *Y. pestis* by standard criteria, including direct fluorescent antibody testing with a species-specific conjugate and by lysis with *Y. pestis* phage at 25°C. The positive and negative control strains used for the direct fluorescent antibody test were *Y. pestis* 97A-7975 and *Franciscella tularensis* 93A-4254. The MICs of 12 antimicrobial agents for *Y. pestis* were determined by Biodisk E test (Remel, Lexena, Kans.). The antimicrobial agents tested included ampicillin, cefixime, ceftazidime, ceftriaxone, chloramphenicol, doxycycline, gentamicin, imipenem, rifampin, trimethoprim-sulfamethoxazole, streptomycin, and trovafloxacin. Stored isolates (−70°C) were reconstituted in heart infusion (HI) broth overnight and resulting cultures were then used to streak sheep blood agar plates (HI based) and incubated at 35°C. Overnight growth was used to make a no. 1 MacFarland suspension (3.5×10^8 CFU/ml) from which a sheep blood Mueller-Hinton plate (100 mm in diameter) was inoculated, and E test strips were applied. After overnight incubation at 35°C, plates were examined and MICs were recorded. Interpretation criteria were based upon National Committee for Clinical Laboratory Standards guidelines for rapidly growing gram-negative rods. Control strains included *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922.

All *Y. pestis* strains were found susceptible to antimicrobial agents traditionally recommended for the treatment of *Y. pestis* infections, including streptomycin, doxycycline, and chloramphenicol (Table 1). Plague bacilli demonstrated in vitro resistance to only two antimicrobials—rifampin and imipenem. For imipenem, 19 of 92 strains tested (20.6%) exhibited in vitro resistance to imipenem. This appeared as a thin film of secondary growth within a primary zone (halo) of clearing. Subculturing of this growth confirmed this film as viable *Y. pestis*. When resistant colonies were regrown in broth and retested, a completely susceptible population resulted. However, the initial phenomenon could be repeatedly reproduced by subculturing strains from the original freezer stock. All strains demonstrating this transient resistance were isolated from fleas or animal carcasses. Fifteen of these 19 strains (79%) were confirmed as resistant to imipenem by disk diffusion (no zone [controls within limits]). Imipenem resistance did not correlate with plasmid carriage, because six strains screened (all rifampin resistant, three imipenem resistant) contained identical plasmids of 110, 70, and 19 kb, with one exception (one strain lacked a 70-kb plasmid). Major differences in MICs at which 50 or 90% (MIC_{50}s or MIC_{90}s) of the isolates were inhibited by

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TABLE 1. MICs of 12 antimicrobial agents for *Y. pestis*

Agent	MIC (μg/ml)[a]		
	Range	50%	90%
Ampicillin	0.094–0.38	0.25	0.38
Cefixime	0.006–0.032	0.016	0.023
Ceftazidime	0.016–0.19	0.094	0.125
Ceftriaxone	0.006–0.032	0.016	0.023
Chloramphenicol	0.25–4.0	1.5	2.0
Doxycycline	0.125–2.0	1.0	1.5
Gentamicin	0.19–1.0	0.38	0.75
Imipenem	0.094–>32	0.5	>32
Rifampin	2–32	8.0	16
Streptomycin	1.5–4.0	3.0	3.0
Trimethoprim-sulfamethoxazole	0.012–0.047	0.023	0.032
Trovafloxacin	0.006–0.047	0.023	0.032

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

antimicrobial agents were not detected regardless of the source of the isolate (human, flea, animals).

The initial reason for undertaking this investigation was increasing concern regarding possible emerging drug resistance in *Y. pestis* (5) and the potential impact such resistance could have if this organism were used as an agent of bioterrorism. The use of a large panel of strains collected over two decades from diverse sources indicates that this bacterium remains highly susceptible not only to drugs traditionally used to treat plague, but also to newer agents, including broad-spectrum cephalosporins and quinolones. Although there is a recent report of a fulminant case of pneumonic plague in a 31-year-old male who succumbed to infection while on ciprofloxacin (2; P. M. Shah, Letter, J. Antimicrob. Chemother. **42:**399, 1998), it is unclear what conclusions can be drawn about the efficacy of quinolone therapy from a single case of advanced illness. Because of the transient resistance to imipenem noted in ~20% of isolates tested, one should be careful in considering the use of carbapenems for treating *Y. pestis* infections. The documentation of multidrug resistance in this species and its potential use in bioterrorism make it important that collections of *Y. pestis* strains continue to be screened for antimicrobial resistance to monitor for potential changes with regard to the susceptibility status of this important species.

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