

## In Vitro Antimicrobial Susceptibilities of Strains of *Yersinia pestis*

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**The in vitro activities of 14 antimicrobial agents were determined for 78 strains of *Yersinia pestis*. The most active antibiotics were ceftriaxone and ciprofloxacin, followed by ofloxacin and ampicillin. The agents traditionally used for the treatment of plague (streptomycin, tetracycline, and chloramphenicol) were considerably less active. Azithromycin showed poor activity against all strains.**

Although plague is best known in history for its devastating epidemics, the infection is still endemic in many parts of the world today. Plague is reported consistently from several countries in Africa, Asia, and South America and has also been reported in the United States. Sporadic outbreaks, such as that reported from India during late 1994, are a reminder of its lethal potential.

Streptomycin, tetracycline, and chloramphenicol are traditionally considered the most effective antibiotics, but there have been no clinical evaluations of different treatment regimens in recent years and no reported experience with newer antimicrobial agents such as the fluoroquinolones and expanded-spectrum cephalosporins.

In this study we have tested the in vitro susceptibilities of *Yersinia pestis* strains isolated in Vietnam to 14 antimicrobial agents including ceftriaxone, ciprofloxacin, ofloxacin, and the new macrolide azithromycin.

The 78 strains of *Y. pestis* used in this study were isolated in the western Central Highlands (Tây Nguyên) area of Vietnam between 1985 and 1993. Fifty-seven strains were from patients with plague, 18 strains were isolated from rats, and three strains were isolated from fleas. MICs were determined for all 78 strains, and MBCs were determined for 10 randomly selected strains.

The antimicrobial agents tested were gifts from their respective manufacturers: ampicillin (SmithKline Beecham Pharmaceuticals, Betchworth, United Kingdom), azithromycin and doxycycline (Pfizer International Ltd., Bangkok, Thailand), ceftriaxone (Roche Products Ltd., Welwyn Garden City, United Kingdom), chloramphenicol (Parke Davis Research Laboratories, Pontypool, United Kingdom), ciprofloxacin (Bayer Ltd., Newbury, United Kingdom), gentamicin and ofloxacin (Roussel Laboratories Ltd., Uxbridge, United Kingdom), penicillin (Government Pharmaceutical Organization, Bangkok, Thailand), rifampin (Merrell Dow Pharmaceuticals Ltd., Uxbridge, United Kingdom), streptomycin (M & H Manufacturing Co. Ltd., Bangkok, Thailand), sulfamethoxazole and trimethoprim (The Wellcome Foundation, Crewe, United

Kingdom), and tetracycline (Lederle Laboratories Ltd., Gosport, United Kingdom).

MICs were determined by an agar dilution method following National Committee for Clinical Laboratory Standards guidelines (7), using Mueller-Hinton agar and a multipoint inoculator with an inoculum of  $10^4$  CFU per spot. MBCs were determined by a microdilution method following National Committee for Clinical Laboratory Standards tentative guidelines (6). The tests were performed in sterile microtiter trays with a final inoculum of approximately  $5 \times 10^5$  CFU/ml in 100  $\mu$ l of Mueller-Hinton broth per well. The trays were incubated at 27 to 30°C for 24 h. Then, 10- $\mu$ l aliquots from wells with no visible growth were cultured onto nutrient agar and incubated for 24 to 48 h. The MBC was defined as the concentration killing 99.9% of the initial inoculum.

MIC and MBC results are shown in Table 1. The most active antibiotics in vitro are ceftriaxone and ciprofloxacin, followed by ofloxacin and ampicillin. The antimicrobial agents traditionally used for treatment of plague (streptomycin, tetracycline, and chloramphenicol) are considerably less active. Gentamicin and doxycycline are more active than streptomycin and tetracycline, respectively. No strain of *Y. pestis* was fully susceptible to azithromycin or rifampin, and intermediate susceptibility occurred in only 2 and 15% of strains, respectively.

MBCs of azithromycin and sulfamethoxazole were not tested. For the remaining antimicrobial agents, MBCs were the same or onefold higher than the corresponding MIC, except for chloramphenicol for which the MBC was twofold higher.

In clinically suspected cases of plague, appropriate antibiotic therapy should be started as soon as specimens have been taken for microbiological confirmation. *Y. pestis* multiplies rapidly in vivo, and even bubonic plague can evolve quickly into a life-threatening disease.

*Y. pestis* remains consistently highly susceptible to a wide range of antibiotics. One strain isolated from a Vietnamese rat was reportedly resistant to streptomycin (25  $\mu$ g/ml) (5, 10), but testing was not done by currently approved methods. Although streptomycin is usually regarded as the treatment of choice for plague, kanamycin has been used successfully for treatment in Vietnamese patients (3). Gentamicin has greater in vitro activity against *Y. pestis* and is now more widely used in other infections than the earlier aminoglycosides.

Tetracycline is often used for the oral treatment of milder cases and is useful when prophylaxis is considered necessary.

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TABLE 1. MICs and MBCs for *Y. pestis* strains

Antibiotic	MIC ( $\mu\text{g/ml}$ ) <sup>a</sup>			MBC ( $\mu\text{g/ml}$ ) <sup>b</sup>		
	50%	90%	Range	50%	90%	Range
Gentamicin	0.5	1	0.25–1	0.5	2	0.5–2
Streptomycin	4	4	4–8	4	8	4–16
Doxycycline	0.5	1	0.25–1	1	2	0.5–2
Tetracycline	2	4	0.5–4	4	8	1–8
Ciprofloxacin	0.031	0.062	0.008–0.062	0.062	0.062	0.016–0.125
Ofloxacin	0.125	0.25	0.031–0.25	0.125	0.25	0.062–0.5
Penicillin	1	2	0.25–2	1	1	0.25–1
Ampicillin	0.25	0.5	0.125–0.5	0.25	0.5	0.125–0.5
Ceftriaxone	0.016	0.031	0.008–0.031	0.016	0.031	0.016–0.031
Azithromycin	8 <sup>c</sup>	32 <sup>c</sup>	4–32 <sup>c</sup>	NT <sup>d</sup>	NT	NT
Chloramphenicol	2	4	0.5–4	4	8	2–16
Rifampin	4	8	2–8	4	8	4–8
Sulfamethoxazole	8	16	2–32	NT	NT	NT
Trimethoprim	0.5	1	0.5–1	2	2	1–4

<sup>a</sup> 50% and 90%, MICs for 50 and 90% of strains tested, respectively. A total of 78 strains were tested.

<sup>b</sup> 50% and 90%, MBCs for 50% and 90% of strains tested, respectively. A total of 10 strains were tested.

<sup>c</sup> MICs tested against 43 strains.

<sup>d</sup> NT, not tested.

Resistance to tetracycline has been reported from one study in Madagascar (8). Doxycycline should be preferable to tetracycline, because it has better activity against *Y. pestis* and requires only one dose a day.

Although it has not been recommended for treatment of plague, ampicillin is very active in vitro against *Y. pestis*. Butler (2) reported that ampicillin was successful in reducing mortality in a murine model of *Y. pestis* infection but was slightly less effective than streptomycin.

The fluoroquinolone compounds and ceftriaxone are the most active agents in vitro against *Y. pestis*. In animal models, the expanded-spectrum cephalosporins and imipenem have failed in the treatment of other yersinioses despite having good in vitro activity (4, 9). In contrast, ceftriaxone was as effective

as ofloxacin or streptomycin in a murine model of *Y. pestis* infection (1). However, only one bacterial strain was used in this study and efficacy was measured by reduction in bacterial counts rather than mortality.

The fluoroquinolone compounds offer the best chance to improve the treatment of plague. They are highly active against all pathogenic yersiniae in vitro and in vivo, are very well tolerated, and can be given by parenteral and oral routes. However, they cannot be recommended generally until they have been proved clinically to be effective.

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