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In Vitro Activities of 14 Antibiotics against 100 Human Isolates of *Yersinia pestis* from a Southern African Plague Focus

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A limited repertoire of antimicrobial agents is currently in use for the treatment of plague. We investigated the in vitro activities of some newer antimicrobial agents against *Yersinia pestis*. Among the injectable agents tested, cefotaxime was the most active, and among the oral agents, both levofloxacin and ofloxacin were highly active, with MICs at which 90% of isolates are inhibited of <0.03 μg/ml. The susceptibilities to the ketolide RU004 and the penem faropenem warrant attention. The enhanced activities of quinolones against *Y. pestis* suggest that these agents should be further investigated for the treatment of human plague in the future.

At about the time of the centennial in 1994 of the discovery of *Yersinia pestis*, the agent of plague (4), outbreaks in India (7) and Mozambique (2) raised the profile of a disease which has a widespread distribution. At least 11 countries in Africa have reported plague since 1978; Madagascar, Tanzania, and Zaire have been areas in Africa with the most plague activity in the last 5 years (19). Another important African plague focus is Ovamboland in northern Namibia (18). The overall case fatality rate from plague is about 10%, although it was higher (15%) among patients with reported cases of the disease in Africa between 1976 and 1990 (10). In view of the high mortality rate among individuals with untreated plague, effective antibiotic treatment is mandatory (4). For the last half of the century which has elapsed since the discovery of the plague bacillus, treatment has relied mainly on streptomycin, chloramphenicol, and tetracycline (13), which are still recommended in current reference books (5). In view of plague's serious complications (septicemia, meningitis, and pneumonic plague), this could be viewed as a limited therapeutic repertoire which is in need of supplementation with newer antibiotics (3). We therefore determined the MICs of 14 antibiotics, chosen to represent conventional as well as newer antimicrobial agents, for 100 *Y. pestis* isolates using the agar dilution method. The strains were isolated between 1982 and 1991 at the South African Institute for Medical Research, Johannesburg, from humans with plague in Ovamboland, northern Namibia. The isolates had been stored in semisolid medium at room temperature; they were plated onto blood agar, and the purity was checked. Identification was reconfirmed by bacteriophage susceptibility testing and carbohydrate fermentation reactions (1).

Escherichia coli ATCC 25922 was used as the control organism. Test and control organisms were grown overnight at 37°C. The growth was standardized so that the turbidity matched that of a McFarland 0.5 standard, and the MICs were determined by the agar dilution method by following the standard methods of the National Committee for Clinical Laboratory Standards (14). All manipulation of the bacteria was done in a class 2 biosafety hood in the P3 laboratory facility at the South African Institute for Medical Research. The MICs at which 50 and 90% of isolates are inhibited (MIC_{50}s and MIC_{90}, respectively) were determined by the "orderly array" method (8).

Levofloxacin, ofloxacin, cefotaxime, roxithromycin, the ketolide RU004, and faropenem were obtained from Hoechst Marion Roussel, Romainville, France; trimethoprim, sulfamethoxazole, tetracycline, doxycycline, streptomycin, rifampin, erythromycin, and chloramphenicol were obtained from Sigma Chemical Company, St. Louis, Mo.; and amoxicillin was obtained from SmithKline Beecham, Brockham Park, United Kingdom.

The results of the in vitro susceptibility tests are summarized in Table 1. Consistent with previous studies of southern African strains (6), all of the isolates were susceptible to streptomycin, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, doxycycline, and amoxicillin. These represent antibiotics which are or which have been used for the treatment of plague. The clinical effectiveness of streptomycin, chloramphenicol, and tetracycline remains high, and resistance has not been a problem to date (5). Agents which have been clinically less effective or more toxic are sulfonamides, trimethoprim-sulfamethoxazole, kanamycin, and ampicillin (5).

The potential of quinolones for treating yersinial infections was proposed some time ago (9). Ciprofloxacin prophylaxis was highly effective against intraperitoneal challenge with *Y. pestis* in outbred mice, but postinfection treatment with ciprofloxacin was less protective (16). Similarly, another comparative study performed postinfection indicated that single injections of ofloxacin, amoxicillin, doxycycline, gentamicin, cefotaxime, ceftriaxone, and streptomycin all reduced mouse spleen colony counts, but did not eliminate *Y. pestis*. With repeated injections, all of these antibiotics reduced spleen colony counts to the limit of detection, with streptomycin, ceftriaxone, and ofloxacin being the most rapidly effective (3). The activity of ofloxacin in the present study also corroborates the in vivo efficacies of fluoroquinolones in murine septicemia caused by *Yersinia pseudotuberculosis* (11). In our study, cefotaxime and the fluoroquinolones levofloxacin and ofloxacin were all highly active against all of the strains tested (MIC_{90}s, <0.03 μg/ml). The ofloxacin MIC_{90} was 0.12 μg/ml for a smaller number of strains tested by Bonacorsi et al. (3). In their study, doxycycline and cefotaxime MICs were similar to the MICs found in our study, while streptomycin MICs for their strains were higher (3). Reduced susceptibility to streptomycin has also been noted in some strains of *Y. pestis* from Madagascar (15).

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TABLE 1. In vitro activities of 14 antibiotics against 100 isolates of *Y. pestis*

Antibiotic	MIC (μg/ml) for 100 isolates		
	Range	50%	90%
Levofloxacin	<0.03–0.06	<0.03	<0.03
Ofloxacin	<0.03–0.12	<0.03	<0.03
Tetracycline	<0.03–2.0	1.0	2.0
Doxycycline	<0.03–4.0	0.25	1.0
Streptomycin	<0.03–2.0	0.5	0.5
Rifampin	<0.03–8.0	2.0	8.0
TMP-SMX[a]	<0.03/0.59–0.06/1.18	0.03/0.59	0.06/1.18
Cefotaxime	<0.03	<0.03	<0.03
Erythromycin	<0.03–>16	4.0	16.0
Roxithromycin	0.25–>16	>16.0	>16.0
RU004	<0.03–0.5	0.12	0.5
Chloramphenicol	0.06–2.0	0.5	1.0
Amoxicillin	<0.03–0.25	<0.03	0.12
Faropenem	<0.03–0.5	0.25	0.5

[a] TMP-SMX, trimethoprim-sulfamethoxazole.

The MIC_{90}s of erythromycin and roxithromycin (≥16 μg/ml) are in keeping with the macrolide antibiotics' general lack of activity against members of the family *Enterobacteriaceae*; in contrast, the low MICs of the ketolide RU004 indicate that it may be worthy of further investigation. The wide range of MICs of the macrolides achieved for the strains suggests that the study encompassed a diverse population of strains. Faropenem is a new oral penem active against a broad range of the family *Enterobacteriaceae*, including those expressing extended-spectrum β-lactamases (12). The low MICs of faropenem are consistent with the penems' established efficacy against members of the family *Enterobacteriaceae.*

The high degree of activity shown by the fluoroquinolones and the extended-spectrum cephalosporins suggests that these may be among the candidates suitable for the treatment of plague in the future. In this context, a Russian report of quinolone-resistant mutants of virulent *Y. pestis*, induced by a single exposure to nalidixic acid, is of interest (17). The mutation frequency was 10^{-10} to 10^{-8}. There was cross-resistance with ciprofloxacin, which resulted in treatment failure in experimental infections in mice. While the implications of that report require further investigation, the high intracellular concentrations achievable by fluoroquinolones, combined with the low MICs found in the present study, suggest that further studies on the clinical usefulness of this class of antimicrobial agents against *Y. pestis* infection are warranted.

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