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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 pIP1202 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 $\sim 5 \times 10^5$ 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

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

Country	No. of isolates	Year(s) represented[a]	No. from source[b]		
			Animal	Human	Flea
Bolivia	3	1965, 1969, 1990	0	3	0
Brazil	6	1966	3	3	0
China	4	1940, 1958, 1983	0	2	0
Democratic Republic of Congo	6	2006	0	6	0
Ecuador	3	2005	3	0	0
India	4	1955, 1994	0	3	1
Indonesia	2	1983, 1998	1	0	1
Iran	1	1961	0	1	0
Kazakhstan	8	1997-1999	4	2	2
Madagascar	53	2004-2007	0	53	0
Nepal	3	1969	0	3	0
Peru	5	1994	2	3	0
Saudi Arabia	5	1984	2	3	0
Uganda	63	2004, 2006, 2008-2009	0	63	0
United States[c]	214	1971-2009	76	77	61
Vietnam	8	1990, 1995, 1998-2000	5	3	1
Zimbabwe	4	1976, 1994	0	4	0

[a] The avirulent lab strain A1122, originally isolated in 1939, was included.

[b] The source of two isolates from China was unknown.

[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.

27853) were tested with every batch of *Y. pestis* isolates to verify that results fell within the acceptable limits.

The distribution of the MICs for the 392 *Y. pestis* isolates with the eight antimicrobials is listed in Table 2. The MIC_{50}s and MIC_{90}s are shown in Table 3. For the eight antimicrobial agents tested, the MICs for 390 strains fell within the susceptible range for *Y. pestis*. The two remaining isolates, one from Peru and one from India, each had a reproducible ($n = 2$) MIC in the intermediate range (8 μg/ml) for streptomycin. The MIC_{50} and MIC_{90} were within one doubling dilution when U.S. ($n = 214$), Madagascar ($n = 53$), and Uganda ($n = 63$) isolates were compared to the entire data set, indicating similar susceptibilities for isolates from different geographic regions (Table 3). Overall, the most active antimicrobial agents *in vitro* were ciprofloxacin and levofloxacin and the least active was streptomycin (for 67% of isolates tested, the MIC of streptomycin was 4 μg/ml, the upper limit of the sensitive range). MICs for 384 isolates (98%) could be determined at 24 h, indicating that for the majority of isolates, susceptibility testing for *Y. pestis* via broth microdilution can be completed in 24 h. Antimicrobial-resistant *Y. pestis* isolates could not be included in this study, as the distribution of the single- and multiple-drug-resistant *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 *Y. pestis* or *in vivo* development of resistance in *Y. pestis*.

Our data indicate no evidence of single-drug resistance or MDR in *Y. pestis* in animals and fleas. This is consistent with the idea that opportunities for plasmid exchange are extremely rare in animals, given that *Y. pestis* infects sterile sites (14). The most likely place for MDR plasmid exchange to occur between MDR pathogens and *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 *Y. pestis* in order to have coinfection with both the MDR pathogen and *Y. pestis* in the flea gut; (iii) conjugative plasmid exchange must occur in the flea coinfected with *Y. pestis* and an MDR pathogen; and (iv) the flea containing MDR *Y. pestis* must feed on another rodent and transmit 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 *Y. pestis* is generally quite low; (ii) host specificity of fleas transmitting *Y. pestis* is generally high, although host shifts may occur during plague epizootics; and (iii) host bacteremia levels of $>10^7$ CFU/ml for *Y. pestis* are required for infection of fleas, which

TABLE 2 Antimicrobial MIC distributions for *Y. pestis* isolates in this study ($n = 392$)

Antimicrobial	No. of isolates with MIC (μg/ml)[a]											
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Gentamicin			3	260	110	18	1					
Streptomycin					2	6	119	263	2			
Tetracycline				11	154	224	3					
Doxycycline			4	66	245	77						
Ciprofloxicin	371	20	1									
Levofloxacin	385	7										
Chloramphenicol					34	86	201	71				
Trimethoprim-sulfamethoxazole	9	333	49	1								

[a] The susceptibility breakpoint for gentamicin, streptomycin, tetracycline, doxycycline is 4 μg/ml, that for ciprofloxacin and levofloxacin is 0.25 μg/ml, that for chloramphenicol is 8 μg/ml, and that for trimethoprim-sulfamethoxazole is 2 μg/ml.

typically results in the death of the host after only a very short infectious period (6, 13).

Although geographic overlap between MDR *Salmonella* and *Y. pestis* occurs in the southwestern United States, identification of bacterial communities within fleas has failed to identify *Enterobacteriaceae*, suggesting that little opportunity exists for MDR enteric pathogens and *Y. pestis* to come into contact in this environment (6, 7, 13, 16, 17). Previous studies demonstrated that the ability of *Xenopsylla cheopis* fleas to transmit *Y. pestis* was severely compromised when the fleas were coinfected with *Salmonella* (8). In addition, retail animals and rodents are not typically bacteremic with enteric pathogens, particularly at the levels required for infection of fleas, thereby limiting the possibility for fleas to become infected with MDR enteric pathogens via this route.

Given that the only case of MDR *Y. pestis* documented to data occurred in Madagascar >15 years ago, there appears to be little or no selective advantage for *Y. pestis* to maintain a MDR plasmid generated in natural cycles. Galimand et al. reported in 2006 a lack of MDR in *Y. pestis* strains isolated from humans, rats, and fleas in Madagascar after 1995 (11). Similarly, we found no evidence of resistance in isolates of *Y. pestis* isolated from patients in Madagascar from 2004 to 2007. The selective advantage for enteric pathogens of maintaining MDR plasmids is clear, as 17 classes of antimicrobial agents are approved by the FDA for retail animal growth promotion and production efficiency (22). In contrast, there is no obvious advantage for *Y. pestis* to maintain MDR plasmids in natural plague cycles, as rodent and vector populations necessary for maintenance of *Y. pestis* are not routinely exposed to antibiotics used for human treatment.

In conclusion, this study demonstrated a lack of antimicrobial resistance in 392 *Y. pestis* isolates from 17 countries to drugs used in the treatment and prophylaxis of plague. Antimicrobial susceptibility monitoring of *Y. pestis* isolates has been carried out routinely in the United States since 1995. Continued monitoring will be important for providing accurate public health information regarding *Y. pestis* and antimicrobial susceptibility.

TABLE 3 MIC_{50}s and MIC_{90}s

Antimicrobial	MIC (μg/ml) for:											
	All strains (392)			U.S. strains (214)			Madagascar strains (53)			Uganda strains (63)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Aminoglycosides												
Gentamicin	0.12–2	0.25	0.5	0.12–1	0.25	0.5	0.25–0.5	0.25	0.5	0.25–2	0.5	1
Streptomycin	0.5–8	4	4	0.5–4	4	4	2–4	4	4	1–4	4	4
Tetracyclines												
Tetracycline	0.25–2	1	1	0.25–2	1	1	0.5–1	1	1	0.25–0.5	0.5	0.5
Doxycycline	0.12–1	0.5	1	0.12–1	0.5	1	0.5–1	0.5	1	0.12–0.25	0.25	0.25
Fluoroquinolones												
Ciprofloxacin	0.03–0.12	0.03	0.03	0.03–0.12	0.03	0.03	0.03–0.06	0.03	0.06	0.03	0.03	0.03
Levofloxacin	0.03–0.06	0.03	0.03	0.03–0.06	0.03	0.03	0.03–0.03	0.03	0.03	0.03	0.03	0.03
Phenicol												
Chloramphenicol	0.5–4	2	4	0.5–4	2	4	1–4	2	4	0.5–2	1	1
Other												
Trimethoprim–sulfamethoxazole	0.03/0.6–0.25/4.75	0.06/1.19	0.12/2.38	0.03/0.6–0.12/2.38	0.06/1.19	0.12/2.38	0.06/1.19–0.12/2.38	0.06/1.19	0.06/1.19	0.06/1.19–0.12/2.38	0.06/1.19	0.12/2.38

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