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In Vitro Efficacy of Antibiotics Commonly Used To Treat Human Plague against Intracellular *Yersinia pestis*▽

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Yersinia pestis **initiates infection as a facultative intracellular parasite in host macrophages; however, little is known about the efficacy of antibiotics commonly used to treat human plague against intracellular *Y. pestis*. Intracellular minimal bactericidal concentrations (MBCs) were determined using a high-throughput broth microdilution assay in which human THP-1 macrophage-like cells were infected with *Y. pestis* strain KIM6-2053.1+ and exposed to 2-fold serial dilutions of antibiotics for 24 h in 96-well plates. The numbers of CFU, upon which minimal bactericidal concentrations were based, were determined by counting "microcolonies" in wells of 96-well plates following lysis of tissue culture cells to release surviving *Y. pestis*, replica dilution, and plating in soft tryptic soy broth agar. For THP-1 cells, streptomycin and ciprofloxacin had comparable efficacies for intra- and extracellular *Y. pestis*, but the MBCs for chloramphenicol, gentamicin, doxycycline, and amoxicillin were two-, three-, four-, and five 2-fold serial dilutions greater, respectively, for intracellular than for extracellular *Y. pestis*. During the initial stage of plague, intracellular *Y. pestis* may be less susceptible to antibiotic killing by particular antibiotics recommended for treatment of plague, such as gentamicin or doxycycline, whereas others, such as streptomycin and ciprofloxacin, may have similar efficacies against extracellular or intracellular *Y. pestis*. This may be of particular importance in the selection of antibiotics for prophylactic treatment in the case of a bioterrorism event.**

Yersinia pestis, a facultative intracellular Gram-negative bacterium, causes vector-transmitted plague epizootics in definitive rodent hosts and sporadic cases in dead-end hosts such as humans (22, 27). In humans, three common clinical presentations for plague are bubonic, septicemic, and pneumonic, with bubonic being the most prevalent and least severe form and pneumonic the least prevalent and most severe of naturally transmitted plague. Unnatural transmission by bioterrorism is anticipated to result in virtually 100% primary pneumonic plague (3, 13, 19). Case fatality rates for bubonic and septicemic plague can be as high as 40%, underlying the potential for the difficulty of successful treatment of plague with antibiotic therapy (10, 17), whereas case fatality rates for pneumonic plague can be 100% if not treated within 24 h of onset of clinical symptoms. Although most strains of *Y. pestis* are sensitive to commercially available antibiotics *in vitro*, treatment failures occur (4, 12). These failures are attributed typically to delayed diagnosis, untimely initiation of treatment, or use of inappropriate antibiotics (4, 7, 11). However, another potential cause is the failure of antibiotics to kill intracellular *Y. pestis* (1, 13). During the initial 36-to-72-h phase of infection, *Y. pestis* invades host macrophages to escape innate immunity (25), and this intracellular *Y. pestis* may be less sensitive to antibiotic killing (1; J. D. Wendte, D. Ponnusamy, and K. D. Clinkenbeard, presented at the 6th ASM Biodefense and Emerging Diseases Research Meeting, Baltimore, MD, 2008). Prophylactic antibiotic treatment for a mass casualty bioterrorism event may benefit from antibiotics that are efficacious against intracellular *Y. pestis*.

Streptomycin, introduced in 1944, continues to be recommended as the first-line treatment of human plague (3, 18), although issues with resistance, the requirement for injectable rather than oral delivery, and limited product availability suggest that newer and more readily available antibiotics might be better choices for treatment of plague. In addition to streptomycin, other antibiotics recommended for treatment of plague are gentamicin, doxycycline, and ciprofloxacin; however, there are limited studies upon which to base these recommendations (4, 23, 26).

We developed a high-throughput (HTP) assay to study antibiotic efficacy against intracellular *Y. pestis* (J. D. Wendte et al., presented at the 6th ASM Biodefense and Emerging Diseases Research Meeting, Baltimore, MD, 2008), with the aim of providing additional data for the rational use of antibiotics for treatment of plague, particularly those with efficacy against intracellular *Y. pestis.* We found that two of four antibiotics recommended for treatment of plague, gentamicin and doxycycline, were markedly less efficacious against intracellular *Y. pestis in vitro*, whereas ciprofloxacin and streptomycin had activity against intracellular *Y. pestis* similar to or better than that against extracellular *Y. pestis* (J. D. Wendte et al., presented at the 6th ASM Biodefense and Emerging Diseases Research Meeting, Baltimore, MD, 2008). The Working Group on

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Civilian Biodefense recommends doxycycline for the mass-casualty setting and postexposure prophylaxis, with ciprofloxacin as an alternative (18), but doxycycline does not appear to be as effective against intracellular *Y. pestis*, the form of the bacteria which may be most prevalent during prophylaxis, whereas ciprofloxacin and streptomycin have similar levels of activity against both extracellular and intracellular *Y. pestis in vitro*.

MATERIALS AND METHODS

Bacterial strains. *Y. pestis* strain KIM6-2053.1+ *hms*$^+$ *psn*$^+$ Psa$^-$ (Δ*psa2053.1*) Ybt$^+$ Lcr$^-$ derived from KIM6-2053.1 (2) and provided by Robert Perry, University of Kentucky, was transformed with a modified green fluorescent protein expression plasmid (pGFPuv; Clontech) by electroporation (9). Plasmid pGFPuv was modified by BsaH1 (New England BioLabs) restriction excision of a portion of the Amp^r selection marker between bp 1402 and 1784 of pGFPuv, and religation followed. Electroporation-transformed bacteria were grown on tryptic soy agar (TSA; Difco), and transformants expressing uvGFP were identified visually using a 360-nm illumination box (VersaDoc; Bio-Rad). A transformant which stably expressed uvGFP was selected (KIM6-2053.1+GFPuv). Growth of KIM6-2053.1+GFPuv in liquid media and in tissue culture cells was indistinguishable from that of the parent KIM6-2053.1+ strain (data not shown).

Tissue culture cells. Human THP-1 and mouse RAW264.7 cells (American Type Tissue Culture Collection) were cultured at 37°C in 5% CO_2 in RMPI 1640 media (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; HyClone), 10 mM HEPES (Sigma-Aldrich), 1 mM sodium pyruvate (Sigma-Aldrich), 4.5 g/liter glucose (Sigma-Aldrich), and 1.5 g/liter sodium bicarbonate (Sigma-Aldrich), with pH adjusted to 7.2, with the exception that media for THP-1 cells also contained 50 μM 2-mercaptoethanol (Sigma-Aldrich).

Infection of tissue culture cells. Prior to use in assays, tissue culture cell viability was assessed by a trypan blue exclusion assay, with >95% viability required for subsequent use. To prepare RAW264.7 cells for infection as attached cells, 5 ml (1×10^6 cells/ml quantified by hemocytometer) RAW264.7 cells was placed in 25-cm^2 tissue culture flasks incubated overnight at 37°C with 5%CO_2. THP-1 cells were infected as suspension cultures. For infection of tissue culture cells, *Y. pestis* strain KIM6-2053.1+ pGFPuv was grown in brain heart infusion (BHI) broth (Difco) at 26°C in an incubator shaker at 120 rpm, and the inocula were quantified at an optical density at 600 nm (OD_{600}) against a standard curve for the number of CFU/OD_{600}. Sufficient inoculum was added to the tissue culture flasks to yield an optimal multiplicity of infection (MOI) of 4:1 for RAW264.7 cells and 6:1 for THP-1 cells, as determined for the infection conditions utilized. Flasks containing RAW264.7 cells were centrifuged at 150 × *g* for 10 min to facilitate bacterial contact with RAW264.7 cells, but THP-1 cell suspensions were mixed with inocula and not centrifuged. The flasks were then incubated for 30 min at 37°C with 5% CO_2, prior to clearing extracellular bacteria by addition of 50 μg/ml gentamicin to flasks, and the incubation described above continued for an additional 2 h. For flasks containing RAW264.7 cells, the gentamicin-containing medium was removed by aspiration, and the flasks were washed twice with phosphate-buffered saline (PBS) to remove residual gentamicin. Subsequently, RPMI 1640 medium containing no antibiotic was added to flasks, and RAW264.7 cells were detached from the flasks using a cell scraper. For THP-1 cells, following 2-h exposure to gentamicin, cells were collected by centrifugation at 200 × *g* for 6 min, gentamicin-containing medium was removed by aspiration, and the cell pellet was washed twice with PBS prior to resuspending the cells in RPMI 1640 medium containing no antibiotics. *Y. pestis* cells were located primarily intracellularly in infected tissue culture cells postinfection as shown in Fig. 1.

Antibiotics. Ciprofloxacin (Serologicals), streptomycin, gentamicin, doxycycline, and amoxicillin (all from Sigma-Aldrich) stock solutions made in sterile water were stored at −80°C and used within 3 weeks of preparation. Chloramphenicol (Sigma-Aldrich) stock solution was made in dimethyl sulfoxide (DMSO) and used the same day due to concerns with stability.

BMA. Broth microdilution assays (BMA) for both intracellular and extracellular *Y. pestis* were conducted in single 96-well plates with two antibiotics tested per plate. Gentamicin was used as a standard antibiotic for efficacy assessment and was included in all BMA plates. To prepare 96-well flat-bottom plates (Corning) for BMA, 80 μl of 2-fold serially diluted test antibiotics in RPMI 1640 medium supplemented with 10% FBS were added to all wells in the top seven rows (A through G) of plates, yielding serial 2-fold dilutions in a series down each column of a plate. The wells in the eighth row (H) of each column were reserved and served as either growth control wells receiving 80 μl of RPMI 1640 media with 10% FBS and no antibiotic or sterility control wells that contained 80 μl of the highest antibiotic concentration in the dilution series in RPMI 1640 medium with 10% FBS, but these sterility control wells were not subsequently inoculated with *Y. pestis* such that the antibiotics could be assessed for contamination. Each plate contained one standard antibiotic (gentamicin) and one test antibiotic, with each antibiotic series dilution repeated in 6 of the 12 columns of the plate. Plates were stored at −20°C until used. To assess antibiotic efficacy against intracellular *Y. pestis*, 20 μl containing 5×10^4 CFU *Y. pestis* in 1×10^5 tissue culture cells was added to the top seven rows (A through G) of three of the six columns for each test antibiotic and to the nonantibiotic-containing growth control wells in the eighth (H) row. In the remaining three columns for each antibiotic, 20 μl of a 1:40 dilution in PBS of a 0.5 McFarland standard suspension *Y. pestis* (5×10^4 CFU *Y. pestis*/well) prepared from bacteria grown on BHI agar (Difco) at 37°C was added to assess antibiotic efficacy against extracellular *Y. pestis* and to serve as comparators for antibiotic efficacy against intracellular *Y. pestis*. Immediately following inoculation of plates, aliquots were taken from two growth control wells per plate for infected tissue culture cells to determine the initial number of CFU/wells. These aliquots containing infected cells were treated with 0.1% Triton X-100 (Sigma-Aldrich) in PBS to lyse cells releasing intracellular bacteria for CFU enumeration by the plating of serial dilutions on 10-cm TSA agar petri dishes. The acceptable inoculum for CFU per well was ≥5×10^4 CFU/well (≥5×10^5 CFU/ml).

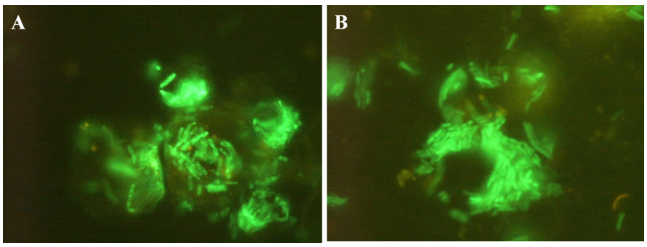

FIG. 1. At 4 h postinfection, *Y. pestis* was located primarily intracellularly in human THP-1 cells (A) and mouse RAW264.7 cells (B) (magnification, ×1,500).

Antibiotic exposure was continued for 24 h at 37°C with 5% CO_2, and the minimal bactericidal concentration (MBC) determined from the number of CFU in wells by a novel HTP microcolony assay in replicate 96-well plates by modification of a method of Nizet and colleagues (21). Briefly, 100 μl sterile water was added to wells in those plate columns that contained the extracellular bacterial inocula, and 100 μl 0.1% Triton X-100 in serial water was added to wells in those plate columns that contained infected tissue culture cells as inocula. Plates were then placed on a rotary shaker for 10 min to allow the lysis of tissue culture cells and resuspension of bacteria in wells. Bacteria in wells were then diluted 10-fold by the transfer of 20 μl of well contents to a replica 96-well dilution plate containing 180 μl sterile water in all wells. Finally, following mixing, 20 μl of well contents in the replica 96-well dilution plate were transferred to a replica 96-well CFU plate, and 180 μl of tryptic soy broth in 0.5% Bacto-agar (Difco) maintained at 45°C was added to all wells. The CFU plates were incubated for 16 h at 37°C. CFU were quantified by visual counting of microcolonies in individual wells using a dissecting microscope (Fig. 2).

MBCs, defined as a 99.9% reduction in the initial inoculum, were determined from the number of CFU/well compared to the growth controls. The minimum inoculum per well was 5×10^4 CFU/well in the exposure plate, which following dilution would be 5×10^2 CFU per well in the CFU plate. Therefore, a 99.9% reduction would be 0.5 CFU/well or no growth observed in wells at the MBC antibiotic concentration in the CFU plate. For MBC determinations, the number of CFU/well was estimated visually by categorization of wells as 0, 1 to 20, 20 to 100, 100 to 1,000, or >1,000 microcolonies per well (Fig. 2). The MBC was the lowest antibiotic concentration with 0 CFU/well. For the MBC to be valid, those wells with lower concentrations of antibiotics than the MBC must have had >20 CFU per well, with no skipped wells. The antibiotic concentration ranges for each test antibiotic were selected by initial testing such that ranges included at least one dilution above and below the low and high MBCs observed.

RESULTS

Antibiotics commonly used for treatment of plague were assessed for *in vitro* efficacy against intracellular *Y. pestis* by

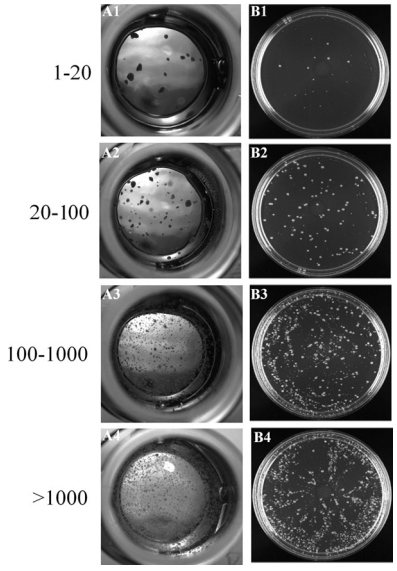

FIG. 2. Visual comparison of microcolonies in wells of 96-well plates (A1 to A4; magnification, ×15) with colonies on 10-cm petri dish for CFU determinations (B1 to B4). Representative examples are shown of single wells for MBC determinations yielding results that were within the four scoring categories used for MBC determinations. The numbers of CFU/plate well or 10-cm petri dish are shown on left.

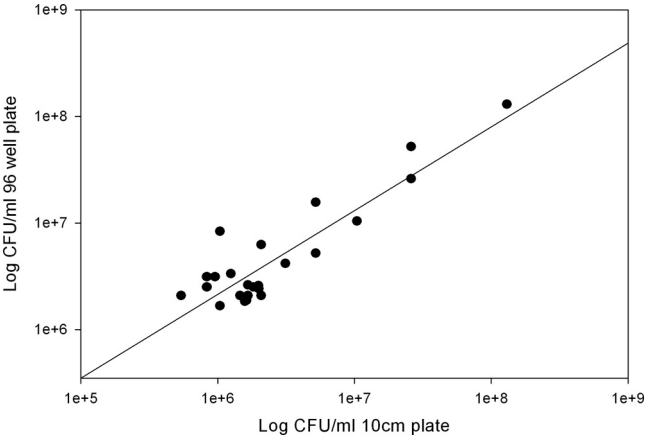

FIG. 3. Comparison of CFU determinations for *Y. pestis* using microcolony visualization with wells of 96-well plates and for colonies on standard 10-cm petri dishes. Identical samples of *Y. pestis* were inoculated in wells of 96-well plates and mixed with TSA soft agar or streaked on 10-cm TSA petri dishes as described in Materials and Methods. Statistical analysis using a simple linear regression (R = 0.979) suggests that these techniques yield similar results within one-order-of-magnitude agreement.

comparison of the MBCs for antibiotics against intracellular bacteria in macrophage cell lines compared to extracellular bacteria using a novel 96-well plate-based method for enumeration of CFU developed by Nizet and colleagues (21). This novel 96-well plate method requires fewer manipulations than the standard serial dilution method for determination of the number of CFU using 10-cm petri dishes and made it feasible to determine the number of CFU over an extended range of 2-fold serial dilutions of antibiotic concentrations, with greater numbers of replicates per antibiotics for the determination of the MBCs of commercially available antibiotics for intracellular *Y. pestis* (21; J. D. Wendte et al., presented at the 6th ASM Biodefense and Emerging Diseases Research Meeting, Baltimore, MD, 2008).

This HTP BMA format allowed three replicates of seven 2-fold serial dilutions of a test antibiotic and a standard antibiotic to be assessed against both intracellular and extracellular *Y. pestis* in a single 96-well plate, reducing the material required and easing the manipulations required for MBC determinations. For this HTP BMA, *Y. pestis*-infected tissue culture macrophage-like cells (5×10^4 CFU *Y. pestis* per 1×10^5 macrophages/100 μl per well) were subjected to antibiotic exposure in 96-well plates for 24 h at 37°C. At the end of the antibiotic exposure, *Y. pestis* was released from macrophages by detergent lysis and diluted in a replica 96-well dilution plate, and finally, the number of CFU was determined by visual microscopic examination of microcolonies following 100-fold dilution and growth in soft TSA in a final 96-well CFU plate. The validity of the microcolony assessment of the number of CFU was confirmed by comparison plating on 10-cm TSA petri dishes with the number of CFU determined by the soft TSA microcolony method in 96-well plates as shown in Fig. 3. There was a 0.5 to ≈1.0 correspondence of CFU for *Y. pestis* strain KIM6-2053.1+GFPuv enumerated by serial dilution and plating on 10-cm TSA petri dishes or inoculation of soft TSA in 96-well plates across three experiments (n = 4 to 8).

Using the HTP BMA, MBCs for five antibiotics (streptomycin, gentamicin, doxycycline, chloramphenicol, and ciprofloxacin) commonly used to treat plague in humans and amoxicillin (included as a test antibiotic with anticipated poor intracellular efficacy) were determined for intracellular *Y. pestis* KIM6-2053.1+GFPuv in THP-1 human tissue culture macrophage cell lines and for extracellular *Y. pestis*. THP-1 cells were undifferentiated and monocyte-like in suspension culture and are phagocytic but have limited antimicrobial activity (24). As shown in Table 1, MBCs for extracellular *Y. pestis* for gentamicin, doxycycline, and ciprofloxacin were 100% within the Clinical and Laboratory Standards Institute ranges for the expected MIC, whereas 73% and 88% of MBCs for chloramphenicol and streptomycin were within this expected range for MICs (8).

Comparisons of MBCs for intracellular and extracellular *Y. pestis* in Table 1 show that for THP-1 cells MBCs for streptomycin were the same or one 2-fold dilution lower for intracellular versus extracellular *Y. pestis*, whereas MBCs for intracellular versus extracellular *Y. pestis* were one 2-fold dilution higher for ciprofloxacin, two 2-fold dilutions higher for chloramphenicol, three 2-fold dilutions higher for gentamicin, four 2-fold dilutions higher for doxycycline, and five 2-fold dilutions higher for amoxicillin.

MBCs were also determined for intracellular *Y. pestis* in mouse RAW264.7 macrophage-like cells. RAW264.7 cells used were not activated by pretreatment and were adherent, phagocytic cells with some antimicrobial activity (20). The MBCs were the same for mouse RAW264.7 and human THP-1 macrophage-like cells for streptomycin, chloramphenicol, ciprofloxacin, and amoxicillin but were two to three 2-fold dilutions lower for RAW264.7 than for THP-1 cells for gentamicin and doxycycline. Microscopic examination of *Y. pestis* KIM-

TABLE 1. Comparison of MBC determinations for extracellular and intracellular *Y. pestis*

Antibiotic	BMA type[a]	No. of assay determinations with an MBC (μg/ml) of:													Total no. of assays[b]
		0.016	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	
Streptomycin	EC							8	37	16	8					69
	THP-1							17	13	5	1					36
	RAW							14	17	8						39
Gentamicin	EC						34	91	38	10						173
	THP-1							3	1	9	27	30	11			81
	RAW						11	28	33	25	4	1				102
Doxycycline	EC						1	11	62	11						85
	THP-1											11	20	8		39
	RAW							3	2	10	13	7	3			38
Chloramphenicol	EC									2	33	7	6			48
	THP-1											7	16	15		38
	RAW										5	4	12	13		34
Ciprofloxacin	EC	16	52													68
	THP-1		5	14	8	5	7									39
	RAW		7	17	13	2										39
Amoxicillin	EC					5	15	6	4							30
	THP-1										1	17	7			25
	RAW								1	3	5	14	1			24

[a] Broth microdilution assay type specifies whether *Y. pestis* was exposed to antibiotics extracellularly (EC), intracellularly in the THP-1 human macrophage cell line (THP-1), or in the RAW264.7 mouse macrophage cell line (RAW).

[b] The plate assay setup resulted in twice as many EC assays as assays for either THP-1 or RAW. Some MBCs were rejected if the inoculum was determined to not be within the range of 5×10^4 to 5×10^5 CFU *Y. pestis*/well.

2053.1+GFPuv demonstrated that the bacteria were located intracellularly in the tissue culture cells (Fig. 1) and that in human THP-1 cells the bacteria were present in tight vesicles in the cytoplasm, whereas in mouse RAW264.7 cells the bacteria were in spacious cytoplasmic vesicles (Fig. 4).

DISCUSSION

Commonly used methods for *in vitro* antibiotic sensitivity testing for intracellular bacteria involves CFU determination by plating serially diluted samples on 10-cm petri dishes, which is a labor-intensive process with limited potential for HTP development. Because of this, the range of antibiotic serial dilutions that can be accomplished in individual assays, as well as the number of replicates of individual MBCs that can be accomplished, is often limited. Alternative methods have been developed that use determinants other than the number of CFU to assess antibiotic efficacy in the HTP format (5, 28), but we report herein an adaptation of a novel method for CFU determination for intracellular bacteria in a 96-well-plate format previously developed by Nizet and colleagues (21) for an HTP BMA for intracellular *Y. pestis* which uses the more traditional CFU determination for assessing antibiotic efficacy. This method allows screening of seven 2-fold antibiotic dilutions to yield 12 MBC determinations per 96-well plate. In addition, the determinations of MBCs for both intracellular and extracellular bacteria can be conducted in the same plate. Although not done for the MBC determinations reported in this study, this method is amenable to the use of robotics to further reduce the labor required for testing.

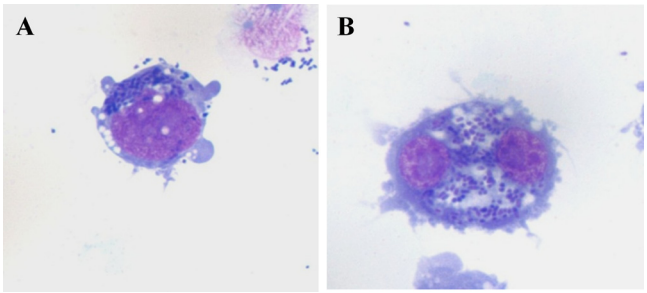

FIG. 4. At 12.5 h postinfection, *Y. pestis* resides in tight cytoplasmic vesicles in human THP-1 cells (A) but in spacious cytoplasmic vesicles in mouse RAW264.7 cells (B) (magnification, ×1,500, Wright-Giemsa stain).

To our knowledge, information on *in vitro* antibiotic sensitivity testing for intracellular *Y. pestis* is not currently available in the literature. The necessity of such information has recently become apparent with the appreciation that during the initial phase in plague, *Y. pestis* resides in host macrophages in which it suppresses immune responsiveness and evades innate immunity (25). Therefore, antibiotics which act effectively against both intracellular and extracellular *Y. pestis* may have the best potential for efficacy. Recommendations for antibiotics for treatment of human plague are based on limited clinical trials and experimental infections in rodents. Of particular recent interest are recommendations for prophylactic antibiotic therapy in a bioterrorism incident involving *Y. pestis*. Because *Y. pestis* uses intracellular parasitism of host macrophages as one mechanism of evasion of host innate immunity during the initial phase of infection, it is reasonable to conclude that antibiotics effective against intracellular *Y. pestis* might be su-

perior as prophylaxis for acute exposure to plague. Using the HTP BMA described, we determined that two recommended antibiotics, streptomycin and ciprofloxacin, had superior efficacy against intracellular *Y. pestis* in human macrophage-like cells, whereas two other recommended antibiotics, gentamicin and doxycycline, had inferior efficacy.

Compared to an abundance of information available on the antibiotic sensitivity of many common bacterial pathogens, only limited information is available for *Y. pestis*. Acceptable limits for antibiotic sensitivity for *Y. pestis* for *in vitro* sensitivity testing are available for only six commercial antibiotics (8). Good antibiotic sensitivity was observed with *in vitro* sensitivity testing conducted on several clinical isolate collections, with the exception that isolates collected more recently have increased MICs (26). *Y. pestis* isolates collected prior to 1988 had MICs <8 μg/ml for 23 of 24 commercially available antibiotics (16). Similarly, a survey of 100 clinical isolates collected prior to 1991 from Africa for 14 commercially available antibiotics found that all were effective at <8 μg/ml except for one macrolide antibiotic (12). For a collection of 78 *Y. pestis* isolates from Vietnam from 1985 to 1993, all were susceptible to 14 commercial antibiotics at 8 μg/ml; however, the authors noted that the MICs for several antibiotics commonly used in Vietnam were less effective (26). The most recently screened collection consisted of 92 isolates (not all are from human cases) from California from 1977 to 1998, and 12 commercially available antibiotics were found to be effective at ≤ 8 μg/ml (29).

The recommended antibiotic of choice for treatment of human plague is streptomycin (14), but with the emergence of streptomycin-resistant strains and the limited availability of streptomycin, reliance on this drug for the treatment of plague has decreased (4, 15). Doxycycline, chloramphenicol, gentamicin, and ciprofloxacin are recommended for treatment of plague and are more commonly used today (4, 14), although inhibitory *in vitro* β-lactams and sulfas may not be effective *in vivo* (6).

As with any *in vitro* assay, the applicability of the results reported for therapeutic decision-making depends on the ability of the *in vitro*-model THP-1 cells and target *Y. pestis* strain KIM6-2053.1+ to accurately reflect the circumstances in actual *in vivo* infections. THP-1 cells are one of several macrophage-like cell lines used to model intracellular parasitism. Several other human macrophage- or monocyte-like cell lines, including HL60 and A529, were assessed with various avirulent strains of *Y. pestis*, with THP-1 cells exhibiting the most consistent intracellular infectivity over a 24-h observation (data not shown). Likewise, of the various avirulent *Y. pestis* strains which are mutants for particular virulence loci, strain KIM6 lacking the plasmid pCD, strain D27 lacking the chromosomal pigment loci, and D1 lacking the MT plasmid, only KIM6 yielded consistent intracellular parasitism of human macrophage-like cells for 24 h (data not shown). Additional studies are needed to determine how well THP-1 cells and *Y. pestis* strain KIM6-2053.1+ mimic the sensitivity of native virulent strains of *Y. pestis* in human macrophages to various antibiotics.

In addition to testing antibiotic efficacy against intracellular *Y. pestis* in a human cell line, antibiotic efficacy against *Y. pestis* in a mouse macrophage cell line, RAW264.7, was also assessed and found to differ for two antibiotics, gentamicin and doxycycline, with these antibiotics being more efficacious in mouse than in human macrophage cell lines. Intracellular antibiotic efficacy is dependent on several pharmacokinetic and pharmacodynamic factors relating to the properties of both the host macrophages and the agent in these host macrophages.

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