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SUPPLEMENT ARTICLE

Postexposure Prophylaxis and Treatment of *Bacillus anthracis* Infections: A Systematic Review and Meta-analyses of Animal Models, 1947–2019

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Background. Anthrax is endemic to many countries, including the United States. The causative agent, *Bacillus anthracis,* poses a global bioterrorism threat. Without effective antimicrobial postexposure prophylaxis (PEPAbx) and treatment, the mortality of systemic anthrax is high. To inform clinical guidelines for PEPAbx and treatment of *B. anthracis* infections in humans, we systematically evaluated animal anthrax treatment model studies.

Methods. We searched for survival outcome data in 9 scientific search engines for articles describing antimicrobial PEPAbx or treatment of anthrax in animals in any language through February 2019. We performed meta-analyses of efficacy of antimicrobial PEPAbx and treatment for each drug or drug combination using random-effects models. Pharmacokinetic/pharmacodynamic relationships were developed for 5 antimicrobials with available pharmacokinetic data. Monte Carlo simulations were used to predict unbound drug exposures in humans.

Results. We synthesized data from 34 peer-reviewed studies with 3262 animals. For PEPAbx and treatment of infection by susceptible *B. anthracis*, effective monotherapy can be accomplished with fluoroquinolones, tetracyclines, β-lactams (including penicillin, amoxicillin-clavulanate, and imipenem-cilastatin), and lipopeptides or glycopeptides. For naturally occurring strains, unbound drug exposures in humans were predicted to adequately cover the minimal inhibitory concentrations (MICs; those required to inhibit the growth of 50% or 90% of organisms [MIC_{50} or MIC_{90}]) for ciprofloxacin, levofloxacin, and doxycycline for both the PEPAbx and treatment targets. Dalbavancin covered its MIC_{50} for PEPAbx.

Conclusions. These animal studies show many reviewed antimicrobials are good choices for PEPAbx or treatment of susceptible *B. anthracis* strains, and some are also promising options for combating resistant strains. Monte Carlo simulations suggest that oral ciprofloxacin, levofloxacin, and doxycycline are particularly robust choices for PEPAbx or treatment.

Keywords. anthrax; treatment; postexposure prophylaxis; drug-resistant; antimicrobial.

Bacillus anthracis, the etiologic agent of anthrax, is distributed worldwide [1]. Naturally occurring anthrax cases usually follow direct contact with infected animals or their contaminated by-products. Because the spores can be aerosolized and natural or laboratory engineered resistance is possible, *B. anthracis* is a potential bioweapon [2–4].

Anthrax mortality is high, particularly for ingestion and inhalation anthrax and anthrax meningitis, even with treatment [5, 6]. Given the high mortality rates of anthrax, timely and effective postexposure prophylaxis (PEP) and treatment are critical to minimizing morbidity and mortality rates. PEP should include anthrax vaccine PEP and antimicrobial PEP (PEPAbx).

To inform clinical guidelines, we conducted a systematic review (SR) and meta-analyses of animal models and leveraged translational pharmacokinetic (PK)/pharmacodynamic (PD) approaches to support robust options for PEPAbx and treatment of anthrax. This article is part of a Centers for Disease Control and Prevention (CDC) effort to determine the most efficacious options for PEPAbx and treatment of anthrax in humans for naturally occurring and multidrug-resistant strains.

METHODS

We searched 9 scientific databases from inception through February 2019 for articles containing terms that captured anthrax, randomized controlled trials in animals, antimicrobials generally, and the specific classes and antimicrobials detailed

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in Supplementary Figure 1. All foreign-language articles were translated. The SR followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for conducting and reporting systematic literature reviews. The system we used to identify full-text articles for inclusion in this review is shown by Maxson et al [7, Supplementary Figure 1]. Data were abstracted from studies that used antimicrobials for PEPAbx or treatment following animal exposures to *B. anthracis*. A time frame exception was made for a single article involving meningitis [8].

Two of the authors (K. C. S. and K. H.) abstracted aggregate data for treatment and control arms on the following topics: *B. anthracis* challenge; antimicrobials used for PEPAbx or treatment (ie, class, dose, route, interval, duration, and trigger for start of treatment), minimal inhibitory concentrations (MICs); PK/PD parameters, and outcome (eg, death and pathology results). After data entry, discrepancies were discussed by the abstractors until they reached consensus.

Exclusions

Study arms were excluded from the main SR analysis if they included sheep, cows, or dogs; added a vaccine or antitoxin to the antimicrobial(s); lacked at least 1 treatment-control pair of arms or a clear antimicrobial dose description; used antimicrobials that were unapproved by the Food and Drug Administration or unavailable in the United States; assessed only preexposure prophylaxis, PK, or PD; or had extremely high *B. anthracis* exposures (eg, ≥750 times the lethal dose in 50% of animals). Some studies meeting exclusion criteria were nevertheless analyzed separately from the main analysis on request of a CDC workgroup that reviewed the data.

Definitions

For studies using susceptible strains of *B. anthracis*, study arms with ciprofloxacin or doxycycline were designated as positive (ie, efficacious) controls. Infection was determined based on the presence of fever, bacteremia, or toxemia. An antimicrobial arm was classified as PEPAbx if the animals lacked infection and if the antimicrobial was started ≤24 hours after exposure. Only aerosol exposures were included for PEPAbx arms. An antimicrobial arm was classified as treatment if the antimicrobial was started for evidence of infection or began >24 hours after exposure. All exposure routes (eg, intravenous, subcutaneous, intranasal, head-only aerosol) were included for treatment arms. Study arms in which vegetative *B. anthracis* was instilled intracranially were classified as treatment.

Analysis

Study quality was assessed on a 24-point scale comprising 4 main topics: (1) animal descriptions, (2) exposures, (3) antimicrobial descriptions, and (4) outcomes (Supplementary Figure 2). Quality scores were categorized as low (0–5 points), fair (6–13 points), good (14–17 points), and high (18–24 points) based on natural breaks in a histogram of the scores and discussions with an internal CDC steering committee.

Within studies, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) for each study arm compared to its nontreated controls; ORs were then visually compared. ORs with overlapping CIs were considered to be not meaningfully different and the corresponding study arms were combined to improve statistical power.

Meta-analyses were performed to combine the results of individual studies on animal mortality data to estimate an overall OR and 95% CI for survival. The meta-analyses used conditional binomial-normal (or generalized linear mixed effects) models with a random-effects model option that allowed the treatment effect to differ from study to study. The conditional binomial-normal model was chosen because it was the most resilient against data challenges, such as 2-by-2 tables with 0 s (zero cells) and imbalanced comparison groups. However, ORs could not be calculated at all if the 2-by-2 table had 2 zero cells. Study arms that prevented OR calculation were removed from the meta-analysis and described separately.

For PK/PD analysis, we incorporated information on the doses, dosing intervals, and routes of administration for published studies in mice, rabbits, and nonhuman primates (NHPs) that evaluated PEPAbx or treatment of anthrax [9]. We assumed plasma protein binding was similar in mice and humans for levofloxacin and ciprofloxacin (approximately 30% bound), as well as for doxycycline (approximately 80%–90% bound) [10]. Known differences in protein binding were incorporated for dalbavancin (93% bound in humans vs 98.4% in mice) [11, 12] and oritavancin (85% bound in humans vs 93.6% in mouse serum) [13, 14].

Based on published human PK data, we performed Monte Carlo simulations with small (20% coefficient of variation) and moderately large (30% coefficient of variation) variability to predict the overall free (ie, non–protein-bound) drug exposures (area under the unbound plasma concentration-time curve [*f*AUC]) in healthy persons exposed to *B. anthracis* and in patients with early anthrax. These *f*AUC values were used to calculate the *f*AUC/MIC and then to calculate the probability of achieving the derived PK/PD target. The PK/PD breakpoint was defined as the highest MIC with a ≥98% probability of attaining the PK/PD target that is associated with near-maximal survival in animal studies. A complete description of the methods is provided in Supplementary Materials.

RESULTS

We identified 62 sources: 53 articles, 5 reports, 2 abstracts, 1 slide set, and 1 other source that described >800 study

arms with >12 000 animals. Following application of exclusion criteria, 34 sources remained that described 27 antimicrobials and 294 study arms with 3262 animals, including 329 NHPs, 807 rabbits, 1328 mice, 138 guinea pigs, and 660 hamsters (Supplementary Table 1).

Meta-analyses results are displayed in 5 groups according to the susceptibility of the infecting *B. anthracis* strain and the type of therapy. Table 1 shows monotherapy for PEPAbx or treatment of infections with susceptible *B. anthracis* strains [12, 14–38, (unpublished data)]. Table 2 shows monotherapy for PEPAbx or treatment of infections with resistant strains [38, 39]. Table 3 shows combination therapies that included ciprofloxacin for resistant and sensitive strains [18, 19, 39]. Table 4 summarizes meta-analyses for anthrax meningitis treatment studies in rabbits [8, 40, 41]. Table 5 shows PEPAbx studies meeting exclusion criteria analyzed on anthrax workgroup request [17, 42–44]. Most studies of monotherapy against susceptible *B. anthracis* strains accrued a quality score of 10–20 points. Studies of monotherapy for resistant strains (Table 2), combination therapy (Table 3), anthrax meningitis (Table 4), and analyses run at the behest of the workgroup (Table 5) accrued 9–18 points. ORs for individual antimicrobials versus no therapy are summarized on logarithmic scales for PEPAbx and treatment in Figure 1 and for combination therapy and anthrax meningitis in Figure 2.

Postexposure Prophylaxis Against Susceptible Strains of *B. anthracis*

For monotherapy PEPAbx following exposures to susceptible *B. anthracis*, ciprofloxacin showed efficacy superior to no therapy (Table 1). Amoxicillin, glycopeptides, and daptomycin all showed efficacy for PEPAbx. Three amoxicillin-clavulanate studies were included in the analysis but could not be combined owing to zero cells. When ORs were calculated, 2 of the 3 studies showed significant efficacy. Tetracyclines also showed efficacy for PEPAbx, with ORs ranging from 5.4 (95% CI, 3.2–9.0) for doxycycline to 1489 (84.4–26 267) for minocycline. Clarithromycin showed no efficacy for PEPAbx. As indicated in Table 1, the presence of zero cells precluded OR calculation for a second study using clarithromycin as PEPAbx. While the 2 azithromycin studies could not be combined (owing to zero cells), 1 showed efficacy while the other did not. Forest plots summarizing ciprofloxacin and doxycycline PEPAbx data appear in Supplementary Figure 3*A* and 3*B*.

For PEPAbx, levofloxacin, procaine penicillin G, daptomycin, doxycycline, and omadacycline had efficacies similar to ciprofloxacin, but oritavancin was less efficacious (OR, 0.1 [95% CI, 0–.8]; Table 1). Ciprofloxacin, procaine penicillin G, omadacycline, and tetracycline had efficacies similar to doxycycline, but minocycline was more efficacious (OR, 99.0 [95% CI, 5.9–1651]). Of PEPAbx study arms initially excluded for non-aerosol exposure route or addition of anthrax vaccine to antimicrobials, the ofloxacin and imipenem arms from 1 study were both efficacious (Table 5) [17].

Postexposure Prophylaxis Against Resistant Strains of *B. anthracis*

Neither doxycycline nor tetracycline were efficacious for PEPAbx of doxycycline-resistant *B. anthracis* (Table 2). In contrast, monotherapy PEPAbx with minocycline was efficacious (OR, 3.9 [95% CI, 2.0–7.8]) against this particular doxycycline-resistant strain [38].

Treatment of Infections from Susceptible Strains of *B. anthracis*

Eleven treatment studies assessed 2 widely used fluoroquinolones (ciprofloxacin and levofloxacin) currently recommended for first-line treatment of anthrax. Ciprofloxacin administration was associated with increased survival compared with the no-treatment control (OR, 12.5 [95% CI, 3.9–40.5]). The meta-analysis OR for levofloxacin did not differ significantly from the no-treatment control (OR, 337 [95% CI, .5–210 268]; Table 1). Forest plots summarizing treatment data for ciprofloxacin and levofloxacin appear in Supplementary Figure 3*C* and 3*D*.

Compared with no treatment, improved survival was found for the following cell-wall synthesis inhibitors: all studied β-lactams and glycopeptides (vancomycin, dalbavancin, and oritavancin; Table 1). One penicillin study in which all animals died was removed from analysis. For anthrax treatment by a protein synthesis inhibitor (PSI), doxycycline and the newer tetracyclines eravacycline (OR, 369 [95% CI, 6.4–21 191]) and omadacycline (9.8 9 [1.9–50.6]) improved survival, as did clindamycin (24.4 [1.03–581]) (Table 1). Some studies had positive controls: Efficacies for dalbavancin, oritavancin, doxycycline, and omadacycline were similar to that of ciprofloxacin (Table 1). Likewise, efficacies for ciprofloxacin and omadacycline were similar to that of doxycycline. The forest plot for doxycycline treatment data is available in Supplementary Figure 3*E*.

In treatment studies evaluating 2 antimicrobial combinations against sensitive strains of *B. anthracis* (Table 3), clindamycin plus ciprofloxacin demonstrated treatment efficacy compared with no treatment in rabbits [18] and NHPs [19]. However, clindamycin plus ciprofloxacin was not superior to ciprofloxacin monotherapy in NHPs.

Treatment of Infections from Resistant Strains of *B. anthracis*

Against a ciprofloxacin-resistant strain, ciprofloxacin monotherapy was not efficacious, as expected (Table 2). In contrast, combination therapy was more effective. Three antimicrobial combination regimens included ciprofloxacin, a β-lactam, and either a PSI or rifampin or ciprofloxacin, a PSI, and rifampin (Table 3). All combinations showed efficacy compared with no treatment. All combinations except ciprofloxacin, meropenem, and doxycycline showed efficacy compared with ciprofloxacin monotherapy [39].

Table 1. Odds of Survival for Monotherapy Studies for Which the Exposures Were Not Antibiotic-Resistant Strains of *Bacillus anthracis*

[Study References] Animal Model[a]	Cidal or Static	Class	Antimicrobials	PEPAbx or Rx	No. of Studies	Quality Score[b]	OR (95% CI) vs No Treatment	I^{2c}	No. Studies	OR (95% CI) vs Ciprofloxacin	I^2	No. Studies	OR (95% CI) vs Doxycycline	I^2
[12]M, [14]M, [15]M, [16]M, [28]NHP, [29]M, [30]M, [31]NHP, [32]M, [33]NHP	C	Fluoroquinolone	Ciprofloxacin	PEPAbx	10	16,10,15,14,18, 12,14,19,13,18	11.5 (5.2–25.3)[d]	N	…	…	…	3	1.1 (0.7–1.8)	N
[12]M, [34]R, [35]M, [unpublished]NHP	C	Fluoroquinolone	Levofloxacin	PEPAbx	4	16,14,8, 19	124.8 (0.4–42 865)	N	1	2.2 (0.2–29.8)	…	…	…	…
[35]M,NHP, [36]NHP	C	Penicillin	Amoxicillin	PEPAbx	1,1	8,14	357 (6.4–19 943)[d] 1345 (76.2–23 762)[d,e]	…	…	…	…	…	…	…
[37]NHP, [unpublished]NHP	C	Penicillin	Penicillin	PEPAbx	2	14,8	4.1 (0.9–19.9)	N	…	…	…	…	…	…
[25]NHP, [28]NHP	C	Penicillin	Procaine penicillin G[f]	PEPAbx	2	14,18	8.2 (0–5 846 972)	N	1	0.3 (0–3.5)		1	0.3 (0–3.1)	
[35]M,NHP, [36]NHP, [36]NHP	C	Penicillin/β-lactamase inhibitor	Amoxicillin clavulanate	PEPAbx	1,1,1	14,18,8	187 (3.2–10 884)[d] 45.0 (0.7–3042) 1617 (91.7–24 498)[d,e]	…	…	…	…	…	…	…
[12]M	C	Glycopeptide	Dalbavancin[g]	PEPAbx	1	16	3381 (63.7–179 559)[d]	…	…	…[h]	…	…	…	…
[14]M	C	Glycopeptide	Oritavancin[g]	PEPAbx	1	10	46.3 (2.6–816)[d]	…	1	0.1 (0–0.8)[d]	…	…	…	…
[30]M	C	Lipopeptide	Daptomycin[g]	PEPAbx	1	14	81.0 (4.4–1505)[d]	…	1	1.9 (0.2–21.5)	…	…	…	…
[15]M, [16]M, [26]M, [28]NHP, [38]H	S	Tetracycline	Doxycycline	PEPAbx	5	15,14,19,18, 12	5.4 (3.2–9.0)[d]	N	3	0.9 (0.6–1.3)	N	…	…	…
[38]H	S	Tetracycline	Minocycline	PEPAbx	1	12	1489 (84.4–26 267)[d]	…	…	…	…	1	99.0 (5.9–1651)[d]	…
[16]M	S	Tetracycline	Omadacycline	PEPAbx	1	14	50.4 (13.9–182)[d]	…	1	0.2 (0–3.6)		1	1.1 (0.4–3.1)	…
[38]M	S	Tetracycline	Tetracycline	PEPAbx	1	12	17.9 (7.8 - 40.9)[d]	…	…	…	…	1	1.1 (0.6–2.0)	…
[35]M, [unpublished]NHP	S	Macrolide	Azithromycin	PEPAbx	1,1	8,19	25.0 (1.1–562.8)[d] 7.5 (0.3–186)[e]	…	…	…	…	…	…	…
[35]M[i], [unpublished]NHP	S	Macrolide	Clarithromycin	PEPAbx	1	8,19	16.2 (0.6–441.7)[h]	…	…	…	…	…	…	…
[12]M, [14]M, [15]M, [16]M [17]GP,R, [18]R, [19]NHP	C	Fluoroquinolone	Ciprofloxacin	Rx	7	16,10,15,14, 11,12,18	12.5 (3.9–40.5)[d]	N	…	…	…	2	0.8 (0.6–1.2)	N
[20]R, [21]R, [22]R, [23]R	C	Fluoroquinolone	Levofloxacin	Rx	4	11,20,14,16	337 (0.5–210 268)	H	…	…	…	…	…	…
[18]R	C	Carbapenem	Imipenem[j]	Rx	1	11	63.0 (2.5–1558)[d]	…	…	…	…	…	…	…
[24]R[i], [unpublished]NHP	C	Penicillin	Penicillin	Rx	1	9,8	24.1 (1.0–567)[d,h]	…	…	…	…	…	…	…
[25]NHP	C	Penicillin	Procaine penicillin[f]	Rx	1	14	24.1 (1.0–567)[d]	…	…	…	…	…	…	…
[18]R	C	Penicillin/beta-lactamase inhibitor	Amoxicillin clavulanate[k]	Rx	1	12	25.0 (1.1–563)[d]	…	…	…	…	…	…	…
[18]R	C	Glycopeptide	Vancomycin	Rx	1	12	52.7 (2.3–1211)[d]	…	…	…	…	…	…	…
[12]M	C	Glycopeptide	Dalbavancin	Rx	1	16	138 (6.8–2780)[d]	…	1	0.7 (0–15.9)	…	…	…	…
[14]M	C	Glycopeptide	Oritavancin	Rx	1	10	39.0 (2.1–734)[d]	…	1	0.3 (0.1–1.1)	…	…	…	…
[16]M, [17]GP,R, [26]M, [27]M	S	Tetracycline	Doxycycline	Rx	4	14,11,19,11	4.9 (2.1–11.6)[d]	N	1	1.6 (0.9–2.8)	…	…	…	…
[unpublished data]R	S	Tetracycline	Eravacycline	Rx	1	19	369 (6.4–21191)[d]	…	…	…	…	…	…	…
[16]M	S	Tetracycline	Omadacycline	Rx	1	14	9.8 (1.9–50.6)[d]	…	1	0.4 (0.1–3.2)		1	0.6 (0.1–4.1)	…
[19]NHP	S	Lincosamide	Clindamycin	Rx	1	18	24.4 (1.0–581)[d]	…	…	…	…	…	…	…

Table 1. Continued

[Study References]	Animal Model[a]	Cidal or Static	Class	Antimicrobials	PEPAbx or Rx	No. of Studies	Quality Score[b]	OR (95% CI) vs No Treatment	I^{2c}	No. Studies	OR (95% CI) vs Ciprofloxacin	I^2	No. Studies	OR (95% CI) vs Doxycycline	I^2
[18]R		S	Macrolide	Clarithromycin[k]	Rx	1	12	5.5 (0.3–111)	…	…	…	…	…	…	…
[18]R		S	Oxazolidinones	Linezolid	Rx	1	12	17.2 (0.8–381)	…	…	…	…	…	…	…
[18]R		C	Rifamycin	Rifampin	Rx	1	12	3.4 (0.2–74.4)	…	…	…	…	…	…	…

Abbreviations: C, bactericidal; CI, confidence interval; OR, odds ratio; PEPAbx, antimicrobial postexposure prophylaxis; Rx, treatment; S, bacteriostatic.

[a]Animal model used in the relevant arms of the above studies: M, mouse; R, rabbit; NHP, nonhuman primate; GP, guinea pig; H, hamster.

[b]Quality score categorization: fair (6–13 points); good (14–17 points); high (18–24 points).

[c]Heterogeneity (I^2) Key: N = 0–24%; L = 25–49%; M = 50–74%; H = 75–100%.

[d]Significant at $P < .05$.

[e]Both ORs are shown as both studies had zero cells and could not be combined.

[f]Only available in the United States as intramuscular formulation for humans, although animals may have received other formulations or routes of administration.

[g]Only available in the United States as intravenous formulation for humans, although animals may have received other formulations or routes of administration.

[h]At least one study removed to allow odds ratio calculation.

[i]This study was removed to allow odds ratio calculation.

[j]Only available in the United States as an intravenous formulation in combination with cilastatin for humans; animals received only imipenem, which may have used another route of administration.

[k]Only available in the United States as oral formulation for humans, although animals may have received other formulations or routes of administration.

Treatment of Anthrax Meningitis in a Rabbit Model

We included all available anthrax meningitis studies due to the extreme mortality rates associated with anthrax meningitis and the limited number of animal studies for this indication. In 2 anthrax meningitis studies using a rabbit model [8, 40], treatment efficacy was shown for penicillins (with or without clavulanate or sulbactam) and clindamycin (Table 4). Meropenem with or without ciprofloxacin was not efficacious in this animal model when dosed intravenously or subcutaneously.

Monte Carlo Simulations to Predict Efficacy of Dosage Regimens

Monte Carlo simulations predicted the human unbound drug exposures for clinically relevant doses of ciprofloxacin, levofloxacin, doxycycline, oritavancin, and dalbavancin. Subsequently, efficacy was predicted based on the probability of PK/PD target attainment (Figure 3). These Monte Carlo–simulated efficacies were comparable for both small variability (representing PEPAbx) and moderately large variability (for treatment).

As described more comprehensively in the Supplementary Materials, for PEPAbx, a high degree of efficacy was predicted for 500 mg ciprofloxacin given every 12 hours (for MICs up to 0.25 mg/L), ciprofloxacin 500 mg every 8 hours (MICs up to 0.5 mg/L), levofloxacin 750 mg every 24 hours (MICs up to 0.5 to 1 mg/L), doxycycline 100 mg every 12 hours (MICs up to 0.0625 mg/L), oritavancin 1200 mg × 1 dose (MICs up to 0.0625 mg/L), and dalbavancin 1000 mg × 1 dose, then 500 mg a week later (MICs up to 0.0625 mg/L). When using the higher PK/PD targets for treatment, the highest MICs with robust probabilities of target attainment were predicted to be approximately 2-fold lower than those for PEPAbx (Figure 3). Of note, ciprofloxacin 500 mg every 12 hours was predicted to be highly efficacious up to an MIC of 0.25 mg/L for treatment.

DISCUSSION

In a wide-area release of *B. anthracis*, effective PEPAbx and treatment are needed to minimize morbidity and mortality rates. Models have estimated the impact of a large intentional aerosol release of *B. anthracis* spores. One kilogram of *B. anthracis* spores (approximately 1×10^{15}) released upwind from 11.5 million city dwellers would infect 1.49 million people [45]. With timely treatment, but without PEP, approximately 670 000 people would be predicted to die (ie, 45% of those infected). With PEP, the casualty estimate could be reduced by as much as 80%, saving 537 000 to 547 000 lives [45]. The risk reduction afforded by PEPAbx begins immediately, while that afforded by vaccine PEP occurs only after immunity has developed. Thus, early PEPAbx is key to effective prophylaxis.

For this series of meta-analyses, we were preferentially interested in NHP, rabbit, mice, and guinea pig studies. The Food and Drug Administration identified NHPs and rabbits as acceptable

Table 2. Odds of Survival for Monotherapy Studies With Antibiotic Resistant Strains of *Bacillus anthracis*

[Study References] Animal Model[a]	Cidal or Static	Class	Antimicrobials	PEPAbx or Rx	Strain	No. of Studies	Quality Score[b]	OR (95% CI) vs No Treatment
[38]M,H	S	Tetracycline	Doxycycline	PEPAbx	H-7 (pBC16)[c]	1	12	0.1 (0–0.4)[d]
[38]M,H	S	Tetracycline	Minocycline	PEPAbx	H-7 (pBC16)	1	12	3.9 (2.0–7.8)[d]
[38]M,H	S	Tetracycline	Tetracycline	PEPAbx	H-7 (pBC16)	1	12	0.6 (0.3–1.2)
[39]M	C	Fluoroquinolone	Ciprofloxacin	Rx	CIP-R Ames	1	13	3.9 (0.3–45.6)

Abbreviations: C, bactericidal; CI, confidence interval; CIP-R, ciprofloxacin-resistant; OR, odds ratio; PEPAbx, antimicrobial postexposure prophylaxis; Rx, treatment; S, bacteriostatic.

[a]Animal model used in the relevant arms of the above studies: M, mouse; H, hamster.

[b]Quality score categorization: fair (6–13 points); good (14–17 points); high (18–24 points).

[c]Strain H-7 has plasmid pBC16 which confers tetracycline resistance.

[d]Significant at $P < .05$.

Table 3. Odds of Survival for Antimicrobial Combinations Including Ciprofloxacin for Treatment of Animals Infected With Sensitive or Resistant *Bacillus anthracis* Strains

	Antimicrobial 2			Antimicrobial 3						
[Study References] Animal Model[a]	Cidal or Static	Class	Antimicrobial	Cidal or Static	Class	Antimicrobial	Strain	Quality Score[b]	OR (95% CI) vs No Treatment[e]	OR (95% CI) vs Ciprofloxacin Monotherapy
[39]M	C	Carbapenem	Meropenem	S	Oxazolidinones	Linezolid	CIP-R Ames	13	81.0 (4.4–1505)[c]	21.0 (1.8–248)[c]
[39]M	C	Carbapenem	Meropenem	S	Lincosamide	Clindamycin	CIP-R Ames	13	81.0 (4.4–1505)[c]	21.0 (1.8–248)[c]
[39]M	C	Carbapenem	Meropenem	C	Rifamycin	Rifampin	CIP-R Ames	13	81.0 (4.4–1505)[c]	21.0 (1.8–248)[c]
[39]M	C	Carbapenem	Meropenem	S	Tetracycline	Doxycycline	CIP-R Ames	13	21.0 (1.8–248)[c]	5.4 (0.8–36.9)
[39]M	C	Penicillin	Penicillin	S	Oxazolidinones	Linezolid	CIP-R Ames	13	36.0 (2.7–476)[c]	9.3 (1.2–7.3)[c]
[39]M	C	Penicillin	Penicillin	S	Tetracycline	Doxycycline	CIP-R Ames	13	81.0 (4.4–1505)[c]	21.0 (1.8–248)[c]
[39]M	C	Rifamycin	Rifampin	S	Oxazolidinones	Linezolid	CIP-R Ames	13	133 (4.8–3674)[c]	45.0 (2.0–1007)[c]
[39]M	C	Rifamycin	Rifampin	S	Lincosamide	Clindamycin	CIP-R Ames	13	36.0 (2.7–476)[c]	9.3 (1.2–7.3)[c]
[18]R, [19]NHP	S	Lincosamide	Clindamycin				Vollum,[d,e] Ames[d]	12,18	32.1 (1.4–752)[c] 24.4 (1.03 - 581)[c]	0.1 (0–2.6)[f]

Abbreviations: C, bactericidal; CI, confidence interval; CIP-R, ciprofloxacin-resistant; OR, odds ratio; S, bacteriostatic.

[a]Animal model used in the relevant arms of the above studies: M, mouse; R, rabbit; NHP, nonhuman primate.

[b]Quality score categorization: fair (6–13 points); good (14–17 points); high (18–24 points).

[c]Significant at $P < .05$.

[d]Antibiotic sensitive.

[e]American Type Culture Collection 14578.

[f]Only the study performed in NHPs [19] was included in the odds ratio calculation.

models for product licensure studies under the Animal Rule [46, 47]. Moreover, mice and guinea pigs are considered acceptable for baseline screening of countermeasures [9], and many drug development programs extensively use dynamic in vitro and murine infection models to develop efficacious dosage regimens for patients [46–48].

Postexposure Prophylaxis

For PEPAbx of susceptible *B. anthracis,* monotherapies with ciprofloxacin, amoxicillin, amoxicillin-clavulanate, lipopeptides, glycopeptides, and tetracyclines were associated with improved survival compared with no-PEPAbx controls. Of the macrolides, only 1 of 2 studies with azithromycin was associated with survival, while clarithromycin was not associated with survival. Dalbavancin, oritavancin, daptomycin, and imipenem (combined with cilastatin) should also be effective but are only available intravenously and would therefore be less practical.

For PEPAbx of tetracycline-resistant *B. anthracis*, minocycline monotherapy improved survival, whereas doxycycline

Table 4. Odds of Survival for Treatment Studies Using a Rabbit Model of Anthrax Meningitis With a Susceptible Vollum Strain of *Bacillus anthracis*

[Study References] Animal Model[a]	Cidal or Static	Class	Antimicrobials	Dosing Route	Animals, No.[b]	Quality Score[c]	OR (95% CI) vs No Treatment[d]
[8]R	C	Penicillin/β-lactamase inhibitor	Amoxicillin-clavulanate	Intravenous/ subcutaneous	12	13	153 (2.6–9077)[e]
[8]R	C	Penicillin	Ampicillin	Intravenous/ subcutaneous	45	13	23.9 (1.2–479.2)[e]
[8]R	C	Penicillin/β-lactamase inhibitor	Ampicillin-sulbactam	Intravenous/ subcutaneous	12	13	45.0 (1.5–1358)[e]
[40]R	C	Fluoroquinolone/ carbapenem	Ciprofloxacin/ meropenem	Intravenous/ ubcutaneous	12	10	1.8 (0.1–54.3)
[40]R	S	Lincosamide	Clindamycin	Intravenous/ subcutaneous	12	10	45.0 (1.5–1358)[e]
[8]R, [40]R	C	Carbapenem	Meropenem	Intravenous/ subcutaneous	50	13,10	4.4 (0.2–87.6)
[40]R	C	Carbapenem	Meropenem	Intrathecal/ Intravenous	12	10	153 (2.6–9077)[e]

Abbreviations: C, bactericidal; CI, confidence interval; OR, odds ratio; S, bacteriostatic.

[a]Animal model used in the relevant arms of the above studies: R, rabbit.

[b]Number of treated and control animals.

[c]Quality score categorization: fair (6–13 points); good (14–17 points); high (18–24 points).

[d]Comparison with no-treatment control from Levy et al [41].

[e]Significant at $P < .05$.

and tetracycline monotherapy did not. Because one of the anthrax toxins, lethal factor, is a metalloprotease, some of minocycline's efficacy might be due to its metalloprotease inhibitory activity [49]. Thus, minocycline might be an option for some tetracycline-resistant *B. anthracis* strains once susceptibility has been established by laboratory testing.

Treatment

For treatment of susceptible *B. anthracis* infections, the odds of survival were increased compared with no-treatment controls for ciprofloxacin; β-lactams (with or without a β-lactamase inhibitor, including amoxicillin-clavulanate (available only orally in the United States), penicillin, procaine penicillin, and imipenem; glycopeptides; tetracyclines; and clindamycin. Although the meta-analysis OR for levofloxacin did not differ significantly from the no-treatment control, this was due to 1 study in which a relatively low dose of levofloxacin was delivered 48 to 96 hours after exposure and only 2 of 20 animals survived [22].

For treatment of ciprofloxacin-resistant *B. anthracis*, combinations of meropenem or penicillin with a PSI antimicrobial (linezolid, clindamycin, or doxycycline) or rifampin showed efficacy in mice. Rifampin combined with a PSI antimicrobial was also effective. Promising results for the combinations are plausible because the primary target site mutations that confer resistance to ciprofloxacin and other fluoroquinolones are not expected to affect β-lactams, rifampin, or PSIs [2]. However, fluoroquinolone use might increase the bacterial efflux of, and decrease susceptibility to, other antibiotics (including tetracyclines).

While doxycycline was the only tetracycline included in the combination studies (Table 3), the promising results for other tetracyclines in monotherapy against susceptible *B. anthracis* suggest that minocycline, omadacycline, and tetracycline might all be used in combination treatments against ciprofloxacin-resistant *B. anthracis* with primary target site mutations [2]. Newer tetracyclines that are less affected by efflux pump overexpression than tetracycline and doxycycline [50] also hold promise against fluoroquinolone-resistant strains, although future research is needed.

Susceptibility of suspected engineered strains should be evaluated swiftly following any intentional release to identify potentially engineered, resistant *B. anthracis* and enable rational treatment decisions [51]. While several combinations showed promising activity against ciprofloxacin-resistant *B. anthracis*, the possibility must be considered that a *B. anthracis* strain resistant to a specific antibiotic could be engineered. Point mutations at the active site of rifampin are common and clinically relevant and can confer high-level rifampin resistance [2], which prevents the use of rifampin as monotherapy. Likewise, older tetracyclines and fluoroquinolones may be affected by efflux-related resistance. Thus, these antimicrobials may prove nonefficacious against engineered, multidrug-resistant strains, and omadacycline, eravacycline, or minocycline may be better alternatives. Based on experience with other pathogens, β-lactams and glycopeptides may be promising choices, because emergence of resistance during therapy with these cell-wall synthesis inhibitors tends to be less common.

A penicillin combined with a β-lactamase inhibitor or a carbapenem (such as meropenem or imipenem) may overcome β-lactamase–related resistance. While susceptibility testing is essential, these cell-wall synthesis inhibitors may be used

Table 5. Odds of Survival for Monotherapy Studies That Met ≥1 Exclusion Criterion but Were Performed at Workgroup Request

[Study References] Animal Model[a]	Cidal or Static	Class	Antimicrobials	PEPAbx or Rx	Reason for Exclusion	Quality Score[b]	OR (95% CI) vs No Treatment
[17]GP	C	Quinolone	Ofloxacin	PEPAbx	Exposure route (intranasal), vaccine	11	171 (7.5–3910)[c]
[17]GP	C	Carbapenem	Imipenem[d,e]	PEPAbx	Exposure route (intranasal)	11	29.9 (1.3–692)[c]
[42]GP	C	Cephalosporin	Cefazolin[f]	PEPAbx	Exposure route (intranasal)	14	8.4 (0.4–177)
[43]M	S	Macrolide	Erythromycin ethylsuccinate	PEPAbx	Exposure route (intraperitoneal)	9	21.0 (1.0–454)
[17]GP	C	Aminoglycocide	Gentamicin[f]	PEPAbx	Exposure route (intranasal)	11	3.0 (0.1–83.4)
[44]M	S	Amphenicol	Chloramphenicol[d]	PEPAbx	Exposure route (intraperitoneal)	10	10.2 (0.6–184)
[42]GP	S	Sulfonamide	Trimethoprim-sulfamethoxazole	PEPAbx	Exposure route (intranasal)	14	15.4 (0.8–305)

Abbreviations: C, bactericidal; CI, confidence interval; OR, odds ratio; PEPAbx, antimicrobial postexposure prophylaxis; Rx, treatment; S, bacteriostatic.

[a]Animal model used in the relevant arms of the above studies: M, mouse; GP, guinea pig.

[b]Quality score categorization: fair (6–13 points); good (14–17 points); high (18–24 points).

[c]Significant at $P < .05$.

[d]Only available in the United States as intravenous formulation for humans.

[e]Only available in the United States in combination with cilastatin for humans; animals received imipenem only, which may have used another route of administration.

[f]Only available in the United States as intramuscular or intravenous formulation for humans, although animals may have received other formulations.

empirically if the status of penicillin-resistance is unknown. Of note, *B. anthracis* is naturally resistant to cephalosporins owing to a cephalosporinase that confers high-level resistance to cephalosporins. There were insufficient animal PK data for anthrax infections to establish PK/PD relationships for β-lactams. However, the half-life of β-lactams is much shorter in small animals (eg, mice) than in humans. Therefore, PEPAbx or treatment efficacy in mice should translate to humans, especially if β-lactams are dosed at short intervals or as prolonged infusions, because both lengthen the time the unbound drug concentrations remain above the MIC [52].

Our PK/PD analyses correlated doses and unbound drug exposures with outcomes in mice, rabbits, and NHPs. The application of PK/PD principles and available animal infection model data allowed us to predict probabilities of target attainment for PEPAbx and treatment in humans using clinically relevant dosage regimens and unbound drug exposures. Subsequent Monte Carlo simulations provided useful guidance and PK/PD breakpoints (ie, the highest MICs predicted to be treatable with good success) for clinically relevant antimicrobials.

Limitations

Our set of meta-analyses has several limitations, some inherent to our statistical approach. The model used in the meta-analyses could not combine study results and generate an OR for antimicrobials when the 2-by-2 table had 2 instances of zero cells. Some antimicrobials had large CIs because of small study sizes. Fewer than a third of the studies mentioned blinding of observers, conditions in which the animals were kept, inoculation dose by arm, humanization of the dose, randomization of exposures, PK data for the chosen antimicrobials in their animal model, use of NHPs, or immunological outcomes. No study accrued 24 quality points, and only 5 accrued >18 [21, 26, (unpublished data), 31]. Most low-quality studies were dropped from the analyses because they met exclusion criteria rather than for their low quality. However, it is possible that authors may have performed their experiments to a higher standard than was described in the respective methods sections. Some moderately high-quality studies were also dropped because of exclusion criteria—the most common reason being a nonaerosol mode of exposure in a PEPAbx study [53–56].

Because data on anthrax meningitis are limited, with only 2 articles describing meningitis in animal models falling within our time frame [40, 41], 1 article from 2020, identified by the workgroup, was included in the analysis [8]. These studies had the same methods and almost the same authors, but several methodologic flaws: 2 lacked control groups [8, 40], so controls from another study were used [41]; the model chosen, intracisternal injection of vegetative *B. anthracis*, may be confounded by trauma; antimicrobial PK data were usually lacking; antimicrobial concentrations were not assessed in cerebrospinal fluid; and the dosing regimen chosen for meropenem (40 mg/kg) was poor, as it provided coverage for only 4 of 24 hours in a day. Therefore, Table 4 should be interpreted with caution.

Some of the animal infection model data sets did not characterize the full exposure response relationships over a range of doses for PEPAbx or treatment. This led to uncertainty for

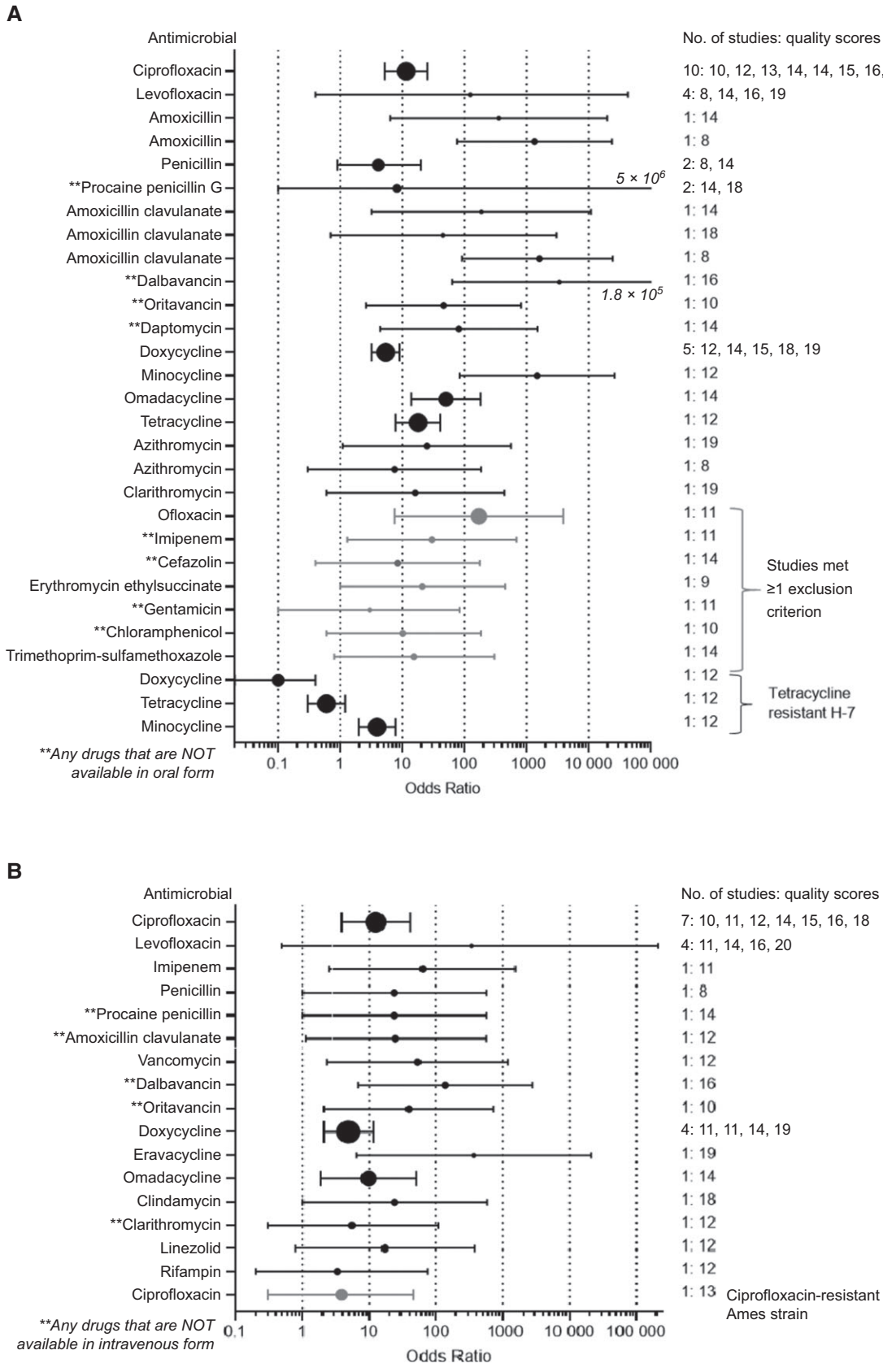

Figure 1. Comparison of efficacies for specified antimicrobials used for postexposure prophylaxis (*A*) or treatment (*B*). *A,* Odds of survival (with 95% confidence interval [CI]) for postexposure prophylaxis monotherapy studies with specified antimicrobial compared with nontreated controls. *B,* Odds of survival (with 95% CI) for treatment monotherapy studies with specified antimicrobial compared with nontreated controls.

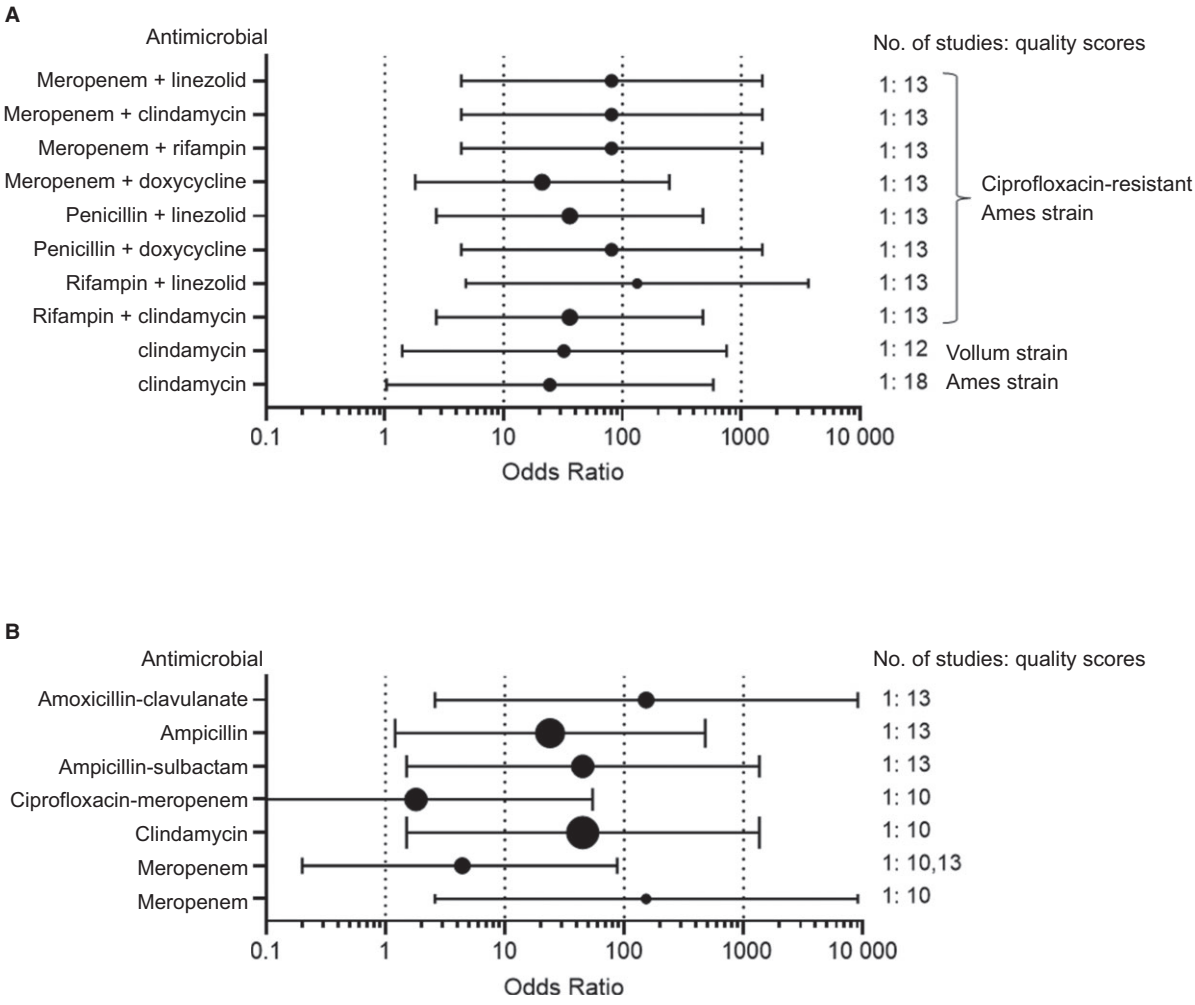

Figure 2. Comparison of efficacies for specified antimicrobial combinations for treatment of susceptible or resistant *Bacillus anthracis* (*A*) and anthrax meningitis (*B*). *A.* Odds of survival (with 95% confidence interval [CI]) for antimicrobial combinations that included ciprofloxacin for treatment of animals infected with either sensitive or resistant strains. *B,* Odds of survival (with 95% CI) for treatment monotherapy studies using a rabbit model of anthrax meningitis with a susceptible strain with specified antimicrobials compared with nontreated controls. Due to limited data availability and high mortality rates, a control arm from one anthrax meningitis paper was used as a comparison for treatment arms in 2 other papers. The 3 papers were analyzed as if they were 1 paper.

the PK/PD target values, which we used during Monte Carlo simulations. Oritavancin displayed a clear exposure response relationship. However, neither ciprofloxacin nor dalbavancin displayed exposure response relationships, since all doses (including the lowest studied doses) provided near-maximal survival. Therefore, the PK/PD predictions for ciprofloxacin and dalbavancin are conservative. The exposure response data for levofloxacin primarily arose from rabbits, which are hypersensitive to anthrax [8]. Thus, the Monte Carlo simulations for levofloxacin borrowed the PK/PD targets from ciprofloxacin. When establishing PK/PD targets, we excluded studies with treatment onset later than 36 hours, because antimicrobial treatment becomes much less effective with late treatment onset [12, 14]. While acknowledging these sources of uncertainty, we simulated clinically relevant unbound drug exposures and used a range of targets values, including conservative (ie, high) PK/PD targets to support treatment choices. Monte Carlo simulations could be performed only for ciprofloxacin, levofloxacin, doxycycline, dalbavancin, and oritavancin because other antimicrobials lacked PK data in anthrax-infected animals.

Conclusions

In summary, these meta-analyses and PK/PD evaluations synthesized the available animal literature on PEPAbx and treatment of anthrax. A wide range of promising antimicrobials are available to combat susceptible strains for natural exposures, and several monotherapy and combination therapy options are available for resistant strains.

Future research is needed on PK/PD relationships for β-lactams, a wider range of tetracyclines, and antibiotic combinations and on anthrax meningitis. Moreover, mechanistic insights can be generated to define the postantibiotic effect for antimicrobials with long

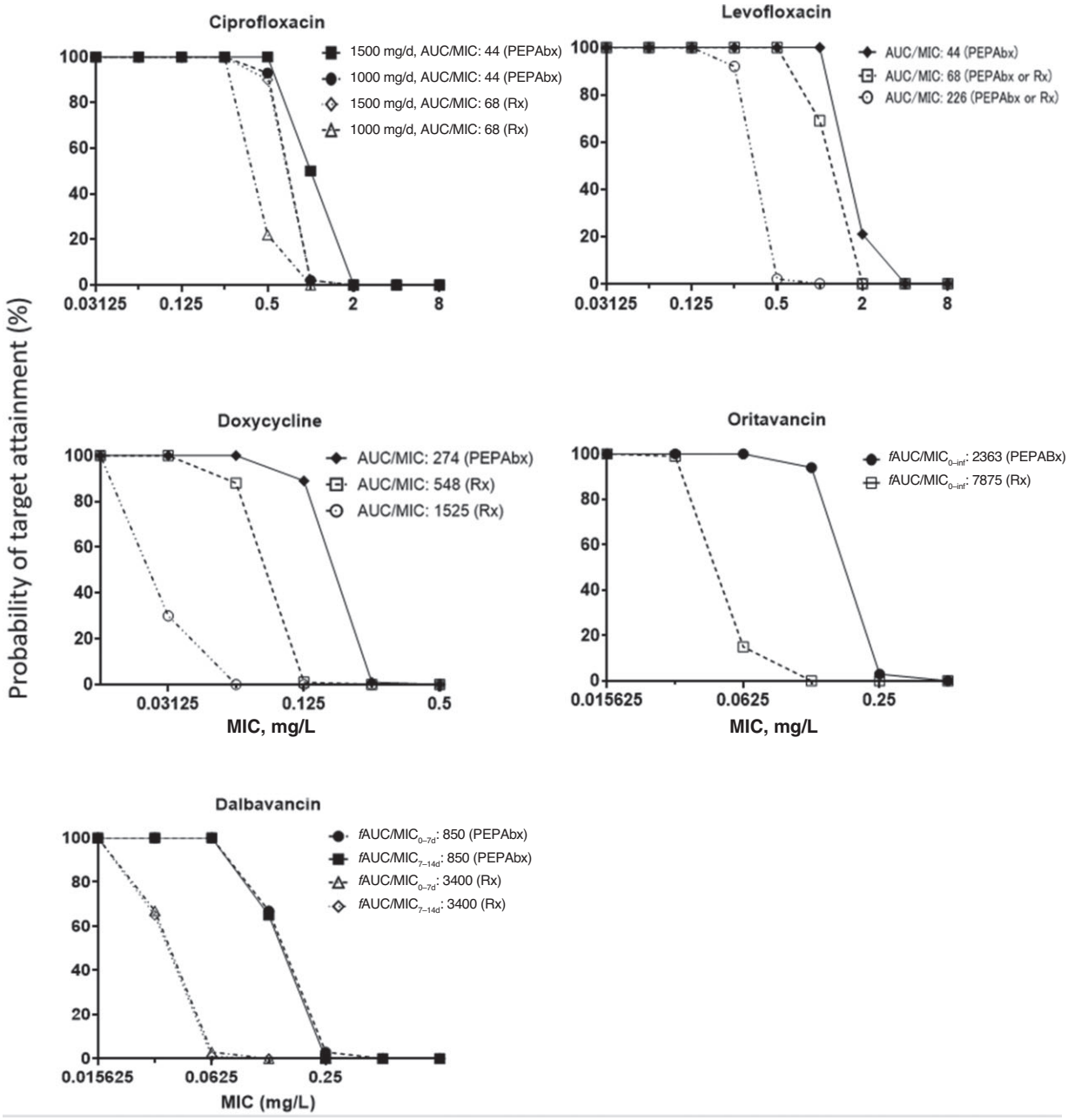

Figure 3. Monte Carlo simulation results on the probability of pharmacokinetic/pharmacodynamic target attainment plotted against the minimum inhibitory concentration (MIC) for clinically relevant dosage regimens of 5 antimicrobials. The exposure targets for antimicrobial postexposure prophylaxis (PEPAbx) and treatment (Rx) are indicated, with more details in the Supplementary Materials. Abbreviations: d, day; AUC, area under the concentration-time curve; *f*AUC, area under the unbound plasma concentration-time curve; inf, infinity.

half-lives, which would help define dosing intervals and treatment durations. Overall, these data will further support the rational design of efficacious dosage regimens to combat anthrax.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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