Effect of Delays in Concordant Antibiotic Treatment on Mortality in Patients with Hospital-Acquired *Acinetobacter* spp. Bacteremia: Emulating a Target Randomised Trial with a 13-year Retrospective Cohort

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Running head: Delays in Concordant Antibiotics for Bacteremia

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Abstract

Delays in treating bacteremias with antibiotics to which the causative organism is susceptible are expected to adversely affect patient outcomes. Quantifying the impact of such delays to concordant treatment is important for decision-making about interventions to reduce the delays and for quantifying the burden of disease due to antimicrobial resistance. There are, however, potentially important biases to be addressed including immortal time bias. Here, we aim to estimate the impact of delays in appropriate antibiotic treatment of patients with Acinetobacter spp. hospital-acquired bacteremia in Thailand on 30-day mortality by emulating a target trial using retrospective cohort data from Sunpasitthiprasong Hospital in 2003 to 2015. For each day, we defined treatment as concordant if the isolated organism was susceptible to at least one antibiotic given. Amongst 1,203 patients with Acinetobacter spp. hospital-acquired bacteremia, 682 had one or more days of delays to concordant treatment. Surprisingly, crude 30-day mortality was lower in patients with delays of ≥3 days compared to those with 1-2 days of delays. Accounting for confounders and immortal time bias resolved this paradox. Emulating a target trial, we found that these delays were associated with an absolute increase in expected 30-day mortality of 6.6% (95% CI: 0.2%, 13.0%), from 33.8% to 40.4%.

Keywords: empirical antibiotic treatment, patient mortality, Acinetobacter spp., bacteremia, causal inference

Abbreviations:
ACI-HAB Acinetobacter spp. hospital-acquired bacteremia
AST antimicrobial susceptibility tests
CCI Charlson comorbidity index
CI confidence interval
ICU intensive care unit
IPWs stabilized inverse-probability weights
LMIC Low and middle-income countries
MDR multidrug-resistant
Initial treatment for suspected bloodstream infections in hospital settings is usually given before the causative organism or its susceptibility to antibiotics is known. Such empiric antibiotic treatment is invariably designed to have broad coverage so there is a high chance that the causative organism is susceptible to one of the prescribed antibiotics; if it is susceptible, the treatment is said to be “concordant”. When designing empiric antibiotic regimes, considerations about concordance must be balanced against concerns about selection for resistance (1,2). In settings where antimicrobial resistance is common, such empirical treatment may often be “non-concordant”, i.e. the causative organism is not susceptible to any of the antibiotics given. The resulting delay in the time until the patient receives appropriate antibiotics may lead to worse patient outcomes (3) and is a key mechanism by which antimicrobial resistance is thought to add to the burden of disease (i.e. health impact due to antibiotic-resistant infections) (2,4). In most high-income settings, a diagnostic microbiology laboratory will attempt to identify the causative organism and its susceptibility to different antibiotics. The process can take 2-4 days from the time the blood sample is taken to getting antibiotic susceptibility testing results, which may prompt a change in the antibiotics given to the patient (5). Empirical antibiotic treatment might also change prior to the availability or in the absence of antibiotic susceptibility results if the patient shows no clinical improvement (5). Many hospitals in low and middle-income countries do not have access to a diagnostic microbiology service and even when they do the threshold of clinical suspicion for taking a blood culture is often higher than in high income settings. Both of these factors can lead to longer delays to concordant antibiotic treatment. Quantifying the impact of delays in concordant antibiotic treatment on patient outcomes is therefore important for understanding the potential benefits of investment in microbiological diagnostic capacity, changes in blood culture practice and improving empirical antibiotic prescribing policies. It is
also important for understanding the potential benefit of new technologies that can reduce the
time taken to obtain antibiotic susceptibility testing results.

Quantifying the causal effect of delays in concordant treatment on patient outcomes poses a
number of challenges. A “gold standard” design would be to randomise patients to different
delays until concordant treatment (6). However, it would clearly be unethical to intentionally
delay the provision of appropriate antibiotic treatment. Therefore, analysis of observational
data is likely to be the best alternative to address the question in practice. However, there are
potential biases that can threaten the validity of inferences made from observational data, and
consideration of many of these biases has been neglected from the considerable literature on
the impact of delays in appropriate antibiotic treatment on patient outcomes. Firstly, key
baseline confounders need to be considered when studying the causal relationship between
antibiotic treatment and patient in-hospital mortality, and should be adjusted for in the
analysis. Secondly, antibiotic treatment may change over the course of an infection (time-
varying exposure), and these changes may be related to the time-varying clinical severity of
the patient, which could be a confounder for the current treatment and a mediator for the
future treatment. Finally, observational studies are vulnerable to bias when patients are
classified into exposure groups after time zero (7-9). As explained in detail in Hernan et al.
2016 these biases can be avoided by specifying a target trial (8). The hypothetical target trial
does not need to be do-able in practice given current technology, ethical concerns, or
financial constraints. Rather, specifying a target trial should be considered an exercise to
make sure that the observational data is designed and analyzed in the most appropriate way
possible (8,10). Note that even when a randomized trial has been performed, analysis of
observational data informed by a target trial will often be of considerable value, for example
due to the much larger sample sizes that can be achieved and the fact that the study
population may be more representative of the population to which we wish to generalize (11).
The aims of this work are to demonstrate how an analysis that attempts to emulate a target randomized trial can address these problems, and to apply the target trial emulation methodology to quantify the impact of delays in concordant antibiotics for treating *Acinetobacter* spp. bloodstream infections in Northeast Thailand. *Acinetobacter* spp. are amongst the leading causes of bloodstream infections in Thailand, and multidrug-resistant (MDR) *Acinetobacter* spp. have been estimated to cause 15,000 excess death per year (12-14).

METHODS

**Emulating two target trials**

Trying to make causal inferences from observational data can be thought of as attempting to emulate a target randomized trial (6). A framework to make the target trial explicit to guide analytic approaches and to help avoid common methodologic pitfalls has been outlined by Hernan and Robins (6). Here, to apply the framework to the study data we first defined two hypothetical target trials to address related questions about the effect of delays to concordant antibiotic treatment on patient outcomes. Both would be unethical to perform in practice, but were intended to guide the analysis (6). Summaries of these protocols are in Web Table 1 and 2. In brief, eligibility criteria are the same in both trials: patients of any age hospitalized for at least 2 calendar days when a blood sample was collected, with *Acinetobacter* spp. identified from the blood sample. The first target trial compares two treatment strategies: at least one-day of delay in concordant antibiotic treatment versus concordant treatment on the same day as blood sample collection (i.e. no delay). In the second target trial, patients are randomly assigned to one of four treatment strategies: i) no delays, ii) one-day of delay, iii) two days of delay, and iv) at least three days of delays in receiving concordant antibiotic treatment. For both target trials, patients are randomly assigned to the treatment strategies on the date of blood sample collection and the concordant antibiotic prescribed would be determined by
physician preference (allowing physicians to select antibiotic regimen is a common approach in antibiotic treatment duration trials (15)). The follow-up period starts at randomization and ends at the day of discharge from the hospital, the day of death within the hospital, or 30 days post-randomization, whichever occurs first. The primary outcome of both trials is survival status at the end of follow-up. Note that there are no practical reasons to prevent such a trial being performed now for an organism such as Mycobacterium tuberculosis where the antibiotic resistance phenotype can be reliably determined within a few hours of taking a sample (16), but the technology to reliably predict antibiotic susceptibility in Acinetobacter spp. from the genotype has not yet been developed. The causal contrasts of interest are the per-protocol effect. We focus on the concordance of empirical antibiotics prescribed within the first three days after a blood sample is taken for microbiology culture without taking into account the dosing and frequency.

Retrospective cohort data to emulate the target trials

We identified, from a 13-year retrospective cohort, patients with Acinetobacter spp. hospital-acquired bacteremia (ACI-HAB) in Sunpasitthiprasong Hospital, Thailand. This is a provincial hospital with 1,500 beds. The hospital has a microbiology laboratory that performs microbial culture for isolate identification and antimicrobial susceptibility tests (AST) on a daily basis. The number of blood cultures performed in 2003 was 11,584 and in 2015 was 56,719. Patients were eligible for inclusion in this study if they had stayed in the hospital longer than 2 calendar days when a blood sample with growth of Acinetobacter spp. was collected. During the study period, bacterial culture was performed using standard methodologies for bacterial identification and AST based on guidelines of the Bureau of Laboratory Quality and Standards, Ministry of Public Health, Thailand (17). AST was performed using the disk diffusion method based on Clinical and Laboratory Standards.
Institute guidelines (18). The first episode of *Acinetobacter* spp. bloodstream infection per eligible patient was included in the analysis. If more than one isolate of *Acinetobacter* spp. with different susceptibility profiles was identified on the same day, only the isolate resistant to the largest number of antibiotics tested was included in the analysis. Data on antibiotic prescription, ICD-10 diagnosis, and demographics were collected for analysis.

The study was approved by the Institutional Review Board of Sunpasitthiprasong Hospital (Ref. 005/2560). STROBE recommendations were followed (Web Table 3).

**Covariate selection**

Potential confounders were identified using a directed acyclic graph to represent the presumed causal relationships between antibiotic treatment and patient mortality (Web Figure 1) (19). The key potential baseline confounders identified were severity of underlying illness (20), antibiotic resistance pattern of the *Acinetobacter* spp. isolated from the blood sample, year in which the blood samples were collected, and specialty of the attending physician. We used the time between date of admission and date of blood sample collection, admission to intensive care unit (ICU) on the day of hospital admission, the number of days on antibiotic treatment prior to blood sample collection, and age-stratified Charlson comorbidity index (CCI) score as surrogates of severity of underlying illness. The CCI scores were calculated from the ICD 10 codes given to each patient by the attending physicians (21). MDR *Acinetobacter* spp. was defined as previously described (22). As data on specialty of the attending physician is not routinely collected in the electronic record, we used the department in which the patient was treated on the day of blood collection as a proxy variable. A time-varying confounder that could affect changes in empirical antibiotic treatment post blood sample collection is severity of infection, which could be affected by the history of treatment...
and itself may influence the decision on future treatment. The prescription of a vasopressor and transfer to ICU during the infection within the analysis time period were used to represent severity of the infection and both coded as binary time varying variables. Patient demographic information, age and gender, were also included as covariates.

**Exposure groups**

We considered any antibiotics prescribed within three days of the date of collection of the first blood sample from which *Acinetobacter* spp. was isolated to represent empirical treatment. This is because microbiological identification and AST usually require at least three days in hospitals in low and middle-income countries (LMICs). During the first three days of blood sample collection, appropriate treatment cannot be guaranteed for each and every case and may be influenced by patient characteristics, physician experiences with antibiotic prescription, and local epidemiology of antimicrobial resistance. An antibiotic regimen was defined as concordant if susceptibility testing indicated that the isolated *Acinetobacter* spp. was susceptible to at least one of the antibiotics given. Otherwise, the regimen was defined as discordant. Concordance of the antibiotic treatment was determined for each eligible patient on the day of blood sample collection (t=0), one calendar day after blood sample collection (t=1), and two calendar days after blood sample collection (t=2). The four exposures groups are: 1) patients with no delays in concordant antibiotic treatment (i.e. patients who had concordant treatment on the day of blood sample collection); 2) patients with one-day delay in concordant treatment (i.e. patients who did not have concordant antibiotic treatment at the least on t=1, which included those who died or were discharged at t=1); 3) patients with two days of delay in concordant treatment (i.e. patients who did not have concordant antibiotic treatment at the least on t=1 and t=2, which included those who...
died or were discharged at t=2); and 4) patients with at least three days of delay in concordant treatment.

Outcomes

The primary outcome is in-hospital all-cause mortality within 30 days of the date of collecting the first blood sample from which *Acinetobacter* spp. was isolated. If a patient was discharged alive from the hospital before Day 30 or remained in the hospital on Day 30, then the patient was considered to have survived in the primary analysis.

It is a common practice in Thailand for moribund patients to be discharged and to die at home. This may cause misclassification of outcomes. To address the issue and to see the impact of assuming no discharged patient dies within 30 days on the estimated effect, we performed a sensitivity analysis (Web Table 4 and 5) and patients who were either discharged without improvement or who rejected treatment and were discharged were classified into the group assumed to have died within 30 days.

Statistical analysis

The effects of delays in concordant empirical antibiotic treatment on 30-day mortality were estimated using marginal structural models (23). We performed two analyses. The first analysis was to evaluate the impact of one or more days of delays in concordant antibiotic treatment (ie. emulating the first target trial). The second analysis was to evaluate the effect of one-day, two-day, and three or more days of delays in concordant antibiotic treatment (ie. emulating the second target trial). Stabilized inverse-probability weights (IPWs) were calculated for the two analyses independently based on methods described elsewhere (9). We applied two sets of IPWs to a marginal structural model. Firstly, a propensity score for each
patient was calculated to represent the probability of being prescribed with a concordant antibiotic treatment on the day of blood sample collection, one day after, and two days after. The propensity scores were then used to calculate stabilized IPWs. Secondly, to emulate the second target trial with treatment regimen assigned on enrolment, we used the three-step procedure described by Hernan (9). Briefly, three duplicates of each observation were created to represent a dataset in which counterfactual treatments were included. For instance, for a patient who had no concordant antibiotic on \( t=0 \) and \( t=1 \), and then died or was discharged from the hospital on \( t=2 \), three clones were created giving four observations for the patient (one observed and three counterfactual treatments with each copy assigned to a different treatment strategy). Then those clones that deviated from their assigned strategy were artificially censored. In the example above, the clones that were assigned to no delays and one day of delay in concordant antibiotic treatment were censored because they deviated from their assigned strategy. Lastly, to address the selection bias due to the censoring process, the uncensored copies were given a weight that is equal to the inverse of the probability of being uncensored (9). We then applied a marginal structural logistic regression model with the stabilized IPWs to estimate the marginal probability of 30-day mortality under each treatment regimen. Statistical analyses were done using STATA, version 15.1 (StataCorp LP, College station, Texas, USA). Detailed descriptions of the statistical analysis and code are provided in Web Appendix 1, Web Figure 2, and Web Table 6. A simulation study was also performed and confirmed that the procedure could recover the expected 30-day mortality associated with delays in concordant antibiotic treatment (Web Appendix 2).

We assessed our study using the Risk of Bias in Nonrandomized Studies of Interventions tools: ROBINS-I. The detailed assessment results are in Web Table 7.
RESULTS

Patients

Between January 1, 2003, and December 31, 2015, 1,203 inpatients had a blood sample collected two or more days after hospital admission yielding *Acinetobacter* spp. (Figure 1). Amongst the eligible patient cohort, 521 patients had no delays in concordant antibiotic treatment (i.e. patients had concordant treatment on the day of blood sample collection); 224 patients had a one-day delay; 119 patients had a two-day delay; and 339 patients had three or more days of delays in concordant antibiotic treatment.

Patient characteristics varied across the four groups of exposures (Table 1). The proportion of patients admitted to ICU wards on the day of hospitalization was highest among those having a one-day delay in concordant antibiotic treatment (159 of 224 patients; 71.0%), followed by those having a two-day delay (72 of 119 patients, 60.5%). The proportion of patients with MDR *Acinetobacter* spp. isolates was highest among those who had a one-day delay in concordant antibiotic treatment (206 of 224; 92.0%), followed by those who had three or more days of delay (302 of 339 patients; 89.1%). The majority of the MDR *Acinetobacter* spp. isolates were also resistant to carbapenem (877 of 975; 90.0%), and most of the non-MDR *Acinetobacter* spp. were susceptible to carbapenem (209 of 228; 91.7%).

The most commonly prescribed antibiotics on the day of blood sample collection were carbapenems (n=312) followed by ceftazidime (n=121). Of 467 patients who died within 30 days after blood sample collection, 294 patients did not have a concordant antibiotic prescription on the day of blood sample collection. Of those patients, 33.7% (99/294) had a prescription of a carbapenem, 16.0% (47/294) did not have an antibiotic prescription, and 9.9% (29/294) had a prescription of a third-generation cephalosporin. Of 736 patients who
survived for at least 30 days after the first positive blood sample was collected, 24.2% (178/736) had a prescription of a carbapenem on the day of blood sample collection, 17.1% (126/736) had a prescription of a third-generation cephalosporin, and 10.1% (74/736) did not have an antibiotic prescription.

**Antibiotic treatment concordance on the day of blood sample collection and 30-day in-hospital mortality**

Based on the analysis to emulate the first target trial, results showed that receiving concordant antibiotic treatment on the day of blood collection was associated with reduced 30-day mortality, after adjusting for the pre-specified confounders. Patients given concordant antibiotic treatment on the day of blood collection had an expected 30-day mortality of 33.8% (95% CI: 29.1%, 38.5%), compared with an expected 30-day mortality of 40.4% (95% CI: 36.1%, 44.7%) in those not treated with concordant antibiotics. The absolute difference was 6.6% (95% CI: 0.2%, 13.0%).

**Days of delays in concordant antibiotic treatment and 30-day in-hospital mortality**

In the analysis to emulate the second target trial, the crude 30-day in-hospital all-cause mortality was highest among those with a one-day delay in concordant antibiotic treatment (133 of 224 patients; 59.4%), and lowest among those with three or more days of delays in concordant antibiotic treatment (102 of 339 patients; 30.1%) (Table 1). The discharge pattern of patients under different treatment groups varied over the three days of the initial treatment period (Figure 2). Amongst the 1,203 eligible patients, 236 (19.6%) either died or were discharged from the hospital one day after blood was collected for culture and, of those patients, 63.6% (150 out of 236) died within the hospital (Figure 2).
The marginal structural model adjusting for baseline confounders, time-varying confounders, and immortal time bias resolved paradoxical observations in the crude data (Table 2). While the crude analysis suggested that patients with three or more days of delays in concordant antibiotic treatment had the lowest mortality, the adjusted analysis found that the expected mortality was lowest if the patients had no delays, though we found no evidence of increasing mortality with increasing delays. However, uncertainty was large. Absolute differences in mortality between no delays in concordant antibiotic treatment and a one-day delay, a two-day delay, and three or more days of delays were 3.0% (95% CI: -12.0%, 18.0%); 11.3% (-3.0%, 25.6%); and 1.1% (-7.8%, 10.0%), respectively.

Sensitivity analysis

Under the assumption that patients discharged within 30 days of the first positive blood culture either without improvement or having rejected treatment died within 30 days, a similar effect of delayed concordant treatment was observed. If patients were given a concordant antibiotic treatment on the day of blood sample collection, the expected marginal probability of developing a detrimental outcome (death or discharge without improvement) would be 58.9% (95% CI: 53.8%, 63.9%), which is lower than if they were not given a concordant antibiotic treatment (62.0% [95% CI: 57.7%, 66.4%]). The difference was 3.2% (95% CI: -3.5%, 9.9%). The estimated marginal probabilities of developing a detrimental outcome (death or discharge without improvement) within 30 days of blood collection were 64.6% (95% CI: 56.8%, 72.4%), 63.2% (95% CI: 50.4%, 76.0%), 68.4% (95% CI: 56.1%, 80.7%), 63.4% (95% CI: 58.5%, 68.3%) for no delays, a one-day delay, a two-day delay, and three or more days of delays in concordant antibiotic treatment respectively (Web Table 4). The estimated impacts of the treatment regimens on detrimental outcomes were similar to the effects on 30-day in-hospital mortality (Web Table 5).
DISCUSSION

After adjusting for measured confounders, we found that delays in concordant antibiotic treatment of one or more days were associated with an absolute increase of 6.6% in 30-day mortality from 33.8% to 40.4% in the first analysis attempting to emulate a target trial with two treatment arms. There was no evidence of a dose response relationship between days of delays in concordant antibiotics and 30-day mortality in the second analysis.

ACI-HAB is associated with increased patient mortality, especially in developing countries (12). The proportion of hospital-acquired Acinetobacter spp. bacteremias that are MDR can be as high as 75%, and attributable mortality has been estimated to range from 18-41% in developing countries (12-14, 24-27). Therapeutic options for treating MDR Acinetobacter spp. infections are limited. Carbapenem, colistin, and tigecycline are currently the last resort antibiotics for drug-resistant Acinetobacter spp. bloodstream infection, and increasing resistance to these antibiotics has been reported in developing countries (28,29). The spread of resistant pathogens can be fueled by the overuse of broad-spectrum antibiotics in hospital settings (2), and Acinetobacter spp. has an ability to assemble different mechanisms of resistance (12). Hence, time to initiation of antibiotic treatment in patients suspected of having bacterial infection is important to both control the spread of resistant infection and to save lives. Hospital antibiotic policies that minimize morbidity and mortality due to infections, while preserving the effectiveness of antimicrobial agents for treatment purposes are important in preventing the spread of microbial infections (1). Moreover, statistics on the impact of delays to concordant antibiotic treatment are important when estimating the potential benefits of diagnostic stewardship and of the efforts to reduce the time taken to obtain antibiotic susceptibility resting results. Our estimates of the impact of delays in concordant empirical antibiotic treatment on patient mortality among those with hospital-
acquired Acinetobacter spp. bloodstream infection will be of particular relevance in settings with a high incidence of drug-resistant Acinetobacter spp. infection.

Immortal time bias is a common phenomenon and needs to be considered in studies comparing treatment regimens where the observed treatment durations vary (7,8). In this study empirical antibiotic treatment over a period of three days after blood collection was considered and only patients who survived up to three days after blood collection could be classified into the group of “≥3 days of delays in concordant treatment”. Hence, by definition, they cannot have died within the first 3 days and for this time period they are effectively “immortal”. This bias will tend to make them appear to survive longer compared to the reference group (no delays in concordant antibiotic treatment). This is reflected in the paradoxical observation that the crude all-cause 30-day mortality was lowest among patients with ≥3 days of delay in concordant antibiotic treatment. This bias can be avoided (and the paradox resolved) by adopting the target trial emulation methodology as described by Hernan et al (8) and employed in our analysis.

Previous analyses have evaluated the impact of delayed antibiotic treatment on outcomes for patients with bacteremia related to Acinetobacter spp., but appropriate adjustment for biases has been lacking (30-37). A study in Taiwan on 252 patients with ICU-acquired bloodstream infections caused by Acinetobacter baumannii suggested appropriate antibiotic therapy (defined as administration of at least one antibiotic treatment that is appropriate in type, route and dosage within 48 hours of bacteremia onset) reduced 14-day mortality (adjusted odds ratio was 0.22 [95% CI: 0.10, 0.50]) (34). The analysis used a multivariable logistic regression model adjusted for APACHE II score measured two days prior to bacteremia onset and for malignancy. In this study, among those with APACHE II score >35 more than 70%
of patients died within 24 hours in the inappropriate antibiotic group and in the appropriate treatment group no patient died within the initial 48 hours treatment period (34). Some of the reported differences in survival probability are therefore expected to be due to immortal time bias.

In most previous studies antibiotic use has been considered as a binary variable and switching of antibiotic regimens due to changes in clinical symptoms has been neglected (5). In hospitals in resource-limited settings, antibiotics are sometimes prescribed even before a clinical specimen is taken for culture, and switching regimen in response to severity of infection is common (5). This change in regimen determined by clinical signs, if not adjusted for using appropriate methods for time-varying confounders, may also lead to biases (6,23). Marginal structural models have been used to adjust for time-varying confounders in a previous study of the association between appropriate antibiotic treatment for bacteremia on the day the blood culture was taken and mortality and discharge (38). The previous study found no evidence for a protective effect of appropriate empirical antibiotic treatment on mortality and discharge, but confidence intervals were wide (38). Differences in bacterial species considered, patient characteristics and clinical setting make direct comparison with the current study inappropriate.

Our study has limitations. Firstly, data on the severity of infections were not routinely collected, and this is typically the case in LMIC settings. We used admission to ICU and prescription of vasopressor as proxy variables for the severity of infection. These proxy variables will only imperfectly represent the severity of infection and residual confounding is to be expected; however, both are specific in representing severe clinical conditions. Secondly, despite the relatively large sample size, the power to detect a dose-response
relationship in the four regimens under evaluation might be low. This is reflected in the wide confidence intervals in our expected mortality under each regimen. Thirdly, our emulation included only ACI-HAB. Initiating empirical antibiotic treatment for patients with hospital-acquired sepsis would include both ACI-HAB and non-ACI-HAB. Nonetheless, point-of-care rapid diagnostic tests for ACI-HAB with AST results are critically needed to differentiate from non-ACI-HAB to avoid overuse of antibiotics that target non-MDR and MDR AS-HAB. Fourthly, residual confounding factors may be present.

In conclusion, we observed a 6.6% (95% CI: 0.2%, 13.0%) absolute increase in mortality among patients with hospital-acquired Acinetobacter spp. bacteremia when concordant antibiotic treatment was delayed for one or more days. Accounting for confounding and immortal time biases is necessary when attempting to estimate causal effects of delayed concordant treatment and, in this case, helped resolve paradoxical results in the initial crude data analysis.

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Table 1. Characteristics of patients with hospital-acquired bloodstream infection related to *Acinetobacter* spp..

| Covariates | No delays in concordant antibiotic treatment (n=521) | One-day of delay in concordant antibiotic treatment (n=224) | Two-day of delay in concordant antibiotic treatment (n=119) | Three or more days of delay in concordant antibiotic treatment (n=339) |
|------------|---------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
| No.        | %                                                 | No.                                                     | %                                                       | No.                                                     |
| Age (years)<sup>a</sup> | 54 (26-69)                                       | 57 (12-70)                                               | 51 (14-69)                                               | 54 (6-70)                                               |
| Female gender | 212 40.7                                         | 103 46.0                                                  | 56 47.1                                                  | 143 42.2                                                |
| Fluoroquinolone-resistance | 309 59.3                                         | 191 85.3                                                  | 93 78.2                                                  | 266 78.5                                                |
| Carbapenem-resistance   | 312 59.9                                         | 195 87.1                                                  | 103 86.6                                                  | 286 84.4                                                |
| Multi-drug resistance   | 364 69.9                                         | 206 92.0                                                  | 103 86.6                                                  | 302 89.1                                                |
| Age-adjusted CCI score on admission<sup>a</sup> | 2 (0-4)                                          | 2 (0-5)                                                   | 2 (0-4)                                                   | 2 (0-4)                                                 |
| Patients with vasopressor prescription on the day of blood sample collected for culture | 211 40.5                                         | 129 57.6                                                  | 50 42.0                                                  | 103 30.4                                                |
| Patients with vasopressor prescription on the second day of blood sample collected for culture | 176 38.7<sup>b</sup> | 22 40.7<sup>c</sup> | 43 36.1 | 117 34.5 |
| Patients with vasopressor prescription on the third day of blood sample | 133 34.1<sup>d</sup> | 17 34.7<sup>e</sup> | 15 38.5<sup>f</sup> | 97 28.6 |

<sup>a</sup> Age adjusted Charlson Comorbidity Index score on admission

<sup>b</sup> p = 0.001

<sup>c</sup> p = 0.007

<sup>d</sup> p = 0.001

<sup>e</sup> p = 0.010

<sup>f</sup> p = 0.004
|                                |     |     |     |     |     |     |
|--------------------------------|-----|-----|-----|-----|-----|-----|
| Patients admitted to ICU on   | 285 | 54.7| 159 | 71.0| 72  | 60.5|
| the day of hospitalisation    |     |     |     |     |     |     |
| Patients in the ICU on the    | 304 | 58.4| 156 | 69.6| 70  | 58.8|
| day of blood sample           |     |     |     |     |     |     |
| collected for culture         |     |     |     |     |     |     |
| Overall in-hospital mortality | 194 | 37.2| 137 | 61.2| 59  | 49.6|
| 30-day in-hospital            | 173 | 33.2| 133 | 59.4| 59  | 49.6|
| mortality since blood         |     |     |     |     |     |     |
| collection                   |     |     |     |     |     |     |
| Length of hospital stay       | 9 (6-19) | 8 (6-15) | 9 (6-15) | 10 (6-17) |
| since admission to blood       |     |     |     |     |     |     |
| sample collected for culture   |     |     |     |     |     |     |
| (days)\textsuperscript{a}      |     |     |     |     |     |     |
| Days on antibiotic prior      | 8 (5-15) | 5 (0-10) | 7 (3-12) | 7 (4-14) |
| blood sample collection       |     |     |     |     |     |     |

ICU=intensive care unit. CCI=Charlson Comorbidity Index Score; defined using ICD10 scores (21).

\textsuperscript{a}Values are expressed as median (interquartile range).\textsuperscript{b}Denominator is 455 as 66 patients with no delays in concordant antibiotic treatment were discharged from the hospital or died after t=0.

\textsuperscript{c}Denominator is 54 as 170 patients with a one-day delay in concordant antibiotic treatment were discharged from the hospital or died after t=0.\textsuperscript{d}Denominator is 390 as 65 more patients with no delays in concordant antibiotic treatment were discharged from the hospital or died after t=1.\textsuperscript{e}Denominator is 49 as 5 more patients with a one-day delay in concordant antibiotic treatment were discharged from the hospital or died after t=1.\textsuperscript{f}Denominator is 39 as 80 patients with a two-day delay in concordant antibiotic treatment were discharged from the hospital or died after t=1.

Multidrug resistance is defined as non-susceptible to ≥1 agent in ≥3 antimicrobial categories (22).
Table 2. Estimated probability of 30-day mortality under each exposure group.

| Treatment regimen                                      | Total No. | Crude 30-days in-hospital all-cause mortality (n) | Expected 30-days mortality (95% CI) under each treatment regimen<sup>a</sup> |
|---------------------------------------------------------|-----------|---------------------------------------------------|---------------------------------------------------------------------------|
| No delays in concordant antibiotic treatment (n=521)    | 521       | 173                                               | 39.8                                                                      |
|                                                         |           |                                                   | 32.3-47.2                                                                 |
| A one-day delay in concordant antibiotic treatment (n=224)| 224       | 133                                               | 42.8                                                                      |
|                                                         |           |                                                   | 29.8-55.7                                                                 |
| A two-day delay in concordant antibiotic treatment (n=119)| 119       | 59                                                | 51.0                                                                      |
|                                                         |           |                                                   | 38.9-63.1                                                                 |
| Three or more days of delays in concordant antibiotic treatment (n=339)| 339       | 102                                               | 40.9                                                                      |
|                                                         |           |                                                   | 36.0-45.8                                                                 |

<sup>a</sup>Estimated from a marginal structural model with stabilized IPWs
Figures

**Figure 1.** Flow chart of patients identified in the hospital microbiology database and included in the analysis.

**Figure 2.** Regimen assignment, all-cause 30-day in-hospital mortality, and discharge patterns over three days post blood sample collection of the study cohort.

**Footnote:** “Treatment starts” indicates the time at which an empirical antibiotic treatment would be initiated for a patient in a hypothetical randomized controlled trial. This is the time at which patients are enrolled into the study and randomized to one of the four treatment strategies.
No. of Days Since Blood Sample Was Collected for Culture

Key
*Groups of Patients That are Discharged or Died Before Reaching the Third Day of Antibiotic Treatment (t = 2)

- Unobserved
- Concordant Antibiotic Treatment
- Discordant Antibiotic Treatment

Crude Mortality

| No. | Total No. | % |
|-----|-----------|---|
| 84  | 342       | 26|
| 23  | 34        | 98|
| 11  | 24        | 46|
| 0   | 3         | 0 |
| 16  | 31        | 48|
| 6   | 21        | 29|
| 34  | 66        | 52|
| 12  | 44        | 27|
| 2   | 5         | 40|
| 3   | 5         | 60|
| 116 | 170       | 68|
| 15  | 39        | 38|
| 44  | 80        | 55|
| 102 | 339       | 30|