Online supplement for "In critically ill patients, anti-anaerobic antibiotics increase risk of adverse clinical outcomes"

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Supplemental Methods

Ethics statement

All analyses of human data were approved by the University of Michigan Institutional Research Board (HUM00102282 and HUM00104714). All animal studies were approved by the Institutional Animal Care and Use Committee at the University of Michigan (PRO00009673). Laboratory animal care policies at the University of Michigan follow the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Study design and rationale

We designed a retrospective cohort study of patients admitted to one of the six intensive care units at the University of Michigan through the period of 2016-2019. We included patients who received mechanical ventilation for at least 72 hours and were treated with intravenous antibiotics up to 72 hours prior to mechanical ventilation. We excluded patients who were mechanically ventilated for less than 72 hours, and patients transferred from an outside medical facility. For patients with multiple hospital admissions during the study period, we recorded data for the first hospitalization only.

Identification of eligible subjects

Eligible patients were identified through a Structured Query Language (SQL) query of the University of Michigan's Research Data Warehouse. We extracted ventilatory support documentation from nursing flowsheets to capture which patients were mechanically ventilated for over 72 hours. We next extracted information regarding medication orders from the medication administration record. We identified all antimicrobial administrations for the population using a list of search terms that captured every antibiotic available on our hospital formulary during the study period (**Supplemental Table S1**).

Identification of outside facility transfers

We performed a text search of the available documentation in the electronic medical record to identify patients who were potentially transferred from an outside facility using the University of Michigan's Electronic Medical Record Search Engine (EMERSE)[1,2]. We used a defined list of search terms to identify patients potentially transferred from an outside hospital (**Supplemental Table S2**). Those patients with the search terms present in either a hospital transfer note or admission History and Physical underwent physician adjudication to confirm that they were not directly admitted to the University of Michigan hospital. Patients confirmed to have been transferred from an outside facility were excluded from the cohort.

Classification of anti-anaerobic activity of administered antibiotics

Antibiotics were classified as "anti-anaerobic" based on widely accepted clinical criteria. Namely, "anti-anaerobic antibiotics" are agents recommended for treatment of anaerobic pathogens isolated in odontogenic infections[3,4], deep-space neck infections[5], diabetic foot ulcers[6], intraabdominal sepsis[7], and necrotizing skin infection[8] based on guidelines from Infectious Diseases Society of America and other major medical associations. Prior studies have found that antibiotics meeting this definition of "anti-anaerobic" activity cause greater disruption to gut microbiota than other antibiotics when measured with both culture-dependent techniques[9–12] and culture-independent (sequencing-based) techniques[13–15]. For agents with variable activity against anaerobic pathogens, we considered pharmacokinetic and pharmacodynamic profiles of the antibiotic and data from observational studies of the impact of these medications on gut microbiota when considering how they should be classified. We ultimately decided to classify agents with variable activity as anti-anaerobic if they met two criteria: 1) intravenous formulations of the medication achieved high concentrations in the gut lumen; and 2) they have consistently associated with significant gut microbiota disruption in observational studies of humans. A complete listing of the antibiotics considered anti-anaerobic is listed in **Supplemental Table 3**. A summary of antimicrobial use by anti-anaerobic classification is presented in **Supplemental Table 5** and **Supplemental Figure 1**.

Primary Outcome: VAP-free survival
As a primary outcome, we used a composite outcome of VAP or in-hospital death, defined as "VAP-free survival": the time from the initiation of mechanical ventilation to the time VAP onset or death. We chose this endpoint because 1) it is a validated measure used in randomized clinical trials for VAP prevention[16–19] and 2) it addresses threats to validity introduced by competing risks (e.g., patients may die before they have the opportunity to develop VAP, or may die due to VAP before it can be diagnosed). Patients were censored from survival analysis at the time of hospital discharge if they were discharged prior to 30 days.

VAP adjudication
VAP was diagnosed using a streamlined version of CDC surveillance criteria for VAP, previously shown to be highly reliable and predictive of patient outcomes[20–22] (**Table 1**, main manuscript). To be eligible to be considered as having developed VAP, an endotracheal aspirate **or** BAL culture must have been collected during a patient's time on mechanical ventilation. The date of collection of this respiratory sample served as a timestamp for VAP development.

All of the following criteria within the 96 hours preceding or following collection of sputum culture had to be fulfilled to be considered a case of VAP:

I. Sustained two days of increasing daily minimum $FiO_2 \geq 0.15$ or PEEP >2.5 mm H_2O as determined by the validated screening algorithm for ventilator-associated complications.
    a. For PEEP
        i. Day -3 Minimum PEEP<= Day -2 Minimum PEEP, if not, then stop and do not advance to next step in algorithm
        ii. Day -2 Minimum PEEP**+2.5 mm** Hg<= Day -1 Minimum PEEP, if not then no VAP do not advance to next step in algorithm
        iii. Day -1 Minimum PEEP **+ 2.5 mm** g<=Day 0 Minimum PEEP (day of sputum collection) Then patient meets criteria for increased PEEP
    b. For FiO2
        i. Day -3 Minimum FiO2<= Day -2 Minimum FiO2, if not, then stop and do not advance to next step in algorithm
        ii. Day -2 Minimum FiO2 **+0.15** <= Day-1 Minimum FiO2 if not then do not advance to next step in algorithm
        iii. Day -1 Minimum FiO2 **+ 0.15**<=Day 0 (day of sputum collection) Minimum FiO2 Then patient meets criteria for increased FiO2
II. One or both of the following systemic symptoms
    a. Fever (>38º C)
    b. Leukocytosis (>12,000 WBC) or Leukopenia (<4000 WBC)

At least one of the following changes in sputum had to be met to be considered VAP:

III. Pulmonary inflammation as evidenced by the presence of alveolar neutrophils:
    a. >26 neutrophils per high power field on gram stain of endotracheal aspirate (read as moderate PMNs on gram stain)
    b. >500 WBC on BAL fluid

IV. Either bacterial growth with known pathogens or growth of Aspergillus mold species on endotracheal aspirate or BAL culture

Patients who met all other criteria for VAP but did not grow a pathogen on respiratory culture were categorized as having “probable VAP,” while patients who met all criteria were categorized as having “confirmed VAP.” Time of VAP onset was defined as time of respiratory culture collection.

Secondary Outcomes: Infection-free Survival and Mortality
In addition to VAP, we characterized all nosocomial infections in the cohort. We defined nosocomial infections as a culture-confirmed infection that was not present on admission, grown from a site typically considered “sterile” (blood, urine, ascites fluid, cerebrospinal fluid, deep tissue culture, or lower respiratory tract specimen), meeting clinical criteria set by major medical societies[8,23–26]. We used the time of culture acquisition, death, or discharge to calculate “infection-free survival.” Nosocomial infections were adjudicated by an Infectious Diseases-trained physician who was blinded to primary exposure (receipt of anti-anaerobic antibiotics). Mortality was adjudicated via chart review. We determined each patient’s vital status at 30 days via review of the electronic medical record, as well via the Michigan Department of Health and Human Services Genealogical Death Indexing Services[27] and CDC National Death Index[28].

Cause of death determination
Death certificates and the discharge summary in the electronic medical record were processed using the CDC National Vital Statistics System’s Instruction for Classification of Underlying and Multiple Causes of Death – 2022[29], to assign a cause of death to the decedents in the cohort. This process that has previously been used to document and report life expectancy and mortality rates in the United States[30].

We first used the EMERSE search engine to identify and pull text from the death note from all decedents in the cohort. The death note in the EMR at the University of Michigan adheres closely to the structure of the U.S. Standard Certificate of Death and provides spaces for the certifying physician to record information concerning the diseases, conditions and injuries which resulted in or contributed to death. Specifically, a physician is requested to report both the immediate cause of death, and the antecedent conditions which gave rise to the listed immediate cause of death.

We followed the selection rules of the General Principle outlined by the National Vital Statistics Instructions for Classification of Underlying and Multiple Causes of Death[29]. The General Principle states, “When more than one condition is entered on the [death] certificate, the condition entered alone on the lowest line of Part I [containing the diseases, conditions, and injury which resulted in or contributed to death] should be selected if it could only have given rise to all conditions entered above it.” Thus, in most cases, a physician adjudicator selected the diagnosis listed last in the death note as the cause of death, as this was the cause of death that the treating medical team deemed to be the condition which gave rise to all the conditions entered above it.

In cases when the General Principle was violated, we followed the selection rules outlined in the Instructions:

I. Rule 1: If the General Principle does not apply and there is a reported sequence terminating in the condition first entered on the certificate, select the originating cause of this sequence. If there is more than one sequence terminating in the condition mentioned first, select the originating cause of the first-mentioned sequence

a. Example provided by Instructions:
    i. Recorded diagnoses
        1. Bronchopneumonia
        2. Cerebral infarction and hypertensive heart disease
    ii. General principle is violated as there are 2 diagnoses on the lowest line used
    iii. Cerebral infarction is selected, since this is the first mentioned originating cause

II. Rule 2: If there is no reported sequence terminating in the condition entered on the certificate, select this first mentioned condition.

a. Example provided by Instructions:
    i. Recorded diagnoses
        1. Pernicious anemia and gangrene of foot
        2. Atherosclerosis
    ii. General principle is violated as Pernicious anemia due to atherosclerosis is not an acceptable sequence.
    iii. The reported sequence of gangrene of the foot due to atherosclerosis does not terminate in the condition first entered in certificate
    iv. Pernicious anemia is selected

III. Rule 3: If the condition selected by the General Principle or by Rule 1 or Rule 2 is obviously a direct consequence of another reported condition, whether in Part I or Part II, select this primary condition

a. A full list of "obvious consequences" can be found at https://www.cdc.gov/nchs/nvss/manuals/2022/2a-sectionii-2022.htm
b. Notable frequent uses of the "obvious consequence guidance" among decedents in the cohort were as follows:
    i. Dehydration as an obvious consequence of intestinal infectious diseases
    ii. Acute renal failure as an obvious consequence of urinary tract infection, provided that renal failure was absent before urinary tract infection.
    iii. Arterial embolism as an obvious consequence of atrial fibrillation
    iv. Secondary or unspecified anemia, malnutrition, or cachexia as an obvious consequence of malignant neoplasm
    v. An operation on a given organ is a direct consequence of any surgical condition (such as malignant tumor or injury) of the same organ reported anywhere on the certificate.

A physician adjudicator followed the instructions outlined and pulled the free text diagnosis listed in the death note as the cause of death. An ICD-10 code was then assigned using computer-assisted coding incorporated as part of the Epic® EMR software (Epic Systems Corporation, Madison, WI) utilized by the University of Michigan. The Epic software integrates clinical documentation and billing information by providing a proprietary reference terminology that allows clinicians to search for and display diagnostic concepts that clinicians find meaningful while maintaining a mapped connection to ICD-10 codes for reporting and financial support purposes. This system is integrated into the EPIC system as a free-text query field, in which a clinician enters an abbreviation or string of characters, which is then used by the EHR to return results based on relevance to the search string and ranks them in list according to differentiating logic for complete word or partial word matching. An example frequently used in the process: a search initiated by typing "CAD" returned diagnostic codes I25* (where * represents 0 or more alphanumeric characters), and an ICD-10 code of I25 was assigned to this free-text search.

After an ICD-10 code was assigned as a diagnosis cause of death, we used the categories previously used to report life expectancy and mortality rates in the United States to bin decedents into broad categories of causes of death, with two notable additions and modifications (**Supplemental Table S4**).

1) We added a category of Liver disease with diagnoses that included ICD-10 codes of K7* representing cirrhosis of the liver
2) We included diagnoses of S06*, which encapsulated traumatic brain injury and diagnoses of intracerebral hemorrhage, as Neurologic conditions.

The S06* addition was added as it was frequently not captured by the classification bins used to report mortality rates. Liver disease was classified as distinct as general gastrointestinal disease, as previous studies have shown that patients with cirrhosis have significant shifts in gut microbiota.

Statistical analysis of clinical data
All analyses were performed using the R programming statistical programming language (v 4·1·2)[31]. We compared baseline demographics of age, race, gender, frequency of medical comorbidities, weighted Charlson Comorbidity Index[32–34], the Acute Physiology and Chronic Health Evaluation Score (APACHE IV) within 24 hours of initiation of mechanical ventilation, and the proportion of patients admitted to each hospital unit between treatment groups with the two sample independent t-test.

We constructed Kaplan-Meier curves to determine the median VAP-free, infection-free, and all-cause survival. We used a stratified log-rank statistic to determine the statistical significance of differences in survival between groups. We built Cox proportional hazards models incorporating early treatment with anti-anaerobic antibiotics, hospital unit of admission, age, gender, race, weighted Charlson comorbidity score, and the APACHE IV calculated within 24 hours of initiation of mechanical ventilation between groups (**Supplemental Table S5, S7, S8**). The proportional hazards assumption for variables included in the model was tested with a goodness of fit test of correlation between Schoenfeld residuals and time[35] (**Supplemental Figure S4**). All survival analysis was done with the *survival*[36] (v 3·1-8) package in R.

We then built a logistic regression model using an outcome 30-day VAP free survival as a binary variable (alive and VAP-free at 30-days or not). We used early treatment with anti-anaerobic antibiotics, hospital unit of admission, age, gender, race, weighted Charlson comorbidity score, and APCHE IV score calculated within 24 hours of admission as the independent predictors of 30-day VAP-free survival in this model **(Supplemental Table S6**). We then used the *margins*[37] package in R to calculate the average marginal effect of all covariates to determine the independent effect of early anti-anaerobic antibiotic treatment on VAP free survival.

To compare rates of VAP independently (rather than within a composite outcome), we compared the cumulative incidence VAP in 30 days for each treatment group and determined the estimated marginal probability of VAP in 30 days. We compared VAP-specific cumulative incidence functions between groups with Gray's test44. Competing risk analysis was performed with the cmprsk (v 2·2-6) 45 package in R.

We compared the overall distributions of bacterial pathogens between anti-anaerobic treated and untreated patients with Chi-Square testing and compared the frequency of individual bacterial pathogens between treatment groups with two sample independent t-testing. All statistical tests used p=0.05 as a threshold for significance.

Microbiome analysis of rectal swab specimens
We performed a secondary analysis of bacterial community data generated for a previously published study[38,39]. Briefly, we characterized the gut microbiota present in rectal swabs collected from 116 hospitalized patients admitted to the University of Michigan Hospital in 2016. The infection control practice throughout the study period was to perform routine surveillance for Vancomycin-Resistant *Enterococcus* (VRE) using rectal swabs on eight adult hospital units, including intensive care units and the bone marrow transplant ward. All hospitalized patients had routine collection of rectal swabs on admission and weekly thereafter to screen for VRE. In the prior study, we studied gut microbiome communities in 232 rectal swab samples from 58 matched pairs of case and control subjects (defined by nosocomial infection of VRE). Patient characteristics, bacterial DNA isolation, bacterial DNA quantification, 16S rRNA gene amplicon sequencing, and microbiome analysis have previously reported[38,39]. We built a mixed effects multivariable linear regression model using, age, gender, race, weighted Charlson comorbidity score, APACHE IV score at admission, and early treatment with anti-anaerobic antibiotics to predict log-transformed bacterial density and the relative abundance of *Enterobacteriaceae* over time. We included an interaction term of anti-anaerobic antibiotic treatment and time in these models to compare the daily change in bacterial density and relative abundance of *Enterobacteriaceae* between anti-anaerobic treated patients and untreated patients. We used the *lme4* package in R[40] **to** build the mixed effects models.

Murine modeling
Mice (8-10 week old female C57BL/6) were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Mice were housed in colony cages at 21°C with a 12:12-hour light-dark cycle and had *ad libitum* access to regularly changed water and standard chow (Envigo Teklad, Indianapolis, IN, USA). Prior to experimental start, mice were allowed to acclimate under specific pathogen-free conditions for one week, with subsequent transfer to BSL2 containment housing for the experimental duration. All experiments were conducted with approval from the University of Michigan Institutional Animal Care and Use Committee.

All antibiotics were suspended in sterile 0.9% saline and administered via intraperitoneal injection of 200 µl, such that mice received the following dosages: 40 mg/kg aztreonam, 30 mg/kg cefepime, 67.5 mg/kg piperacillin/tazobactam total (8 parts piperacillin to 1 part tazobactam), or saline alone (sham).

Experimental details

To avoid direct antibiotic interactions with the inoculum, mice were pre-treated with either sham, cefepime, or piperacillin/tazobactam daily for three days and allowed a washout period of 24 hours prior to inoculation. *S. aureus* or *K. pneumoniae* inocula were prepared as follows: 1) freezer stock was streaked on an LB agar plate (Lennox formulation [5 g/L NaCl] for *S. aureus* and Miller formulation [10 g/L NaCl] for *K. pneumoniae*) and incubated at 37°C for 24 hours, 2) five colonies were used to inoculate 50 mL of LB broth (same formulation as agar plates for respective species), which was incubated overnight (16-18 hours), and 3) 10 mL of overnight bacterial batch culture was centrifuged at 3,000 rpm for 10 min to pellet cells and washed twice with sterile PBS before diluting to the final concentration for the inoculum.

To inoculate each mouse, 10^7 CFU (range: $2.9 \times 10^7 - 5.8 \times 10^7$) of methicillin-resistant *S. aureus* (MRSA USA300, strain NRS384) or 10^6 CFU (range: $4.5 \times 10^6 - 9.0 \times 10^6$) was suspended in 50 ul of sterile phosphate-buffered saline (PBS) and instilled intratracheally via oropharyngeal aspiration under ketamine/xylazine anesthesia. Mock-infected control mice underwent a similar procedure, using 50 µl of sterile PBS instead of the bacterial suspensions. Following anesthesia, mice were then monitored until ambulatory and harvested 24 hpi to collect either bronchoalveolar lavage (BAL) fluid or whole lung tissue.

For the hyperoxia experiments, oxygen was administered to mice by placing their cages in a sealed chamber (BioSpherix) with medical-grade 100% oxygen (0.1 to 99.9 ± 0.1%) delivered continuously via a ProOx110 controller to maintain chamber oxygen levels. Antibiotics were delivered concurrently with oxygen exposure on days 0, 1, and 2 (for 3 day endpoint experiments) or days 0, 1, 2, and 3 (for 4 day endpoint experiments). To control for cage effect, antibiotic and sham-treated mice were cohoused, either in the oxygen chamber at 95% FiO_2 or on the standard housing rack at 21% FiO_2 (room air controls). Mice were removed from the chamber for about 20 minutes to complete the antibiotic injections and placed immediately back into the oxygen chamber to resume oxygen administration. Mice were harvested on day 3 or 4 to collect BAL fluid.

To assess survival during concurrent antibiotic and hyperoxia exposure, mice were monitored for moribundity and mortality every 12 hours during the 4 day timecourse. Weight loss at time of intraperitoneal antibiotic administration was recorded and visual inspection to assess general appearance, respiration, locomotion, and behavior was conducted every twelve hours. A threshold of >20% body weight loss, visual inspection revealing marked dyspnea and loss of locomotive control to the extent requiring humane euthanasia, or death within 10 minutes upon removal from oxygen chamber during cage transport at the endpoint was considered time of death. All other mice were considered as surviving and euthanized at 4 day endpoint for tissue collection. Tissue samples from non-surviving mice were not analyzed to avoid survivor bias.

Murine tissue collection and processing

Both types of lung samples collected for this study were harvested and processed according to previously published protocols[41–43]. Briefly, murine whole lung tissue was excised using sterile instruments, placed in tubes containing 1 mL sterile water, and mechanically homogenized using a Tissue-Tearor (Biospec Products, Bartlesville, OK). The tissue homogenizer was cleaned and rinsed in ethanol and water between each tissue sample. Water control specimens from homogenization rinsed with clean instruments were included as controls when plating whole lung tissue to determine *S. aureus* load.

Bronchoalveolar lavage fluid was collected via (1) sterile dissection to expose and make a small incision in the trachea, (2) insertion of sterile 0.58 mm tubing and connected 23-gauge sterile syringe needle into the incision, (3) tightening of sterile surgical thread around the intubated trachea to seal, and (4) two rounds of instillation and retrieval of 1 mL of sterile PBS into the lungs using a sterile 1 mL syringe. Each tubing-needle-syringe setup was rinsed thoroughly with sterile PBS between the collection of each sample. Sterile PBS used for lavage and PBS rinses of the tubing-needle-syringe setup (pre- and post-lavage) were collected as controls. BAL fluid was prepared by pooling the two serial lavages from each mouse, yielding up to 2 mL total BAL fluid per mouse. Pooled BAL fluid was centrifuged at 13,000 rpm for 30 min. For hyperoxia experiments, supernatant was removed and stored in aliquots at -20°C until further analysis. For *S. aureus* inflammation experiments, the BAL fluid cell pellet was used for leukocyte cell counts and differentials.

Murine tissue analysis

Lung injury was assessed using quantification of total protein and IgM in bronchoalveolar lavage fluid. Protein was quantified colorimetrically using the Bradford assay (Bio-Rad, Hercules, CA) with bovine serum albumin as the standard. Alveolar IgM was quantified using the IgM Mouse Uncoated enzyme-linked immunosorbent assay (ELISA) Kit (Thermo Fisher Scientific, Waltham, MA).

Lung bacterial culture was performed using up to eleven 10-fold serial dilutions of lung homogenate from *S. aureus- or K. pneumoniae*-infected mice with sterile PBS as the diluent. 10 µl of each serial dilution and the original lung homogenate were plated on square LB agar plates in duplicate using an adjustable electronic multichannel pipette (limit of detection: 50 CFU) . Plates were incubated overnight at 37°C, and colony-forming units (CFU) were counted the following day in the first dilution where defined colonies were visible. Control samples (e.g., mock-infected lung homogenate, water from homogenization, PBS from homogenate dilution) were also plated to identify sources of contamination. Colonies with morphology consistent with normal respiratory tract bacteria identified in the mock-infected controls were excluded from CFU determination.

Alveolar leukocytes were resuspended in 100 µl of RPMI media supplemented with 5% fetal bovine serum. Leukocytes were then further diluted with red blood cell lysis buffer and then counted using a standard hemocytometer. Slides for alveolar leukocyte differential counts were prepared by Cytospin of 100 µl of 1:3 dilution of resuspended cell pellet in RPMI + 5% FBS, allowed to dry, and then stained using a modified Wright-Giemsa staining protocol. Two hundred cells were counted per mouse. Leukocyte counts were divided by paired total cell count to obtain estimates of alveolar leukocyte counts for monocytes/macrophages, neutrophils, lymphocytes, eosinophils, and basophils.

Supplemental Tables and Figures

Supplemental Table S1. Antibiotic search terms

Cephalexin [2231]	Ciprofloxacin [2551]	Aztreonam [1272]
Cephradine [2239]	Levofloxacin [82122]	Linezolid [190376]
Cefdinir [25037]	Ofloxacin [7623]	Rifaximin [35619]
Cefuroxime [2194]	Moxifloxacin [139462]	Rifampin [9384]
Cefpodoxime [20489]	Norfloxacin [7517]	Rifabutin [55672]
cefTRIAXone [2193]	Besifloxacin [819911]	Rifapentine [35617]
Cefepime [20481]	Gatifloxacin [228476]	Isoniazid/Rifampin [6038/9384]
ceFAZolin [2180]	Gemifloxacin [138099]	Isoniazid/Pyrazinamide/Rifampin [6038/8987/9384]
Cefadroxil [2177]	Trovafloxacin [115552]	Sulfamethoxazole/Trimethoprim [10180/10829]
Cefixime [25033]	Floxuridine [4488]	Sulfamethoxazole [10180]
cefTAZidime [2191]	Ertapenem [325642]	sulfiSOXAZOLE [10207]
Cefprozil [19552]	Meropenem [29561]	Fidaxomicin [1111103]
Cefaclor [2176]	Meropenem/Vaborbactam	GENTAMICIN IVPB [142438/1596450]
Ceftaroline [1040005]	Cilastatin/Imipenem [2540/5690]	Polymyxin B/Trimethoprim [10829/8536]
cefoTEtan [2187]	Cilastatin/Imipenem/Relbactam	Bacitracin/Polymyxin B [1291/8536]
Cefotaxime [2186]	Doripenem [119771]	Neomycin/Polymyxin B [7299/8536]
cefOXitin [2189]	Azithromycin [18631]	Polymyxin B [8536]
Cefditoren [83682]	Clindamycin [2582]	DALBAVANCIN IVPB [1539239]
ceftolozane-tazobactam [1597609/37617]	Erythromycin [4053]	Telavancin [473837]
Ceftibuten [20492]	Vancomycin [11124]	Aztreonam [1272]
cefTAZidime-avibactam [1603834/2191]	Tobramycin [10627]	Linezolid [190376]
Ceftizoxime [2192]	Clarithromycin [21212]	Rifaximin [35619]
ceftaroline fosamil [1040004/1040005]	Neomycin [7299]	Rifampin [9384]
Cefiderocol	Fosfomycin [4550]	Rifabutin [55672]
Amoxicillin [723]	DAPTOmycin [22299]	Rifapentine [35617]
Penicillin V Potassium [7984]	Natamycin [7268]	Isoniazid/Rifampin [6038/9384]
Amoxicillin/Clavulanate [48203/723]	Paromomycin [7934]	Isoniazid/Pyrazinamide/Rifampin [6038/8987/9384]
Ampicillin [733]	Streptomycin [10109]	Tigecycline [384455]
Dicloxacillin [3356]	tobramycin [10627/9853/9863]	Oxytetracycline [7821]
Piperacillin/Tazobactam [37617/8339]	ERYTHROMYCINS/MACROLIDES	metroNIDAZOLE [6922]
Penicillin G Potassium [7980]	Lincomycin [6398]	Clavulanate/Ticarcillin [10591/48203]
Nafcillin [7233]	Telithromycin [274786]	cloxacillin 125 mg/5 mL Recon Soln [2625]
Ampicillin/Sulbactam [10167/733]	Doxycycline [3640]	Bacampicillin [18687]
Piperacillin [8339]	Minocycline XR [6980]	Tetracycline [10395]
Oxacillin [7773]	Ticarcillin [10591]	Demeclocycline [3154]

Supplemental Table S2. Search terms used to identify outside hospital transfers

“Outside hospital transfer”	“Another facility”
“Outside hospital”	“Transfer from outside facility”
“OSH transfer”	“Transfer from another facility”
“OSH txf”	“OSH”
“Outside facility”	“Transfer”

Supplemental Table S3. Antibiotic classification

Antibiotic class	Antibiotic	Anti-anaerobic
Nitroimidazole	Metronidazole	X
Beta-lactam + beta-lactamase inhibitor	Piperacillin-tazobactam	X
	Ampicillin-sulbactam	X
	Ceftolozane-tazobactam	
	Ceftazidime-avibactam	
Cephalosporin	Cefazolin	
	Cefoxitin	X
	Cefpodoxime	X
	Ceftriaxone	X
	Cefuroxime	X
	Ceftazidime	
	Cefepime	
	Ceftaroline	
Carbapenem	Meropenem	X
	Ertapenem	X
Glycopeptide	Vancomycin	
Penicillin	Penicillin	X
	Amoxicillin	X
	Ampicillin	X
	Nafcillin	
	Oxacillin	
Quinolone	Ciprofloxacin	
	Levofloxacin	X
	Moxifloxacin	X
Lincomycin	Clindamycin	X
Oxazolidinones	Linezolid	
Lipopeptide	Daptomycin	
Macrolide	Clarithromycin	
	Erythromycin	
	Azithromycin	
Monobactam	Aztreonam	
Sulfonamide and Folic acid inhibitor	Trimethoprim-Sulfamethoxazole	
Tetracycline	Doxycycline	X
	Minocycline	X
	Tigecycline	X
Rifamycin	Rifabutin	
	Rifaximin	X
	Rifabutin	
Aminoglycoside	Tobramycin	
	Gentamicin	
Phosphonic	Fosfomycin	

Supplemental Table S4. Cause of death classification

Cause of Death Category	ICD-10 Codes
Cardiovascular	Diseases of the circulatory system (I00-I99)
Pulmonary	Diseases of the respiratory system (J00-I98)
Renal	Renal Failure (N17-N19)
Gastrointestinal	Diseases of the digestive system (K00-K60; K80-K92)
Infection	Infectious and parasitic diseases (A00-B99)
Hepatic	Diseases of the Liver (K70-K76)
Neurologic	Diseases of the nervous system (G00-G98) Intracranial injury (S06)
Poisoning or overdose	Accidental drug poisoning (X40-X45) Intentional self-harm (X60-X84)
Malignancy	Neoplasms (C00-D48)
Unknown	Other ill-defined condition (R69) or missing death note

Supplemental Table S5. Total antibiotic use in study cohort

Antibiotic	Anti-anaerobic coverage (N = 1,942)	No anaerobic coverage (N = 1,090)	Difference in proportion (95% CI)	*P* value
Cefazolin	0.09 (170)	0.23 (254)	0.15 (0.12 - 0.17)	1.54e-23
Cefepime	0.272 (529)	0.55 (596)	0.27 (0.24 - 0.31)	5.25e-49
Vancomycin	0.76 (1477)	0.76 (828)	-0.001 (-0.033 - 0.031)	9.54e-01
Clindamycin	0.07 (136)	0 (0)	-0.07 (-0.08 - -0.06)	1.70e-32
Piperacillin-Tazobactam	0.57 (1107)	0 (0)	-0.57 (-0.59 - -0.55)	0.00e+00
Ceftriaxone	0.115 (224)	0 (0)	-0.12 (-0.13 - -0.10)	1.17e-53
Metronidazole	0.273 (530)	0 (0)	-0.27 (-0.29 - -0.25)	1.61e-136
Rifaximin	0.039 (76)	0 (0)	-0.04 (-0.04 - -0.03)	1.35e-18
Azithromycin	0.23 (442)	0.46 (504)	0.24 (0.2 - 0.27)	6.71e-38
Ampicillin-Sulbactam	0.14 (262)	0 (0)	-0.14 (-0.15 - -0.12)	4.06e-63
Cefuroxime	0.02 (44)	0 (0)	-0.02 (-0.03 - -0.016)	2.58e-11
Aztreonam	0.03 (61)	0.027 (29)	-0.01 (-0.02 - 0.01)	4.44e-01
Levofloxacin	0.03 (49)	0 (0)	-0.03 (-0.03 - -0.02)	1.89e-12
TMP-SMX	0.04 (84)	0.036 (39)	-0.0` (-0.02 - 0.01)	3.05e-01
Meropenem	0.05 (90)	0 (0)	-0.05 (-0.06 - -0.04)	8.32e-22
Cefoxitin	0.01 (26)	0 (0)	-0.01 (-0.02 - -0.01)	3.15e-07
Doxycycline	0.03 (53)	0 (0)	-0.03 (-0.04 - -0.02)	2.34e-13
Rifampin	0.02 (30)	0 (0)	-0.02 (-0.021 - -0.01)	3.88e-08
Linezolid	0.03 (50)	0.007 (8)	-0.02 (-0.03 - -0.01)	3.32e-05
Erythromycin	0.01 (15)	0.003 (3)	-0.005 (-0.01 - 0)	5.07e-02
Ciprofloxacin	0.01 (23)	0.01 (7)	-0.005 (-0.012 - 0.001)	1.16e-01
Gentamicin	0.01 (21)	0.02 (20)	0.01 (-0.002 - 0.02)	1.09e-01
Minocycline	0.002 (4)	0 (0)	-0.002 (-0.004 - 0)	4.55e-02
Ampicillin	0.03 (55)	0 (0)	-0.03 (-0.04 - -0.02)	8.23e-14
Fosfomycin	0.002 (3)	0.004 (4)	0.002 (-0.002 - 0.01)	2.97e-01
Penicillin	0.004 (8)	0 (0)	-0.004 (-0.01 - -0.001)	4.65e-03
Moxifloxacin	0.005 (10)	0 (0)	-0.01 (-0.01 - -0.002)	1.55e-03
Tobramycin	0.01 (27)	0.004 (4)	-0.01 (-0.02 - -0.004)	1.54e-03
Amoxicillin	0.004 (8)	0.003 (3)	-0.001 (-0.01 - 0.003)	5.25e-01
Daptomycin	0.012 (24)	0.003 (3)	-0.01 (-0.02 - -0.004)	1.22e-03
Ceftazidime	0.001 (2)	0.004 (4)	0.003 (-0.001 - 0.01)	1.81e-01
Nafcillin	0.01 (17)	0.006 (7)	-0.002 (-0.01 - 0.004)	4.68e-01
Rifabutin	0.001 (2)	0 (0)	-0.001 (-0.002 - 0)	1.57e-01
Tigecycline	0.001 (1)	0 (0)	-0.001 (-0.002 - 0)	3.17e-01
Ceftaroline	0.003 (5)	0 (0)	-0.003 (-0.01 - 0)	2.53e-02
Ceftolozane-Tazobactam	0.002 (4)	0 (0)	-0.002 (-0.004 - 0)	4.55e-02
Ertapenem	0.002 (3)	0 (0)	-0.002 (-0.003 - 0)	8.33e-02
Oxacillin	0.001 (1)	0 (0)	-0.001 (-0.002 - 0)	3.17e-01
Cefpodoxime	0.001 (1)	0.001 (1)	0 (-0.002 - 0.002)	7.02e-01
Clarithromycin	0.002 (3)	0 (0)	-0.002 (-0.003 - 0)	8.33e-02

Values reported as proportion (N).

Supplemental Table S6. Culture-identified pathogens in VAP

Organism	Anti-anaerobic coverage (N = 1,942)	No anaerobic coverage (N = 1,090)	*P* value
No growth ("Probable VAP")	5 (0.07)	7 (0.25)	0.049
Staphylococcus spp.	11 (0.15)	8 (0.29)	0.17
Corynebacterium spp.	3 (0.04)	1 (0.04)	0.90
Enterobacteriaceae spp.	35 (0.48)	6 (0.21)	0.009
Pseudomonas spp.	8 (0.11)	4 (0.13)	0.67
Streptococcus spp.	7 (0.10)	2 (0.071)	0.69
Stenotrophomonas spp.	3 (0.04)	0 (0)	0.083
Acinetobacter spp.	1 (0.01)	0 (0)	0.32

Supplemental Table S7. Species-level identity of VAP organisms

Family	Species	Anti-anaerobic coverage	No anaerobic coverage	Total
Enterobacteriaceae	*Escherichia coli*	9 (0.12)	3 (0.11)	12
	Klebsiella pneumoniae	8 (0.11)	1 (0.04)	9
	Klebsiella oxytoca	1 (0.01)	1 (0.04)	2
	Klebsiella (Enterobacter) aerogenes	2 (0.03)	0 (0)	2
	Proteus mirabilis	2 (0.03)	0 (0)	2
	Proteus vulgaris	1 (0.01)	0 (0)	1
	Enterobacter cloacae complex	5 (0.07)	1 (0.04)	6
	Serratia marcescens	6 (0.08)	0 (0)	6
	Citrobacter species	1 (0.01)	0 (0)	1
Staphylococcus	*Staphylococcus aureus*	11 (0.15)	8 (0.29)	19
Pseudomonas	*Pseudomonas aeruginosa*	7 (0.1)	4 (0.14)	11
	Pseudomonas stutzeri	1 (0.01)	0 (0)	1
Streptococcus	*Streptococcus pneumoniae*	3 (0.04)	2 (0.07)	5
	Streptococcus anginous group	2 (0.03)	0 (0)	2
	Streptococcus (not Group A)	1 (0.01)	0 (0)	1
	Beta-hemolytic Streptococcus	1 (0.01)	0 (0)	1
Corynebacterium	*Corynebacterium striatum*	2 (0.03)	1 (0.04)	3
	Corynebacterium amycolatum	1 (0.01)	0 (0)	1
Acinetobacter	*Acinetobacter baumanii*	1 (0.01)	0 (0)	1
Stenotrophomonas	*Stenotrophomonas maltophilia*	3 (0.04)	0 (0)	3
	Probable VAP (no culture growth)	5 (0.07)	7 (0.25)	12
	Total	**73 (1)**	**28 (1)**	**101**

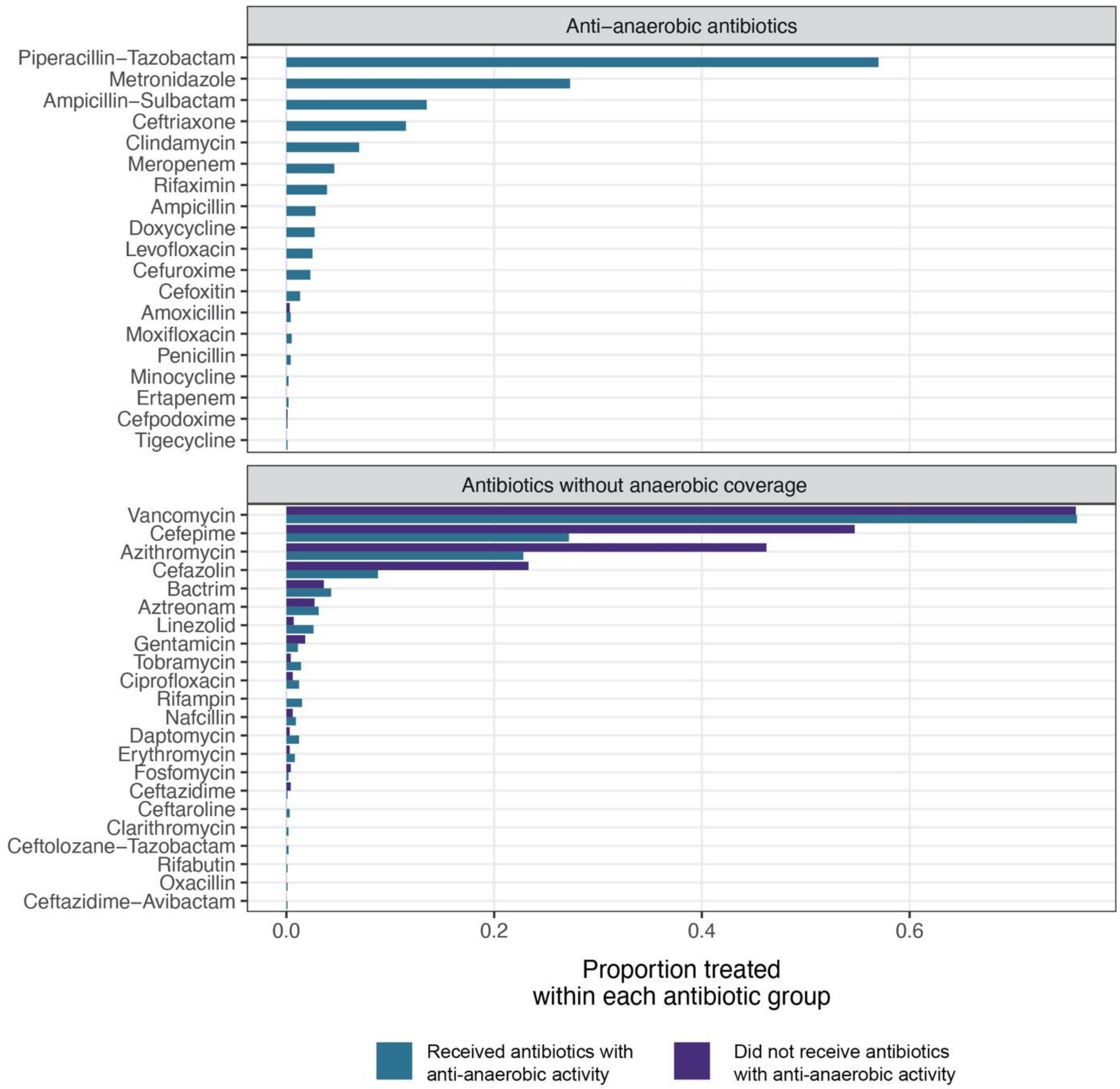
Supplemental Table S8. Comparison of causative organism for fatal nosocomial infections

Organism	Anti-anaerobic coverage (N = 1,942)	No anaerobic coverage (N = 1,090)	*P* value
Streptococcus spp.	0 (0)	0.03 (1)	0.32
Enterobacteriaceae spp.	0.29 (15)	0.03 (1)	<0.001
Staphylococcus spp.	0.02 (1)	0.32 (11)	<0.001
Candida spp.	0.1 (5)	0.09 (3)	0.91
Pseudomonas spp.	0.06 (3)	0.18 (6)	0.11
Enterococcus spp.	0.1 (5)	0.06 (2)	0.53
C. difficile	0.44 (23)	0.29 (10)	0.16

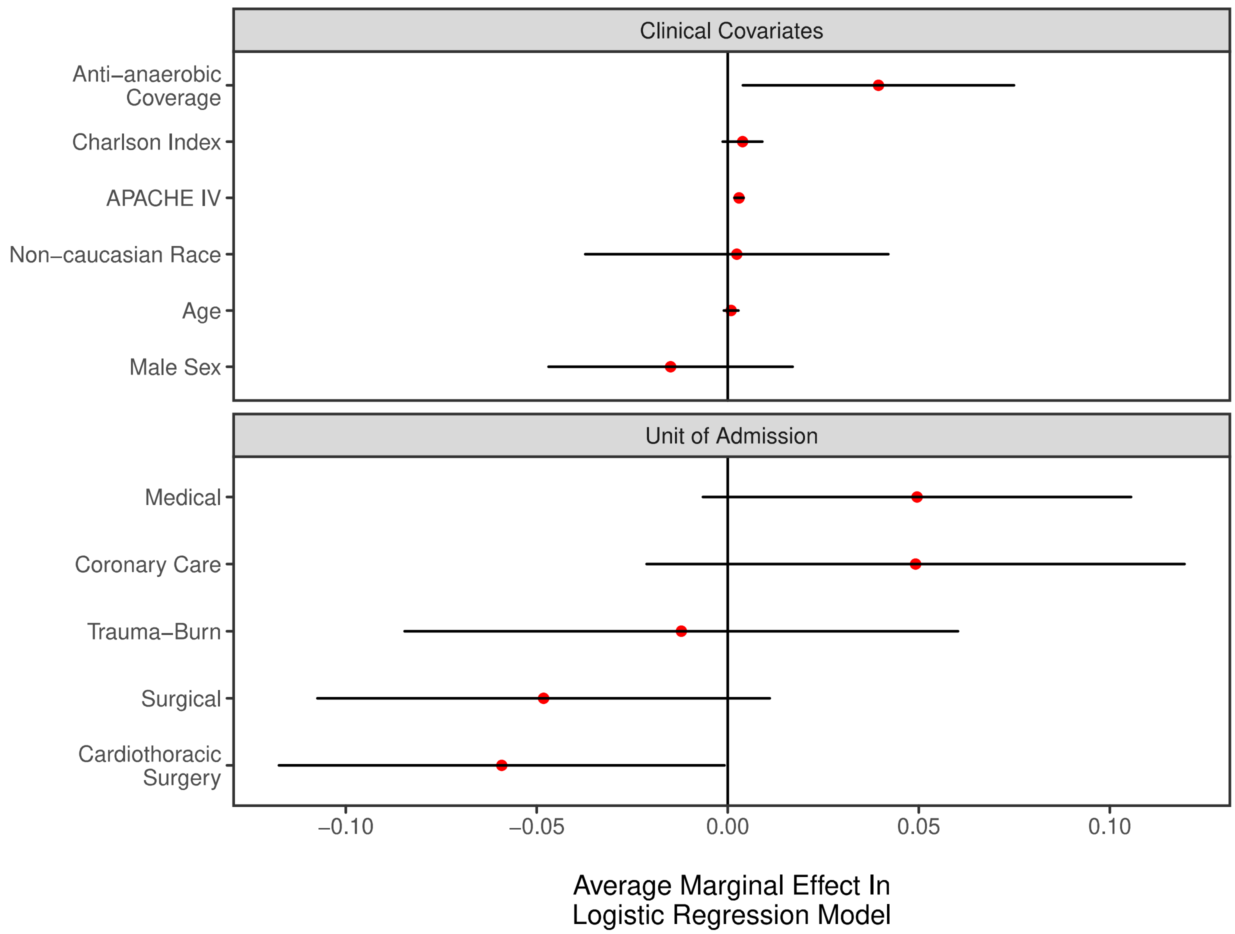
$p<2.2 * 10^{-6}$ by Chi-square test

Supplemental Table S9. Causative organisms for fatal nosocomial infection

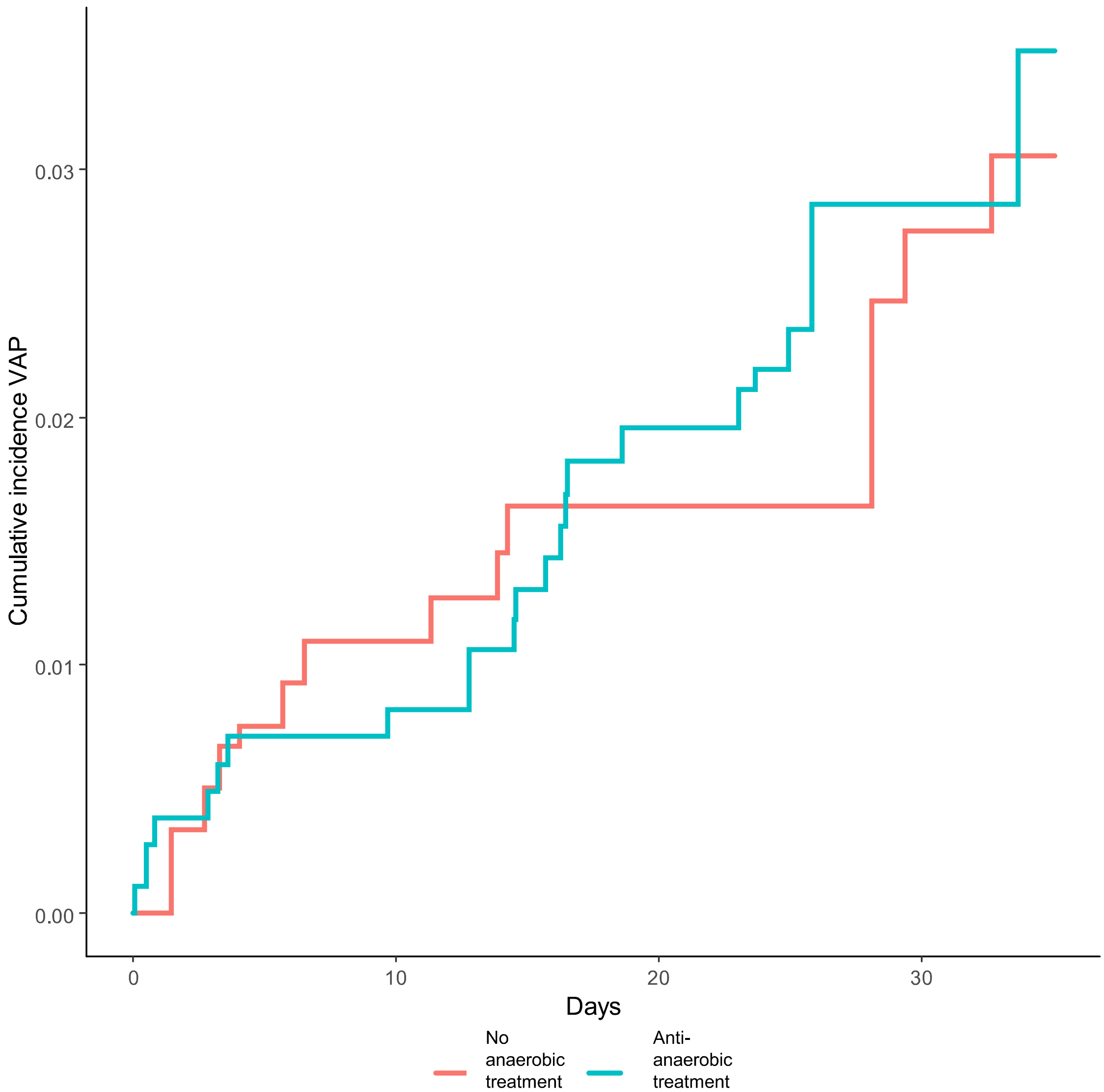
Antibiotic Coverage	Infection site	Organism	N
Without anaerobic coverage	Bacteremia	*Staphylococcus aureus*	11
	Stool	*C. difficile*	10
	Bacteremia	*Pseudomonas aeruginosa*	6
	Bacteremia	*Candida*	3
	Bacteremia	*Enterococcus faecalis*	1
	Bacteremia	*Enterococcus faecium*	1
	Bacteremia	*Klebsiella pneumoniae*	1
	Bacteremia	*Streptococcus anginosus*	1
With anaerobic coverage	Stool	*C. difficile*	23
	Bacteremia	*Candida* spp.	5
	Bacteremia	*Escherichia coli*	4
	Bacteremia	*Enterococcus faecalis*	3
	Bacteremia	*Klebsiella pneumoniae*	3
	Bacteremia	*Enterobacter cloacae*	2
	Bacteremia	*Enterococcus faecium*	2
	Bacteremia	*Pseudomonas aeruginosa*	2
	Ascites Fluid	*Citrobacter freundii*	1
	Urine Culture	*Escherichia coli*	1
	Tissue culture	*Pseudomonas aeruginosa*	1
	Bacteremia	*Klebsiella oxytoca*	1
	Bacteremia	*Proteus mirabilis*	1
	Bacteremia	*Serratia marescens*	1
	Bacteremia	*Staphylococcus aureus*	1



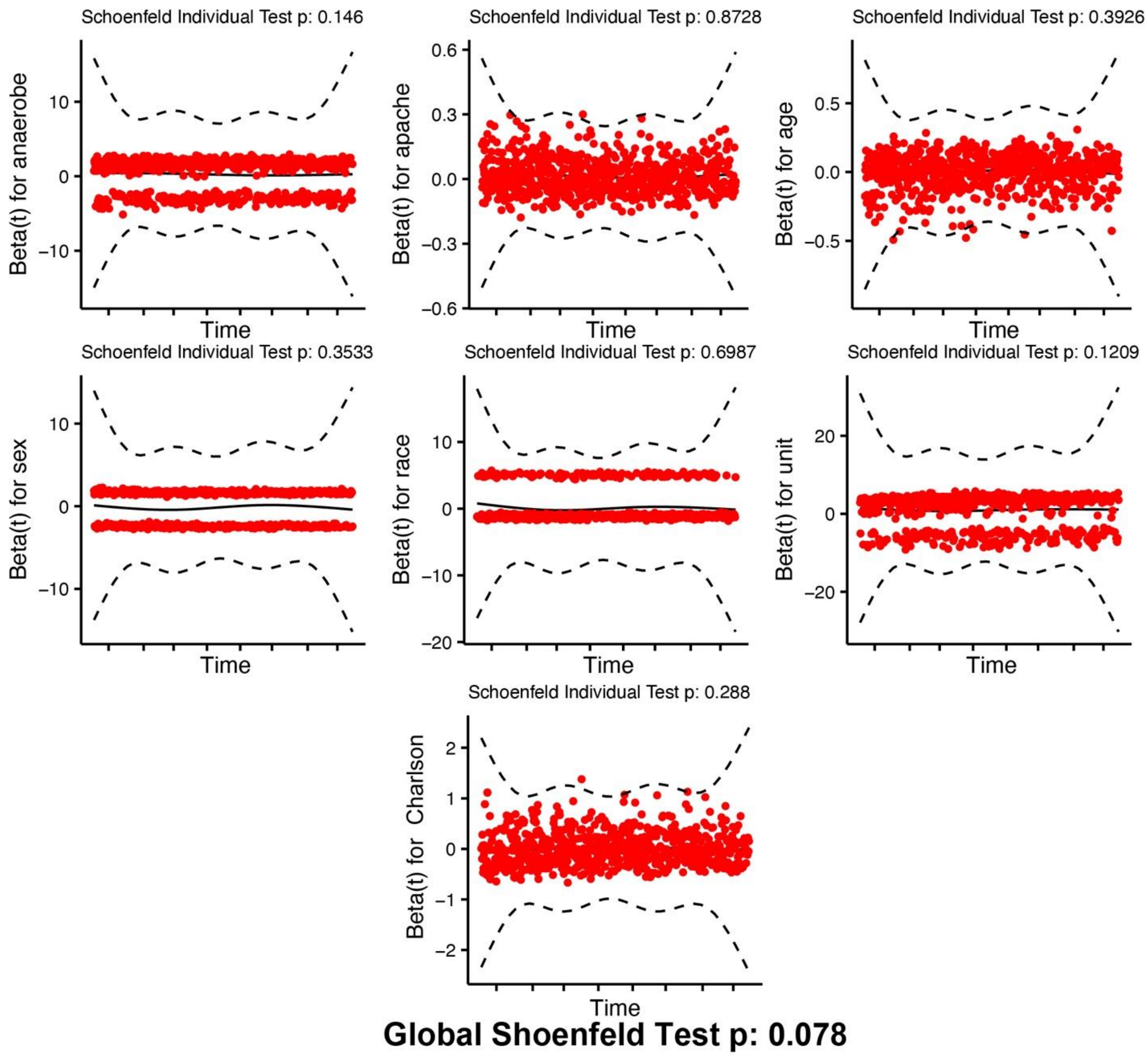
Supplemental Figure 1. Antibiotic use in study cohort. We characterized the antibiotic use in the eligible patient population classified by whether agents had significant anaerobic activity. Piperacillin-tazobactam, metronidazole, ampicillin-sulbactam, ceftriaxone, and clindamycin represented most anti-anaerobic antibiotic use. Vancomycin, cefepime, azithromycin, and cefazolin were the most commonly used antibiotics without anti-anaerobic activity.



Supplemental Figure 2: Average marginal effect of covariates in a logistic regression model. We built a multi-variable logistic regression model of VAP or death within 30 days of mechanical ventilation. When adjusting for other covariates, anti-anaerobic antibiotic treatment was independently associated with an average marginal effect of 0.039 (95% CI 95% 0.0040-0.0075, p=0.029)



Supplemental Figure 3. No difference in the cumulative Incidence of VAP within 30 days. We calculated the cumulative incidence of VAP over 30 days for each treatment group. The average marginal probability of VAP at 30 days was 3.1% for patients who did not receive anti-anaerobic treatment and 3.5% for patients who did receive anti-anaerobic treatment (p=0.60 by Gray's test)



Supplemental Figure 4: The proportional hazards assumption for variables included in the Cox-regression model were tested **by** a goodness of fit test of correlation between Schoenfeld residuals and time as proposed by Grambsch and Therneau. The proportional hazards assumption was satisfied for the model as a whole (p=0.078) and all individual variables.

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