

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: The Canadian Critical Care Trials Group. A randomized trial of diagnostic techniques for ventilator-associated pneumonia. *N Engl J Med* 2006;355:2619-30.

Table E1: Antibiotics Utilized in the 24 hours Prior to Randomization

	ETA (n=374)	BAL (n=365)	Total (n=739)
Antibiotics			
Cefazolin	66 (17.6%)	43 (11.8%)	109 (14.7%)
Piperacillin/tazobactam	26 (7.0%)	23 (6.3%)	49 (6.6%)
Ceftriaxone	19 (5.1%)	28 (7.7%)	47 (6.4%)
Vancomycin	16 (4.3%)	27 (7.4%)	43 (5.8%)
Cefuroxime	18 (4.8%)	18 (4.9%)	36 (4.9%)
Cefotaxime	13 (3.5%)	15 (4.1%)	28 (3.8%)
Clindamycin	9 (2.4%)	18 (4.9%)	27 (3.7%)
Ticarcillin/clavulanic acid	9 (2.4%)	11 (3.0%)	20 (2.7%)
Cloxacillin	13 (3.5%)	6 (1.6%)	19 (2.6%)
Levofloxacin	7 (1.9%)	10 (2.7%)	17 (2.3%)
Penicillin G	9 (2.4%)	6 (1.6%)	15 (2.0%)
Ceftazidime	5 (1.3%)	10 (2.7%)	15 (2.0%)
Ampicillin	10 (2.7%)	5 (1.4%)	15 (2.0%)
Gentamicin	10 (2.7%)	3 (0.8%)	13 (1.8%)
Trimethoprim/Sulfamethoxazole	7 (1.9%)	5 (1.4%)	12 (1.6%)
Erythromycin	3 (0.8%)	7 (1.9%)	10 (1.4%)
Azithromycin	4 (1.1%)	4 (1.1%)	8 (1.1%)
Ciprofloxacin	3 (0.8%)	4 (1.1%)	7 (0.9%)
Amoxicillin	3 (0.8%)	2 (0.5%)	5 (0.7%)
Ticarcillin	3 (0.8%)	2 (0.5%)	5 (0.7%)
Imipenem/cilastatin	2 (0.5%)	3 (0.8%)	5 (0.7%)
Piperacillin	1 (0.3%)	3 (0.8%)	4 (0.5%)
Tobramycin	1 (0.3%)	2 (0.5%)	3 (0.4%)
Cefepime	1 (0.3%)	1 (0.3%)	2 (0.3%)
Moxifloxacin	1 (0.3%)	1 (0.3%)	2 (0.3%)
Gatifloxacin	0 (0.0%)	2 (0.5%)	2 (0.3%)
Amikacin	1 (0.3%)	0 (0.0%)	1 (0.1%)
Ampicillin/sulbactam	1 (0.3%)	0 (0.0%)	1 (0.1%)
Cephalothin	1 (0.3%)	0 (0.0%)	1 (0.1%)
Amoxicillin/clavulanate	0 (0.0%)	1 (0.3%)	1 (0.1%)
Clarithromycin	0 (0.0%)	1 (0.3%)	1 (0.1%)
Number of antibiotics per patient (p=0.83)			
0	171 (45.7%)	175 (47.9%)	346 (46.8%)
1	146 (39.0%)	128 (35.1%)	274 (37.1%)
2	55 (14.7%)	55 (15.1%)	110 (14.9%)
3	2 (0.5%)	5 (1.4%)	7 (0.9%)
4	0 (0.0%)	2 (0.5%)	2 (0.3%)

Legend: The antibiotics used in the 24 hours preceding enrollment. There were no differences in the number of antibiotics used between groups (p=0.83). ETA- endotracheal aspirates; BAL- Bronchoalveolar lavage.

Table E2. Mortality

	ETA (n=374)	BAL (n=365)	RR* BAL/ETA	p-value*
Died within 14 days	49 (13.1%)	43 (11.8%)	0.88 (0.60-1.30)	0.53
Died within 28 days	69 (18.4%)	69 (18.9%)	1.01 (0.75-1.37)	0.94
Died in ICU	65 (17.4%)	58 (15.9%)	0.90 (0.65-1.25)	0.54
Died in Hospital	98 (26.2%)	84 (23.0%)	0.87 (0.67-1.12)	0.27

Legend:

RR-Relative Risk

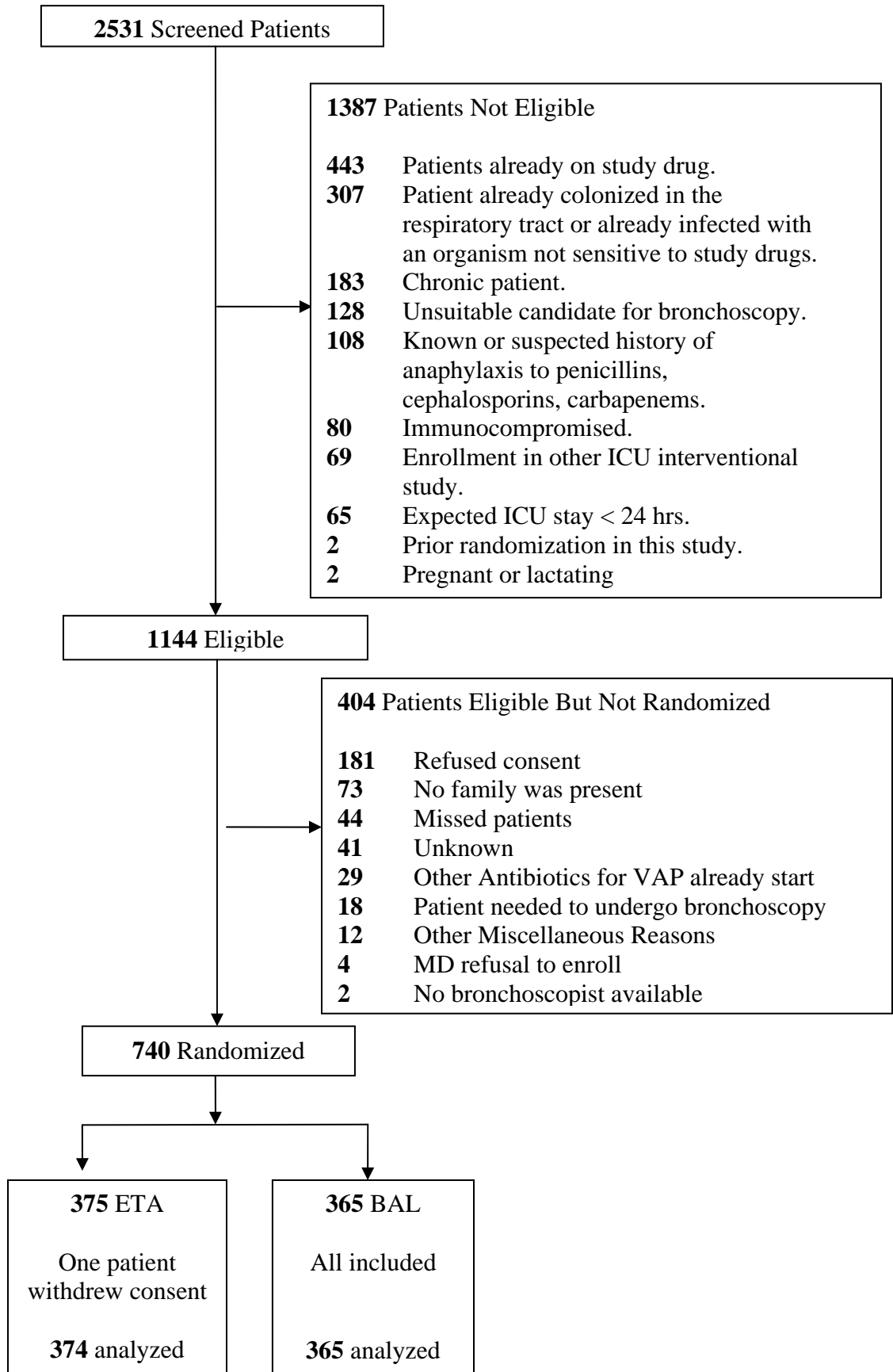
BAL – Bronchoalveolar Lavage

ETA – Endotracheal Aspirates

ICU- Intensive Care Unit

* Stratified by Mantel-Haenszel method for APACHE II score (≤ 24 vs. >24) and antibiotic therapy (mono vs. combination). For each outcome, the treatment interaction between diagnostic technique and antibiotic therapy was non-significant (all $p > 0.1$).

Figure E1 Flow of Study Participants



Legend:

ETA- Endotracheal Aspirates BAL- Bronchoalveolar Lavage

ICU- Intensive Care Unit MD- Doctor

VAP- Ventilator Associated Pneumonia

Appendix: Definitions of Clinical and Microbiological Outcomes

Clinical Outcomes:

Clinical resolution: the elimination of fever, purulence of secretions, and leukocytosis, improved oxygenation and radiographic improvement within 14 days of enrollment.

Delayed resolution: the patient improved but persisted on mechanical ventilation more than 14 days after enrollment.

Relapse or Recurrent infection: after initial improvement, the patient suffered a clinical and radiographic deterioration with the same organism that was responsible for the initial infection.

Superinfection: similar to relapse or recurrent infection but involves a different or new organism.

Clinical failure: death or persistence of clinical and radiographic features of infection throughout the study period requiring additional antibiotics.

Indeterminate: If while on treatment for respiratory symptoms the patient developed a requirement for additional antibiotics for non-respiratory tract infections (e.g., line sepsis requiring vancomycin).

Microbiological Outcomes:

Microbiological resolution: the elimination of the putative pathogen from repeated culture of lower respiratory tract.

Relapse or Recurrent infection: after initial eradication, the patient suffered a clinical and radiographic deterioration with the same organism that was responsible for the initial infection.

Superinfection: similar to relapse or recurrent infection but involved a different or new organism.

Failure: Persistence of the enrollment microorganism from secretions of the lower respiratory tract throughout the study period.

Colonization: the acquisition (after enrollment) of yeast or bacteria not associated with features of infection.

Indeterminate: If a patient died early and no subsequent cultures were available they were considered indeterminate.

Adequacy of Empiric Therapy: The organism(s) that grow in the enrollment specimen show in vitro susceptibility to meropenem or ciprofloxacin. If Pseudomonas species were isolated, 2 drugs were necessary for empiric therapy to be considered adequate.

Classification of VAP:

1) Definite bacterial pneumonia- if at least one of the following three criteria was fulfilled:

-positive result of pleural fluid culture

-rapid cavitation of the lung infiltrate as determined by computed tomography or

-histopathologic demonstration of pneumonia (presence of consolidation with intense polymorphonuclear leukocyte accumulation in bronchioles and adjacent alveoli involving several adjacent low-power microscopic fields, with or without tissue necrosis) during biopsy or autopsy.

2) Probable bacterial pneumonia- if none of the above criteria were met yet patient had cultures of specimens obtained using a bronchoalveolar lavage which grew at least one organism in significant concentration ($>10^4$ cfu/ml).

3) Possible pneumonia- if none of the above criteria were met yet patient's chest radiograph, sputum culture, temperature, white blood cell count and clinical course were consistent with pneumonia.

4) No pneumonia- if in the opinion of the study investigator, the patient's course was not compatible with pneumonia.

Protocol for Bronchoscopy and BAL

Patient prepared for bronchoscopy with 100% FiO₂, adequate sedation with or without paralysis. Patient on assist mode on the ventilator (RR 16-20) with continuous monitoring and oximetry. Suction through the endotracheal tube prior to starting bronchoscopy.

Sampling area is selected on the basis of the location of the new or progressive infiltrate seen on CXR. When passing the bronchoscope down into the lung, avoid suctioning secretions in the ETT, trachea or large airways to minimize contamination of the working channel.

Do not use lidocaine spray.

Tip of the bronchoscope is wedged into the subsegment of the lung and 20 ml sterile saline solution are injected, aspirated, and discarded. A new trap is positioned and additional 20-60 ml aliquots are injected slowly and aspirated. The total amount of fluid injected should be around 140 ml.

Note in chart percent retrieved fluid, presence or degree of haemorrhage or purulent secretions and location of sampling.

Send labelled specimens to lab immediately for Gram stain on cytospun fluid and quantitative culture.

Protocol for Endotracheal Suctioning

Patient prepared for suctioning with 100% FiO₂, bagging (if necessary), adequate sedation.

A sterile suction catheter (not a closed, inline system) and suction trap will be used.

3-5 ml of sterile saline will be instilled if an adequate specimen not obtained.

Note in chart quantity and nature of the specimen.

Send labelled specimens to lab immediately for Gram stain and culture.

Protocol for BAL Sample Processing

POTENTIAL PATHOGENS

Any of the following may be pathogenic if greater than 1×10^6 CFU/L:
Haemophilus influenzae, *S. pneumoniae*, *S. aureus*, *M. catarrhalis*, Gram negative bacilli, anaerobes, *N. meningitidis*

NORMAL FLORA

Coagulase negative Staphylococci, Corynebacterium species, Viridans streptococcus group, Neisseria species (not *N. meningitidis*)

INTERPRETATION OF CULTURES

Quantitative cultures are used to assess the concentration of organisms present in the lower respiratory tract and to help distinguish between low level contamination and a significant concentration of bacteria. (These numbers are for use in patients not on antibiotic therapy).

A significant colony count in BAL specimens is $\geq 10^4$ CFU/mL ($\geq 10 \times 10^6$ CFU/L). However, because many of our patients may have been on antibiotic therapy at the time of sampling (not always known to the laboratory), and because our technique allows us to isolate organisms at a one log lower level, our threshold for work up of potential pathogens in a BAL is $\geq 10^3$ CFU/ml ($\geq 1 \times 10^6$ CFU/L).

Day 1:

1. If less than 1×10^6 CFU/L of an organism per plate, no work-up. Reincubate plates.
2. If greater than or equal to 1×10^6 CFU/L of any potential pathogen per plate perform full ID and susceptibilities. See "Interpretation of Count".

Day 2:

1. Re-examine all aerobic plates
2. Examine anaerobic culture if requested, determine count and workup as per Day 1 protocol.
3. Correlate aerobic to anaerobic growth if anaerobic culture was requested.

INTERPRETATION OF COUNT (BAL)

Volume Plated	Colony Count	Report
0.01 ml (10 µl)	1 - 9	<1 X 10 ⁶ CFU/L -----no further work-up (Report: <10 x 10 ⁶ CFU/L; No significant growth)
	10 - 100 (confirm count from 0.001 ml volume)	<10 X 10 ⁶ CFU/L(normal flora)---no further work up <10 x 10 ⁶ CFU/L(potential pathogen)---ID & STF
0.001 ml (1 µl)	1 - 9	<10 x 10 ⁶ CFU/L(normal flora)---no further work up <10 x 10 ⁶ CFU/L(potential pathogen)---ID & STF
	≥10	≥10 x 10 ⁶ (normal flora)---no further work up ≥10 x 10 ⁶ (potential pathogen)---ID & STF

REPORTING GUIDELINES

Note: ! significant aerobic counts should be reported within 24 hours.

! non-significant aerobic counts should not be reported until anaerobic counts are available if anaerobic culture was requested

1. No growth observed.
2. Less than 10 x 10⁶ CFU/L; No significant growth (LIS=L10106 NG)
3. Less than 10 x 10⁶ CFU/L of _____ (Organisms consistent with normal respiratory flora) (LIS=NRF: L10106)
4. Less than 10 x 10⁶ CFU/L of _____ (Potential Pathogen)] ID and STF {record as 10³CFU/ml in the case report form
5. Greater than 10 x 10⁶ CFU/L of _____] ID and STF {record as 10⁴ CFU/ml in the case report form