

**An Open-label Study into the Efficacy and Dosing of Probiotic *Escherichia coli* Nissle 1917 for the Prevention of Gram-negative Gastric Colonisation in Ventilated Intensive Care Patients**

By

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*Keep an open mind toward pneumonia. Our grandchildren will be interested and are likely to have as many differences of opinion regarding the disease as we have.*

—Sir William Osler, 1849-1919

## Abstract

Twenty-five percent of hospital infections occur in critical care patients. Up to 30% of these develop ventilator associated pneumonia, increasing length of stay, morbidity, mortality and cost. Causative bacteria are predominantly Gram-negative, and with increasing multidrug-resistant strains, we must look towards non-antibiotic strategies in prevention and treatment. Probiotics are one option in this quest; however, efficacy and mechanisms of action are unclear. The randomised controlled trials of probiotics to date, have predominantly used Gram-positive bacteria and varied in their inclusion criteria, sample size and population studied, in addition to formula, dosing and route of administration. These studies have yielded conflicting results, however, there is some evidence that ventilator associated pneumonia can be prevented by probiotic treatment.

This thesis demonstrates successful gastric colonisation by probiotic *Escherichia coli* Nissle 1917 in ventilated patients. Trends toward dose-dependent incidence, and time to colonisation were observed, but these were not statistically significant. In the 15 millilitre treatment group, there was a significant reduction in the burden of pathogenic Gram-negative bacteria in gastric aspirates after 48 hours. Probiotic *Escherichia coli* Nissle 1917 can be safely administered to a selected group of critically ill patients. As a reduction in pathogenic Gram-negative gastric colonisation has been demonstrated, further study is warranted to determine if this leads to a reduction in the incidence of ventilator associated pneumonia.

*This thesis is dedicated to my parents, for their unfailing support throughout my career.*

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## **Declaration**

This thesis is submitted to the University of Birmingham to support my application for the degree of Doctor of Medicine. I certify it has been entirely composed by myself and contains no material, which, has been accepted for the award of any other degree, or diploma in my name, in any university or other tertiary institution. To the best of my knowledge and belief the thesis contains no material previously published or written by another person, except where the due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for another degree or diploma.

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## List of Abbreviations

ACCP	American College of Chest Physicians
AIDS	Acquired Immune Deficiency Syndrome
APACHE II	Acute Physiology and Chronic Health Evaluation Score
APACHE III	Acute Physiology, Age and Chronic Health Evaluation Score
ARDS	Acute Respiratory Distress Syndrome
<i>b.d.</i>	bis in die (twice a day)
b.p.	Base pairs
Balti-2	Beta-Agonist Lung injury Trial-2: a multicentre, randomised, double-blind, placebo-controlled trial and economic evaluation of intravenous infusion of salbutamol versus placebo in patients with acute respiratory distress syndrome
BAL	Bronchoalveolar Lavage
CAP	Community Acquired Pneumonia
CDC-NHSN	US Centers for Disease Control National Healthcare Safety Network
CFU	Colony-forming Units
CFU g <sup>-1</sup>	Colony-forming Units per gram
CFU ml <sup>-1</sup>	Colony-forming Units per millilitre
CPIS	Clinical Pulmonary Infection Score
CSU	Catheter Specimen of Urine
DOH	Department of Health
DSI	Daily Interruption of Sedation
ECN	<i>Escherichia coli</i> Nissle 1917
EPIC Study	European Prevalence of Infection in Intensive Care
ETT	Endotracheal Tube



FiO <sub>2</sub>	Fraction of (Fractional) Inspired Oxygen
GI(T)	Gastro-intestinal (Tract)
GPICS	Guidelines for the Provision of Intensive Care Services
H2RA	Histamine 2 Receptor Antagonist
HAI	Hospital Acquired Infection
HAP	Hospital Acquired Pneumonia
HCAP	Healthcare Associated Pneumonia
HEFT	Heart of England NHS Foundation Trust
HELICS	Hospitals in Europe Link for Infection Control through Surveillance Criteria
HPLV	High-Pressure/Low-Volume
HVLP	High-Volume/Low-Pressure
ICCQIP	Infection in Critical Care Quality Improvement Programme
ICS	Intensive Care Society
ICU	Intensive Care Unit
Ig	Immunoglobulin
IHI	Institute for Health Improvement (US)
IL	Interleukin
IMP	Investigational Medicinal Product
INICC	International Nosocomial Infection Control Consortium
IVAC	Infection-Related Ventilator-Associated Complication
LAB	Lactic Acid Bacteria
Lp299	Lactobacillus plantarum 299
LR-	Negative Likelihood Ratio
LR+	Positive Likelihood Ratio

MALDI-TOF	Matrix Assisted LASER Desorption/Ionization-Time of Flight Mass Spectrometry
MDR	Multi-Drug Resistant
MODS	Multiple organ dysfunction syndrome
MRSA	Methicillin-Resistant Staphylococcus aureus
NDM-1	New Delhi metallo-beta-lactamase-1
NHS	National Health Service
NHSN	National Healthcare Safety Network
NICE	National Institute for Health and Care Excellence
NPSA	National Patient Safety Agency
NPV	Negative Predictive Value
PAC-Man	Assessment of the clinical effectiveness of pulmonary artery catheters in management of patients in intensive care: a randomised controlled trial
PaO <sub>2</sub>	Arterial Partial Pressure of Oxygen
PaO <sub>2</sub> /FiO <sub>2</sub>	Arterial Partial Pressure of Oxygen/ Fraction of Inspired Oxygen Ratio
PCR	Polymerase Chain Reaction
PECaN-ED Study	Probiotic Escherichia coli Nissle – Efficacy and Dosing Study
PEEP	Positive End Expiratory Pressure
PHE	Public Health England
PLR	Personal Legal Representative
PPI	Proton Pump Inhibitor
PPV	Positive Predictive Value
ProLR	Professional Legal Representative
PSB	Protected Specimen Brushing
PTC	Protected Telescopic Catheter

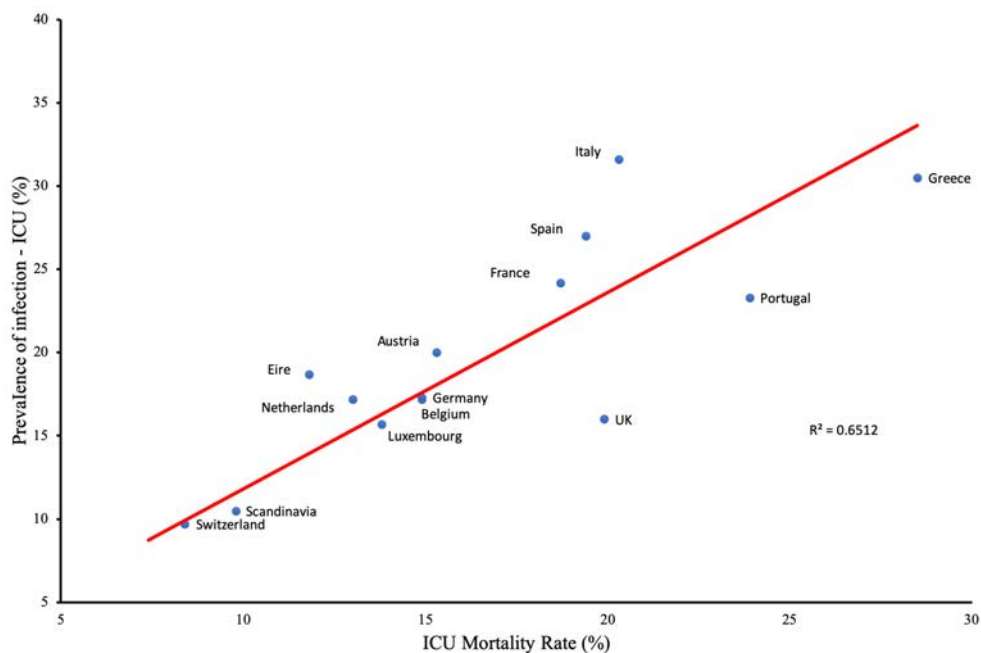
PVC	Polyvinylchloride
RCT	Randomised Controlled Trial
SAPS II	Simplified Acute Physiology Score
SDD	Selective Decontamination of the Digestive tract
Sens	Sensitivity
SOAP Study	Sepsis Occurrence in Acutely Ill Patients
SOD	Selective Oral Decontamination
Spec	Specificity
SSD	Subglottic Secretion Drainage
Study ID	Study Identity (patient number)
T3SS	Type 3 Secretion System
TA	Tracheal aspirate
TA	Tracheal Aspirates
TNF- $\alpha$	Tumour Necrosis Factor Alpha
TracMan	Effect of early vs late tracheostomy placement on survival in patients receiving mechanical ventilation: the TracMan randomized trial
TMG	Trial Management Group
TT	Tracheal Tube
UK	United Kingdom
US/USA	United States/United States of America
VAC	Ventilator Associated Complications
VAE	Ventilator Associated Events
VAP	Ventilator associated pneumonia
VARTI	Ventilator Associated Respiratory Tract Infection

# **Chapter 1**

## **Introduction**

## 1.1 Infection in critical care

There are at least 100,000 cases of hospital acquired infection (HAI) per year in England, causing around 5,000 deaths, and costing the UK NHS somewhere in the region of £1 billion each year (1). Patients in Intensive Care Units (ICUs) are a small subgroup of hospitalised patients, yet they account for approximately 25% of all hospital infections (2). HAI rates among ICU patients are 5-10 times higher than among general ward patients (2). Approximately 30% of ICU patients suffer from infection as a serious complication of critical illness (3), increasing ICU length of stay and incurring excess costs of £10,000 - £30,000 per patient. Furthermore, there is a relationship between the prevalence of ICU acquired infection and mortality (figure 1.1)(4).



**Figure 1.1 Correlation between prevalence of ICU acquired infection and mortality rate by country (4)**

In addition to demonstrating the scale of the problem of ICU acquired infection, the European Prevalence of Infection in Intensive Care (EPIC) study also highlighted some interesting points about the geographical trends in infection rates across Europe. The prevalence of ICU acquired infection ranged from 10% in Switzerland to 32% in Italy. Of note, the mortality rate in Italy is similar to that in the UK yet the prevalence of infection in Italy is more than twice as high. This may reflect differences in patient selection and ICU practices between different countries. For example, the relatively low number of ICU beds in the UK compared to other European countries is likely to result in a more severely ill ICU population.

ICU acquired (nosocomial) infection is defined as an infection that is not present or incubating when the patient is admitted to an intensive care unit (5). The time frame for diagnosis of an ICU acquired infection will thus clearly be dependent upon the incubation period of the specific infection; 48–72 hours after admission is generally deemed indicative of nosocomial, rather than community-acquired, infection. The peak of incidence of ICU acquired infection occurs around the fifth day after admission (6). The predominant risk factors for ICU acquired infection are length of ICU stay, requirement for mechanical ventilation, and the presence of central venous, pulmonary artery, and urinary catheters (4).

Among ICU patients, the six most common sites of infection are pneumonia, bloodstream infections (including infective endocarditis), intravascular catheter-related sepsis, intra-abdominal infections, urosepsis, and surgical wound infections (7).

## **1.2 Ventilator associated pneumonia**

### **1.2.1 Overview**

Ventilator associated pneumonia (VAP) is a nosocomial pneumonia that develops more than 48 hours after tracheal intubation and mechanical ventilation (8). VAP occurs because potentially pathogenic bacteria from the sinuses, oropharynx, subglottis and gastrointestinal (GI) tract gain access to the lower respiratory tract in the critically ill patient. This is facilitated by the presence of the tracheal tube (TT), which allows microaspiration of secretions and biofilm formation, by-passing the body's natural defence mechanisms (9).

Making a diagnosis of VAP is difficult due to its associated non-specific signs *e.g.* chest radiograph infiltrates, fever, leucocytosis and purulent respiratory secretions. The differential diagnosis includes pulmonary oedema, atelectasis, pulmonary embolism, ARDS and pulmonary haemorrhage.

VAP is the most common HAI in the adult critical care environment. In the EPIC study, pneumonia and lower respiratory tract infections accounted for more than 50% of ICU acquired infections (4). Subsequently, in the Sepsis Occurrence in Acutely Ill Patients (SOAP) study, the respiratory tract was again found to be the predominant site of ICU acquired infection (10).

The precise incidence of VAP is somewhat elusive and depends on the type of ICU population studied, the duration of mechanical ventilation and the diagnostic criteria used. Bearing this in mind, the evidence suggests that VAP occurs in 9-27% of mechanically ventilated patients, with approximately 5 cases per 1000 ventilator days (11). The attributable mortality from VAP

is also difficult to define, but it is clear that VAP makes a significant contribution to mortality, morbidity, length of ICU and hospital stay and financial cost (9).

The diagnosis of VAP is made on a combination of clinical, radiological and microbiological criteria. However, there is still no consensus definition, as no combination of criteria offers enough diagnostic accuracy or confidence to justify their use in defining VAP. The commonly used 'gold standard' diagnosis of VAP is made using the American College of Chest Physicians (ACCP) clinical criteria and quantitative cultures of distal airways samples obtained by non-bronchoscopic bronchoalveolar lavage (BAL) using a protected catheter. The ACCP clinical criteria require a new and persistent infiltrate on chest radiographs with two out of three supporting findings: fever ( $>38.5^{\circ}\text{C}$  or  $<35.0^{\circ}\text{C}$ ), leucocytosis (white blood cells  $>10,000\text{ mm}^{-3}$  or  $<3,000\text{ mm}^{-3}$ ) and/or purulent sputum (12).

Hospital acquired infections in the critically ill population have been the focus of much attention in recent years, in particular, ventilator associated pneumonia. There is no doubt that VAP is a major cause of morbidity and mortality, with HAP and VAP together accounting for 22% of hospital acquired infections (HAIs) (13). In addition, there is concern about the role of multi-drug resistant (MDR) organisms in their pathogenesis, combined with the 'indiscriminate' use of broad-spectrum antibiotics. Not only have clinical concerns been highlighted; these events also confer a financial burden to health care systems, with an episode of HAP increasing hospital stay by an average of 7-9 days and costing more than \$40,000 per patient in the United States (14).



### 1.2.2 Definitions

The main VAP scoring systems and definitions currently in use are the Clinical Pulmonary Infection Score (CPIS), the US Centers for Disease Control National Healthcare Safety Network (CDC-NHSN) definition and the Hospitals in Europe Link for Infection Control through Surveillance (HELICS) criteria. None of these systems are without the drawbacks of inter-individual variability of reporting and use of subjective clinical criteria; thus, the reported incidences of VAP vary according to the diagnostic criteria used.

To complicate the ‘VAP’ issue, the terminology used in discussions about VAP is not straightforward and various definitions have been used in connection with respiratory HAIs. The term healthcare-associated pneumonia (HCAP) was used together with HAP and VAP in the 2005 American Thoracic Society Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare-associated Pneumonia (14). The thinking behind the use of the term, HCAP, was that this group of patients were at particular risk of developing infections with MDR pathogens by virtue of their contact with the healthcare environment – not only in the acute care setting but in a variety of community care environments. This would obviously dictate antibiotic treatment as such patients may not develop a straightforward community acquired pneumonia (CAP). Subsequent evidence has called this idea into question (15,16). Although interaction with healthcare environments may be a risk factor for acquiring infections with MDR organisms, many of these patients are not at *high risk* from these pathogens, with underlying patient characteristics playing an important role in the development of infection (15). Therefore, in the 2016 guidelines (Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice

Guidelines by the Infectious Diseases Society of America and the American Thoracic Society) the term HCAP has been removed (13).

The Centers for Disease Control (CDC) 2008 document ‘Surveillance definition of health care–associated infection’ was designed as an HAI surveillance tool and the pneumonia (PNEU) definitions used therein were not intended for the clinical classification of pneumonia or VAP (17). When they were applied to the diagnosis of VAP, they were shown to have good sensitivity and positive predictive value, but low specificity when compared with criteria that include microbiological results from bronchoscopy. The flow diagram for the adult pneumonia algorithm which was originally designed to be used as a data collection tool is shown in Figure 1.2.

Subsequently, in 2013, the National Healthcare Safety Network (NHSN), the CDC’s HAI surveillance system, revised the pneumonia (PNEU) definitions after convening a VAP Surveillance Definition Working Group (18). It was felt that the previous PNEU definitions were useful for internal quality improvement purposes and surveillance but were limited by their subjectivity and complexity. Therefore, the 2008 terminology and criteria were amended to have objective, reliable surveillance definitions for use in public reporting and for inter-institution comparisons of event rates.

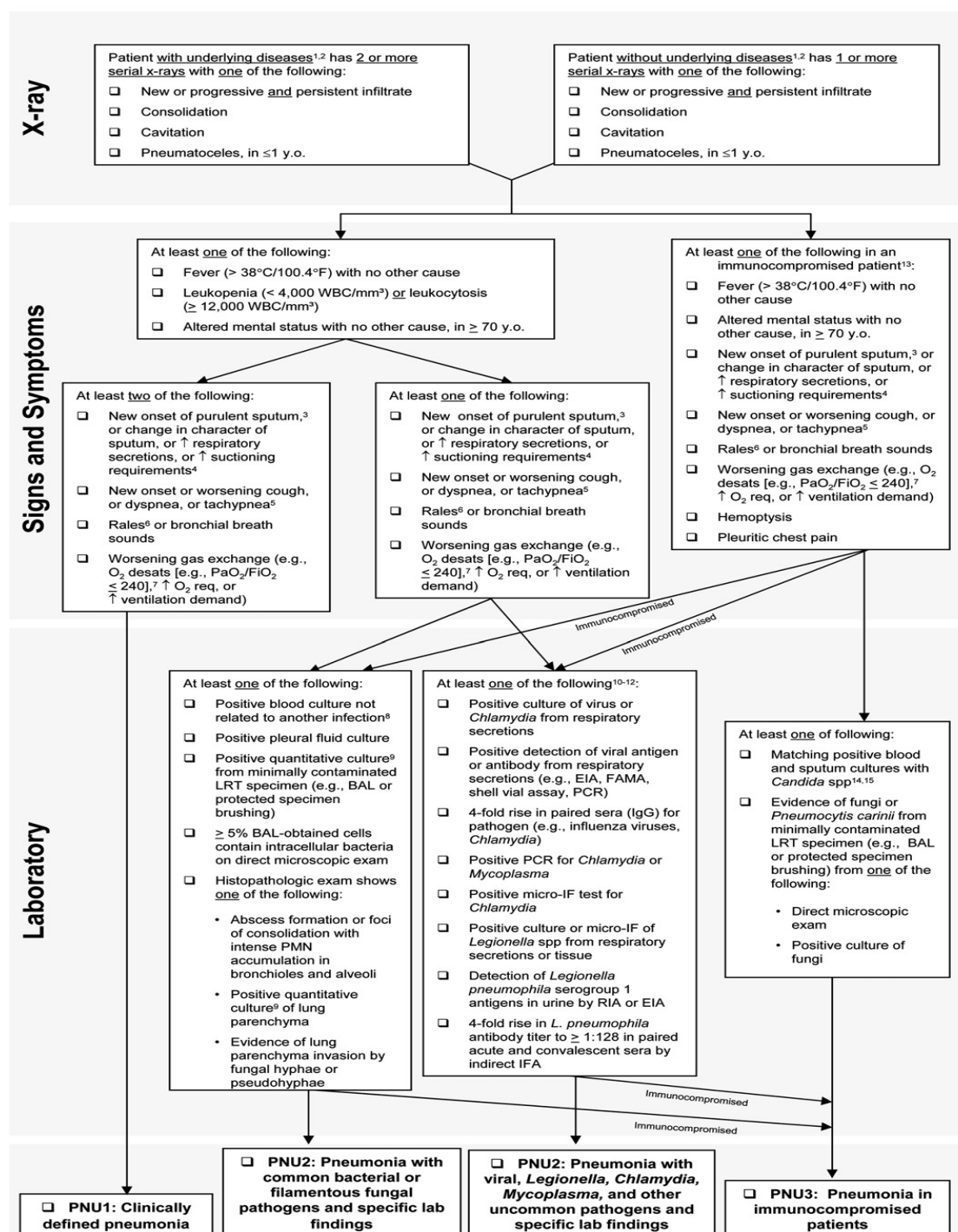
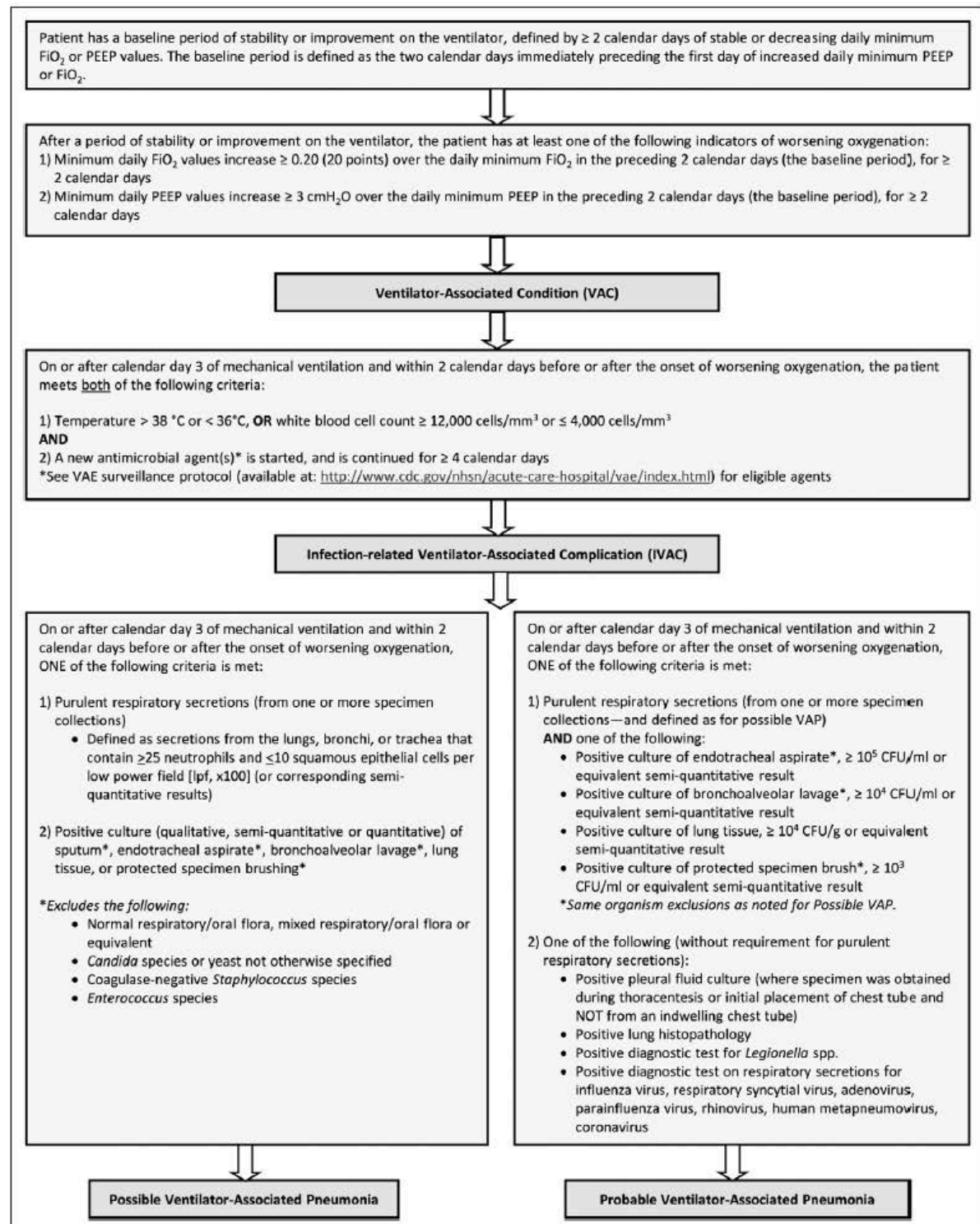


Figure 1.2 The Pneumonia Flow Diagram from the 2008 CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting (17)

It was highlighted, once again by the Working Group, that there is currently no valid, gold standard, reliable definition of VAP. Even the most widely used VAP definitions are neither sensitive nor specific. The Working Group therefore decided to develop a surveillance definition algorithm for detection of ventilator-associated events (VAEs). This algorithm was designed to detect a broad range of conditions and/or complications arising during the mechanical ventilation of adult patients (figure 1.3). It is from *this* algorithm that the new battery of terms, alluded to below, originated. The key differences which were brought about by this review are (18): -

- i. The use of VAE as the ‘catch-all’ surveillance term.
- ii. The introduction of a significant change with regard to timing. Previously, *any* pneumonia in a patient receiving mechanical ventilation was considered a VAP - even if this occurred within the first 24 hours of admission. For example, pneumonia developing from an episode of aspiration in a critically ill patient just prior to intubation could be considered a VAP. In the new surveillance algorithm, at least 48 hours of stability on a ventilator must be documented before a VAE can be considered.
- iii. An increase in positive end-expiratory pressure (PEEP) or fraction of inspired oxygen (FiO<sub>2</sub>) must be present for more than 2 days. It was envisaged that this would give greater validity to a VAE diagnosis.
- iv. The chest radiograph is now absent from the diagnostic protocol. With the wide variability in interpretation of new infiltrates in mechanically ventilated patients, the reliability and reproducibility of chest radiographs as a criterion for diagnosis of VAP has often raised questions and was therefore excluded.



**Figure 1.3 Ventilator-associated events surveillance definition algorithm produced by the VAP Surveillance Definition Working Group, illustrating the criteria and terminology used (18)**

Interpretation of the literature is hampered by the variable use of the terms hospital acquired pneumonia (HAP) and VAP. In the 2005 American Thoracic Society Guidelines, *pneumonia* was defined as the presence of a ‘new lung infiltrate plus clinical evidence that the infiltrate is of an infectious origin, including the new onset of fever, purulent secretions, leucocytosis, and worsening oxygenation’ (14). This definition continues to be used in the 2016 American Thoracic Society Guidelines. Similarly, the terms HAP and VAP are still widely used and their definitions have also been upheld. HAP refers to a pneumonia that is not incubating at the time of hospital admission and occurring 48 hours or more after admission. VAP is ‘a pneumonia occurring >48 hours after endotracheal intubation’ (13). For clarity, they are regarded as separate entities, therefore avoiding expressions such as ‘ventilator associated HAP’.

Other terms, such as ventilator associated events (VAE), including ventilator-associated conditions (VAC), infection-related ventilator-associated complications (IVAC) and possible or probable VAP, have now gained widespread use in the literature. The term ventilator associated complications (VAC) has been coined by US Centers for Disease Control and Prevention (CDC) as potential metric to assess the quality of care provided to ventilated patients (19). These terms may be useful from an epidemiological study perspective but do not necessarily aid classification from a clinical perspective and may confuse clinical study of these entities. Furthermore, the term VAC is now being used with two potential meanings; ventilator associated complications (19) or as part of the CDC first tier definition, ventilator-associated conditions (VAC) (18).

### **1.2.3 Incidence and attributable mortality**

VAP is the most common HAI in adult critical care patients (10) and is undeniably an entity with which critical care physicians are familiar. However, the lack of a consensus definition for VAP remains a problem, as does the lack of a consensus on a gold standard diagnostic test or diagnostic criteria. This presents obvious difficulties in trying to present exact incidence data. To add to an already complicated situation which the American Thoracic Society have attempted to simplify in their 2016 guidelines, we are also presented with statements such as ‘VAP contributes to approximately half of all cases of hospital-acquired pneumonia’ (20).

Despite this, the US Institute of Healthcare Improvements identified ‘VAP’ as one of six areas to target in their 2006 ‘100,000 Lives’ Campaign (21). Specifically, this was to be approached using the ‘ventilator bundle’ in an attempt to tackle the problem using an evidence-based approach to modifiable risk factors.

VAP is variably estimated to occur in 9-27% of all mechanically ventilated patients (14,22). In one US publication looking at VAP incidence, the estimated rate varied from 1.2 to 8.5 per 1,000 ventilator days depending on the definition used (23).

In Europe, the incidence is estimated to be 12.2 per 1000 ventilator days (24). Data is also available for Scotland where a VAP rate of 6.5 per 1000 ventilator days has been observed (25). This is markedly different to the data from Wales which reported a VAP incidence of 1.2-2.2 per 1000 ventilator days (26). There are no national data available for VAP rates in England or the UK as a whole. Based on this interesting variation in VAP rate, Pugh *et al* carried out an

investigation into episodes of ventilator-associated respiratory tract infection (VARTI) in Welsh ICUs during two study periods, each of 14 days duration, from 2012 – 2014.

The reported rate of 1.2-2.2 per 1000 ventilator days was based on the use of the HELICS surveillance definitions over the 2009 – 2013 period. In the Pugh *et al* study of 282 patients who were invasively ventilated for 48 hours or more, 10 episodes of VAP were identified according to the HELICS definition. This equates to 4.2 per 1000 ventilator days and is clearly more in keeping, at least, with the Scottish data. Interestingly, the cases of HELICS-defined VAP represented less than a third of the VARTIs for which antibiotics were initiated during the study period. This was due to the fact that almost half of the VARTIs were treated with antibiotics without radiological evidence being sought.

The VARTI estimate from the study by Pugh *et al* was 11.3%. This is in keeping with a figure of 12% from a point prevalence study of UK antimicrobial use (27). Although the study had some drawbacks in terms of size and relatively limited periods of data collection, it raises interesting points not only regarding VAP definitions but also about critical care practices in the UK.

The concept of attributable mortality has gained popularity in recent years. Any nosocomial pneumonia or VAP occurs in patients who already possess a mortality risk associated with their underlying disease process. Therefore, the risk of death associated with the nosocomial pneumonia will be in addition to this. Attributable mortality is defined as total mortality minus the mortality associated with the underlying disease process (28). The attributable mortality from VAP has been estimated at 13% with surgical patients, and those with mid-range acute



physiology scores at the time of admission, being at greater risk (28,29). Attributable mortality appears to be related to risk of dying due to increased length of ICU stay (29), however the relationship between VAP and mortality is not clear cut.

#### **1.2.4 Clinical criteria and scoring systems**

The differences in approach to the definitions and classification of VAP have come about in part due to the variation in diagnostic strategies applied. On one hand the ‘clinical’ strategy aims to achieve early, effective treatment of patients with VAP while the ‘microbiological’ approach emphasises certainty of diagnosis and the avoidance of overtreatment with antibiotics. The potential VAP patient on an ICU will undergo the routine clinical assessment which examines new or progressive infiltrates on the chest radiograph, coupled with two or more of the following –  $\text{PaO}_2/\text{FiO}_2$  ratio, fever, leucocytosis or leucopenia and purulent (or change in quality and/or quantity) of secretions. This is regarded as a reliable group of criteria on which to commence empirical antibiotic therapy. Clearly, adopting fewer criteria will lead to greater sensitivity in the diagnosis of VAP at the expense of specificity, with a concomitant increased frequency of antibiotic therapy. Conversely, applying broader set of criteria before embarking upon therapy will lead to cases of pneumonia being missed which should otherwise have been treated.

The disadvantage of the clinical approach is that it consistently leads to more antibiotic use compared to decisions made with the benefit of microbiological results. The clinical approach can even lead to non-infective processes, such as pulmonary oedema, atelectasis or pulmonary haemorrhage, being treated inadvertently with antibiotics.

In 1991 Pugin *et al* developed the Clinical Pulmonary Infection Score (CPIS) in order to facilitate the diagnosis of VAP using clinical parameters (30). The system gives a score of 0–3 for temperature, leucocytosis, PaO<sub>2</sub>/FiO<sub>2</sub> ratio, chest radiography, tracheal secretions and culture of tracheal aspirate. The maximum score that can be obtained is 12, with a score >6 being categorised as diagnostic of VAP (table 1.1). Drawbacks of the CPIS score are inter-observer variability, particularly regarding interpretation of the tracheal secretions and chest radiographs, and inclusion of respiratory tract secretion microbiology data which may take up to 72 hours to yield a result. This cut-off at a score of >6 was determined by correlation with quantitative bronchoscopic and non-bronchoscopic BAL specimens.

Temperature (°C)	≥ 36.5 ≤ 38.4	0
	≥ 38.4 ≤ 38.9	1
	<36 or ≥ 39	2
White blood count (cell µl <sup>-1</sup> )	≥ 4,000 ≤ 11,000	0
	< 4,000 > 11,000	1
	+ band forms ≥500	2
Secretions	≤ small/day	0
	Moderate/large	1
	Purulent	2
Chest radiograph	No infiltrate	0
	Diffuse/patchy infiltrate	1
	Localised infiltrate	2
PaO <sub>2</sub> /FiO <sub>2</sub> (mmHg)	> 240 without ARDS	0
	< 240 without ARDS	2
Culture	< 10,000 bacteria or no growth	0
	> 10,000 bacteria	1
	+ Positive Gram stain	2

**Table 1.1 The Clinical Pulmonary Infection Score developed by Pugin *et al* in 1991 (30)**

In 1999 Fàbregas *et al* examined the cadavers of 25 patients who had undergone mechanical ventilation. Histological and quantitative microbiological testing was conducted on post mortem lung samples which demonstrated that CPIS had a sensitivity of 77% and a specificity of 42% (31). This study made a comparison with the Johansen Criteria and found these to have a sensitivity and specificity of 69% and 75% respectively. The Johansen Criteria, which are a rudimentary form of CPIS, include the presence of infiltrates on the chest radiograph and two of three clinical criteria (leucocytosis, purulent secretions or fever) (32).

Other studies have been carried out looking at the diagnostic accuracy of the CPIS criteria and these have produced a wide range of results (sensitivity 72-89%, specificity 17-85%). These are summarised in Table 1.2 (33).

Direct comparison of studies is hampered by variation in the diagnostic gold standard that is applied. Nevertheless, these results are markedly different to the sensitivity and specificity (93% and 96% respectively) reported by Pugin *et al* in the original study.

Another interesting point about the original description of the CPIS criteria is the inclusion of the bacterial index as a standard for defining VAP. Bacterial index was defined as the sum of quantitative cultures (log CFU ml<sup>-1</sup>) of organisms obtained and an index >5 was used to define VAP. This is not a widely used indicator, nor is it used in other studies. Furthermore, in the study by Schurink *et al*, a bacterial index of >6 rather than >5 was applied for the diagnosis of VAP (34). In the same study, the diagnosis of VAP using the CPIS criteria by two different intensivists was examined and this demonstrated poor correlation (k=0.16), providing further evidence of the inter-observer variability when applying these criteria.

Source	Criteria	Gold standard	Sens (%)	Spec (%)	PPV (%)	NPV (%)	LR+ (95% CI)	LR- (95% CI)
Papazian <i>et al</i> 1995(35) (n=38)	CPIS>6	PM histology	72	85	-	-	4.8 (1.6-14)	0.33 (0.15-0.70)
Fabregas <i>et al</i> (36) (n=25)	CPIS>6	PM histology + culture	77	42	-	-	1.3 (0.75-2.3)	0.55 (0.17-1.8)
Fartoukh <i>et al</i> (37)(N=79)	BAL	BAL>10 <sup>4</sup> CFU	85	49	63	76	1.67	-
	PTC	BAL>10 <sup>4</sup> CFU	78	56	65	71	1.77	-
Luyt <i>et al</i> (38) (n=201)	CPIS>6	BAL>10 <sup>4</sup> CFU or PSB>10 <sup>3</sup> CFU	89	47	57	84	-	-
Schurink <i>et al</i> (34) (n=99)	CPIS>5	BAL>10 <sup>4</sup> CFU	83	17	-	-	-	-
Miller <i>et al</i> (39) (n=292)	NHSN	BAL>10 <sup>5</sup> CFU	84	69	83	70	-	-

Sens=sensitivity; Spec=specificity; PPV=positive predictive value; NPV=negative predictive value

LR+ = negative likelihood ratio; LR- = negative likelihood ratio

BAL=bronchoalveolar lavage; PTC=protected telescopic catheter; PSB=protected specimen brushing  
CFU=colony forming units

**Table 1.2 Table summarising studies examining the diagnostic accuracy of the CPIS and CDC-NHSN criteria (33)**

Fartoukh *et al* investigated the diagnostic accuracy of three modifications of the CPIS — the first omitting the microbiological component (not included here), the second using Gram stain of BAL fluid for the microbiological component, the third using PSB Gram stain for the microbiological component (37).

As the original CPIS criteria had a prerequisite for microbiological data, they could not be used as a VAP screening tool. Modifications to CPIS were therefore proposed by Singh *et al* which used only the first 5 CPIS criteria, excluding the score for ‘culture of tracheal aspirate’ (40). This was in an attempt to devise a strategy to avoid prolonged use of unnecessary antimicrobial therapy.

Another approach to improve the rather low sensitivity and specificity of CPIS was the addition of a Gram stain of deep respiratory tract culture (obtained from BAL or blind protected telescoping catheter sample), devised by Fartoukh *et al* (37). With this method, the CPIS for confirmed VAP was significantly higher than the score in patients with unconfirmed VAP.

The Hospitals in Europe Link for Infection Control through Surveillance (HELICS) criteria are used for surveillance of VAP in Europe (41). HELICS uses a similar set of criteria to that of CDC-NHSN with a combination of clinical, radiological and microbiological criteria. These are illustrated in tables 1.3. The microbiological criteria are subdivided into 5 categories (PN1 - 5) depending on the microbiological method used.

Morris *et al* used paired samples from 53 patients and found that if a BAL-based diagnosis (PN1) was used in preference to quantitative endotracheal aspirates (ETA)(PN2), VAP rates would be 59% lower in their unit (42). The criteria also allow for a diagnosis of VAP to be made with non-quantitative ETA culture, other indirect cultures and without any positive microbiology.

Rx	Two or more serial chest X-rays or CT-scans with a suggestive image of pneumonia for patients with underlying cardiac or pulmonary disease. In patients without underlying cardiac or pulmonary disease one definitive chest X-ray or CT-scan is sufficient.
	<u>and</u> at least one of the following
Symptoms	<ul style="list-style-type: none"> <li>▪ Fever &gt; 38 °C with no other cause</li> <li>▪ Leukopenia (&lt;4000 WBC/mm<sup>3</sup>) or leucocytosis (≥ 12 000 WBC/mm<sup>3</sup>)</li> </ul> <p><u>and</u> at least one of the following (or at least two if clinical pneumonia only = PN 4 and PN 5)</p> <ul style="list-style-type: none"> <li>▪ New onset of purulent sputum, or change in character of sputum (color, odor, quantity, consistency)</li> <li>▪ Cough or dyspnea or tachypnea</li> <li>▪ Suggestive auscultation (rales or bronchial breath sounds), ronchi, wheezing</li> <li>▪ Worsening gas exchange (e.g., O<sub>2</sub> desaturation or increased oxygen requirements or increased ventilation demand)</li> </ul> <p>and according to the used diagnostic method</p>
Microbiology	<p><b>a – Bacteriologic diagnostic performed by :</b></p> <p><i>Positive quantitative culture from minimally contaminated LRT<sup>1</sup> specimen</i> <b>(PN 1)</b></p> <ul style="list-style-type: none"> <li>▪ Broncho-alveolar lavage (BAL) with a threshold of ≥ 10<sup>4</sup> CFU<sup>2</sup>/ml or ≥ 5 % of BAL obtained cells contain intracellular bacteria on direct microscopic exam (classified on the diagnostic category BAL).</li> <li>▪ Protected brush (PB Wimberley) with a threshold of ≥ 10<sup>3</sup> CFU/ml</li> <li>▪ Distal protected aspirate (DPA) with a threshold of ≥ 10<sup>3</sup> CFU/ml</li> </ul> <p><i>Positive quantitative culture from possibly contaminated LRT specimen</i> <b>(PN 2)</b></p> <ul style="list-style-type: none"> <li>▪ Quantitative culture of LRT specimen (e.g. endotracheal aspirate) with a threshold of 10<sup>6</sup> CFU/ml</li> </ul> <p><b>b – Alternative microbiology methods</b> <b>(PN 3)</b></p> <ul style="list-style-type: none"> <li>▪ Positive blood culture not related to another source of infection</li> <li>▪ Positive growth in culture of pleural fluid</li> <li>▪ Pleural or pulmonary abscess with positive needle aspiration</li> <li>▪ Histologic pulmonary exam shows evidence of pneumonia</li> <li>▪ Positive exams for pneumonia with virus or particular germs (<i>Legionella</i>, <i>Aspergillus</i>, mycobacteria, mycoplasma, <i>Pneumocystis carinii</i>) <ul style="list-style-type: none"> <li>○ Positive detection of viral antigen or antibody from respiratory secretions (e.g., EIA, FAMA, shell vial assay, PCR)</li> <li>○ Positive direct exam or positive culture from bronchial secretions or tissue</li> <li>○ Seroconversion (ex : influenza viruses, <i>Legionella</i>, <i>Chlamydia</i>)</li> <li>○ Detection of antigens in urine (<i>Legionella</i>)</li> </ul> </li> </ul> <p><b>c – Others</b></p> <ul style="list-style-type: none"> <li>▪ Positive sputum culture or non-quantitative LRT specimen culture <b>(PN 4)</b></li> <li>▪ No positive microbiology <b>(PN 5)</b></li> </ul> <p>Note: PN 1 and PN 2 criteria were validated without previous antimicrobial therapy</p>

<sup>1</sup> LRT = Lower Respiratory Tract

<sup>2</sup> CFU = Colony Forming Units

**Table 1.3 The HELICS case definition of ICU acquired pneumonia, 2004 (43)**

Patients must fulfil radiological, systemic and pulmonary criteria to meet the HELICS definition of pneumonia. For Intubation associated pneumonia, an invasive respiratory device must have been present for at least part of the preceding 48 hours.

### **1.2.5 Microbiological sampling and testing methods**

Histopathologically, VAP represents lung parenchymal inflammation which arises as a result of inoculation of bacteria into the airways, largely attributed to microaspiration of secretions which have accumulated around the tracheal tube (TT). The composition of these secretions is variable and includes a microbiota of commensals and healthcare associated organisms, some of which may exhibit antibiotic resistance (44).

On a microscopic level, ventilator associated respiratory tract infection may be anywhere on the spectrum of tracheobronchitis, peribronchial pneumonitis, through to bronchopneumonia, with the pneumonia itself being largely heterogenous in nature (45).

As discussed previously, relying solely on the clinical signs which result from this inflammatory process leads to overdiagnosis of VAP. However, the best microbiological method for identifying the causative bacteria in VAP is uncertain, adding a further layer of complexity and controversy when making the diagnosis.

Various invasive and non-invasive sampling techniques are used to obtain respiratory secretions, including tracheal aspirates (TA), protected specimen brushings (PSB), protected telescopic catheter (PTC) sampling and directed and non-directed bronchoalveolar lavage (BAL).

The samples obtained can be analysed quantitatively (with a threshold count of bacterial growth to differentiate between infection and colonisation of the bronchial tree), semi-quantitatively (subjective quantitative scoring method of bacterial load in a Gram stain sample or culture plate by a technician) or qualitatively (the binary presence or absence of pathogenic organisms in culture).

The rationale for using quantitative cultures of respiratory secretions in suspected VAP patients is to differentiate the organisms causing infection (those with a higher concentration) from colonising organisms (those with lower concentration), in order to direct antibiotic therapy. However, there is no clear consensus on the use of quantitative *versus* qualitative cultures in this patient group.

The bacterial concentration in lung *tissue* from a histopathologically confirmed pneumonia is  $\geq 10^4$  CFU g<sup>-1</sup> which corresponds to a bacterial concentration in respiratory *secretions* of  $\geq 10^5$  CFU ml<sup>-1</sup>. A series of threshold levels can be established for the samples obtained using the different techniques described above (46) and these are illustrated in table 1.4.

According to the NHSN definitions, VAP is deemed *possible* when microscopically purulent secretions are noted and *probable* when a quantitative culture of lower respiratory material is above a designated threshold described in the table. The clinical role for these remains uncertain, in particular samples obtained bronchoscopically.

Klompas *et al* reviewed a number of studies looking at pulmonary secretions obtained by a variety of techniques from ICU patients (47). These were compared to post-mortem



histopathological samples alone, or in combination with post-mortem lung culture as a gold standard. The objective was to assess diagnostic accuracy of pulmonary secretion microbiology and the results of this comparison are shown in table 1.5.

One should still bear in mind that even interpretation of histopathological samples is not without controversy. The microscopic appearance of these specimens is affected by the time between initial clinical suspicion of VAP and subsequent post-mortem analysis, during which time some healing will have taken place and antibiotic exposure may have had an effect (47).

Specimen	Collectors	Site(s) sampled	Sample amount	Diluent volume	Dilution Factor	Quantitative threshold <sup>a</sup>	Semiquantitative equivalent <sup>b</sup>	Comment(s)
Lung tissue	Trained physicians	Lung parenchyma	~ 1.0 g	~ 1.0 ml	1	≥ 10 <sup>4</sup> g	Few-moderate or 2-3+	Often obtained post-mortem, is not representative of all patients
Bronchoscopic <sup>c</sup> protected specimen brushing	Trained physicians	Bronchioles	0.001 – 0.01 ml	1.0 ml	1/100 – 1/1000	≥ 10 <sup>3</sup> ml	Rare-few or 1-2+	Low-volume sampling restricts utility
Bronchoscopic bronchoalveolar lavage fluid	Trained physicians	Bronchioles & alveoli	~ 1.0 ml	10 – 100 ml	1/10 – 1/100	≥ 10 <sup>4</sup> ml	Few-moderate or 2-3+	Adequate volume for multiple analyses, including microscopy
Non-bronchoscopic protected specimen brushing	Respiratory therapists or trained personnel	Bronchioles	0.001 – 0.01 ml	1.0 ml	1/100 – 1/1000	≥ 10 <sup>3</sup> ml	Rare-few or 1-2+	Low-volume sampling restricts utility
Nonbronchoscopic bronchoalveolar lavage fluid	Respiratory therapists or trained personnel	Bronchioles & alveoli	~ 1.0 ml	10 – 100 ml	1/10 – 1/100	≥ 10 <sup>4</sup> ml	Few-moderate or 2-3+	Comparable to BAL but often lower in lavaged volume
Endotracheal aspirate	Respiratory therapists or nurses	Tracheal secretions	1-10 ml	1 – 10 ml	1 – 1/10	≥ 10 <sup>5</sup> ml	Moderate-numerous or 3-4+	Easily obtained; primarily reported semiquantitatively with microscopy

<sup>a</sup> Number of colonies meeting threshold value varies with calibrated loop volume.

<sup>b</sup> Assuming an ~0.01ml inoculum and a quadrant streak method with the following observations: rare or 1+ is growth in first quadrant, few or 2+ is growth in second quadrant, moderate or 3+ is growth in third quadrant, and numerous or 4+ is growth in fourth quadrant.

<sup>c</sup> Bronchoscopic sampling is performed via fiber optic bronchoscopy and incurs greater costs but allows targeted sampling.

**Table 1.4 Comparison of various sampling methods used for National Healthcare Safety Network (NHSN) microbiologic thresholds (39)**

Finding and source	Gold standard	Sens (%)	Spec (%)	LR+ (95%CI)	LR- (95%CI)
<b>&gt;50% neutrophils in BAL fluid</b>					
Kirtland <i>et al</i> (n=39)(48)	Histology	100	53	2.0 (1.4-3.0)	0.09 (0.01-1.4)
<b>Neutrophils with intracellular bacteria in BAL fluid</b>					
Kirtland <i>et al</i> (n=39)(48)	Histology	33	60	0.83 (0.3-2.3)	1.1 (0.64-1.9)
Papazian <i>et al</i> 1997 (n=28)(49)	Histology	54	53	1.2 (0.55-2.4)	0.87 (0.41-1.8)
<b>Summary</b>				1.0 (0.59-1.9)	1.0 (0.64-1.6)
<b>Positive gram stain (Papazian <i>et al</i> 1997)(49)</b>					
Tracheo-bronchial aspirate	Histology + culture	56	74	2.1 (0.81-5.5)	0.60 (0.28-1.3)
Mini BAL fluid	Histology + culture	56	89	5.3 (1.3-22)	0.50 (0.24-1.0)
BAL fluid	Histology + culture	44	100	18 (1.1-302)	0.56 (0.32-0.99)
<b>Culture - tracheo-bronchial aspirate &gt;10<sup>5</sup> CFU ml<sup>-1</sup></b>					
Papazian <i>et al</i> 1995 (35)	Histology + culture	56	95	11 (1.6-78.5)	0.47 (0.28-0.79)
Fabregas <i>et al</i> (36)	Histology + culture	69	92	8.3 (1.2-56.2)	0.34 (0.15-0.77)
<b>Summary</b>				9.6 (2.4-38)	0.42 (0.27-0.67)
<b>Culture - BAL fluid &gt;10<sup>4</sup> CFU ml<sup>-1</sup></b>					
Torres <i>et al</i> (50)	Histology	50	42	0.86 (0.44-1.7)	1.2 (0.53-2.7)
Papazian <i>et al</i> 1995 (35)	Histology + culture	50	95	10 (1.4-71.4)	0.53 (0.33-0.84)
Kirtland <i>et al</i> (48)	Histology	11	80	0.56 (0.08-4.0)	1.1 (0.83-1.5)
Fabregas <i>et al</i> (36)	Histology + culture	77	58	1.8 (0.89-3.8)	0.40 (0.13-1.2)
<b>Summary</b>				1.4 (0.76-2.5)	0.78 (0.51-1.2)

Sens = sensitivity; Spec = specificity; LR+ = positive likelihood ratio; LR- = negative likelihood ratio; BAL = bronchoalveolar lavage; CFU = colony forming units

**Table 1.5 The diagnostic accuracy of pulmonary secretion microbiology as determined by comparison of invasive and non-invasive sampling techniques and quantitative and qualitative culture against a defined gold standard - from Klompas *et al* (47)**

In the two studies that examined culture of blind tracheobronchial aspirate, using  $>10^5$  CFU-ml<sup>-1</sup> as a threshold, the diagnosis of VAP was significantly increased. The utility of quantitative culture from BAL fluid was less definitive, with a summary positive likelihood ratio of only 1.4 (0.76-2.5) compared to 9.6 (2.4-38) for blind tracheobronchial aspirate.

Several randomised controlled trials (RCTs) have been carried out in an attempt to establish if the more invasive sampling techniques (e.g. BAL or PSB) confer any patient outcome benefit when compared to the less invasive methods such as TA. The RCTs by Ruiz *et al* and Sanchez-Nieto *et al* (51,52) looked at quantitative invasive *versus* quantitative non-invasive sampling techniques. Quantitative invasive *versus* non-quantitative non-invasive techniques were dealt with in another three RCTs (53–55). Of these five RCTs, only the study by Fagon *et al* (54) demonstrated a significant reduction in mortality at 14 and 28 days in the invasive treatment arm. Subsequently, a Cochrane meta-analysis of these five studies showed that there was no significant difference in mortality, length of ICU stay or duration of mechanical ventilation between the invasive and non-invasive groups or the quantitative and qualitative groups (56).

### **1.2.6 Summary**

The debate continues with regard to what constitutes best practice in the diagnosis of VAP. The decision to treat or not to treat a patient with potential VAP rests with the treating intensivist and, for the time being, this decision will be based on the clinical, radiological and microbiological information available. Bacterial presence in respiratory secretions, confirmed by Gram-stain or growth above a quantitative threshold lends weight to the diagnosis of VAP. However, clinicians should keep an open mind in these patients and if not improving after 48 hours of antibiotic therapy an alternative diagnosis should be considered.

### **1.3 Prevention of ventilator associated pneumonia**

Ventilator associated pneumonia is a preventable condition and, as such, the incidence of VAP is deemed to be an important marker of quality of care on ICU (57). The development of VAP can be mitigated by a number of interventions which are aimed at reducing microaspiration and biofilm formation. These have been grouped together and are delivered as a ‘care bundle’.

A care bundle, as defined by the Institute for Health Improvement (IHI) in the US, is ‘a structured way of improving the processes of care and patient outcomes: a small, straightforward set of (generally three to five) evidence-based practices that, when performed collectively and reliably, have been proven to improve patient outcomes’ (58).

The original ventilator care bundle was an early example of the IHI care bundle methodology in the US. Care bundle methodology was adopted early in the UK, initially by the NHS Modernisation Agency and then via multiple agencies including the National Patient Safety Agency (NPSA). In 2007, ‘High Impact Intervention No 5 – Care bundle for ventilated patients’ was incorporated into the Department of Health (DOH) ‘Saving Lives: reducing infection, delivering clean and safe care’ campaign.

#### **1.3.1 Evidence-based care bundles**

The ventilator care ‘bundle’ or package of care was described by Resar *et al* in 2005 and looked at the effect of implementing this concept in 61 institutions from 2002 – 2004 (58). The results were striking and an average reduction in VAP of 44.5% was observed. This early bundle comprised ‘peptic ulcer disease prophylaxis, deep vein thrombosis prophylaxis, elevation of the head of the bed, and a sedation vacation’. In the US, the ventilator care bundle was one of

the interventions in the ‘100,000 Lives Campaign’ and subsequently in the ‘5 Million Lives Campaign’(59).

The bundle concept was adopted and introduced in the UK by the, now defunct, NHS Modernisation Agency and subsequently the National Patient Safety Agency (NPSA). The 2007 NHS campaign ‘Saving Lives: reducing infection, delivering clean and safe care’ cited the care bundle as a VAP prevention strategy in their ‘High Impact Intervention No 5’ (60). The 2007 document included daily sedation holds, elevation of the head of the bed, gastric ulcer prophylaxis and oral care.

The bundle was subsequently updated in 2010 to include extended oral hygiene measures (using ‘adequate strength’ antiseptics), subglottic aspiration and monitoring of TT cuff pressure (to maintain a cuff pressure of  $\geq 20$  and  $\leq 30$  cm H<sub>2</sub>O) (61). The six elements of the ‘recommended good practice’ are described in table 1.6.

An additional point, although one that was not included as an auditable component, was that ‘ventilator tubing should be managed and positioned effectively to ensure condensate flows away from the patient and does not enter the patients’ airways’ (14).

A single-centre before and after study by Morris *et al* examined the use of the original four high impact interventions when applied to an 18 bed Scottish ICU (62). Although there was only 70% overall compliance with the bundle, they were able to demonstrate a reduction in VAP incidence from 32 per 1000 ventilator days to 12 per 1000 ventilator days post bundle

implementation. They were, however, unable to demonstrate a reduction in length of ICU stay or duration of mechanical ventilation.

1. Elevation of the head of the bed
<ul style="list-style-type: none"> <li>The head of the bed is elevated to 30-45° (unless contraindicated).</li> </ul>
2. Sedation level assessment
<ul style="list-style-type: none"> <li>Unless the patient is awake and comfortable, sedation is reduced/held for assessment at least daily (unless contraindicated).</li> </ul>
3. Oral hygiene
<ul style="list-style-type: none"> <li>The mouth is cleaned with chlorhexidine gluconate (<math>\geq 1-2\%</math> gel or liquid) 6-hourly (as chlorhexidine can be inactivated by toothpaste, a gap of at least 2 hours should be left between its application and tooth brushing).</li> <li>Teeth are brushed 12-hourly with standard toothpaste.</li> </ul>
4. Subglottic aspiration
<ul style="list-style-type: none"> <li>A tracheal tube (endotracheal or tracheostomy) which has a subglottic secretion drainage port is used if the patient is expected to be intubated for &gt;72 hrs.</li> <li>Secretions are aspirated via the subglottic secretion port 1-2 hourly.</li> </ul>
5. Tracheal tube cuff pressure
<ul style="list-style-type: none"> <li>Cuff pressure is measured 4-hourly, maintained between 20-30cm H<sub>2</sub>O (or 2cm H<sub>2</sub>O above peak inspiratory pressure) and recorded on the ICU chart.</li> </ul>
6. Stress ulcer prophylaxis
<ul style="list-style-type: none"> <li>Stress ulcer prophylaxis is prescribed only to high-risk patients according to locally developed guidelines.</li> <li>Prophylaxis is reviewed daily.</li> </ul>

**Table 1.6 The six elements of the care process described in the 2010 Department of Health ‘High Impact Interventions’ for reducing healthcare associated infections (61)**

The current UK ventilator care bundle guidelines are published in the first edition of Guidelines for the Provision of Intensive Care Services (GPICS) (63). This document makes recommendations on measures that should be implemented to reduce the incidence of VAP.

The current recommendations are:

1. ICUs should have standardised systems to monitor VAP rates and antibiotic resistance patterns.
2. Patients should be nursed in a semi-recumbent position (30° – 45°).
3. Mechanically-ventilated patients should be tracheally intubated with an orotracheal tube (as opposed to nasotracheal tube which increases risk of maxillary sinusitis (64)) and cuff pressure maintained above 20 cmH<sub>2</sub>O.
4. Subglottic suction tubes should be considered for use in patients who it is anticipated will be mechanically ventilated for more than 72 hours.
5. Ventilator tubing and suction systems should only be changed if specifically indicated, such as by visible soiling, to avoid unnecessary changes.
6. Stress ulcer prophylaxis should be used judiciously, and only in patients considered to be at high risk of upper gastrointestinal (GI) bleeding.

These recommendations and the evidence on which they are based will now be discussed in more detail.

### **1.3.2 VAP rate monitoring**

Audit forms part of the overall quality improvement strategy in ICU and should be targeted at areas where improvements can be made. One process and outcome that can be measured is



compliance with ventilator care bundles and local VAP rates. This is recommended in GPICS as part of the framework to assist in the delivery of quality improvement in Critical Care (63).

### **1.3.3 Patient positioning**

As discussed above, measures to prevent VAP are aimed at reducing microaspiration of gastric and oropharyngeal contents. It was demonstrated by Torres *et al* that there was significantly less aspiration of a technetium (Tc)-99m labelled sulphur colloid from the stomach, in patients nursed at 45° compared to those nursed in a supine position (65). Patient position in relation to VAP was also examined in an unblinded, randomised trial by Drakulovic *et al* (66). Bearing in mind the potential for bias, a significant reduction in VAP was demonstrated in the 45° group.

In reality, it is unusual for patients to be nursed fully supine and so a further trial by van Nieuwenhoven *et al* compared 10° to 45° bed head elevations (67). The authors were unable to demonstrate a significant reduction in VAP, but the trial did illustrate what is known anecdotally – that achieving 45° elevation is difficult in practice. These studies demonstrate that there is an association between nursing ventilated ICU patients in the supine position and the development of VAP. However, the most beneficial degree of bed head elevation in the prevention of VAP is unclear, with the recommended minimum being 30°.

### **1.3.4 Tracheal tube design**

A number of VAP prevention strategies have focused on the tracheal tube as this provides the conduit from the oropharynx to the bronchial tree. These include devices to control the pressure in the tracheal tube cuff, modifications which allow drainage of subglottic secretions, variations in design of the cuff itself and tracheal tube coatings designed to impede biofilm formation.

There are a number of devices currently on the market that aim to provide continuous control and monitoring of tracheal tube cuff pressure. These include devices such as Cuff Controller (VBM Medizintechnik GmbH), the Nosten® device (Leved, St-Maur, France), IntelliCuff® (Hamilton Medical) and the PressureEasy® Cuff Pressure Monitor (Smiths Medical).

The optimal inflation pressure for the tracheal tube cuff is thought to be 20–30 cm H<sub>2</sub>O. Pressures below 20 cm H<sub>2</sub>O permit passage of secretions from the oropharynx into the trachea and pressures above 30 cm H<sub>2</sub>O cause injury to the tracheal mucosa by compromising the microcirculation (68). The paper by Rello *et al* is frequently cited regarding the potential complications of inadequate tracheal tube cuff pressure (69). In this study, there was a trend towards higher risk of VAP (RR = 2.57; 95% CI = 0.78 to 8.03) among patients whose cuff pressures were persistently less than 20 cm H<sub>2</sub>O. Rello *et al* concluded that leakage of colonised sub-glottic secretions around the cuff was the most important risk factor for VAP within the first 8 days of mechanical ventilation.

In a study of 122 patients, the Nosten® device was found to reduce the rate of microaspiration as defined by a reduction in pepsin found in tracheal secretions (18% vs 46%; P=0.002; OR [95% confidence interval], 0.25 [0.11-0.59]). Reduced bacterial concentration in tracheal aspirates (mean ± SD 1.6 ± 2.4 vs 3.1 ± 3.7 log<sub>10</sub> CFU ml<sup>-1</sup>, P=0.014), and a reduced VAP rate (9.8 vs 26.2%; P=0.032) were also demonstrated as secondary outcomes (70).

Valencia *et al* also looked at the maintenance of tracheal tube cuff pressure with an unspecified mechanical device in 142 patients (71). Cuff pressures were maintained in the target range 80% of the time in the treatment group, with pressure falling below 20 cm H<sub>2</sub>O in 0.7% of the

treatment group compared to 45.3% in the control group ( $P=0.001$ ). A statistically significant difference in the VAP rate between the two groups was not demonstrated, although this is not surprising given the size of the study.

The description above gives a rather simplistic view of cuff pressure and micro-aspiration. Control of cuff pressure is important, but it will only reduce, not completely eliminate, the unwanted flux of oropharyngeal and gastric secretions into the bronchial tree. The situation is not a static one, with a simple air-filled cushion forming a barrier around the tracheal tube. Instead the situation is dynamic, with constant changes in cuff pressure and the cuff-trachea interface brought about by coughing, movement and nursing interventions (68).

Although mechanical devices may deliver a constant pressure, they may not afford a constant seal. It has been suggested that varying cuff pressure with inspiration and expiration may, in fact, be more effective than maintaining a constant cuff pressure. This was highlighted in a small study ( $n=10$ ) of porcine models (72). This study also appeared to show that dynamic cuff pressure modulation might reduce the incidence of tracheal injury – although the model applied a period of hypoxic ventilation, which could itself predispose the tracheal mucosa to injury.

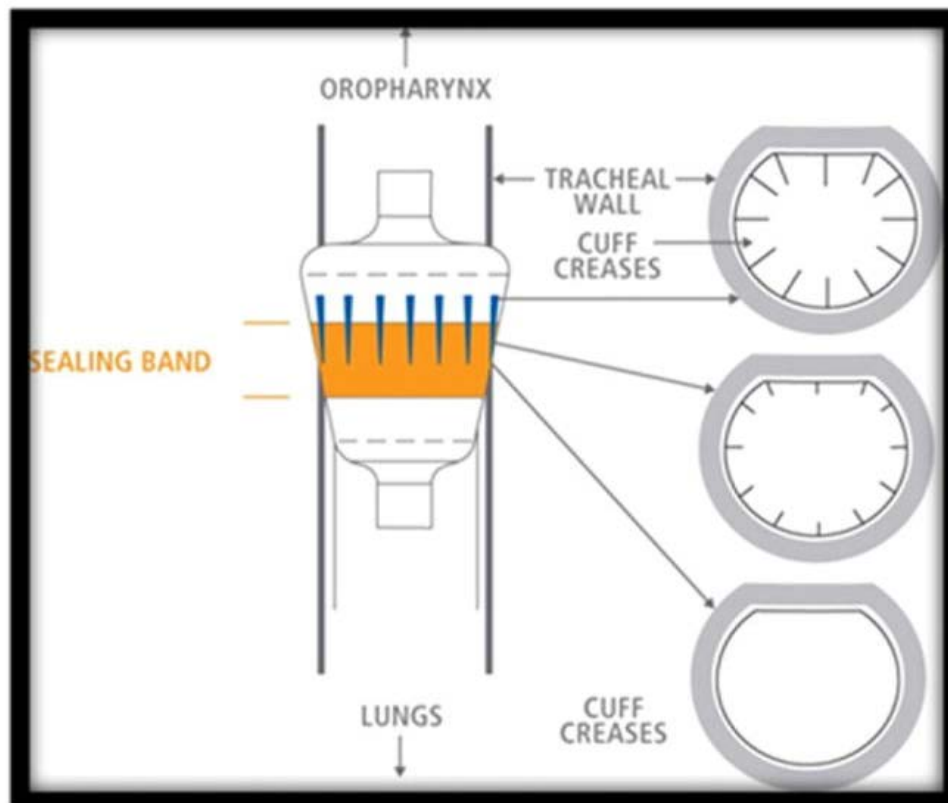
The cuff of the modern tracheal tube is of a high-volume/low-pressure (HVLP) design, made of pliable medical grade polyvinylchloride (PVC), which forms to the airway as it is inflated. This is in contrast to the older high-pressure/low-volume (HPLV) cuff that could cause significant pressure injury to the tracheal wall (73).

In order for the HVLP cuff to function correctly, it must have a deflated diameter greater than that of the trachea into which it will be inserted. This is to allow some slack in the cuff wall so that there is no tension when the cuff is inflated (73). Hence, all the intra-cuff pressure is transmitted to the tracheal wall allowing direct pressure measurement and monitoring.

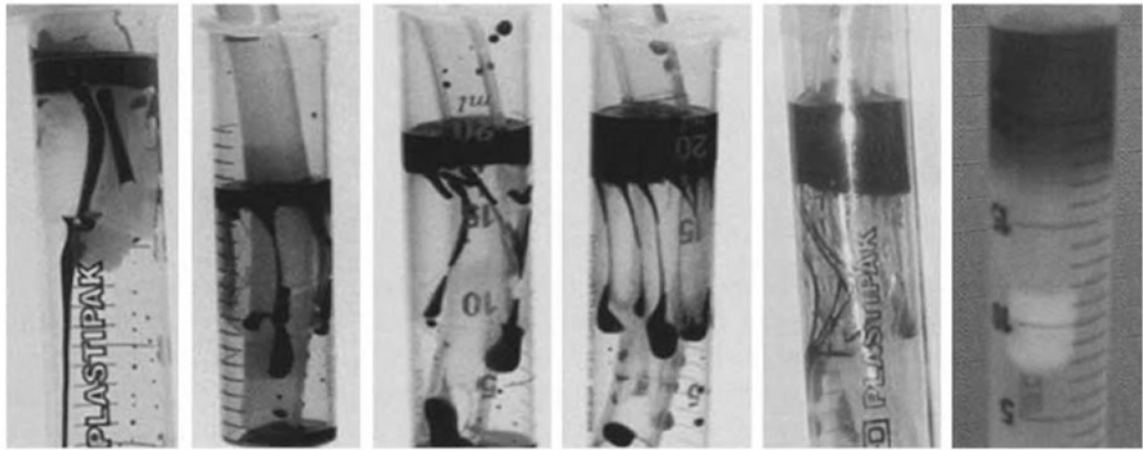
Unfortunately, even when inflated correctly, these cuffs allow some degree of aspiration to occur. The reason for this ongoing risk of aspiration, despite an appropriately inflated HVLP cuff, is the presence of microchannels. Microchannels exist because these cuffs will not be fully distended even when inflated to the recommended pressure. Patient and TT movement in addition to anatomical variations may exacerbate the passage of secretions via these microchannels (figure 1.4) (68).

In this static depiction, the cuff creases gradually become smaller to form a proximal sealing band. In practice this is not the case due to patient and TT movement and anatomical variation.

The risk of aspiration *is* reduced by having TT cuffs with thinner cuff walls or by completely eliminating cuff folds. This is illustrated in the model shown in figure 1.5.



**Figure 1.4 Diagrammatic representation of microchannels in an HVLP tracheal tube cuff(68)**



**Figure 1.5 Model illustrating the effect of a reduction in the size of TT microchannels (74)**

The first five images are of HVLP cuffs of reducing thickness from left to right. The channel folds increase in size with thickness of cuff material, hence the amount of ink bypassing the cuff reduces from image one to image five. The sixth image is a silicone Lotrach® cuff, in which there is no leak as there are no folds.

Other modifications to TT design have been proposed and implemented, such as silver-coating. Silver-coated TTs slowly release silver cations, which are known to have an antimicrobial effect. However, there is insufficient evidence at present to recommend their use outside clinical trials (75).

### **1.3.5 Management of secretions**

Secretions containing potential pathogens pool in the area above the TT cuff and the true vocal cords. These may pass into the bronchial tree via the microchannels described above or at the time of cuff deflation. This area is inaccessible to conventional suction methods, hence

specifically designed TTs (and tracheostomy tubes) are available that permit drainage of these 'subglottic' secretions (9).

Three meta-analyses have examined the role of subglottic secretion drainage (SSD) in ICU patients, all of which gave a signal towards a reduction in VAP (9). In a subsequent RCT of 352 patients, SSD resulted in a reduction in the rate of microbiologically confirmed VAP (9.6 vs 19.8 of 1,000 ventilatory days ( $p=0.0076$ )) and a significant reduction in the total number of antibiotic days (76). SSD is therefore suggested in patients who are likely to be mechanically ventilated for more than 72 hours.

#### **1.3.6 Timing of ventilator circuit changes**

Frequent ventilator circuit changes are thought to be a risk factor for VAP (77). This is due to the risk of introducing contaminated secretions or condensate to the patient via the TT by unnecessary manipulation of the tubing. However, in two RCTs (which used different methods of humidification) routine seven-day tubing changes were compared to no routine changes (78,79). No significant differences in VAP rates were demonstrated between the two-trial arms in either trial.

#### **1.3.7 Stress ulcer prophylaxis**

Stress ulcer prophylaxis has traditionally formed part of the ventilator care bundle, however, pharmacologically reducing the acidic environment of the stomach risks promoting colonisation with potentially pathogenic organisms (80).

The use of proton pump inhibitors (PPI) is widespread in ICU despite the relatively low incidence of GI bleeding (81). Maclaren *et al* carried out an observational study of 35,312 ventilated ICU patients receiving PPI or H<sub>2</sub> receptor blockers (H2RA). PPI use was associated with more GI bleeding compared to H2RA (5.9% vs 2.1%), more VAP (38.6% vs 27%) and more *Clostridium difficile* infection (3.8% vs 2.2%) (82).

Hence the GPICS recommendation state that stress ulcer prophylaxis should only be used in patients thought to be at high risk of upper GI bleeding (9).

### **1.3.8 Oral chlorhexidine**

The recommendation for the use of oral chlorhexidine to decontaminate the oropharynx has been removed from the most recent guidelines. It is no longer recommended outside of cardiac ICU by the Intensive Care Society (ICS) or by the National Institute for Health and Care Excellence (NICE) (9).

The previous recommendation for the use of chlorhexidine had been based on the results of two large meta-analyses which showed a reduction in VAP in the treatment groups (83,84). These meta-analyses included both general and cardiac ICU patients and a subsequent meta-analysis by Klompas *et al* demonstrated that the VAP reduction was not significant in the general ICU patients. Furthermore, there was a non-significant trend towards increased mortality in this group (85).

In a further meta-analysis of GI decontamination practices, the use of oral chlorhexidine was associated with an increase in mortality with an odds ratio of 1.25 (95% CI 1.05–1.50)



compared to selective decontamination of the digestive tract (SDD) and selective oral decontamination (SOD) (86).

### **1.3.9 Sedation practices**

Studies looking at ICU sedation practices tend to focus on duration of mechanical ventilation and length of ICU stay. However, there is some evidence that sedation holds (or daily interruption of sedation (DSI)) may reduce VAEs (87). As DSIs are associated with a reduction in duration of mechanical ventilation, this follows suit (9). intubation and mechanical ventilation predispose patients to VAP, hence, reducing the duration of mechanical ventilation should reduce the time they are at risk for developing VAP.

Although DSI is not in the current GPICS recommendations for the prevention of VAP, there is sufficient evidence to support the implementation of DSI to prevent over-sedation and earlier liberation from mechanical ventilation.

## **1.4 Emergence of resistant organisms in critical care**

Jean-Louis Vincent's review article, published in *Lancet* 2003, provides a coherent statement regarding the pathophysiology of ICU acquired infection (3). 'The development of ICU-acquired infection is dependent on two key pathophysiological factors: decreased host defences and colonisation by pathogenic, or potentially pathogenic, bacteria. Although these two factors can arise independently, for infection to result both must be present to some degree'.

#### 1.4.1 Reduced host defences

Critical illness is associated with decreased host immune defence making this patient group more susceptible to nosocomial infection. Obviously, the administration of immunosuppressive drugs can contribute to this state, however, critical illness *per se* and mechanical ventilation are likely to have a detrimental effect on a patient's immunocompetence.

Morris *et al* have demonstrated neutrophil dysfunction and impaired phagocytosis in mechanically ventilated patients in whom there was a high clinical suspicion of VAP. They found significantly reduced phagocytic activity in these patients due to overexpression of the inflammatory anaphylatoxin C5a (88). Excess C5a is known to cause neutrophil dysfunction and this appears to precede, rather than occur as a result of, nosocomial infection (89) This is relevant to the ICU population as they are highly susceptible to HAIs and neutrophil dysfunction may play a role in their relative immunosuppression.

Immunosuppression due to release of mediators such as interleukin 10 and interleukin-1-receptor antagonist has also been demonstrated. If significant and prolonged, this acquired immunodeficiency state has been referred to as 'immunoparalysis'. It is associated with an increased risk of infection-related complications, therefore placing such individuals in a particularly high-risk group for nosocomial infection (90).

Immune defence also includes mechanical host-defence mechanisms; for example, coughing, sneezing, saliva production and mucociliary clearance are all important in the prevention of respiratory infection (91). Tracheal intubation is a common intervention on ICU, which

bypasses these protective mechanisms hence reducing local defences and predisposing to respiratory tract infection in mechanically ventilated patients.

#### **1.4.2 Colonisation by pathogenic bacteria**

Colonisation is the phenomenon of active bacterial growth on a body surface without the bacterial growth leading to immune response and infection. Colonisation is, however, an essential step in the development of infection. The bacteria in question may arise from the normal microbiological flora of the individual or they may have been introduced by another mechanism.

It is common for ICU patients to be colonised by a variety of bacterial species. This occurs for a number of reasons including impaired host defences, the high intensity of antimicrobial use and the presence of invasive devices, *e.g.* catheters, that bypass normal protective mechanisms and act as a nidus for colonisation by microorganisms (3).

Endogenous colonisation occurs when the colonising organism arises from the host's own microbiota (92). Antibiotic use exerts selective pressure on patients' normal microbiological flora, modifying it to promote potential pathogenic colonisers. This selective pressure will depend not only on the duration of antibiotic therapy but on the choice of agent. Exogenous colonisation is said to occur if the organism is acquired from the hospital environment or by cross-infection from another host (92). This occurs due to cross-transmission via direct contact, droplet, or aerosol spread.

The efficiency of intestinal barrier function is demonstrated by the fact that the faecal bacterial concentration approaches  $10^{12}$  organisms  $\text{ml}^{-1}$  in the caecum, while tissues one cell deep to the intact intestinal mucosa are usually sterile (93). In contrast to the large bowel, the stomach, duodenum and jejunum have a relative paucity of bacteria ( $10^3$  to  $10^4$  organisms  $\text{ml}^{-1}$ ). The presence of enteric organisms in gastric aspirates is therefore abnormal and represents gastric colonisation. In the context of critical illness, this colonisation is the result of bacterial overgrowth in the proximal gastro intestinal tract (93).

Increased adherence of microorganisms may also play an important role in colonisation of the critically ill patient. There are a number of reasons why this may occur: increased bacterial adherence to the oral and respiratory tract mucosa of the invasively ventilated patient is facilitated by reduced mucosal immunoglobulin A production and increased protease production; exposure of, or trauma to mucous membranes; increased airway pH; and increased numbers of airway receptors for bacteria due to acute illness and antimicrobial use (94).

Microbial adherence factors (adhesins) can exist as polypeptides or polysaccharides. Adhesin-receptor interactions are important in defining the bacterial population found on any particular surface, with the apparent predilection of certain microorganisms for a particular host tissue, determined partly by the adhesin-receptor interaction on host cell surfaces (95). Changes in adhesins associated with antibiotic-resistant organisms, or in the host-adhesin interaction, may account for the pathogenicity of certain bacteria commonly implicated in nosocomial infections (96).

The genetic makeup of bacterial genomes is subject to rapid and dramatic change through a variety of processes collectively referred to as 'horizontal gene transfer'. This flux plays a major role in the molecular evolution of novel bacterial pathogens.

Horizontal gene transfer refers to the incorporation of genetic elements transferred from a donor organism directly into the genome of the recipient organism, where they form genomic islands. Genomic islands may contain large blocks of virulence determinants (adhesins, invasins, toxins, protein secretion systems, antibiotic resistance mechanisms), and thus are referred to as pathogenicity islands (97).

For example, *Pseudomonas aeruginosa* has an array of virulence factors. The main adhesins are flagella and type 4 pili which are capable of binding to the host epithelial gangliosides, asialoGM1 and asialoGM2. As with lipopolysaccharide, these surface appendages are highly inflammatory. Once contact with the host epithelium has taken place, the T3SS (Type 3 Secretion System) can be activated. This system enables direct injection of cytotoxins into the host cell (98).

The most common reservoirs for nosocomial colonisers are the oropharynx, the gastrointestinal tract and the urinary tract (99). The oropharynx and gastrointestinal tract are most relevant with regard to VAP. Due to the alterations in host defences and the resulting changes in mucosal bacterial adherence discussed above, the oropharynx rapidly becomes colonised with aerobic Gram-negative bacteria. The resulting microaspiration of these contaminated oropharyngeal secretions is the key factor in the majority of cases of VAP (100).

### **1.4.3 Gram-negative infection: a new threat**

There is now a major emphasis on infection control and prevention in UK hospitals. This has become a key quality improvement and patient safety issue in ICU. Government initiatives such as the Health Care Improvement's 100,000 Lives Programme in the United States have also made an impact in the UK, with emphasis now placed on reductions in VAP and catheter-related blood stream infections (101). Rates of ICU acquired infection are regarded as a quality marker and in the US rates of infection will even influence healthcare reimbursement.

ICU acquired infection continues to be a major concern for trusts throughout the UK and the rest of the world. There has been a reduction in UK MRSA transmission and infection in recent years, linked to the mandatory MRSA bacteraemia surveillance scheme (102). However, the increasing prevalence of infection and sepsis caused by Gram-negative bacteria in critically ill adults is a major concern. The International Study of the Prevalence and Outcomes of Infection in Intensive Care Units (EPIC II) in 2007 found that Gram-negative bacteria were the predominant cause of infection accounting for 62% of culture-positive cases. Gram-positive organisms and fungi were isolated in 47% and 19% of cases respectively (103). More recently, Morrow *et al* (104) reported a 10% higher incidence of VAP with Gram-negative organisms than Gram-positive (22.8% vs 12.8%) in their own ICU (Creighton University School of Medicine, Omaha, Nebraska). Prevention of Gram-negative infection is therefore likely to become a focus for improving ICU outcomes in the future.

Despite the widespread discussion about importance of VAP and other HAI's, it is disappointing to note the paucity of published evidence or surveillance data relating to ICU's

in the UK as a whole. VAP surveillance data is collected in Scotland and Wales and published findings and limitations are discussed further in section 1.2.3.

It is encouraging, however, to see the development of the Infection in Critical Care Quality Improvement Programme (ICCQIP) (105). This is a collaboration between various organisations covering adult, paediatric and neonatal intensive care, microbiology, infection control and Public Health England. Participation in this surveillance system is voluntary but is being offered to all acute Trusts in England. The initial focus has been on blood stream infections, but in time this will be rolled out to include other ICU-related HAI's.

The ICCQIP Collaboration also highlights the importance of multi-resistant Gram-negative bacteria and their importance for critically ill patients of all ages, mirroring the emphasis placed in this thesis (105). However, that is not to underestimate the importance of Gram-positive organisms in ICU-related infection and VAP. Again, UK specific data on this is lacking, but Hunter cites the distribution of organisms isolated from cases of VAP by bronchoscopic techniques from 24 studies which include 1689 VAP episodes (11). Approximately 34% of VAP episodes were caused by Gram-positive organisms, predominantly Staphylococci and Streptococci. In late onset VAP, the Gram-positive culprits are more likely to be MDR organisms *e.g.* MRSA.

#### **1.4.4 Multiple drug resistance in critical care**

In addition to the increasing prevalence of Gram-negative infections, a major challenge for healthcare professionals is the emergence of multi-drug resistant (MDR) Gram-negative bacteria, *e.g.* Enterobacteriaceae with carbapenem resistance conferred by New Delhi metallo-

beta-lactamase 1 (NDM-1) (106). The number of antibiotics available to treat such infections is limited and, paradoxically, research into new antibiotics and novel mechanisms of action has been decreasing. This has been attributed to high costs for development and a poor return on investments (107).

The rise of MDR bacteria is most commonly attributed to the widespread use of broad-spectrum antibiotics. This is of particular relevance to ICU with over 60% of all ICU patients receiving antibiotics during their admission (108). However, the emergence and transmission of MDR organisms is more complicated, with the recognition that some antibiotics appear to carry a higher risk of promoting antimicrobial resistance than others *e.g.* third-generation cephalosporins, vancomycin, imipenem, and intravenous fluoroquinolones (109).

Bonten and Mascini have described four main methods of emergence and spread of MDR organisms, outlined below (110):

1. Induction of resistant strains – Development of resistant bacteria in response to antimicrobial treatment.
2. Selection of resistant strains – Treatment with antimicrobials leading to selection of pre-existing resistant organisms.
3. Introduction of resistant strains – This can occur from the community reservoir, or *via* carriers or vectors (healthcare workers).
4. Dissemination of resistant strains – Spread facilitated by suboptimal infection control.

Infection control measures, such as hand washing, are important in controlling nosocomial infection, but these have only been partially successful in preventing the increase in incidence of drug-resistant bacterial strains both nationally and worldwide. In a Vietnamese study by Schultz *et al*, a multi-faceted infection control approach, using barrier precautions, hand



washing and antibiotic cycling, was highly effective in reducing the prevalence of MRSA, but *not* of MDR Gram-negative microorganisms (111). This study was carried out in an infectious diseases hospital tetanus ICU. The reduction in MRSA is likely to reflect differences in the predominant acquisition routes between MRSA and GNB. The MRSA colonisation was largely *via* the exogenous route and was easily controlled by simple infection control interventions. It is unclear why these interventions did not result in a concomitant reduction in MDR GNB, but may reflect the high carriage rates of these bacteria in the local community, where Enterobacteriaceae resistant to third-generation cephalosporins and/or gentamicin were present in stool samples from up to 90% of healthy people (111).

The focus of therapeutic interventions to date has largely been on treatment after sepsis has become established (112–114). Despite over three decades of intense research no silver bullet has yet been identified, although agents aimed at reducing the impact of sepsis have come and gone, *e.g.* activated protein C, without any clear evidence of outcome benefit (115); preventative measures therefore remain vitally important.

In the absence of universally effective pharmacological treatments, alternative strategies aimed at preventing the development of ICU-acquired infection have been sought. Ventilator care bundles target modifiable risk factors for colonisation and aspiration, but despite having had a substantial impact on infection rates have not eliminated VAP.

Selective digestive tract decontamination (SDD) and selective oropharyngeal decontamination (SOD) with antibiotics are strategies that have been shown to significantly reduce the development of Gram-negative infections and improve patient outcome in a large randomised

controlled trial (116) and several meta-analyses (117,118). Selective decontamination is effective as it reduces the presence of potentially pathogenic bacteria in the intestinal tract.

However, Oostdijk *et al* demonstrated a statistically significant increase in intestinal colonisation with Gram-negative bacteria resistant to ceftazidime, tobramycin or ciprofloxacin during ICU stay and after ICU discharge ( $P < 0.05$ ) (119). These concerns were borne out by a large-cluster, randomised cross-over trial of selective decontamination of the digestive tract that showed a marked increase resistance to ceftazidime in faecal Enterobacteriaceae, together with a small but significant increase in bacterial resistance from the respiratory tract (120). In a previous trial, the use of cefotaxime as part of selective decontamination of the digestive tract regime was found to select for an outbreak of extended-spectrum  $\beta$ -lactamase-producing *E. coli* and *Klebsiella pneumonia* (121). Therefore, despite clear evidence of effectiveness, concerns regarding the negative consequences of antibiotic overuse and bacterial resistance have limited the widespread uptake of SDD and SOD (122).

New antimicrobial agents are in limited supply. In 2010 an EU-US taskforce called for a commitment to the development of 10 new antibacterial agents by 2020 (The 10 × '20 Initiative: Pursuing a Global Commitment to Develop 10 New Antibacterial Drugs by 2020) (123). This will require a substantial financial investment and will need to be sustained in the long-term, as continued antibiotic use will maintain the pressure on organisms to evolve new resistant strains. Therefore, the identification of an alternative method for reducing colonisation with Gram-negative bacteria, without inherent concerns about precipitating antimicrobial resistance, is an attractive prospect.

## **1.5 Probiotics**

### **1.5.1 Probiotics, prebiotics and synbiotics**

Probiotics are defined as ‘live microorganisms that confer a health benefit on the host when administered in adequate amounts’ (124). Prebiotics are non-digestible food components that stimulate the growth and/or activity of bacteria in the digestive tract in ways that may be beneficial to health (125). Synbiotics are a combination of probiotics and prebiotics. There has been an explosion of interest in probiotics and their potential health benefits since 2000, with initial attention focusing on the gastrointestinal tract.

### **1.5.2 Probiotics in critical care**

During an episode of critical illness, a number of significant changes occur in the microbiota of the human gut. These changes occur due to alterations in the stress hormone profile, impairment of blood supply to the gut, immunosuppression, antibiotic use and nutrient deficiency (126). In experimental models, these changes have been shown to occur within 6 to 8 hours, with endogenous *Lactobacillus* strains being replaced by pathogenic bacteria (127). This change can lead to a breakdown in the intestinal barrier function and is likely to play a significant role in the pathogenesis of the multiple organ dysfunction syndrome (MODS) (128,129).

Redressing this balance and exploiting the beneficial effects of probiotic bacteria is understandably an area of considerable interest. However, the mechanisms by which these microorganisms exert their effects are various and depend upon the dose used, the route(s) of administration and the dosing frequency (130). Furthermore, a number of these effects are strain specific. For example, lactic acid bacteria (LAB), such as *Lactobacillus* and *Bifidobacterium*,

are commonly used as probiotics. Campana *et al* demonstrated that individual LAB showed strain-specific abilities to reduce the invasion of intestinal pathogens *e.g.* by antimicrobial activity, co-aggregation with pathogens and adherence (131).

The human intestine is home to hundreds of species of bacteria, archaea and eukarya, many of which are non-culturable but can now be identified using metagenomic techniques. The bacterial load tends to be highest in the large intestine (up to  $10^{11}$  CFU g<sup>-1</sup>), and while the healthy human gut is dominated by *Bacteroides*, Firmicutes and Actinobacteria, each individual has their own distinct stool bacterial composition determined by environmental and genetic factors. This bacterial profile remains relatively constant over time unless altered by disease state or antibacterial treatment (132,133).

Culture-based and molecular detection methods have demonstrated that it is possible to significantly alter the composition of gut flora in adults and infants by treatment with probiotics. Sepp *et al* treated 15 neonates with *Lactobacillus rhamnosus* GG for their first two weeks of life. They found that *L. rhamnosus* GG persisted for 1 month in eight of these neonates. There were also significant differences in the bacterial composition of the stool in the probiotic treatment compared with the control group, with increased numbers of coliforms, *Lactobacillus* and *Bifidobacterium* species (134).

Benno *et al* demonstrated a statistically significant increase in bifidobacteria in adults treated with *L. rhamnosus* GG for a four-week period. They also found an increase in Lactobacilli and a decrease in the proportion of *Clostridium* species. (135). As these techniques are based on

faecal profiling, they tend to reflect the large bowel bacterial composition with little information being available on the small bowel effects of probiotics.

### **1.5.3 Mechanisms of action of probiotics**

Much of the information available on the mechanisms of action of probiotics has been obtained from animal work and *in vitro* studies; hence we must be careful in extrapolating this to humans. What is clear, however, is that there are multiple mechanisms by which different probiotic bacteria exert their effects. These effects may vary with the probiotic strain and population studied. Table 1.7 summarises the main mechanisms by which probiotics exert their effects, and table 1.8 presents details of commonly used probiotic preparations.

Probiotics may alter the local environment within the lumen of the gut, producing antimicrobial effects on pathogenic organisms. Lactic acid and acetic acid producing probiotics reduce the luminal pH resulting in an unfavourable milieu for pathogens. This has been demonstrated *in vitro*, with pathogen growth being reduced in a pH-dependent manner by *Lactobacillus* species (136). Venturi *et al* demonstrated a significant luminal pH reduction, *in vivo*, in ulcerative colitis patients treated with the probiotic mixture VSL#3 (137). This may be a factor in maintaining remission in ulcerative colitis patients.

Probiotics can also exert a direct antimicrobial effect via the production of bacteriocins. Bacteriocins are proteins produced by bacteria that inhibit the growth and virulence of other pathogenic bacteria. Probiotic bacteria which are deficient in the bacteriocin gene have diminished probiotic activity. This was demonstrated in a murine model where a mutant form of *Lactobacillus salivarius* UCC118 failed to protect against infection with *Listeria*

*monocytogenes* (138). A wide variety of bacteriocins are recognised, and their spectrum of action ranges from antagonism of similar bacterial strains to the inhibition of a wide range of Gram-positives, Gram-negatives, yeasts and moulds (139). One such example of a broad-spectrum bacteriocin is that produced by a subspecies of *L. salivarius*. The ABP-118 bacteriocin inhibits growth of *Bacillus*, *Staphylococcus*, *Enterococcus*, *Listeria* and *Salmonella* species. (140).

Bacteria communicate with each other using a mechanism known as quorum sensing. This involves the production and secretion of signalling molecules known as autoinducers. In their *in vitro* study, Medellín-Peña *et al* demonstrated that *Lactobacillus acidophilus* La-5 secretes molecules that disrupt this inter-bacterial communication, reducing expression of virulence-related genes by *Escherichia coli* O157:H7 (141).

It has also been demonstrated that probiotics can enhance intestinal barrier function. Intestinal barrier function is complex, and its control involves cellular stability at a cytoskeletal and tight junction level, as well as mucus, chloride and water secretion. Probiotics have been shown to exert an effect, *in vitro* and *in vivo*, via these mechanisms (130). For example, *Lactobacillus plantarum* 299v can enhance mucus production and secretion in human intestinal epithelial cells (142). The probiotic strain *Escherichia coli* Nissle 1917 appears to enhance mucosal barrier function by production of human  $\beta$ -defensin 2 (139). It has also been demonstrated *in vitro*, that *E. coli* Nissle can reduce adhesion and invasion of intestinal epithelial cells by an enteroinvasive *E. coli*.

Mechanism of Action		Specific Probiotic Example
Luminal pH Modification	Production of lactic acid and acetic acid reduces luminal pH resulting in unfavourable milieu for pathogens	<i>Lactobacillus</i> spp.: pH-dependent reduction in pathogen growth (136) VSL#3: <i>in vivo</i> luminal pH reduction in ulcerative colitis patients (137)
Bacteriocin Production	Bacteriocins are proteins produced by bacteria that inhibit the growth and virulence of other microorganisms. They may be narrow spectrum (inhibit related bacterial strains) or broad spectrum (inhibit a wide range of bacteria, yeasts and moulds) (139)	Mutant <i>Lactobacillus salivarius</i> deficient in bacteriocin gene are unable to protect mice against <i>Listeria monocytogenes</i> infection (138)  <i>L. salivarius</i> subspecies produce broad-spectrum bacteriocins (140)
Disruption of interbacterial communication	Autoinducers are the signalling molecules produced and secreted by bacteria that form the basis of quorum sensing (bacterial communication)	<i>Lactobacillus acidophilus</i> La-5 disrupts quorum sensing and expression of virulence-related genes by <i>E. coli</i> O157:H7 (141)
Enhanced mucosal barrier function	<p>Increased intestinal epithelial cell mucus production and secretion</p> <p>Reduced adhesion and invasion of intestinal epithelial cells by enteroinvasive bacteria resulting in reduced translocation</p> <p>Increased production of human <math>\beta</math>-defensin 2 by epithelial cells</p> <p>Stabilisation of intracellular tight junctions and reduced chloride/water secretion</p> <p>Epithelial cell regeneration and reduced apoptosis</p>	<p><i>Lactobacillus plantarum</i> 299v: increased mucin gene expression <i>in vitro</i> (142) and adherence to colonic cells via a mannose-specific adherence mechanism (143)</p> <p><i>Lactobacillus casei rhamnosus</i> adheres to colonic cells <i>in vitro</i> (144)</p> <p><i>E. coli</i> Nissle 1917: increase in mucin gene expression (145) and production of human <math>\beta</math>-defensin 2 by colonic cells (146)</p> <p><i>Streptococcus thermophiles</i> and <i>L. acidophilus</i> reduce water and chloride secretion in response to pathogenic bacteria (147,148)</p> <p><i>Lactobacillus</i> pretreatment of intestinal epithelium reduces disruption of epithelial tight junctions by pathogenic <i>E. coli</i> (149). Probiotic preparation VSL#3 (see Table 2) prevents redistribution of epithelial tight junction proteins on exposure to pathogenic bacteria (145). <i>L. rhamnosus</i> GG prevents cytokine-mediated apoptosis of intestinal epithelial cells (150). <i>L. casei</i> and <i>Clostridium butyricum</i> both stimulate gut epithelial proliferation in rats (151)</p>

Colonisation resistance	The probiotic competes with pathogen for nutrients and adhesion in a microbiological niche (130)	<i>L. casei rhamnosus</i> adheres to colonic cells, reduces pathogenic bacterial growth and can persist within the gastrointestinal tract (144,152)  <i>E. coli</i> Nissle 1917 inhibits growth of Shiga-toxin producing <i>E. coli</i> (153)
Immunological effects	<p>Bacterial–epithelial cross-talk enables luminal probiotic organisms to influence gut-associated lymphoid tissue and innate and adaptive host responses (154,155). Toll-like receptors play a central role in mediating this process (156)</p> <p>Increased promotion of B cells to plasma cells and increased production of immunoglobulins(130)</p> <p>Activation and modulation of macrophages, T cells and natural killer cells</p>	<p>VSL#3 has been associated with increased anti-inflammatory and reduced proinflammatory cytokine activity, reduced inducible nitric oxide synthase and matrix metalloproteinase activity in patients with pouchitis (157). <i>L. plantarum</i> 299v increases IL-10 secretion from macrophages and T cells in patients with ulcerative colitis (158). <i>L. casei</i> and <i>L. bulgaricus</i> significantly reduce TNF<math>\alpha</math> release from inflamed mucosa in Crohn's disease (159). <i>E. coli</i> Nissle 1917 shows local and systemic anti-inflammatory effects in a murine model of lipopolysaccharide-induced sepsis (160)</p> <p><i>L. rhamnosus</i> GG: increased circulating IgA, IgG and IgM concentrations in children with gastroenteritis (161,162). Pretreatment with probiotic prior to typhoid vaccination leads to increased anti-typhoid antibody titres (163)</p> <p><i>L. casei</i> Shirota: cell wall structure potently induces IL-12 production and the probiotic differentially controls the inflammatory cytokine responses of macrophages, T cells and natural killer cells (164–166). <i>L. casei</i> Shirota and <i>B. breve</i> administered preoperatively to biliary cancer patients significantly reduce postoperative IL-6, C-reactive protein and white cell count concentrations (164). <i>L. acidophilus</i> and <i>B. longum</i> increased macrophage phagocytic activity in a murine model (167)</p>

**Table 1.7 Summary of probiotic mechanisms of action**



Probiotic	Constituents	Administration example and dosing comments
Antibiophilus (Lcr35) (Germania Pharmazeutika GesmbH, Vienna, Austria)	<i>L. casei rhamnosus</i>	10 <sup>9</sup> CFU twice daily via nasogastric tube (144)
Ecologic 641 (Winclove Bio Industries, Amsterdam, the Netherlands)	Six different strains of bacteria: <i>L. acidophilus</i> , <i>L. casei</i> , <i>L. salivarius</i> , <i>L. lactis</i> , <i>B. bifidum</i> , and <i>B. lactis</i> (previously classified as <i>B. infantis</i> ), plus cornstarch and maltodextrins	Administered twice daily via nasojejunal tube to a total daily dose of 10 <sup>10</sup> bacteria (168)
Ergyphilus (Nutergia, Capdenac, France)	Predominantly <i>L. rhamnosus</i> GG, but also <i>L. casei</i> , <i>L. acidophilus</i> and <i>B. bifidum</i>	One capsule contains 2×10 <sup>10</sup> lyophilised bacteria. Capsules can be broken and given via enteral feeding tube. Five capsules administered over 24 hours in critically ill patients (169)
Mutaflor (Ardeypharm GmbH, Herdecke, Germany)	<i>E. coli</i> Nissle 1917	2.5×10 <sup>9</sup> to 25×10 <sup>9</sup> bacteria per capsule. Adult dose 1 or 2 capsules per day (170)
Proviva (Skanemejerier, Malmo, Sweden)	<i>L. plantarum</i> 299v and oatmeal 500ml	Oatmeal-based drink containing 5×10 <sup>7</sup> CFU ml <sup>-1</sup> . Dose of 500 ml day <sup>-1</sup> used by McNaught <i>et al</i> (171)
Synbiotic 2000 (Medipharm, Kagerod, Sweden and Des Moines, IA, USA)	A probiotic mixture comprising <i>Pediacoccus pentosaceus</i> 5-33:3, <i>Leuconostoc mesenteroides</i> 77:1, <i>Lactobacillus paracasei</i> ssp., paracasei F19, <i>L. plantarum</i> 2362 plus β-glucan, inulin, pectin and resistant starch	Administered twice daily via feeding tube or orally (172)
Synbiotic 2000 Forte (Medipharm, Kagerod, Sweden and Des Moines, IA, USA)	A probiotic mixture comprising <i>P. pentosaceus</i> 5-33:3, <i>L. mesenteroides</i> 32-77:1, <i>L. paracasei</i> ssp. paracasei 19 and <i>L. plantarum</i> 2362, plus inulin, oat bran, pectin and resistant starch	Sachet for reconstitution containing 10 <sup>10</sup> bacteria plus 10g prebiotic fibre. Administered in doses of 12 g (1 sachet) per day for a 15-day study period (173)
Trevis (Christen Hansen, Hørsholm, Denmark)	<i>L. acidophilus</i> La5, <i>L. bulgaricus</i> , <i>B. lactis</i> Bb-12 and <i>Strep. thermophilus</i>	4×10 <sup>9</sup> CFU/capsule. One capsule three times daily (174,175)
VSL#3 (Ferring Pharmaceuticals, West Drayton, UK)	Four strains of <i>Lactobacillus</i> ( <i>L. acidophilus</i> , <i>L. casei</i> , <i>L. plantarum</i> , <i>L. delbrueckii</i> ), three strains of <i>Bifidobacterium</i> ( <i>B. infantis</i> , <i>B. longum</i> , <i>B. breve</i> ) and one strain of <i>Strep. salivarius</i> subsp. <i>Thermophilus</i>	Powder for reconstitution with water or to be mixed with cold foods prior to consumption. One sachet contains 4.5×10 <sup>11</sup> lactic acid bacteria. Also available as a capsule containing 2.25×10 <sup>11</sup> bacteria. Adult dose 0.5 to 8 sachets (2 to 32 capsules) per day depending upon disease activity. Six grams once a day for 12 months administered by Venturi <i>et al</i> (137)

**Table 1.8 Summary of commonly studied probiotic preparations**

Furthermore, by competing with pathogens for nutrients and adhesion in a microbiological niche, probiotics can prevent pathogen replication through a phenomenon known as colonisation resistance (130). Probiotics can thus promote the integrity of the gut defence barrier and create an unfavourable environment for pathogen colonisation.

Probiotics also exert a range of immunological effects. The interaction between the luminal bacteria and the underlying epithelial and mucosal lymphoid cells is referred to as bacterial–epithelial cross-talk. Crosstalk enables probiotics to have an effect on both the innate and adaptive host immune system (154) – for example, promotion of B cells into plasma cells, increased production of secretory immunoglobulin A and prevention of activation of the pro-inflammatory nuclear transcription factor NF- $\kappa$ B (130). Other immunological mechanisms include alteration of the cytokine profile and activation of macrophages to present antigen to B lymphocytes and increase immunoglobulin production (176).

#### **1.5.4 Probiotics in the prevention of non-respiratory infection**

Probiotics have been studied in the prevention of postoperative infection. Three trials in patients undergoing major colorectal surgery have shown no significant reduction in postoperative infection rates (171,174,175). In each trial, however, the effectiveness may have been limited by a relatively short postoperative period of probiotic administration (4 to 5 days).

In contrast, several studies in patients undergoing pancreatic resection (177,178) and hepatic resection (164,179) have shown significant reductions in postoperative infection rates of up to 30%. These patients received probiotic for 8 to 14 days post operatively.

Liver transplant patients have multiple risk factors for infection, including immunosuppression. Two randomised trials have shown probiotics to be safe and effective in this group of patients. In the first trial, 95 patients were randomised to receive standard enteral feed plus selective bowel decontamination, fibre-containing enteral feed plus live *Lactobacillus plantarum* 299 (Lp299) or fibre-containing enteral feed plus heat-killed Lp299 (180). The live Lp299 group developed significantly fewer infections than the other two groups (48% vs. 13% vs. 34%, respectively). In addition, the mean duration of antibiotic therapy, mean total hospital stay, and length of ICU stay were also shorter in the live Lp299 than in the groups with inactivated Lp299 and selective bowel decontamination. These differences, however, did not reach statistical significance. The second trial compared only Synbiotic 2000 and prebiotic fibre, reporting postoperative infection rates of 3% and 48%, respectively (172). No serious side effects or infections caused by the probiotics were noted in either trial.

Oláh *et al* randomised 45 patients with severe acute pancreatitis to receive enteral oat fibre and live Lp299, or enteral oat fibre and heat-killed Lp299 (181). In the group treated with the live probiotic, only one patient required surgery for an infective complication involving the pancreas, compared with seven such complications in the control group ( $P=0.02$ ). There was also a non-significant trend towards a shorter length of hospital stay (13.7 days vs. 21.4 days, respectively). The same group carried out a single-centre, double-blind, randomised placebo-controlled trial using Synbiotic 2000 in a further 62 patients with severe acute pancreatitis (182). This trial showed no statistically significant differences in the incidence of mortality, septic complications or development of multiorgan failure between the two groups. However, the total incidence of systemic inflammatory response syndrome (SIRS), multiorgan failure and

rate of complications was significantly less in the treatment group versus the control group (8 vs. 14,  $P < 0.05$  and  $P < 0.05$ , respectively).

The trial that has raised most concern with regard to adverse outcomes and the use of probiotics is the PROPATRIA trial (168). In this multicentre, placebo-controlled trial, 296 patients with predicted severe acute pancreatitis were randomised to receive the synbiotic preparation Ecologic 641 or placebo. This was administered together with fibre-enriched enteral feed via the nasojejunal route for 28 days or until discharge. The rate of infective complications was similar in both groups (30% vs. 28%) but the mortality rate was higher in the synbiotic group. Nine patients in the synbiotic group developed bowel ischaemia, eight of these being small bowel ischaemia. There were no cases of bowel ischaemia in the placebo group. One possible explanation for this outcome is a difference in the two groups, with more patients in the synbiotic group having established organ failure at the time treatment began. An alternative theory is that such a significant intestinal burden of bacteria and high-fibre feed could result in increased oxygen consumption and local bowel ischaemia. Nevertheless, this is the first time such a complication has been reported.

The results of the PROPATRIA trial were surprising as they went against the findings of the two previous studies by Oláh *et al* (181,182), the concern being that the increased mortality was a direct result of the treatment, *i.e.* that the prebiotic-probiotic-fibre mixture led to fatal small bowel ischaemia.

The PROPATRIA investigators delivered high doses of enteral feed, fibre and bacteria directly into the small bowel of the patients. Of the nine patients who developed gut ischaemia, six

received enteral nutrition while receiving vasopressor support for hypotension. This could have produced a combination of gut hypoperfusion, reduced absorption of nutrients, fermentation-related gas production leading to gaseous distension compounding the mucosal ischaemia.

There are a number of other similarities and differences between the three studies worthy of mention. All three studies used twice daily nasojejunal administration of the probiotics and all three studies used a prebiotic component (*i.e.* oat fibre, corn starch, pectin) in addition to the probiotic formula which already contained fibre. However, the patients in the PROPATRIA trial were on average 15 years older than the Oláh studies and there was a higher proportion of biliary as opposed to alcohol induced pancreatitis.

The patients in the Dutch trial had higher Imrie scores and CRP levels but lower APACHE II scores and less pancreatic necrosis on CT scan compared to the studies by Oláh *et al.*

Furthermore, the patients in the Dutch trial received a higher number of probiotic species (six species of both *Lactobacillus* and *Bifidobacteria* at  $10^{10}$  CFU ml<sup>-1</sup>) compared to one to four *Lactobacillus* species in the Oláh studies, the duration of treatment was longer (four weeks compared to one week) and the Dutch group were more aggressive with enteral nutrition and probiotic administration.

These differences raise important questions, for example, regarding the potential effects of the type or strain of organism used and their interaction with prebiotics or fibre, and whether the route of administration has a bearing on the response to probiotic therapy.

### **1.5.5 Probiotics in the prevention of ventilator associated pneumonia**

Colonisation of the stomach by pathogens or potential pathogens is believed to occur due to a combination of poor gut motility, increased gastric pH (due to acid suppression) and the use of broad-spectrum antibiotics. This combination of factors leads to an overgrowth of bacteria in the duodenum, which reflux into the stomach and are ultimately regurgitated and aspirated into the lungs (183).

The normal intestinal microbiota of critically ill patients is altered and replaced by pathogens for the reasons alluded to earlier. Therefore, it would seem logical to consider that redressing this balance with probiotic organisms may have a role in reducing gastrointestinal colonisation by pathogens, and thus, in the prevention of respiratory infection in this population. In addition, the restoration of gut barrier function may confer additional immunomodulatory benefits. A number of studies have provided evidence that probiotics reduce the incidence of VAP, however, none of the studies published to date have looked at *E. coli* Nissle in this context.

To date, the studies of probiotics in the prevention of VAP have been relatively small and have yielded conflicting results in subsequent systematic reviews and meta-analyses (184). The systematic review by Watkinson *et al* in 2007 examined the use of prebiotics, probiotics and synbiotics in 999 adult critical care patients from eight randomised controlled trials. The authors concluded that there was no benefit in probiotic prophylaxis for VAP (185).

However, in 2010 Siempos *et al* examined five randomised controlled trials of 689 patients and showed that probiotic administration was associated with a lower incidence of VAP when compared with standard care (odds ratio=0.61; 95% confidence interval=0.31 to 0.91) (186).

Importantly, both of these were published before the studies by Morrow (104), Oudhuis (187) and Barraud (169).

In the literature review conducted prior to commencing the PECaN-ED trial, eight randomised controlled trials of probiotic therapy as a strategy to prevent VAP (104,169,173,187–191) were identified. The inclusion criteria, sample size (range 50 to 259), populations studied and diagnostic criteria for VAP varied widely between the studies. The probiotic formulae, dosing and route of administration also varied but all trials contained *Lactobacillus* species. (see Table 1.9).

Six of the eight RCTs demonstrated a lower incidence of VAP in the probiotic group (104,173,187,188,190,191), however, this difference was statistically significant in only three of the trials (104,173,188). Interestingly, one trial used chlorhexidine oral disinfection as a control and found that probiotic Lp299 was at least as effective as the antiseptic in preventing oropharyngeal colonisation (61.9% vs. 34.8% new colonisation, respectively;  $P=0.13$ ) (190). The trial by Forestier *et al* demonstrated no difference in incidence of VAP between groups but did demonstrate a median delay in respiratory colonisation with *Pseudomonas aeruginosa* of 50 days versus 11 days in controls ( $P=0.01$ ) (189). This is the most commonly isolated antibiotic-resistant Gram-negative species in VAP (192).

The trial by Morrow *et al* is unique in that it included oropharyngeal slurry as one of the routes of administration for the probiotic (104). The group randomised 146 ventilated patients, who were considered at high risk for VAP, to receive probiotic *L. rhamnosus* GG or placebo (inulin) within 24 hours of intubation until extubation, tracheostomy or death. The primary outcome

was microbiologically confirmed VAP based on quantitative culture of distal airway samples obtained by bronchoscopy. The incidence of VAP was significantly reduced in the probiotic group (19.1% with probiotic vs. 40.0% with placebo,  $P=0.007$ ).

The rates of oral colonisation with pathogenic species at 72 hours (70% for placebo vs. 38.2% for *Lactobacillus*,  $P<0.001$ ) correlated with development of VAP (Pearson correlation coefficient=0.22,  $P=0.009$ ). Interestingly, the probiotic treatment appeared to preferentially reduce rates of infection caused by Gram-negative pathogens (22.8% for placebo vs. 8.8% for *Lactobacillus*,  $P=0.02$ ) while having no statistically significant effect on Gram-positive species (12.8% vs. 5.8%,  $P=0.16$ ) (104).

The question regarding the use of probiotics for the prevention of VAP has generated sufficient discussion and debate to form the subject of a Cochrane Review by Bo *et al* (184) (appendix VI). Prior to this, the meta-analysis by Siempos *et al* (186), mentioned earlier in this section, showed that probiotics reduced the incidence of VAP. However, the results of this meta-analysis were questioned by Van Silvestri *et al* due to the selection methodology applied (193). Following the Siempos meta-analysis, three further RCTs looking at probiotics in the prevention of VAP, were published by Baraud, Morrow and Tan *et al* (104,169,194), which all showed that probiotics were safe and effective in preventing VAP.

The Cochrane Review included RCTs and excluded quasi-RCTs, controlled clinical trials, controlled before and after studies, interrupted time series studies, cross-over studies and cluster-RCTs. It was restricted to adult ICU patients 18 years of age or older who were mechanically ventilated. The primary outcome measures examined were the Incidence of VAP,



all-cause mortality (including ICU mortality, 28/30-day mortality, hospital mortality or mortality at an unspecified time) and safety (including incidence of diarrhoea). Secondary outcomes included length of ICU stay and duration of mechanical ventilation.

Eight RCTs were identified which met criteria for inclusion in the analysis and compared a probiotic treatment group to a control. The sample size in these studies ranged from 50 to 264 (total 1083 patients), all of which reported VAP incidence as an outcome (104,169,173,188–191,194). The Cochrane analysis of VAP incidence included 1018 patients. This showed that the use of probiotics decreased the incidence of VAP (OR 0.70, CI 0.52-0.95), however, the evidence on which it was based was graded as low quality.

In terms of ICU mortality, the combined results were uncertain, with OR 0.84 (CI 0.58 to 1.22). This was based on evidence graded as very low quality (184). Similarly, the analysis of hospital mortality (OR 0.78, CI 0.54-1.14), length of ICU stay (mean difference -1.60, CI -6.53-3.33) and duration of mechanical ventilation (mean difference -6.15, CI -18.77-6.47) were all based on very low quality evidence (184).

The evidence from the available studies analysed in the Cochrane review suggests that use of probiotics is associated with a reduction in the incidence of VAP. However, the quality of the evidence is low. The trial by Spindler-Vesel *et al* did not provide a robust definition of VAP (188) which adds a degree of uncertainty to this previous finding. Had this trial been excluded from the primary outcome of VAP incidence, the result would no longer be statistically significant (OR 0.76, CI 0.56-1.05,  $P=0.10$ ). The available evidence was felt to be insufficient

to give a definitive answer regarding effect of probiotics on length of ICU stay or hospital mortality.

The principal findings of the Cochrane Review were in keeping with the previous meta-analyses by Siempos *et al* (186) and Petrof *et al* (195), but contradicted the results of the meta-analysis by Gu *et al* (196). The meta-analysis by Siempos *et al* included five studies with a total of 689 patients and showed that probiotics appeared to be associated with a lower incidence of VAP. The meta-analysis by Petrof *et al* included 23 studies with a total of 2153 patients and again demonstrated a lower VAP incidence in the probiotic group. However, the meta-analysis by Gu *et al* comprising seven studies and a total of 1142 patients showed no evidence of a reduction in VAP.

There are several notable differences between these publications. Gu *et al* excluded two studies that were included in the Cochrane Review (Spindler-Vesel *et al*; Tan *et al* (188,194)), and included one trial that was excluded from (Oudhuis *et al* (197)). In the trial by Oudhuis *et al* the control group received SDD using an antibiotic regime comprising oral paste and enteral solution (polymyxin E, gentamicin, amphotericin B) and intravenous cefotaxime.

The earlier review by Siempos *et al* did not include data from the studies by Barraud *et al*, Morrow *et al* or Tan *et al* (104,169,194). The subsequent review by Petrof *et al* did not include data from the trial by Spindler-Vesel *et al* (188).

Studies of probiotics in the critically ill have trialled a number of different species, with lactobacilli featuring frequently. It is currently unknown, however, whether one species is

superior in the prevention of infection associated with critical illness. Similarly, the optimum administration route, dosage and duration of treatment are not clear. Further research is undoubtedly warranted, perhaps considering Gram-negative probiotic species.

#### **1.5.6 *Escherichia coli* Nissle as a potential candidate in the prevention of ventilator associated pneumonia**

Gram-negative bacteria are the causative organisms in the majority of cases of ventilator-associated pneumonia, particularly Enterobacteriaceae and *Pseudomonas aeruginosa* (198). They are also the potential pathogens colonising the stomach of ventilated patients, and therefore, probiotic species of Enterobacteriaceae would appear worthy of consideration and further investigation (22,183).

The most extensively studied and widely available probiotic from this family is *Escherichia coli* strain Nissle 1917 (serotype 06:K5:H1). It is a single, flagellated *E. coli* strain comprising 5324 genes and two cryptic plasmids. *Escherichia coli* Nissle produces the bacteriocins Microcin M and H47 (199).

The organism was first isolated during the First World War from a soldier who did not develop enterocolitis during a shigellosis outbreak (200). *Escherichia coli* Nissle 1917 has already been used in a number of studies looking at ulcerative colitis. These studies demonstrated that the probiotic bacteria were as effective as mesalazine in the maintenance of remission (170).

The Nissle bacterium has an excellent safety record with no reports of systemic infection in adults. This is due to its production of a semi-rough lipopolysaccharide, which results in serum

sensitivity (bactericidal effects of normal human serum), together with the complete absence of any genes encoding exotoxins, pathogenicity islands and other virulence factors (201).

The Nissle strain does not acquire any pathogenic bacterial DNA and expresses two broad host range microcins which interfere with colonisation of mucosal surfaces by other bacteria as well as producing factors that prevent adherence and invasion of the intestinal wall by pathogenic *E. coli* and most probably other Gram-negative *Enterobacteriaceae* (201).

The study by Leatham *et al* demonstrated that pre-colonisation of the mouse gastrointestinal tract with human commensal *E. coli* encouraged elimination of pathogenic bacteria (*E. coli* O157:H7) (202). In this study, commensal *E. coli* were first introduced to the mice followed by pathogenic *E. coli* EDL933 (O157:H7) on day 10. Five different human commensals were tested, and of these *E. coli* Nissle 1917 and *E. coli* EFC1 limited the growth of the pathogenic strain (202).

In addition to its strong pathogen displacing properties, *E. coli* Nissle has been shown to exhibit a favourable effect on the inflammatory response in a number of animal models. In a murine model of severe sepsis, it was demonstrated that prior administration of *E. coli* Nissle resulted in significantly reduced TNF- $\alpha$  production 24 hours after induction of septic shock (160). The *E. coli* Nissle treated mice also showed decreased IL-2, IL-5 and IgG expression and increased IL-10 expression compared to controls. Myeloperoxidase activity (as an indirect measure of neutrophil invasion) was significantly higher in the lungs and colon of the control as compared to probiotic treated mice. Serum IgG was raised in the control group following induction of septic shock, but healthy and probiotic treated mice had indistinguishable IgG levels. This

sepsis model is particularly interesting as it suggests that *E. coli* Nissle may have a role in immunomodulation. It could even be suggested that *E. coli* Nissle potentially has a role to play as a novel therapy in Gram-negative sepsis as it promotes the host immune response towards anti-inflammatory pathways.

Although the interest in *E. coli* Nissle, to date, has focused largely on gastrointestinal pathology, other potential roles are being investigated in the prevention of device-associated bacterial infections. For example, Chen *et al* have demonstrated that *E. coli* Nissle biofilms on silicone substrates reduced colonisation by pathogenic enterococci (203).

However, as I am focusing on the activity of *E. coli* Nissle in the stomach, one limitation of this trial is the lack of information or a functional assay examining its behaviour and replication in a low pH environment.

#### **1.5.6 Administration of probiotics and monitoring of their effects**

Probiotics are commercially available in various preparations including yoghurt-based products, capsules, powders and suspensions. The studies in critically ill patients discussed above involve enteral administration of a variety of probiotic strains using different dosing regimens. In eight of the nine studies involving mechanically ventilated patients (table 1.9), probiotic powder or capsule contents were dissolved in water and delivered via a feeding tube into the stomach. Morrow *et al* used an oropharyngeal slurry of *L. rhamnosus* GG (suspended in a sterile water-based surgical lubricant) in addition to nasogastric administration (104). After 72 hours, the patients receiving this regime were found to have lower rates of oral (38.2% vs. 70%,  $P=0.001$ ) and gastric (32.3% vs. 45.7%,  $P=0.03$ ) colonisation with pathogenic species

than those receiving placebo. Klarin *et al* used topical application of Lp299 to the oral cavity alone and found it to be at least as effective as chlorhexidine 0.1% in reducing oropharyngeal pathogenic load (190).

Testing for colonisation of the gastrointestinal tract with the probiotic species is reported in only a minority of studies. McNaught *et al* collected gastric aspirates at induction of anaesthesia in elective surgical patients who had received at least one week of oral Lp299 (171). The probiotic species was not isolated in any subject. In the trial by Forestier *et al*, however, gastric aspirates were taken at inclusion, at day 7 and at discharge. *Lactobacillus casei rhamnosus* was detected in 52 out of 102 patients on probiotic treatment after a median of 13 days (189).

In the trial by Klarin *et al* described above, the probiotic species Lp299 was detected in all oropharyngeal cultures and in the tracheal cultures from 56% of patients in the probiotic arm (190). Knight *et al* demonstrated detection of probiotic species in stool culture after 3 days treatment with Synbiotic 2000 Forte, indicating its survival from the stomach to the distal gut (204). However, they did not routinely analyse stool samples in their more recent trial (191). None of the other studies cited in Table 1.9 reported detection of probiotic species in any microbiological specimens.

Study	n	Study Design	Study Population	Probiotic Regimen	Primary Endpoint	Additional Findings	Limitations
<b>VAP/respiratory tract infection as primary outcome</b>							
Forestier <i>et al</i> (188)	208	DB, SC, RCT	Mixed ICU, >18 years old, requiring MV >48 hours	<i>L. casei rhamnosus</i> , 10 <sup>9</sup> CFU vs placebo ng/og twice daily until ICU discharge or death	Time to first colonisation/infection of the gastric and respiratory tracts with <i>P. aeruginosa</i> strains. Median delay 50 days probiotic vs 11 days placebo ( $p=0.01$ )	<i>P. aeruginosa</i> VAP reduced in probiotic group: 2.9% vs 7.5% ( $p=NS$ ). No adverse effects.	Single centre. More patients in probiotic group than placebo received anti-pseudomonal anti-biotics during their admission (55% vs 43%)
Knight <i>et al</i> (190)	259	DB, SC, RCT	Mixed ICU, >16 years old, requiring MV >2 days	Synbiotic 2000 Forte: 10 <sup>10</sup> CFU vs placebo ng/og twice daily until day 28, ICU discharge or death	Incidence of VAP: 9% probiotic vs 13% placebo ( $p=0.42$ )	Hospital mortality: 27% vs 33% ( $p=0.39$ ). No adverse effects.	Single centre. Overall VAP rate lower than anticipated
Morrow <i>et al</i> (104)	146	DB, SC, RCT	Mixed ICU, >18 years old, expected to require MV >72 hours	<i>L. rhamnosus</i> GG 109 CFU vs placebo <i>per os</i> and ng twice daily, started within 24 hours until death, extubation or tracheostomy	Incidence of VAP: 19.1% probiotic vs 40% placebo ( $p=0.007$ )	Significant reduction in <i>C. difficile</i> associated diarrhoea and ICU-associated diarrhoea, fewer antibiotic days, delay in onset of VAP, reduction in gastric and oral colonisation with pathogenic species, preferential reduction in VAP caused by Gram-negative pathogens. No adverse effects.	Single centre. Small sample size. High-risk population, selected: mean APACHE II score 23, mean days ventilated 10 days. Extensive exclusions: pregnancy; immuno-suppression; prosthetic heart valve or trauma; history of rheumatic fever, endocarditis or congenital heart defect; gastro-oesophageal or intestinal injury or foregut surgery; oropharyngeal injury; tracheostomy
<b>VAP/respiratory tract infection as secondary outcome</b>							
Kotzampassi <i>et al</i> (172)	65	DB, two centre, RCT	Severe multiple Trauma patients, >18 years old, requiring MV	Synbiotic 2000 Forte: 10 <sup>11</sup> CFU vs placebo once daily via gastrostomy or ng for 15 days	Systemic infection rate during ICU stay or the development of SIRS and MODS. Overall infection rate: 63% probiotic vs 90% placebo ( $p=0.01$ )	VAP rate reduced in probiotic group: 54% vs 80% in placebo group ( $p=0.03$ ) Central line and urinary tract infections also significantly reduced. Severe sepsis: 17% vs 40% ( $p=0.04$ ). Ventilation days ( $p=0.001$ ) and ICU length of stay ( $p=0.01$ ) significantly reduced with probiotics. Reduction in mortality (14.3% vs 30%) non-significant ( $p=0.12$ ). No adverse effects	Small sample size, severe trauma patients only

**Table 1.9 Probiotic trials in mechanically ventilated patients for prevention of ventilator-associated pneumonia**

VAP/respiratory tract infection as secondary outcome (table 1.9 cont'd)							
Spindler-Vessel <i>et al</i> (187)	132	SC, RCT	Severe multiple trauma patients requiring MV, at least 4-day ICU stay	Synbiotic 2000 Forte, 10 <sup>10</sup> CFU vs glutamine or fermentable fibre or peptide diet once daily ng/og for 15 days or until ICU discharge or death	Effect on intestinal permeability reduced on day 7 in probiotic group only ( <i>p</i> <0.05)	Probiotic group also had fewer pneumonias ( <i>p</i> =0.03) and total infections ( <i>p</i> =0.003). No adverse effects	Single centre. Small sample size, comparing multiple interventions, no placebo group
Klarin <i>et al</i> (189)	50	Open label, SC, RCT	Mixed ICU, 18 years old, requiring MV >24 hours	<i>L. plantarum</i> 299, 10 <sup>10</sup> CFU vs 0.1% chlorhexidine <i>per os</i> twice daily until ICU discharge or death	Pathogenic bacterial load in oropharynx. New colonisation rate: 34.8% probiotic vs 61.9% chlorhexidine ( <i>p</i> =0.13)	Emerging bacteria largely Gram-negative species. Non-colonised patients had lower ventilator days ( <i>p</i> <0.001). Incidence of VAP: 4% probiotic groups 14% chlorhexidine group ( <i>p</i> =NS). No adverse effects	Single centre. Not powered for incidence of VAP as primary outcome. Small sample size
Barraud <i>et al</i> (168)	167	DB, SC, RCT	Medical ICU, >18 years old, MV > 48 hours	Ergyphilus 2x10 <sup>10</sup> lactic acid bacteria, mostly <i>L. rhamnosus</i> GG, once a day vs placebo via enteral feeding tube.	28-day mortality. No difference: 25.3% probiotic vs 23.7% placebo ( <i>p</i> =0.8)	Mortality rates in ICU and at 90 days were also unaffected by the treatment. Incidence of ICU-acquired infections including VAP not significantly different except for catheter-related bloodstream infections that were lower in probiotic group. Reduced 28-day mortality in severe sepsis patients given probiotics ( <i>p</i> =0.035) but higher mortality rate in non-severe sepsis patients ( <i>p</i> =0.08)	Single centre. Small sample. Stopped early
Oudhuis <i>et al</i> (186)	254	Two centre, open label, cross over	Mixed ICUs, consecutive ICU patients With expected MV ≥48 hours and/or expected ICU stay ≥72 hours	<i>L. plantarum</i> 299/299v in a dose of 5x10 <sup>9</sup> CFU together with 6g of rose-hip, twice daily via ng vs SDD	ICU-acquired infection rate: 31% probiotic vs 24% SDD ( <i>p</i> =0.10)	No significant difference in VAP rate (7.7% vs 7.2%), 28-day or ICU mortality between groups	Small sample size. Stopped early. Cross-over of units not completed, resulting in unequal mix of patients and disease burden. Not DB. Infections defined retrospectively

APACHE, Acute Physiology and Chronic Health Evaluation; CFU, colony-forming units; DB, double blind; MODS, multiorgan dysfunction syndrome; MV, mechanical ventilation; ng, nasogastric; NS, not significant; og, orogastric; RCT, randomised controlled trial; SC, single centre; SDD, selective decontamination of digestive tract; SIRS, systemic inflammatory response syndrome; VAP, ventilator-associated

**Table 1.9 Probiotic trials in mechanically ventilated patients for prevention of ventilator-associated pneumonia (cont'd)**



### 1.5.7 Quality and safety of probiotics

Probiotics are now widely available and are being consumed daily in large quantities. Overall, they appear to have an excellent safety record, but there are some concerns that are likely to lead to caution in their widespread use in clinical practice.

The availability of different probiotics varies from country to country and there can be lack of consistency between manufacturers, and even batches, in terms of density of bacteria, adhesion characteristics, stability and viability (205). Strain-specific adhesion properties and viability have been shown to vary between batches from the same manufacturer, which could lead to conflicting clinical trial results (206).

There have been a number of publications reporting serious infections caused by *Lactobacillus* species related to those used as probiotics (207). The Finnish group of Salminen *et al* examined 89 cases of *Lactobacillus* bacteraemia. In 11 cases, the strain was identical with the probiotic *L. rhamnosus* GG, but they could not directly relate these cases to probiotic consumption (208). Salminen *et al* also examined trends in *Lactobacillus* bacteraemia in Finland over the period 1990 to 2000. This period coincided with a rapid increase in the consumption of probiotic *L. rhamnosus* GG. However, the group concluded that increased probiotic use had *not* led to an increase in *Lactobacillus* bacteraemia (209).

There are some case reports in the literature of *Lactobacillus* infection and bacteraemia that *do* appear to be directly related to probiotic consumption (210–213). All of the patients involved were immunocompromised to some degree and the causative organism was linked to the probiotic by molecular techniques.

Infections caused by *Lactobacillus* species from probiotics have also been reported in immunosuppressed patients – including those with acquired immunodeficiency syndrome (AIDS) and those following lung and liver transplantation (214–216). *Lactobacillus* bacteraemia has been associated with structural heart abnormalities, valve prosthesis or prior endocarditis (217). However, the majority of clinical trials using *Lactobacillus* species probiotics have reported few adverse effects.

The only reported infection associated with *E. coli* Nissle 1917 is in a premature neonate (gestational age 28 weeks) (218). The child had an extremely low birth weight of 935g and developed gastroenteritis due to rotavirus and adenovirus 14 days into the postnatal period. *E. coli* Nissle treatment initially led to improvement but the child developed severe sepsis 10 days later and subsequently *E. coli* Nissle 1917 was isolated in blood cultures. The child was treated with antibiotics and made a full recovery.

An additional aspect to the trial by Morrow *et al* was the data collected on the incidence of *Clostridium difficile* and ICU-associated diarrhoea (diarrhoea not caused by *C. difficile*) (104). The probiotic group had significantly less *C. difficile* cytotoxin-positive diarrhoea compared to the placebo group (5.6% vs. 18.6%,  $P=0.02$ ), although the duration of *C. difficile* cytotoxin-positive diarrhoea in those patients receiving probiotics was not significantly less than those with *C. difficile* cytotoxin-positive diarrhoea in the placebo group. However, patients treated with probiotic did receive fewer days of antibiotics for *C. difficile*-associated diarrhoea ( $0.5\pm2.3$  days vs.  $2.1\pm4.8$  days in placebo group,  $P=0.02$ ). The duration of ICU-associated diarrhoea was also significantly less in the probiotic group ( $4.1\pm3.7$  days vs.  $5.9\pm3.8$  days in placebo group,  $P=0.03$ ).

Adverse events were examined as a secondary outcome in the Cochrane review by Bo *et al* (184). The three trials which reported on the incidence of diarrhoea (which was initially included as an adverse outcome) were examined and the pooled results indicated no clear evidence of a difference (OR 0.72, CI 0.47-1.09) (184). Six studies, with a total of 430 probiotic treated patients, reported no adverse events in the form of nosocomial probiotic infections (104,169,173,189,191,194). However, the authors concluded that the results of this meta-analysis did not provide sufficient evidence to draw firm conclusions on the efficacy and safety of probiotics for the prevention of VAP in ICU patients.

A wide range of probiotic species is being investigated for an increasing number of indications. There has been little work carried out on the rationale behind which probiotics are used and in what combination. Timmerman *et al* attempted to address this issue by examining specific strains in an attempt to produce an effective multi-species mixture (219). The synbiotic preparation Ecologic 641 was used in the PROPATRIA trial. This group selected six strains of *Lactobacillus* based on survival in a simulated gastrointestinal environment, antimicrobial activity and ability to induce IL-10, highlighting the point that there should be a disease-specific rationale for selection of probiotics.

We are still far from fully understanding the probiotic–host interaction, however, given the potential benefits that probiotic bacteria have to offer and the association with reduction in VAP, further study is clearly warranted. Careful consideration should be given to further well-powered studies addressing the questions of which probiotic by what route, in what dose and at what time.

## **1.6 Hypothesis**

It was hypothesised that twice daily nasogastric administration of 5 ml or 15 ml of *E. coli* Nissle 1917 suspension to invasively ventilated ICU patients would lead to successful gastric colonisation, and significantly reduce ICU acquired gastric colonisation by pathogenic or potentially pathogenic Gram-negative bacteria compared to a non-treatment control.

## **1.7 Aims of the Thesis**

This thesis is a report of the proof of concept, open-label, randomised, non-treatment control feasibility study PECaN-ED (Probiotic *E. coli* Nissle – Efficacy and Dosing).

The specific aims of this study were:

1. To determine whether gastric colonisation with *E. coli* Nissle could be demonstrated in invasively ventilated ICU patients.
2. Investigate whether a higher dose of *E. coli* Nissle would allow gastric colonisation to occur within a shorter time period or in a higher proportion of patients.
3. Determine whether gastric colonisation by pathogenic or potentially pathogenic Gram-negative bacteria could be reduced by administration of *E. coli* Nissle.
4. Evaluate the safety and tolerability of *E. coli* Nissle administered *via* the nasogastric route in a selected group of ICU patients.

## **Chapter 2**

### **Methods**

## **2.1 Ethical and MHRA approval**

The trial was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. Approval from a Research Ethics Committee (REC) (reference 11/SC/0423) and Clinical Trial Authorisation from the Medicines and Healthcare products Regulatory Agency (MHRA) (reference 24698/0010/001-0001), were obtained before starting the study.

Research and Development approval was also obtained from the Heart of England NHS Foundation Trust (HEFT) (reference 2011074AN) which acted as sole sponsor for this study. The study was registered with the European Clinical Trials Database, EudraCT reference number 2011-002343-99.

Details of the trial were submitted to EudraCT, The European Clinical Trials Register. The information submitted provided a summary of the clinical trial and contact details if further information was required. As this was a feasibility study for an MD thesis, no protocol publication was undertaken. The registration of the trial on the European Clinical Trials Register fulfilled the ethics requirement for details of the trial to be available on an accessible database. The Eudract registration is available at the following URL: <https://www.clinicaltrialsregister.eu/ctr-search/trial/2011-002343-99/GB>.

The patients who participated in this study were, by definition, unable to provide informed consent as they were sedated and ventilated. Therefore, consent was sought from the patients' legal representatives. Ethical approval was obtained for this process, and for the use of either a Personal Legal Representative (PLR) or a Professional Legal Representative (ProLR). The PLR

was defined as a person who is close to the patient, willing and capable to act in this role for the purpose of this study. The ProLR was defined as the doctor primarily responsible for the medical treatment provided to the patient and not connected with the conduct of this study. In both instances the representative was provided with an information sheet explaining the study. The representative was given adequate time to read the document, discuss with relatives or colleagues and ask questions about the research itself or conduct of the study. The representative provided consent for the patient to take part in the study, believing that the patient would not have objected to being part of this research.

It is acknowledged that a representative is providing consent on behalf of a patient for the purposes of a CTIMP and that this is performed in accordance with current International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP). This technique has been used successfully in other clinical trials (e.g. PAC-Man, TracMan and Balti-2 trials) undertaken in the ICU setting.

Retrospective consent was sought if and when the patient was able to provide fully informed consent (usually following ICU discharge) The patient was provided with an information sheet and asked to provide their consent to be included in the study. If the representative withdrew consent or the patient declined, no further study treatment would be administered, and the patient's data would not be entered into the analysis.

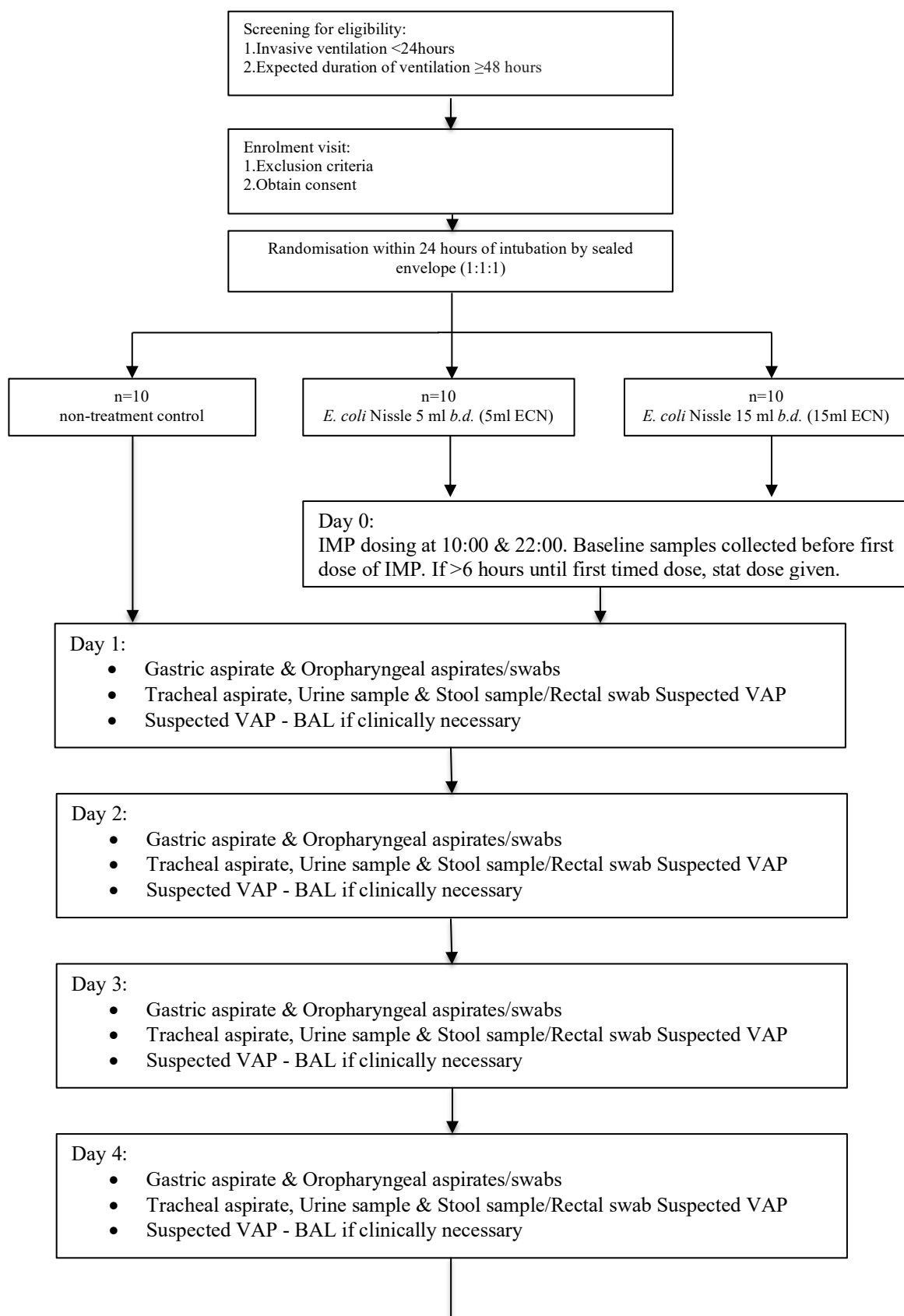
## **2.2 Trial management**

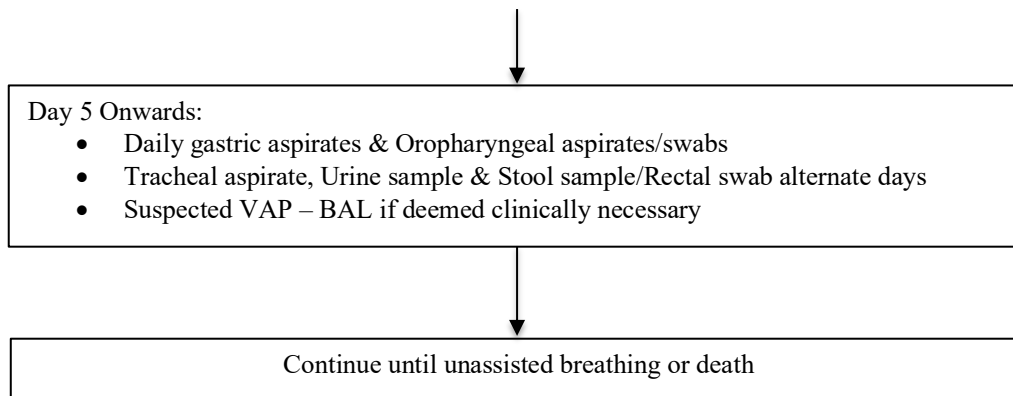
As agreed with the study sponsor, the trial was overseen by the Trial Management Group (TMG). This comprised the Chief Investigator, Principal Investigator, Professor of Microbiology, Senior Research Nurse, and other members of the Academic Department of Critical Care. As a single centre, feasibility study it was decided that there was no requirement for a separate Data Safety and Monitoring Committee. The TMG met monthly and reviewed patient data for safety throughout the trial. The TMG had access to the full data set and were not blinded to any data or information.

## **2.3 Study protocol**

This proof of concept study was designed to establish the efficacy and dosing range of *E. coli* Nissle by addressing the aims outlined in section 1.7, and to establish feasibility in this patient population. The study protocol is described in the consort flow diagram, figure 2.1.







**Figure 2.1 Consort flow diagram showing PECaN-ED Study Protocol**

The following primary and secondary endpoints were used to establish if the hypothesis was true: -

The primary endpoint was the detection of successful gastric colonisation with *E. coli* Nissle following nasogastric administration in ventilated ICU patients. The primary end point was defined as the presence of *E. coli* Nissle in two consecutive gastric aspirates, although the treatment was continued for entire duration of ventilation. The presence of *E. coli* Nissle strain was confirmed by PCR analysis against a standard.

The secondary endpoints examined in the study were:

- The effect of dose variation on the frequency of gastric colonisation by *E. coli* Nissle
- The effect of dose variation on the time to gastric colonisation by *E. coli* Nissle
- The proportion of gastric aspirates colonised by potentially pathogenic Gram-negative bacteria after 48 hours ICU stay compared to baseline

- The proportion of oropharyngeal aspirates colonised by potentially pathogenic Gram-negative bacteria after 48 hours ICU stay compared to baseline
- The proportion of tracheal aspirates colonised by potentially pathogenic Gram-negative bacteria after 48 hours ICU stay compared to baseline
- The incidence of ventilator-associated pneumonia in the study population
- An analysis of antibiotic days for the study population
- The cumulative incidence of ICU-acquired infection; defined as positive microbiological results from all types of samples
- The ICU, hospital and 28-day mortality for the study population

## 2.4 Patient recruitment

The study took place in the Intensive Care Unit (ICU) at Birmingham Heartlands Hospital. This is a university affiliated teaching trust with a total of 1449 in-patient beds spread over three sites that receives patients from the north and east of Birmingham. Heartlands Hospital is situated in east Birmingham and has a 19-bed general ICU.

The Intensive Care National Audit and Research Centre (ICNARC) HEFT database showed that there were 1214 adult ICU admissions at Birmingham Heartlands Hospital in 2012. The number of patients receiving invasive ventilation was 465 and, of these, 248 patients (53%) were ventilated for 48 hours or more.

Based on the trial by Forestier *et al* (189), who demonstrated a 30% recruitment rate from the eligible patients, it was estimated that approximately 74 patients per year could be randomised.

We anticipated recruiting approximately 3 patients per month, allowing recruitment of 30 patients within 10-12 months, which translated to 10 patients per arm. This was deemed to be achievable within the timelines of the study and large enough to see an effect of the IMP, to dose the IMP and to determine if it was feasible to undertake a larger trial of this nature.

Potential study participants were screened for eligibility based on the following inclusion and exclusion criteria. These were based on criteria from similar probiotic studies and also on the consensus of clinical opinion as to which patients may be in higher risk groups for developing a VAP. This was based on clinical prediction of duration of invasive ventilation as opposed to a screening marker of risk, such as lymphopaenia.

Although *E. coli* Nissle has an excellent safety record, this was the first time that it had been administered to critically ill patients; hence a high degree of caution was used.

Inclusion criteria:

- age  $\geq 18$  years, and
- Invasive ventilation for  $\leq 24$  hours, and
- expected duration of mechanical ventilation of  $\geq 48$  hours.

Exclusion criteria:

- imminent treatment withdrawal
- pregnancy or lactation
- immunosuppressant therapy
- absolute neutrophil count  $< 500 \text{ mm}^{-3}$

- gastrointestinal bleeding
- prosthetic cardiac valve or vascular graft
- cardiac trauma
- history of rheumatic fever, endocarditis or congenital heart disease
- contraindication to enteral feeding
- gastro-oesophageal or intestinal injury or foregut surgery during the current admission
- acute pancreatitis
- participation in an interventional clinical trial within the last 30 days

Intensive care patients, who had been intubated by the oro-tracheal route, were reviewed to determine if they fulfilled the eligibility criteria for the study. A screening log was kept of all patients who were reviewed for the study, this detailed if they were eligible for inclusion or not, and reasons for non-recruitment. The most common reason for non-recruitment was uncertainty about duration of mechanical ventilation. It is recognised that the accuracy of early clinical prediction of duration of mechanical ventilation is limited (220). This is an important factor to take into consideration and has an important bearing on a study such as this.

## **2.5 Randomisation**

Patients were randomised to one of three study groups:

1. to receive *E. coli* Nissle suspension 5ml *bis in die* (*b.d.*)
2. to receive *E. coli* Nissle suspension 15ml *b.d.*
3. no *E. coli* Nissle, routine clinical care and sampling undertaken

Randomisation was in a 1:1:1 ratio using the sealed envelope technique. The sealed envelopes were prepared by a Critical Care Research Administrator, who was independent of the study, and kept securely in the Critical Care Research Department. Only one envelope was released after each informed consent was received. Those patients randomised to a treatment group received the first dose of probiotic suspension within 12 hours of randomisation.

## **2.6 The Investigational Medicinal Product**

Full details of the Investigational Medicinal Product (IMP) are given in the Summary of Product Characteristics in Appendix V.

Active ingredient:	<i>Escherichia coli</i> Nissle 1917 suspension
Concentration:	10 <sup>8</sup> viable cells per ml
Container:	5ml polyethylene ampoules
Manufacturer:	Pharma-Zentrale (Ardeypharm) GmbH, Germany
Supplier:	Sharp Clinical Services (UK) Limited (formerly Bilcare GCS (Europe))

The IMP was received by the Clinical Trials Pharmacy at the Heart of England NHS Foundation Trust, directly from the importers Sharp Clinical Services (UK) Limited. The IMP was imported from Pharma-Zentrale GmbH, Germany, in boxes of 32 x 5ml ampoules. These boxes were packed down, assembled and labelled as agreed by the Clinical Trials Pharmacy in accordance with annex 13 of EU legislation and under regulation 37 of the Medicines for Human Use (Clinical Trials) regulations. The IMP was stored between 2-8°C and this was monitored by the Clinical Trials Pharmacy at HEFT in compliance with the Medicines and For Human Use (Clinical Trials) Regulations 2004.

Following randomisation, a clinical trial prescription was prepared, according to the randomisation arm; this was then taken to the Clinical Trials Pharmacy at HEFT for the relevant IMP pack to be dispensed. As patients were recruited and randomised into the study outside normal pharmacy hours, drug packs were available on ICU at all times. These were temperature monitored in accordance with the relevant regulations.

## **2.7 Probiotic administration**

Patients who were randomised to probiotic therapy received  $5 \times 10^8$  (1 x 5ml ampoule) or  $15 \times 10^8$  (3 x 15ml ampoules) viable cells per ml of *E. coli* Nissle on a twice daily basis. The *E. coli* Nissle suspension was administered via standard 8 French 105 cm polyurethane nasogastric feeding tube (Merck Corflo™) at 10:00 and 22:00. After administration, the nasogastric tube was flushed with 10ml sterile water and feeding recommenced. Gastric aspirate samples were obtained prior to the 10:00 dose of probiotic (see section 2.10). The third group, non-treatment control group, received routine clinical care.

Patients remained in the trial, with treatment groups receiving the IMP, until reaching the stage of unassisted breathing or death. This is in keeping with similar studies of probiotics. As per the trial protocol, unassisted breathing was defined as one of the following: -

1. Extubation to face mask, nasal prong oxygen, or room air
2. T-tube breathing
3. Tracheostomy mask breathing
4. CPAP of 5cm H<sub>2</sub>O without pressure support or assistance from intermittent mandatory ventilation.

As this was the first time that the IMP had been used for this purpose and in this group of patients, one of the questions that I set out to examine was that of dosing. Therefore, two different dosing regimens were selected, which it was felt would deliver an adequate quantity of viable cells to allow demonstration of gastric colonisation. This decision, and the doses used, were based on a combination of expert microbiological opinion, information obtained from the manufacturer, Ardeypharm GmbH, and comparison with the dosing regimens used in previous probiotic studies. For example, Morrow *et al* used a dose of  $2 \times 10^9$  CFU *b.d.* In this study  $5 \times 10^8$  and  $15 \times 10^8$  CFU *b.d.* were selected.

All patients received enteral nutrition via the nasogastric tube according to local protocols and was not affected by probiotic administration. PPIs or H2RAs were also administered routinely according to the local ventilator care bundle.

The research team and clinical staff were aware of the treatment that the patient was randomised to receive; hence the trial was open label. As this was a feasibility and dosing study, an open label design was used to help the research team identify any difficulties with administration. In addition, it was felt to be important for staff administering the IMP to be cognisant that they were handling a concentrated solution of bacteria, in order to avoid cross contamination. As this was a feasibility study, an open label design was acceptable. The control group for this study did not receive any treatment in relation to the trial, purely sample collection. The randomisation of patients ensured there was no bias as to which treatment arm the patients were allocated.



The study was open label in terms of the clinical interventions and drug administration for the reasons described above. This raises the obvious point about the inherent bias of an open-label study. However, we sought to mitigate against this by blinding the outcome assessment. Only two microbiologists carried out all of the laboratory work. Neither had access to or knowledge of the patient identity or the study group allocation until all sample analysis had been completed for each patient.

## **2.8 Data collection**

Data collected from the study patients included demographic details, primary ICU diagnosis, co-morbidities, antibiotic history for the current admission, observations on admission, baseline haematology and biochemistry results. These are shown in table 2.1.

Data was also collected daily for the duration of the study which included physiological, haematological and biochemical data. Details of antibiotic therapy, routine clinical microbiology and organ support requirements were also collected. In addition, the following outcome data were collected – length of ICU and hospital stay and ICU, hospital and 28-day mortality.

## **2.9 Severity of illness scores**

Data collected from the study, together with information from the Intensive Care National Audit and Research Centre (ICNARC) database were used to calculate severity of illness scores for the enrolled patients. These scoring systems were used to ensure that the patients across the three groups were well-matched in terms of severity of illness. Scores were recorded at the time of admission for all three groups.

Demographics	Age Sex Date & time of ICU admission/intubation Ethnicity Co-morbidities Antibiotic history ICU diagnosis
Baseline Physiology	Temperature Heart rate Blood pressure & Mean arterial pressure (mmHg) Respiratory rate (Breaths per minute) Partial pressure arterial Oxygen/Carbon Dioxide ( $P_aO_2/CO_2$ ) Fractional inspired Oxygen ( $FiO_2$ ) Glasgow Coma Scale (GCS) Urine output ( $ml\ hour^{-1}$ ) Vasoactive drugs
Baseline Investigations	White cell count ( $\times 10^9\ l^{-1}$ ) Haematocrit (%) Urea & creatinine ( $\mu mol\ l^{-1}$ ) Sodium ( $mmol\ l^{-1}$ ) Potassium ( $mmol\ l^{-1}$ ) pH/Bicarbonate ( $mmol\ l^{-1}$ ) Bilirubin ( $\mu mol\ l^{-1}$ ) Albumin ( $g\ l^{-1}$ ) Glucose ( $mmol\ l^{-1}$ )

**Table 2.1 Data collected on patients enrolled into the study**

### **2.9.1 APACHE II Score**

The acute physiology and chronic health evaluation (APACHE) score is a severity of illness scoring system which is applied to patients within 24 hours of admission to ICU (table 2.2). The original score was described in 1981 and has undergone several revisions since then. The APACHE II score was described in 1985 and is the most frequently used intensive care severity of illness scoring system. It incorporates 12 routinely measured parameters, and these are weighted for degree of derangement with a score from 0-4. Age, chronic health conditions and surgery are also taken into account. A score from 0-71 is generated and this is integrated with the patient's diagnosis to calculate an estimate of hospital mortality. Higher scores correspond to increased severity of illness and a higher probability of death (221).

### **2.9.2 APACHE III Score**

This is the latest version of the APACHE scoring system, introduced in 1991, which attempts to improve the risk prediction of the APACHE II system (table 2.2). APACHE III stands for acute physiology, age and chronic health evaluation. It consists of a numerical score from 0-299 obtained from the scores assigned to the physiological measurements, chronological age (with the emphasis reflected by the change in the second 'A') and chronic health status. APACHE III uses data from 16 physiological measurements as well as a modified chronic health component. APACHE III also uses 78 disease definitions to group patients according to the principal reason for ICU admission (222).

### **2.9.3 SAPS II Score**

The simplified acute physiology score (SAPS) II is a severity of illness scoring system which uses 12 variables weighted according to age and underlying disease (table 2.2). It is used in a

similar way to the APACHE scoring systems. A score from 0-163 is generated and probability of death is calculated using logistic regression. SAPS II is superior to APACHE II for the comparison of disease severity in patients with different pathologies (223).

## **2.10 Sample collection**

A full schedule of assessments and samples collected for the study patients is shown in table 2.3.

A routine set of ICU blood tests was taken on a daily basis from ventilated patients using the indwelling arterial or central venous cannula. From these blood tests it was possible to obtain the required haematological and biochemical results.

Gastric aspirates were obtained from patients immediately prior to administration of the 10:00 dose of *E. coli* Nissle suspension and were collected daily for the duration of the study. The gastric aspirate sample was obtained by attaching a sterile 20 ml Enteral-IsoSaf™ syringe to the nasogastric tube. If less than 2 ml of gastric aspirate was obtained, the patient was turned to

APACHE II	APACHE III	SAPS II
Temperature	Temperature	Temperature
Heart rate	Heart rate	Heart rate
Mean arterial pressure	Mean arterial pressure	Systolic blood pressure
Respiratory rate P <sub>a</sub> O <sub>2</sub> /F <sub>i</sub> O <sub>2</sub> ratio	Respiratory rate P <sub>a</sub> O <sub>2</sub> A-a D O <sub>2</sub>	P <sub>a</sub> O <sub>2</sub> /F <sub>i</sub> O <sub>2</sub> ratio
Serum creatinine	Serum creatinine & Urea Urine output	Serum urea Urine output
Serum sodium	Serum sodium	Serum sodium
Serum potassium	Serum albumin	Serum potassium
Arterial pH/Serum Bicarbonate	pH/pCO <sub>2</sub>	Serum Bicarbonate
White cell count	White cell count	White cell count
Glasgow Coma Scale	Neurological score	Glasgow Coma Scale
Haematocrit	Haematocrit	Admission type
Age	Age	Age
Chronic health	Chronic health	Chronic health
-	Serum Glucose	-
-	Serum Bilirubin	-
-	Origin/Reason for ICU admission	-

P<sub>a</sub>O<sub>2</sub>/F<sub>i</sub>O<sub>2</sub> - partial pressure of oxygen/fractional inspired oxygen. A-a D O<sub>2</sub> - Alveolar- arterial oxygen difference.

pCO<sub>2</sub> - partial pressure of carbon dioxide

**Table 2.2 Components of the Acute Physiology and Chronic Health Evaluation score (APACHE II), Acute Physiology, Age and Chronic Health Evaluation (APACHE III) and Simplified Acute Physiology Score (SAPS II)**

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Hours <sup>a</sup>	BASE LINE	48	72	120	168	240	350	504	672																			
Inclusion/exclusion criteria	X																											
Written informed consent	X																											
Randomisation	X																											
5ml/15ml suspension E.coli Nissle	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Demographics	X																											
Medical History	X																											
Current Drug Therapy	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Drug history	X																											
APACHE II	X																											
VAP clinical assessment <sup>b</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SOFA score	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse effects	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ventilator free days <sup>c</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Antibiotic days <sup>d</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ICU/Hospital length of stay (hours)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ICU/Hospital mortality	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Gastric aspirate/Oropharyngeal Swab <sup>e</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tracheal aspirate/Rectal swab/Urine	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
WCC/CRP	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Table 2.3 Schedule of assessment and sample collection

the left and a further attempt was made after 20 minutes. If insufficient aspirate was obtained after turning, 20 ml of sterile water was instilled and aspirated. Up to 10 ml of gastric aspirate was then placed in a sterile 30ml Sterilin™ polystyrene universal container (ThermoFisher Scientific, USA) and transported to the laboratory for processing and analysis.

In retrospect this is a limitation of the study methodology. Data on nasogastric aspirates which were difficult to obtain or required diluted gastric sampling were not recorded. This would have had a more significant bearing if fully quantitative analysis of the samples was carried out. However, it is an important point to consider for future work.

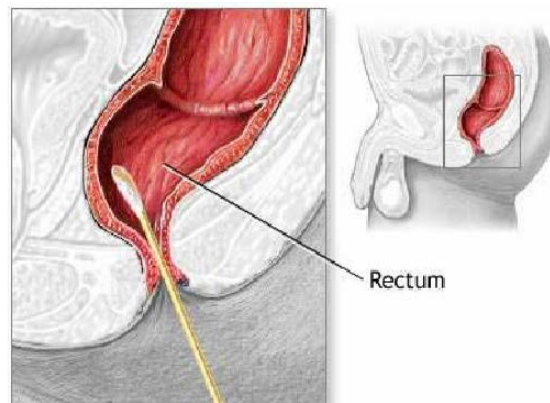
Oropharyngeal swabs or aspirates were obtained from patients on a daily basis, prior to administration of the 10:00 dose of *E. coli* Nissle suspension and prior to routine mouth care, for the duration of the study. A sterile Yankauer suction device (LY-3600, Pennine Healthcare) or sterile 14 French suction catheter (Ref 2141 Covidien™ Argyle™ Gentle Flow suction catheter) was attached to a sterile sputum trap (Trachea Suction Set ref 24006182, Unomedical, ConvaTec limited) and a sample obtained from the oropharynx. If the sample of oropharyngeal secretions was inadequate, a bacterial swab (eSwab™ Copan Italia SpA) was used to obtain a sample from the oropharynx.

Dry tracheal aspirates were obtained in a standard fashion using a 14 French closed endotracheal suction circuit (reference 227-5 Turbo-Cleaning Closed Suction System for adults, Halyard, Belgium) prior to administration of the 10:00 dose of *E. coli* Nissle suspension and prior to routine mouth care. The tracheal aspirate sample was collected in a sterile sputum trap (Trachea Suction Set ref 24006182, Unomedical, ConvaTec limited). Samples were

collected on enrolment, on the three subsequent days and thereafter on alternate days for the duration of the study.

A catheter specimen of urine (CSU) was collected in a sterile 30ml Sterilin™ polystyrene universal container (ThermoFisher Scientific, USA) using a standard technique. Samples were collected on enrolment, on the three subsequent days and thereafter on alternate days for the duration of the study.

A stool sample (formed or unformed) was collected in a sterile 30ml Sterilin™ polystyrene universal container with spoon (ThermoFisher Scientific, USA) on enrolment, on the three subsequent days and thereafter on alternate days for the duration of the study. If a stool sample could not be obtained, a rectal swab was collected according to the protocol shown in figure 2.2.



**Figure 2.2 Procedure for obtaining a rectal swab (Adapted from Shropshire County and Telford and Wrekin Primary Care Trusts Policy No. 30, Version no: 2 – 11/05)**



- 1) Obtain a rectal swab by carefully inserting a swab 2.5 cm (1 inch) beyond the anal sphincter (eSwab™ Copan Italia SpA)
- 2) The swab should be rotated gently & withdrawn; if no faecal staining is seen, gently re-introduce a little further
- 3) Patients who have had a low colorectal anastomosis should not have a rectal swab taken unless a surgical consultant has given permission

If VAP was suspected, then bronchoscopic BAL with quantitative culture was performed if deemed clinically appropriate. This was based on the modified criteria described by Singh *et al* (40). A score of >6 based on the first five CPIS criteria (fever, leucocytosis, quality of secretions, oxygenation and radiographic changes) was considered to be suggestive of VAP.

A standardised BAL technique was used, based on that described by Morris *et al* (224). The bronchoscope was wedged in a subsegment corresponding to the area of focal radiological involvement. In cases where the radiographic change was more diffuse, the bronchoscope was wedged in a subsegment producing visible purulent secretions or, in the absence of purulent secretions, in the posterior segment of the right lower lobe. A 20 ml aliquot of sterile saline was instilled and the aspirate (bronchiolar sample) discarded, then further 20 ml aliquots of sterile saline were instilled and the aspirate (alveolar sample) collected for microbiological analysis in a sterile sputum trap (Trachea Suction Set ref 24006182, Unomedical, ConvaTec limited).

On completion of the study, the HEFT microbiology results system was interrogated for any relevant positive results from routine clinical sampling, including tracheal aspirates, urine samples, stool samples, blood cultures and wound swabs (section 3.3.5). Any positive results

which arose from this search were correlated with clinical data to determine if there was any infection or evidence of colonisation relevant to the study.

## **2.11 Sample processing**

All microbiology samples were processed (including the MALDI-TOF and PCR analysis) in the Public Health England (PHE) Laboratory at Birmingham Heartlands Hospital. This laboratory is registered with the United Kingdom Accreditation Service (UKAS), details of which can be found at the following URL: [https://www.ukas.com/wp-content/uploads/schedule\\_uploads/00007/8213%20Medical%20Single.pdf](https://www.ukas.com/wp-content/uploads/schedule_uploads/00007/8213%20Medical%20Single.pdf).

Samples were processed during working hours, Monday to Friday. If samples were collected outside these hours, they were stored in the designated microbiology refrigerator until the next working day.

All samples were inoculated onto the following plates - blood agar, CPS ID2 (BioMérieux Ltd.), Sabouraud (SAB) and FANEO. Blood agar plates were used to establish the total enumeration of bacteria and identification of streptococci and staphylococci. CPS ID2, is a chromogenic media that enables the identification of *E. coli*, enterococci, *Proteus* and preliminary identification of *Klebsiella*, *Enterobacter* and *Citrobacter*; it was used to determine the presence of *E. coli* in samples. SAB plates were used to determine the presence of candida, the agar has a low pH that inhibits the growth of most bacteria; it also contains gentamicin to inhibit the growth of Gram-negative bacteria. FANEO agar was used to identify the presence of anaerobes in samples.

### *Gastric aspirates*

On receipt of the gastric aspirate in the laboratory, 50µL was directly plated onto whole plates of CPS ID2 and blood agar using a plastic hockey stick to make a lawn. Serial dilutions of the aspirate to  $10^{-1}$  and  $10^{-2}$  were made and the plating repeated as above. The minimum detectable bacterial count was 20 CFU ml<sup>-1</sup> and the maximum number of countable colonies was greater than 10<sup>5</sup> CFU ml<sup>-1</sup>. If *E. coli* was presumptively identified, a representative number of isolates were selected for confirmation depending on the total colony count. Isolates were confirmed as *E. coli* by matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) and confirmed as the Nissle strain by polymerase chain reaction (PCR).

### *Oropharyngeal aspirates, Tracheal aspirates and Urine samples*

Oropharyngeal aspirates, tracheal aspirates and urine samples were all inoculated directly onto the four plates using a cotton swab.

### *Rectal swabs*

Rectal swabs were plated out using a semi-qualitative technique on CPS-ID2, blood agar, and anaerobic plates. At least 2 colonies of *E. coli* were selected for PCR analysis to look for the presence of *E. coli* Nissle.

### *Bronchoalveolar lavage specimens*

BAL samples were processed using a standard operating procedure (SOP) in accordance with the SOP for the processing of BAL issued by the UK Health Protection Agency (HPA). Following vortexing of the sample, serial dilutions were made and 50 µL of each were plated

out. The limit of detection using this technique is  $2 \times 10^3$  CFU ml<sup>-1</sup> and the maximum countable number is  $2 \times 10^6$  CFU ml<sup>-1</sup>. VAP is defined as growth of  $\geq 10^4$  CFU ml<sup>-1</sup>.

All aerobic plates were incubated at 37°C in air and read at 24 hours and 48 hours. The anaerobic plates were read after 48 hours incubation in the anaerobic cabinet. Having noted the plate dilution, where necessary, the bacterial growth on the aerobic plates was described as follows:

1. The qualitative appearance of the overall growth on the plate was recorded using the classification system in table 2.4
2. The presence or absence of *E. coli* was noted, based on colony morphology, usually on the chromogenic plate.
3. A qualitative assessment of the *E. coli* growth was made using the classification system in table 2.4
4. The overall bacterial growth was reported as either pure or mixed, with the growth of the dominant species, if any, noted. If there was mixed dominance it was recorded as 50:50
5. Preliminary species identity was recorded using the chromogenic plate, and the presence of other species and growth noted.

Growth	Classification
Scanty growth (visible individual colonies)	Very Light
Lawn growth in primary inoculum	Light
Growth in primary and secondary inoculum	Medium
Growth in all quadrants of inoculum	Heavy

**Table 2.4 Growth classification for reading plates**

Representative colonies were saved from the blood and CPS-ID2 plates or sweeps of the original blood plates on beads. Multiple representatives of *E. coli* were saved depending on the number of colonies on the plate. These were grouped as 1, 2-5, 6-10 and >10 colonies with 1-4 colonies saved respectively.

For other species, one representative colony was saved for the dominant species. If there was a 50:50 mix, a representative of each was saved. If the culture was a heavy mixed growth, a sweep from the original blood plate was saved.

Yeast growth was classified as positive or negative classified according to the system in table 2.4. No representative yeast colonies were saved.

After the 24-hour read of the plates was completed, they were reincubated for a further 24 hours and read again. Only changes from that of first read were recorded. The plates were stored in the refrigerator and kept for comparison with the anaerobic plates, then discarded.

The anaerobic plates were read after incubation for 48 hours. They were compared to the aerobic plates to help distinguish facultative anaerobes, which were not recorded, from obligate anaerobes. The bacterial growth on the anaerobic plates was described according to the system in table 2.5. No representative colonies were saved, and all plates were discarded after reading.

Overall Growth	Anaerobic Pos/Neg	Comments
Qualitative comment on overall bacterial growth using classification in table 2.4	Positive or Negative for presence of obligate anaerobes by colony morphology/odour	Comment on anaerobic growth and appearance of colonies

**Table 2.5 System for describing growth on anaerobic plates**

## **2.12 Identification of bacteria**

Identification of representative bacteria was carried out using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). MALDI-TOF MS is an accurate and rapid technique used for the identification of bacteria, fungi, and mycobacteria isolated in clinical microbiology. MALDI is an ionisation technique that uses a LASER energy-absorbing matrix to create ions from biological molecules. It is used as it tends to cause less fragmentation of large organic molecules than other ionisation techniques. A TOF mass spectrometer is used in conjunction with MALDI. In TOF MS an ion's mass-to-charge ratio is determined by a time measurement following acceleration of the ion by an electric field of known strength. From this ratio, the identity of the molecule, and therefore bacteria, can be determined.

## **2.13 Polymerase chain reaction assay for identification of *Escherichia coli* Nissle 1917**

A sample of *E. coli* Nissle strain was provided by Pharma-Zentrale (Ardeypharm) GmbH, Germany. This was used as a control for the Polymerase Chain Reaction (PCR) assay. Using the technique described by Blum-Oehler *et al* (225) the primer sets and amplicons in table 2.6 were used for identification of *E. coli* Nissle by PCR assay.

Representative *E. coli* isolates from patient samples were tested to identify whether they were the *E. coli* Nissle strain. Bacterial DNA was extracted from culture and two PCR assays were performed. The PCR assay targeted chromosomal and plasmid regions specific to the *E. coli* Nissle strain. All of the strains identified as *E. coli* Nissle had the amplicons in table 2.6 detected.

Primer	Sequence	Product Size
Muta5	AACTGTGAAGCGATGAACCC	361 bp
Muta6	GGACTGTTCAGAGAGCTATC	
Muta7	GACCAAGCGATAACCGGATG	427 bp
Muta8	GTGAGATGATGGCCACGATT	
Muta9	GCGAGGTAACCTCGAACATG	313 bp
Muta10	CGGCGTATCGATAATTCACG	
EcN1	GCATTCGCCCCAGAGGAATAA	400 bp
EcN2	GTGTGCCTGAGACCCCAACAT	

bp=base pairs

**Table 2.6 Primer Sets and DNA sequences used for PCR identification of *E. coli* Nissle**

## 2.14 Statistical analysis

As this was an efficacy and dosing feasibility study, it was not designed or powered to detect a reduction in VAP; therefore, no sample size calculation was undertaken for this purpose. The sample size was based on a pragmatic approach working within the time, resource and financial framework available - as detailed in section 2.4. The sample size was, however, sufficient to detect gastric colonisation with *E. coli* Nissle.

Using a primary comparison of control versus any *E. coli* Nissle dose, the study used a 1:2 randomisation allocation. At 80% power, with  $p=0.05$  and using Pearson's Chi-squared test

then 10 control vs 20 *E. coli* Nissle would detect an increase from 0.5% to 55% colonisation or more, assuming no loss to follow-up. There would be minimal power to detect any difference between the two doses – at 80% power a difference of 15% vs 85% would be needed to reach statistical significance.

On reflection there was a limitation to this analysis plan. As this was a secondary exploratory analysis, it would have been more appropriate to use an analysis of variance (ANOVA) technique to analyse the data from the groups rather than within group analysis.

Statistical analysis was performed using R Version 3.4.2 (R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>). This was carried out as an intention to treat analysis.

Continuous data were tested for normality by the Kolmogorov-Smirnov method. Parametric data were analysed using a Student's t-test for independent samples or paired t-test as the data dictated.

Non-parametric data were analysed with Pearson's Chi-squared test or a Wilcoxon Rank Sum test as the data dictated. The null hypothesis was rejected when  $p \leq 0.05$ .



## **2.15 Safety and adverse events management**

In compliance with the Medicines for Human Use (Clinical Trials) Regulations (2004) and current International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP), adverse events were monitored and recorded for the duration of patients' participation in the study.

An adverse event (AE) was defined as any untoward medical occurrence in a patient who was receiving the IMP, and which did not necessarily have a causal relationship to the treatment. Expected AEs were recorded on the case report form for each participant. These events were included as part of the safety analysis for the study only and were not required to be reported to the Sponsor.

A serious adverse event (SAE) was defined as an adverse event that fulfils one or more of the following criteria:

- 1) Results in death
- 2) Is immediately life-threatening
- 3) Requires hospitalisation or prolongation of existing hospitalisation
- 4) Results in persistent or significant disability or incapacity
- 5) Results in congenital abnormality or birth defect (not expected in this patient population)
- 6) Requires medical intervention to prevent one of the above or is otherwise considered medically significant

Suspected Unexpected Serious Adverse Reactions (SUSARs) were defined as unexpected, serious events assessed by the sponsor and/or TMG as having a reasonable possibility of a causal relationship to the IMP and not consistent with the Summary of Product Characteristics.

Patients recruited into the PECaN-ED study were already critically ill, therefore, it was expected that many of the participants would experience adverse events which would be expected in this patient population. Events that would be expected in this population, along with those that were collected as study outcomes, were not reported as SAEs. This included:

- 1) Death
- 2) Organ failure

Patients were monitored for up to 30 days after the end of study intervention for any safety events (AE, SAE or SUSAR). Any SAEs or SUSARs that occurred between study recruitment and 30 days after the end of the study intervention were reported to the study sponsor in accordance with the sponsor's safety reporting policy.

SAEs and SUSARs were reported using the SAE form in the patient's CRF, this was in accordance with Sponsor's safety reporting policy. The Principal Investigator has a duty to report any SAEs and SUSARs to the sponsor within 24 hours of becoming aware of them. The Principal Investigator's assessment of causality of SAEs (i.e. their relationship to trial treatment) is recorded on the Serious Adverse Event form. Subsequently, the Principal Investigator would be required to submit a full report on the resolution of the event. The sponsor is responsible for reporting suspected unexpected serious adverse events to the MHRA and ethics committee within required timelines.

If there had been any indication that the IMP was causing harm, or increasing the risk of harm to study participants, this would have been identified and the appropriate action taken. This may have included discontinuing the treatment for the participant or early termination of the study.

## **Chapter 3**

### **Results**

### **3.1 Recruitment and study participants**

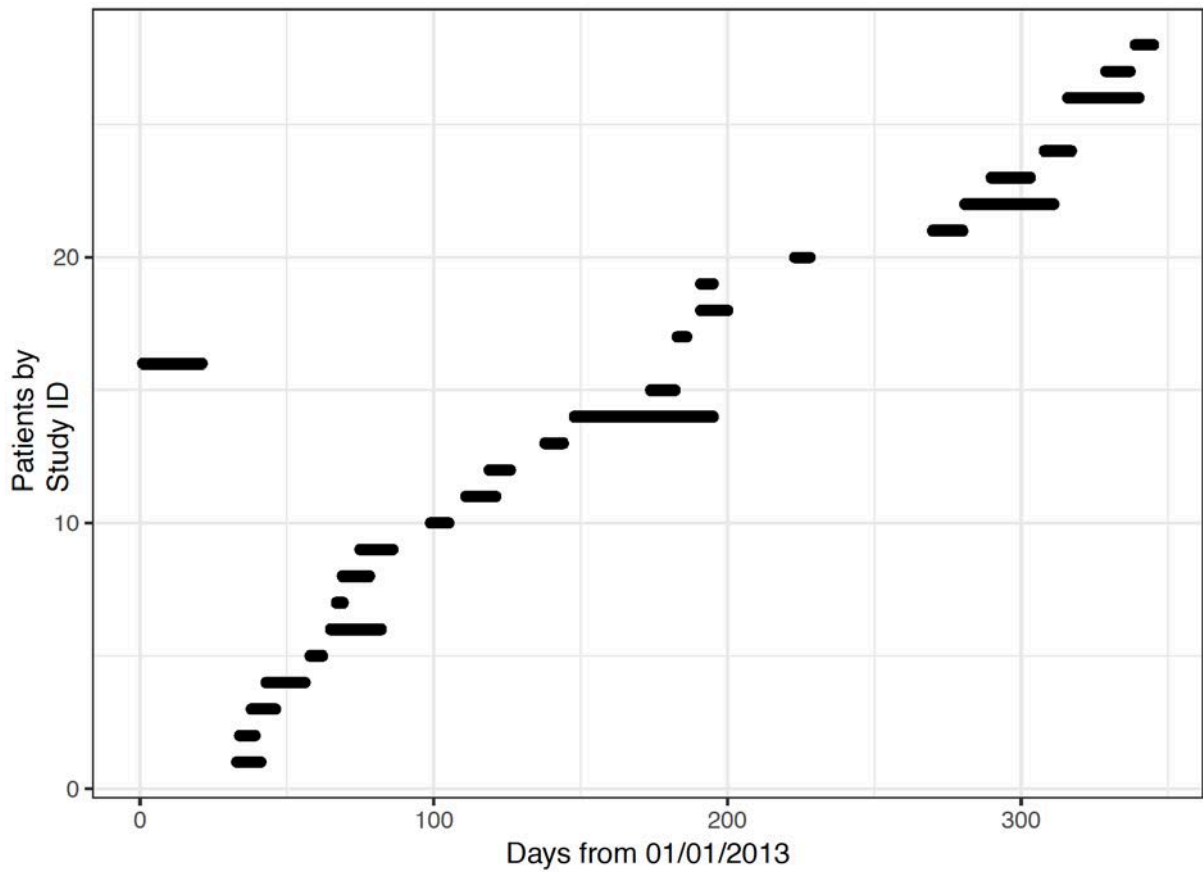
The study took place on the Intensive Care Unit, Birmingham Heartlands Hospital, during the period 02/01/2013 until 12/12/2013 (figure 3.1).

A total of 111 patients were screened for the study with 27 being enrolled (figure 3.2). The original target of 30 patients was not achieved due to time constraints and difficulties obtaining further supplies of *E. coli* Nissle 1917.

The remaining 84 patients were not enrolled due to lack of consent from the personal consultee, consent not being obtained within 24 hours of commencement of mechanical ventilation, patients having exclusion criteria or clinician uncertainty about the requirement for mechanical ventilation for more than 48 hours.

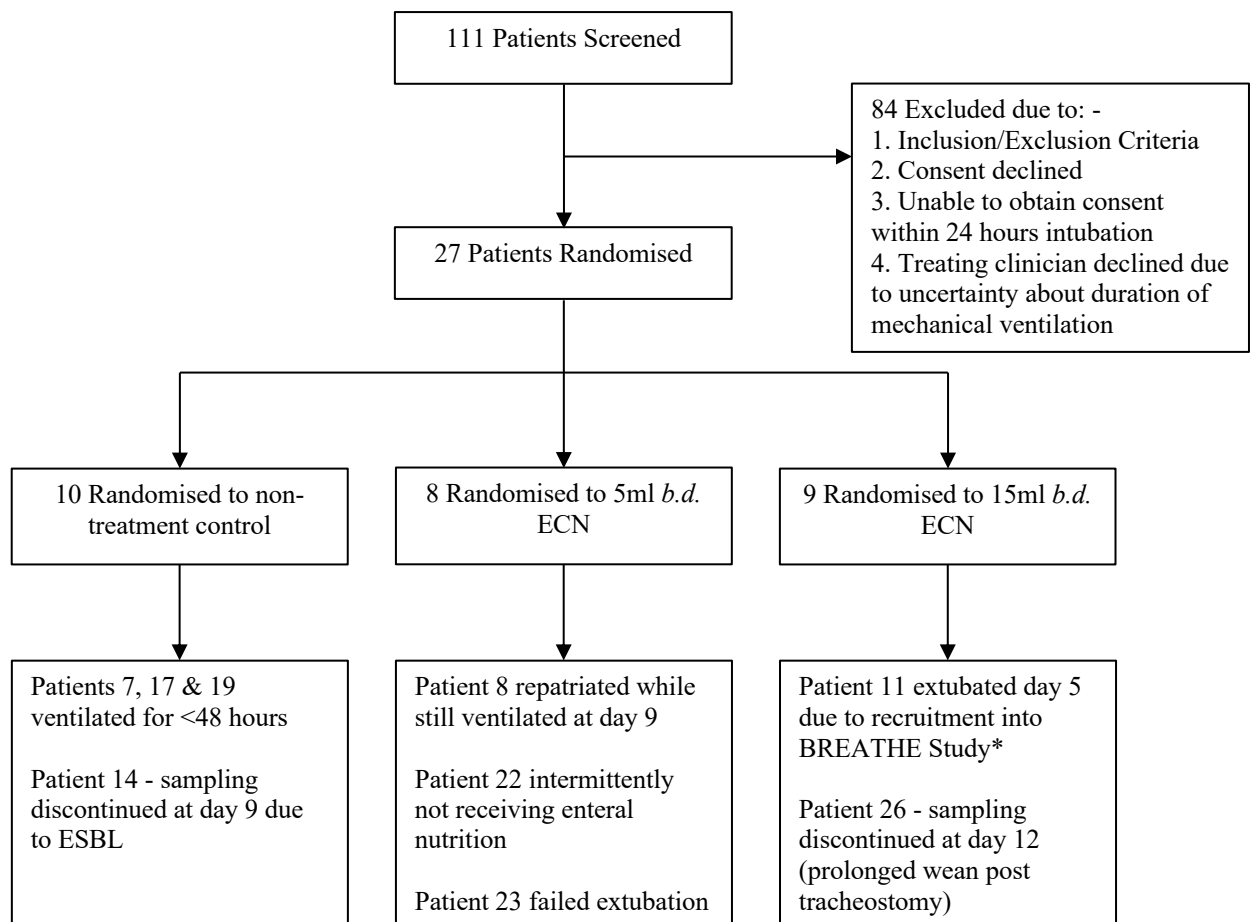
The expected duration of mechanical ventilation was determined by the treating consultant intensivist.

A timeline for each patient is presented in appendix III. This details the clinical history, antibiotic therapy and acid suppression used as well as the timing of probiotic administration, gastric aspirate sampling and microbiology results from these samples.



**Figure 3.1 Recruitment period for PECaN-ED Study**

The horizontal lines represent the period for which each patient was in the study. The patient with study identification number 16 was the first to be recruited into the study due to an administrative error. A patient was not allocated to the number 25, again due to an administrative error.



\*BREATHE Study - Protocolised trial of invasive and non-invasive weaning off ventilation

**Figure 3.2 Consort flow diagram for patient recruitment into PECaN-ED Study**

The setting for the study was a busy general, mixed medical-surgical ICU which does not accept major trauma or cardiac surgical patients. Taking this into account, the spectrum of reasons for ICU admission was representative of the normal ICU patient population for this unit.

Patients were evenly distributed between groups based on age, sex and three different severity of illness scores (table 3.1). Statistical analysis was applied to the baseline data using a Welch T test using R (226). As the numbers involved in this feasibility study were small, statistical analysis was not applied to the groups for race, VAP risk factors or reason for ICU admission.

	<b>Control</b>	<b>5ml ECN</b>	<b>15ml ECN</b>
Total number of patients	10	8	9
Female	6	5	3
Age, mean $\pm$ SD (range), yr	62.8 $\pm$ 23.69 (22-89)	61.9 $\pm$ 18.76 (31-87)	61.1 $\pm$ 12.1 (41-75)
APACHE II score $\pm$ SD (range)	21.0 $\pm$ 6.38 (11-32)	21.6 $\pm$ 4.41 (15-30)	24.3 $\pm$ 6.42 (17-36)
APACHE III $\pm$ SD (range)	85.0 $\pm$ 16.29 (51-108)	84.1 $\pm$ 14.34 (66-103)	86.4 $\pm$ 13.97 (68-112)
SAPS II $\pm$ SD (range)	44.5 $\pm$ 11.46 (26-62)	44.5 $\pm$ 10.01 (32-62)	47.3 $\pm$ 7.37 (37-60)
<b>Race</b>			
Caucasian	8 (80.0%)	7 (87.5%)	8 (88.9%)
Asian	2 (20.0%)	0 (0.0%)	1 (11.1%)
Afro-Caribbean	0 (0.0%)	1 (12.5%)	0 (0.0%)
<b>VAP risk factors</b>			
Smoker	5 (50.0%)	6 (75.0%)	5 (55.6%)
COPD	0 (0.0%)	1 (12.5%)	2 (22.2%)
Chest trauma	0 (0.0%)	1 (12.5%)	0 (0.0%)
Nursing home resident	0 (0.0%)	0 (0.0%)	0 (0.0%)
Alcohol abuse	2 (20.0%)	0 (0.0%)	2 (22.2%)
<b>Reason for ICU admission</b>			
Trauma	0 (0.0%)	1 (12.5%)	0 (0.0%)
Respiratory failure (including pneumonia)	4 (40.0%)	3 (37.5%)	3 (33.3%)
Non-Resp Infection	2 (20.0%)	0 (0.0%)	1 (11.1%)
Cardiology	3 (30.0%)	0 (0.0%)	4 (44.4%)
Neurology	1 (10.0%)	2 (25.0%)	0 (0.0%)
Gastrointestinal	0 (0.0%)	2 (25.0%)	1 (11.1%)

**Table 3.1 Demographics and baseline characteristics of PECaN-ED study population**



### 3.2 Primary outcome

The primary outcome of the study was to demonstrate gastric colonisation with *E. coli* Nissle. For the purposes of this study, colonisation was simply defined as the presence of *E. coli* Nissle in two consecutive gastric aspirates without a specific CFU threshold. The term colonisation is used variably in the literature and is generally taken to mean the presence of an organism without signs of associated infection. However, there is no universally accepted definition of colonisation with regard to the presence of an organism in the stomach, and the criteria used vary between publications. In the trial by Morrow *et al* (104), colonisation was simply taken to mean organism presence at a given time point.

The trial by Garrouste-Orgeas *et al* offers a more detailed definition of gastric colonisation and considers colonisation as ICU-acquired only when there was no colonisation on admission and when the same potentially pathogenic microorganism was detected from at least two consecutive samples at a concentration  $\geq 10^2$  CFU ml<sup>-1</sup> (227).

The results for *E. coli* Nissle colonisation in gastric aspirates are presented in table 3.2. *Escherichia coli* Nissle was not detected in any of the gastric aspirates from the control group. The bacteria would not have been expected to be present in this group, however cross-contamination between treatment and control patient samples could have theoretically occurred during sample collection or handling. *E. coli* Nissle could have been present in a control group patient if that individual was carrying Nissle as a commensal organism. Again, this would have been extremely unlikely as the organism is found in the colon rather than the stomach.

In the 5ml ECN group, colonisation was achieved in five of the eight patients studied (62.5%). In the 15ml ECN group, colonisation was achieved in seven of the nine patients studied (77.7%). The presence of *E. coli* Nissle was confirmed by PCR analysis of *E. coli* colonies.

Therefore, based on these findings, it was possible to demonstrate gastric colonisation with *E. coli* Nissle in a selected group of ventilated ICU patients.

	<b>Control (10 patients)</b>	<b>5ml ECN (8 patients)</b>	<b><i>P</i> value</b>
<b>Patients colonised (%)</b>	0 (0%)	5 (62.5%)	0.0033

	<b>Control (10 patients)</b>	<b>15ml ECN (9 patients)</b>	<b><i>P</i> value</b>
<b>Patients colonised (%)</b>	0 (0%)	7 (77.7%)	0.00045

	<b>5ml ECN (8 patients)</b>	<b>15ml ECN (9 patients)</b>	<b><i>P</i> value</b>
<b>Patients colonised (%)</b>	5 (62.5%)	7 (77.7%)	0.4902

**Table 3.2 Frequency of gastric colonisation with *Escherichia coli* Nissle in the 5ml and 15ml treatment groups**

### 3.3 Secondary outcomes

#### 3.3.1 Dose variation and time to gastric colonisation

*Escherichia coli* Nissle 1917 is marketed as Mutaflor® in Germany and is licensed for administration to infants, children and adults for a variety of indications. This is the first time that it has been administered to ventilated ICU patients. As such two different doses were selected to determine if this would affect gastric colonisation or time to gastric colonisation.

Gastric colonisation was demonstrated in both the 5ml ECN and 15ml ECN treatment groups, however, there was no statistically significant difference between the two doses in terms of the number of patients colonised ( $p=0.4902$ ) (table 3.2).

The time to colonisation was measured in days, with the second consecutive day of *E. coli* Nissle presence in gastric aspirates signifying the time of colonisation. The period of time, in days, to colonisation was recorded for each patient. Although there was a tendency toward a shorter time to colonisation in the 15 ml ECN group (table 3.3), this was not statistically significant ( $p=0.18$ ).

	<b>Control (0 patients)</b>	<b>5ml ECN (5 patients)</b>	<b>15ml ECN (7 patients)</b>	<b><i>P</i> value</b>
Time to colonisation, mean $\pm$ SD (range), days	n/a	5.6 $\pm$ 4.16 (2-11)	2.6 $\pm$ 1.13 (2-5)	0.18

**Table 3.3 Time to gastric colonisation by *Escherichia coli* Nissle**

In the 5ml *b.d.* group, a sample on day four was not obtained for one of the patients. Had this sample been positive for *E. coli* Nissle, the time to colonisation for the 5ml *b.d.* group would have been reduced to 5.4 days. This result would not have altered the statistical significance between the two groups ( $p=0.22$ ).

### **3.3.2 Analysis of gastric aspirates**

Tables 3.4 and 3.5 show the detailed breakdown of the bacteria identified in the gastric aspirates at baseline and after 48 hours (all post 48-hour aspirates per study arm grouped together). The baseline aspirates were obtained at the earliest opportunity following enrolment and prior to administration of *E. coli* Nissle. Initial aspirates were obtained up to 24 hours post intubation depending upon the timing of consent.

The overall proportion of gastric aspirates containing potential pathogens in the baseline group, including both Gram-positives and Gram-negatives was 59.2%. This was defined as gastric aspirates containing organisms with the potential to cause VAP.

The bar charts in figure 3.3 show the relative proportions of organisms present in the combined baseline aspirates and the post 48-hour aspirates by study group. The proportion of Gram-negative organisms remains relatively constant in the post 48-hour control and 5ml ECN groups (26.7% & 22.0% vs 22.2%). However, the proportion of Gram-negative organisms decreases to 4.3% in the 15ml ECN group. There is no statistically significant difference between the post 48-hour 5ml ECN and control groups ( $p=0.58$ ). There is, however, a statistically significant difference in the proportion of Gram-negatives between the post 48-hour 15ml ECN and control groups ( $p<0.01$ ), and post 48-hour 15ml ECN and 5ml ECN groups ( $p=0.01$ ).

The overall proportion of potential pathogens in the gastric aspirates, both Gram-positive and Gram-Negative, is again relatively constant in the baseline and post 48-hour control and 5ml ECN groups (59.2%, 48.9%, 54.2%), decreasing to 15.2% in the post 48-hour 15ml ECN group. There was no significant difference between the post 48-hour control and 5ml ECN groups and baseline ( $p=0.39$  and  $0.66$  respectively), however, there was a significant difference between the 15ml ECN group and baseline ( $p<0.01$ ).

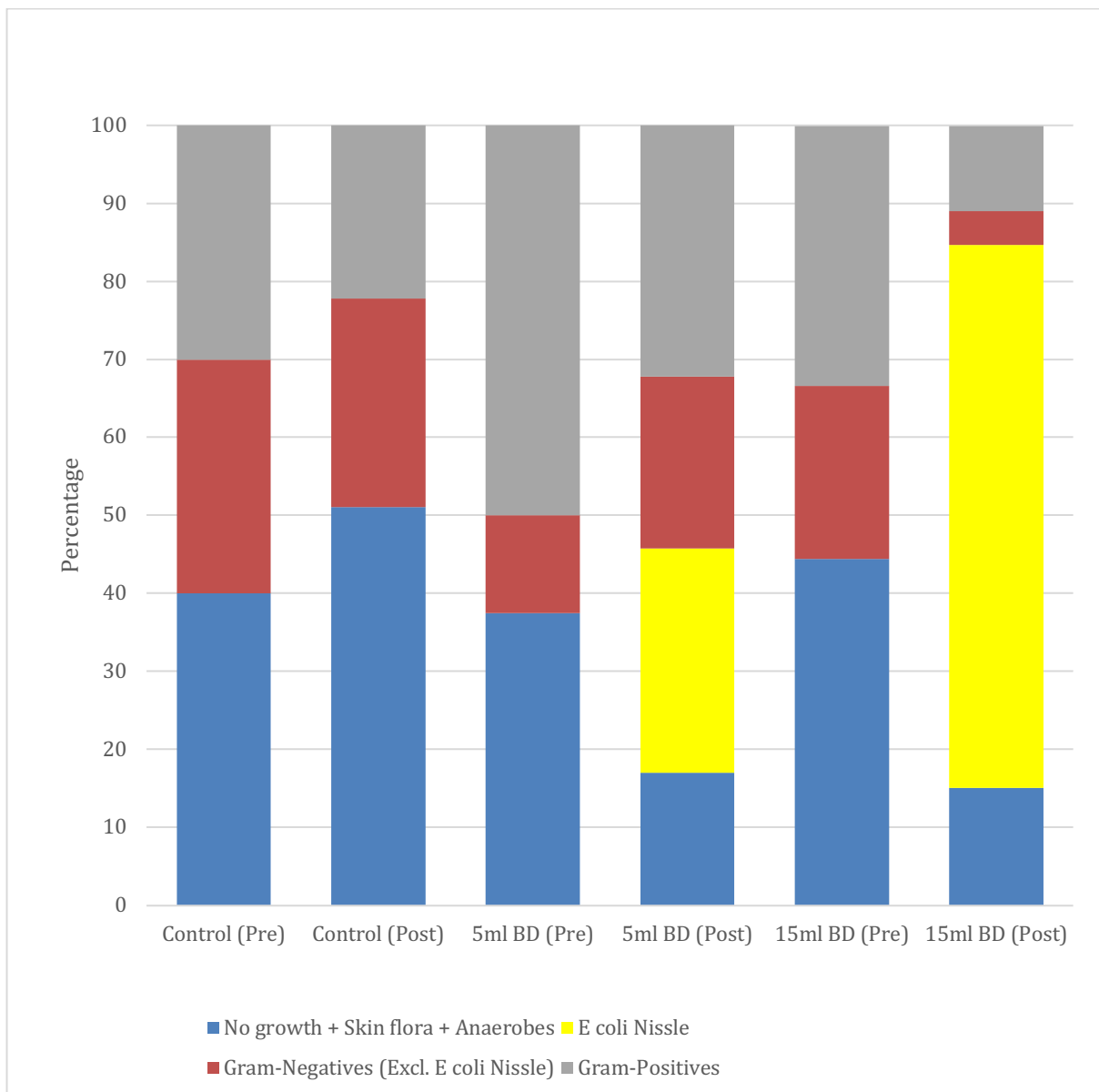
	<b>Control</b>	<b>5ml ECN</b>	<b>15ml ECN</b>	<b>Total</b>
<b>Number NG aspirates</b>	10	8	9	27
<b>No growth</b>	3 (30.0%)	1 (12.5%)	1 (11.1%)	11 (40.7%)
<b>Skin flora (Coagulase-negative Staphylococci/Micrococci)</b>	1 (10.0%)	2 (25.0%)	3 (33.3%)	
<b><i>Escherichia coli</i> Nissle</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Non-Nissle <i>Escherichia coli</i></b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Other Coliforms</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Enterococci</b>	0 (0%)	2 (25.0%)	2 (22.2%)	4 (14.8%)
<b>Streptococci</b>	1 (10.0%)	0 (0%)	1(11.1%)	2 (7.4%)
<b>Staphylococci</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci</b>	1 (10.0%)	1 (12.5%)	0 (0%)	2 (7.4%)
<b>Staphylococci/Corynebacteria</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci/Enterococci</b>	1 (10.0%)	0 (0%)	0 (0%)	1 (3.7%)
<b>Staphylococci/Enterococci</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Streptococci/Enterococci</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Anaerobes</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Total Gram-Negatives</b>	3 (30.0%)	1 (12.5%)	2 (22.2%)	<b>6 (22.2%)</b>
<b>Total Gram-Positives</b>	3 (30.0%)	4 (50.0%)	3 (33.3%)	<b>10 (37.0%)</b>

**Table 3.4 Bacteria identified in gastric aspirates at baseline**

	<b>Control</b>	<b>5ml ECN</b>	<b>15ml ECN</b>
<b>Number NG aspirates</b>	45	59	46*
<b>No growth</b>	13 (28.9%)	7 (11.9%)	1 (2.1%)
<b>Skin flora (Coagulase-negative Staphylococci/Micrococci)</b>	9 (20.0%)	3 (5.1%)	6 (13.0%)
<b><i>Escherichia coli</i> Nissle</b>	<b>0 (0%)</b>	<b>17 (28.8%)</b>	<b>32 (69.6%)</b>
<b>Non-Nissle <i>Escherichia coli</i></b>	10 (22.2%)	5 (8.5%)	1 (2.1%)
<b>Other Coliforms</b>	2 (4.4%)	8 (13.6%)	1 (2.1%)
<b>Enterococci</b>	4 (8.9%)	13 (22.0%)	2 (4.3%)
<b>Streptococci</b>	4 (8.9%)	2 (3.4%)	0 (0%)
<b>Staphylococci</b>	0 (0%)	1 (1.7%)	0 (0%)
<b>Staphylococci/Streptococci</b>	1 (2.2%)	0 (0%)	0 (0%)
<b>Staphylococci/Corynebacteria</b>	1 (2.2%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci/Enterococci</b>	0 (0%)	0 (0%)	1 (2.1%)
<b>Staphylococci/Enterococci</b>	0 (0%)	1 (1.7%)	2 (4.3%)
<b>Streptococci/Enterococci</b>	0 (0%)	2 (3.4%)	0 (0%)
<b>Anaerobes</b>	1 (2.2%)	0 (0%)	0 (0%)
<b>Total Gram-Negatives (Excl. <i>Escherichia coli</i> Nissle)</b>	<b>12 (26.7%)</b>	<b>13 (22.0%)</b>	<b>2 (4.3%)</b>
<b>Total Gram-Positives</b>	<b>10 (22.2%)</b>	<b>19 (32.2%)</b>	<b>5 (10.9%)</b>

\*One aspirate excluded as unable to separate colonies

**Table 3.5 Bacteria identified in gastric aspirates after 48 hours**



**Figure 3.3 Gastric colonisation in the probiotic and control groups (proportion of gastric aspirates) pre and post ECN administration**



### 3.3.3 Analysis of oropharyngeal aspirates

Oropharyngeal (OP) aspirates were obtained at baseline in the control group and before administration of *E. coli* Nissle in the treatment groups. Daily OP aspirates were obtained for the following three days and on alternate days thereafter. Results from the OP aspirate cultures were analysed and the proportion of potentially pathogenic species determined for combined baseline samples (table 3.6) and individual study groups for the subsequent samples (table 3.7).

In the results from the combined baseline OP aspirates, the proportion of pathogenic Gram-negative and Gram-positive potential pathogens is 25.9% and 55.6% respectively. The proportion of Gram-negatives remains relatively constant in the post 48-hour control and 15ml ECN groups but drops to 16.6% in the 5ml ECN group. There was no significant difference between the proportion of pathogenic Gram-negatives in the control, 5ml ECN or 15 ml ECN groups compared to baseline ( $p=0.99$ ,  $p=0.28$  and  $p=0.85$  respectively).

	<b>Control</b>	<b>5ml ECN</b>	<b>15ml ECN</b>	<b>Total</b>
<b>Total OP aspirates</b>	10	8	9	27
<b>No growth</b>	1 (10.0%)	1 (12.5%)	0 (0%)	2 (7.4%)
<b>Skin flora (Coagulase-negative Staphylococci/ Micrococci)</b>	0 (0%)	1 (12.5%)	1 (11.1%)	2 (7.4%)
<i>Escherichia coli</i> Nissle	0 (0%)	0 (0%)	0 (0%)	0(0%)
<b>Non-Nissle <i>Escherichia coli</i></b>	1 (10.0%)	1 (12.5%)	2 (22.2%)	4 (14.8%)
<b>Other Coliforms</b>	1 (10.0%)	0 (0%)	2 (22.2%)	3 (11.1%)
<b>Enterococci</b>	2 (20.0%)	2 (25.0%)	1 (11.1%)	5 (18.5%)
<b>Streptococci</b>	3 (30.0%)	0 (0%)	1 (11.1%)	4 (14.8%)
<b>Staphylococci</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci</b>	0 (0%)	1 (12.5%)	0 (0%)	1 (3.7%)
<b>Staphylococci/Corynebacteria</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci/ Enterococci</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci/ Corynebacteria</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Enterococci</b>	0 (0%)	0 (0%)	1 (11.1%)	1 (3.7%)
<b>Streptococci/Enterococci</b>	2 (20.0%)	2 (25.0%)	0 (0%)	4 (14.8%)
<b>Anaerobes</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Bacilli (excluded from totals)</b>	0 (0%)	0 (0%)	1 (11.1%)	1 (3.7%)
<b>Total Gram-Negatives (Excl <i>Escherichia coli</i> Nissle)</b>	2 (20.0%)	1 (12.5%)	4 (44.4%)	<b>7 (25.9%)</b>
<b>Total Gram-Positives</b>	7 (70.0%)	5 (62.5%)	3 (33.3%)	<b>15 (55.6%)</b>

**Table 3.6 Bacteria identified in oropharyngeal aspirates at baseline**

	<b>Control</b>	<b>5ml ECN</b>	<b>15ml ECN</b>
<b>Total OP aspirates</b>	46	45	46
<b>No growth</b>	13 (28.3%)	2 (4.4%)	4 (8.7%)
<b>Skin flora (Coagulase-negative Staphylococci/Micrococci)</b>	6 (13.0%)	5 (11.1%)	0 (0%)
<b><i>Escherichia coli</i> Nissle</b>	0 (0%)	3 (6.7%)	1 (2.2%)
<b>Non-Nissle <i>Escherichia coli</i></b>	7 (15.2%)	1 (2.2%)	2 (4.3%)
<b>Other Coliforms</b>	5 (10.9%)	6 (13.3%)	9 (19.6%)
<b>Enterococci</b>	3 (6.5%)	13 (28.9%)	7 (15.2%)
<b>Streptococci</b>	3 (6.5%)	4 (8.9%)	7 (15.2%)
<b>Staphylococci</b>	1 (2.2%)	1 (2.2%)	1 (2.2%)
<b>Staphylococci/Streptococci</b>	1 (2.2%)	1 (2.2%)	0 (0%)
<b>Staphylococci/Corynebacteria</b>	1 (2.2%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci/Enterococci</b>	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci/Corynebacteria</b>	1 (2.2%)	0 (0%)	0 (0%)
<b>Staphylococci/Enterococci</b>	0 (0%)	5 (11.1%)	1 (2.2%)
<b>Streptococci/Enterococci</b>	2 (4.3%)	3 (6.7%)	3 (6.5%)
<b>Anaerobes</b>	3 (6.5%)	1 (2.2%)	7 (15.2%)
<b>Bacilli (excluded from totals)</b>	0 (0%)	0 (0%)	4 (8.7%)
<b>Total Gram-Negatives (Excl <i>Escherichia coli</i> Nissle)</b>	<b>12 (26.1%)</b>	<b>7 (15.6%)</b>	<b>11 (23.9%)</b>
<b>Total Gram-Positives</b>	<b>12 (26.1%)</b>	<b>27 (60.0%)</b>	<b>19 (41.3%)</b>

**Table 3.7 Bacteria identified in oropharyngeal aspirates after 48 hours**

### 3.3.4 Analysis of tracheal aspirates

A baseline tracheal aspirate was obtained for each patient prior to administration of the first dose of probiotic, followed by daily aspirates for the next three days and alternate days thereafter. An identical pattern of sampling was applied to the control group. The results from the baseline tracheal aspirates are shown in Table 3.8. Potential Gram-positive and Gram-negative pathogens were demonstrated in 37.0% and 14.8% of combined baseline samples respectively.

The results for the post 48-hour tracheal aspirates are shown for the three study groups in table 3.9. Potential Gram-negative and Gram-positive pathogens persist through the three groups. Of note, there is a higher proportion of potentially pathogenic Gram-negative organisms in the 15ml ECN group (28.9%) compared to baseline (14.8%) and compared to the 5ml ECN (9.4%) and control groups (19.4%). However, there is no statistically significant difference between the control, 5ml ECN or 15ml ECN and baseline ( $p=0.63$ ,  $p=0.52$  and  $p=0.18$  respectively).

*Escherichia coli* Nissle was detected in two tracheal aspirates from the treatment groups. This may represent sample contamination or possibly reflects the passive aspiration of gastric contents into the bronchial tree.

	<b>Control</b>	<b>5ml ECN</b>	<b>15ml ECN</b>	<b>Total</b>
<b>Total tracheal aspirates</b>	10	8	9	27
<b>No growth</b>	3 (30.0%)	3 (37.5%)	1 (11.1%)	8 (29.6%)
<b>Skin flora (Coagulase-negative Staphylococci/Micrococci)</b>	0 (0%)	0 (0%)	1 (11.1%)	
<i>Escherichia coli</i> Nissle	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Non-Nissle <i>Escherichia coli</i></b>	1 (10.0%)	0 (0%)	1 (11.1%)	2 (7.4%)
<b>Other Coliforms</b>	0 (0%)	0 (0%)	2 (22.2%)	2 (7.4%)
<b>Enterococci</b>	1 (10.0%)	1 (12.5%)	1 (11.1%)	3 (11.1%)
<b>Streptococci</b>	4 (40.0%)	1 (12.5%)	0 (0%)	5 (18.5%)
<b>Staphylococci</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci</b>	0 (0%)	1 (12.5%)	0 (0%)	1 (3.7%)
<b>Staphylococci/Corynebacteria</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci/Enterococci</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Enterococci</b>	0 (0%)	0 (0%)	1 (11.1%)	1 (3.7%)
<b>Streptococci/Enterococci</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Anaerobes</b>	1 (10.0%)	2 (25.0%)	2 (22.2%)	5 (18.5%)
<b>Bacilli</b>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<b>Total Gram-Negatives</b>	1 (10.0%)	0 (0%)	3 (33.3%)	<b>4 (14.8%)</b>
<b>Total Gram-Positives</b>	5 (50.0%)	3 (37.5%)	2 (22.2%)	<b>10 (37.0%)</b>

**Table 3.8 Bacteria identified in tracheal aspirates at baseline**

	<b>Control</b>	<b>5ml ECN</b>	<b>15ml ECN</b>
<b>Total tracheal aspirates</b>	36	32	38
<b>No growth</b>	15 (41.7%)	15 (46.9%)	13 (34.2%)
<b>Skin flora (Coagulase-negative Staphylococci/ Micrococci)</b>	2 (5.6%)	3 (9.4%)	6 (15.8%)
<i>Escherichia coli</i> Nissle	0 (0%)	1 (3.1%)	1 (2.6%)
<b>Non-Nissle <i>Escherichia coli</i></b>	5 (13.9%)	0 (0%)	3 (7.9%)
<b>Other Coliforms</b>	2 (5.6%)	3 (9.4%)	8 (21.1%)
<b>Enterococci</b>	3 (8.3%)	1 (3.1%)	2 (5.3%)
<b>Streptococci</b>	2 (5.6%)	3 (9.4%)	1 (2.6%)
<b>Staphylococci</b>	2 (5.6%)	1 (3.1%)	1 (2.6%)
<b>Staphylococci/Streptococci</b>	2 (5.6%)	0 (0%)	0 (0%)
<b>Staphylococci/Corynebacteria</b>	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Streptococci/ Enterococci</b>	0 (0%)	0 (0%)	0 (0%)
<b>Staphylococci/Enterococci</b>	0 (0%)	2 (6.3%)	1 (2.6%)
<b>Streptococci/Enterococci</b>	1 (2.8%)	3 (9.4%)	0 (0%)
<b>Anaerobes</b>	1 (2.8%)	0 (0%)	0 (0%)
<b>Bacilli (excluded from totals)</b>	0 (0%)	0 (0%)	2 (5.3%)
<b>Total Gram-Negatives (Excl <i>Escherichia coli</i> Nissle)</b>	<b>7 (19.4%)</b>	<b>3 (9.4%)</b>	<b>11 (28.9%)</b>
<b>Total Gram-Positives</b>	<b>10 (27.8%)</b>	<b>10 (31.3%)</b>	<b>5 (13.2%)</b>

**Table 3.9 Bacteria identified in tracheal aspirates after 48 hours**

### **3.3.5 VAP, antibiotic days, ICU infection and surveillance data**

As this was the first time *E. coli* Nissle had been administered to an ICU population, regular surveillance samples were obtained for microbiological culture as part of the study protocol. The results of these samples were reviewed in conjunction with the routine clinical samples that were obtained (BAL, tracheal aspirate, blood cultures, wound swabs, catheter specimen urine, stool).

Although the study was not powered to detect a reduction in the incidence of VAP, data were collected in order to determine the incidence of VAP in the study population. Based on the criteria described previously (section 2.10), six patients were diagnosed as having microbiologically confirmed VAP (table 3.10). Three of the study patients were mechanically ventilated for less than 48 hours, which gives a VAP incidence of 25%. In five of the six patients who developed a VAP, the causative organism was a Gram-negative species.

ICU patients are frequently treated with multiple courses of broad-spectrum antibiotics. This reflects severity of illness, diagnostic uncertainty about source of infection or causative organism and concern about resistant species – particularly when the patient has had a prolonged stay on ICU or in in refractory multi-organ failure.

Total antibiotic days, for both ICU stay and hospital stay, were calculated for the study patients as a comparison of overall antibiotic use in the three groups (table 3.11). Calculation of antibiotic days involves adding the number of antibiotics administered daily across all days when antibiotics were prescribed. For example, a patient receiving three different antibiotics

for five days has a total of 15 antibiotic-days. Incomplete administration is recorded as a fraction of the prescribed daily dose (i.e. 2 out of 3 doses is recorded as 0.66 for that day).

Study ID	Group	Diagnostic criteria	Organism
18	Control	Fever, purulent secretions, inflammatory markers, CXR infiltrates	<i>Staphylococcus aureus</i>
20	Control	Fever, purulent secretions, inflammatory markers, CXR infiltrates	<i>Escherichia coli</i>
6	5ml ECN	Fever, purulent secretions, inflammatory markers, CXR infiltrates	<i>Enterobacter aerogenes</i>
13	15ml ECN	Purulent secretions, inflammatory markers, CXR infiltrates	<i>Acinetobacter baumannii</i> ; <i>Klebsiella oxytoca</i>
26	15ml ECN	Fever, purulent secretions, inflammatory markers, CXR infiltrates	<i>Escherichia coli</i>
28	15ml ECN	Fever, purulent secretions, inflammatory markers, CXR infiltrates	<i>Serratia marcescens</i>

**Table 3.10 Details of study patients with microbiologically confirmed VAP**

The 5ml ECN group had the highest mean total antibiotic days for both ICU stay, and hospital stay (16.98 days (range 6.0-35.83), 24.13 days (range 6.5-77.93)). This is unlikely to be related to probiotic administration and it would be difficult to draw any firm conclusions as it represents such a heterogeneous group of patients. Furthermore, the data are likely to be skewed by outlying patients with multiple or prolonged courses of antibiotics. The data for total antibiotic days for ICU stay and hospital stay were analysed using a Wilcoxon Rank-Sum Test and no statistically significant difference any of the groups was identified (ITU: control vs 5ml ECN



$p=0.20$ ; control vs 15ml ECN  $p=0.80$ ; 15ml ECN vs 5ml ECN  $p=0.26$ ), (Hospital: control vs 5ml ECN  $p=0.15$ ; control vs 15ml ECN  $p=0.81$ ; 15ml ECN vs 5ml ECN  $p=0.09$ ).

Cumulative incidence of ICU-acquired infection was also measured as a secondary outcome. In addition to the six patients detailed above who developed VAP, four other episodes of ICU-acquired infection were recorded. Two patients with indwelling urinary catheters developed culture positive urine with single organisms (patient 4 - *E. coli*, patient 19 - *Citrobacter freundii*).

Patient 4 had a fully sensitive urinary *E. coli*. The patient was also found to be positive for *E. coli* Nissle in the stool and surveillance urine samples at the same time. Therefore, this may represent a contaminant, but in view of the diagnostic difficulties in ICU patients this was treated with a short course of antibiotics without further sequelae.

Patient 24 (from the 15 ml ECN group) was treated for cellulitis of the left hand, which may have been related to a cannula site. No causative organism was identified for this infection. This is not likely to be related to the IMP but is included as part of the analysis of all ICU-related infection in the study population.

Patient 27 (from the 15 ml ECN group) was initially admitted to ICU for renal support. He deteriorated and required intubation due to a hospital acquired pneumonia. *Haemophilus influenzae* and *Pseudomonas aeruginosa* were identified in tracheal secretions obtained post-intubation. This would be classified as a HAP leading to respiratory failure requiring ventilatory support, rather than a VAP.

Overall, the episodes described above represent an incidence of 40.0% for cumulative ICU-acquired infection.

Two patients (study ID 9,14 – both control group) were found to be positive for *E. coli* (blood and urine respectively) as part of their clinical presentation. Neither patient had any *E. coli* Nissle positive surveillance cultures and did not receive the IMP.

Four patients (study ID 4,6,24,28) were found to have *E. coli* Nissle in culture of stool or rectal swab. Patient 6 was from the 5 ml ECN group; the other three patients were from the 15 ml ECN group. Patient 4 has already been discussed earlier in this section. Patient 6 had two *E. coli* Nissle positive surveillance urine cultures but no *E. coli* positive clinical urine cultures. Patients 24 and 28 were not found to have *E. coli* Nissle in any other surveillance cultures or *E. coli* in any clinical cultures.

Supplementary secondary outcome data relating to duration of mechanical ventilation and length of ICU and hospital stay were collected (table 3.11). Although the study was not powered to detect difference in these outcomes, they are an excellent illustration of the heterogeneity of the ICU population which I have attempted to study, with wide ranges in all of these criteria across all study groups.

	<b>Control</b>	<b>5ml ECN</b>	<b>15ml ECN</b>
<b>Mean/Median duration of MV (range) Days*</b>	10.4/4.2 (1.7-42.4)	9.0/8.2 (2.7-18.6)	7.5/4.6 (2.3-22.1)
<b>Mean ICU LOS in days* (range)</b>	12.7 (1.6-46.0)	15.1 (5.5-29.8)	11.8 (6.1-24.3)
<b>Median ICU LOS in days*</b>	7.0	13.1	8.0
<b>Mean Hospital LOS in days** (range)</b>	34.3 (4.0-79.0)	32.0 (10.0-53.0)	30.8 (11.0-68.0)
<b>Median Hospital LOS in days**</b>	19.5	31.0	22.0
<b>Mean abx days (ICU) (range)</b>	10.06 (0-35.0)	16.98 (6.0-35.83)	8.92 (0-17.83)
<b>Mean total abx days (incl pre-ICU) (range)</b>	10.47 (0-35.0)	24.13 (6.5-77.93)	9.61 (0-18.5)

\*Patients who survived to ITU discharge; \*\*Patients who survived to hospital discharge

**Table 3.11 Supplementary Secondary Outcome Data**

### **3.3.6 ICU, hospital and twenty-eight day mortality**

Eleven of the patients in the study died within 28 days of ICU admission (table 3.12). Ten patients died while on ICU and one patient died following ICU discharge. The death of this patient was expected. Deaths came from all three trial groups – 4 patients (control), 2 patients (5ml ECN), 5 patients (15 ml ECN).

The causes of death for these patients are listed in table 3.13. The causes of death were diverse although it is notable that 5 of the deceased patients were from the 15ml ECN group. None of the causes of death were linked in any way to the administration of probiotic. Patient 6 did die due to the complications of small bowel ischaemia. This is worthy of mention as it was the

cause of excess deaths in the treatment group patients from the PROPATRIA trial (168). This was not felt to be linked to probiotic administration as it was noted at time of surgery that the small bowel changes appeared to be chronic rather than acute.

	<b>Control</b>	<b>5ml ECN</b>	<b>15ml ECN</b>
<b>28 Day Mortality (% by group)</b>	4 (40%)	2 (25%)	5 (55.5%)

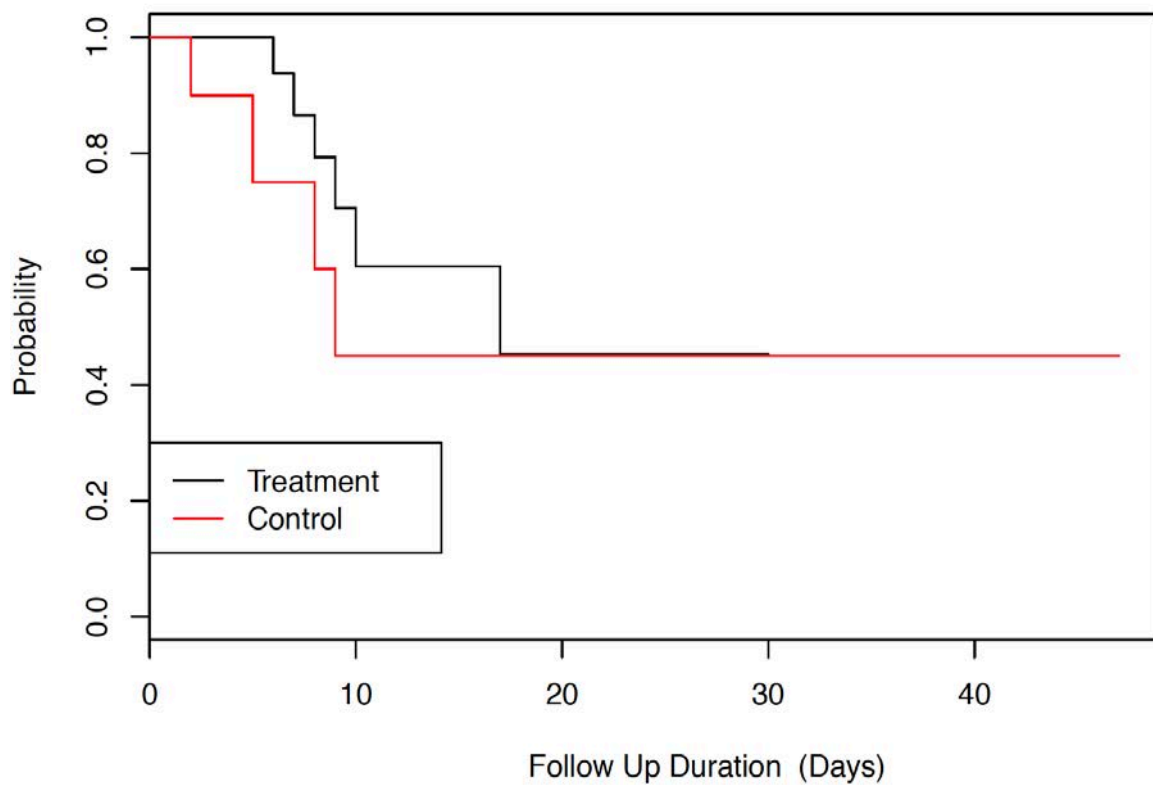
**Table 3.12 Twenty-eight Day Mortality by Group**

Four SAEs were reported to the trial sponsor by the research team. Three of these patients died (study ID 3, 6, 26) and as discussed, the deaths were not related to the IMP. The fourth patient (study ID 23) deteriorated two days after extubation and required re-intubation due to septic shock and cardiovascular instability. This was not related to the IMP and the patient survived to hospital discharge.

Study ID	Study Group	Cause of Death
7	Control	i. Hypoxic-ischaemic encephalopathy ii. Cardio-respiratory arrest iii. Acute myocardial infarction
15	Control	i. Paraneoplastic encephalitis ii. Metastatic lung cancer
18	Control	i. Left ventricular failure ii. Ischaemic heart disease
20	Control	i. Hypoxic-ischaemic encephalopathy ii. Hypoxic Cardiac arrest iii. Fall -> C1/2 fracture
6	5ml ECN	i. Small bowel ischaemia ii. Bleeding duodenal ulcers (chronic) iii. Traumatic broncho-pleural fistula
12	5ml ECN	i. Respiratory failure ii. H <sub>1</sub> N <sub>1</sub> Pneumonia iii. Laparotomy for small bowel obstruction
3	15ml ECN	i. Myocardial infarction secondary to <i>S. aureus</i> endocarditis ii. H <sub>1</sub> N <sub>1</sub> Pneumonia
11	15ml ECN	i. Septicaemia ii. Empyema iii. End stage renal failure
24	15ml ECN	i. Hypoxic-ischaemic encephalopathy ii. Cardio-respiratory arrest iii. Acute myocardial infarction
26	15ml ECN	i. Respiratory failure ii. Hospital acquired pneumonia iii. Severe critical illness polyneuropathy iv. COPD
28	15ml ECN	i. Multi-organ failure ii. Cardiac arrest iii. Ischaemic heart disease

**Table 3.13 Twenty-eight day mortality - Causes of death**

A Kaplan-Meier plot (figure 3.4) was compiled for all treatment patients *versus* control. This demonstrated no significant difference between the survivorship curves. This was carried out using R with a total sample size of 27 patients and a Chi-square value of 0.09 with one degree of freedom, giving a  $p$ -value of 0.76.

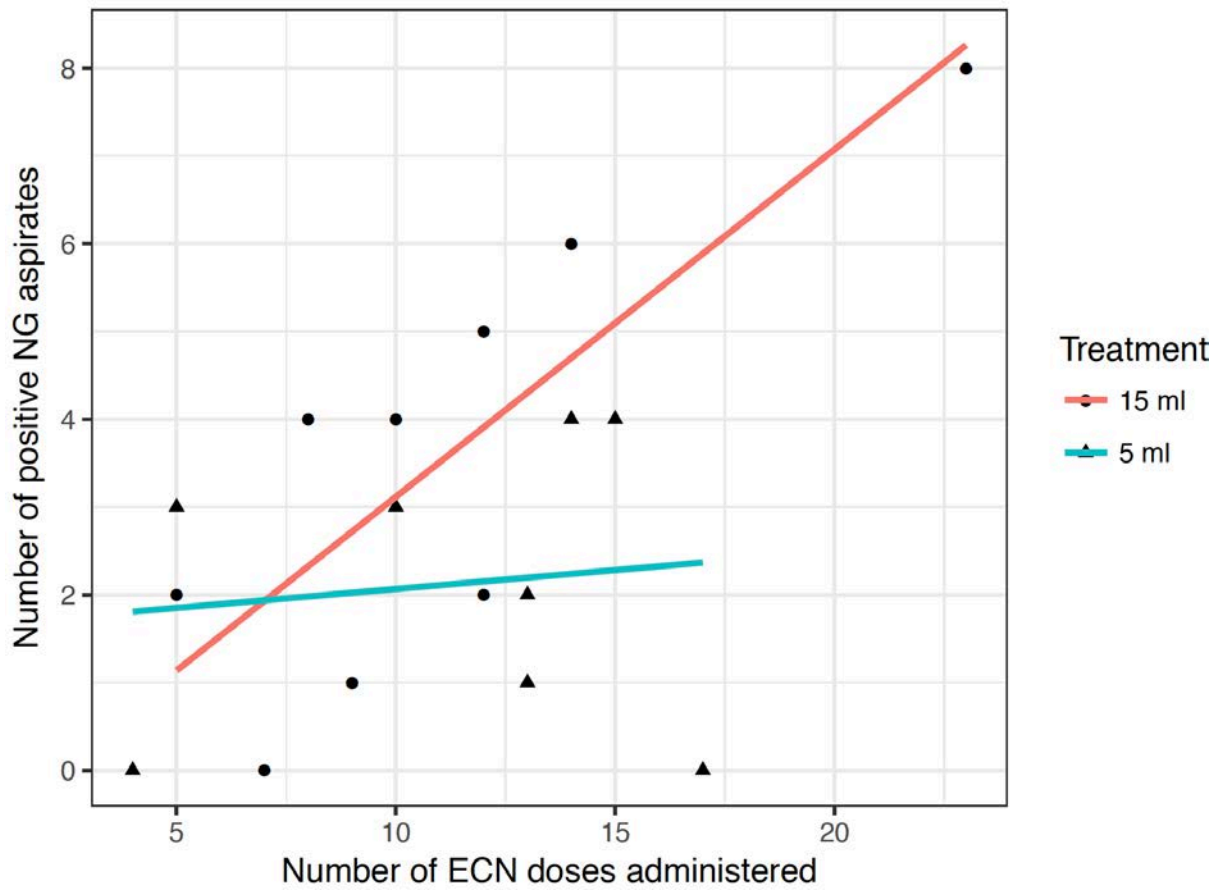


**Figure 3.4 Kaplan-Meier Survivorship curves for treatment *versus* control**

### 3.3.7 Administration and sampling

Obtaining NG aspirates is not without technical difficulty and the presence of a nasogastric tube does not guarantee that a sample can be obtained. It can also be a time-consuming process. Despite the difficulties of NG sampling 180 samples were obtained out of 186 planned samples. Sampling was discontinued electively in two patients, one from the control group (study ID 14) and one from the 15ml *b.d.* group (study ID 26), as it was felt unlikely to yield further useful results.

As a surrogate measure of colonisation, a best fit line was drawn between the number of ECN doses administered and the number of ECN positive NG aspirates for both treatment groups, using the Least Square method. This shows a positive correlation between duration of dosing and number of ECN positive gastric aspirates for the 15 ml ECN group and a weakly positive correlation for the 5 ml ECN group (figure 3.5).



**Figure 3.5** Lines of best fit for number of ECN doses administered and number of positive gastric aspirates for 5 ml and 15 ml ECN treatment groups



## **Chapter 4**

### **General Discussion and Conclusions**

#### 4.1 Discussion

Hospital acquired infections continue to present a significant challenge to healthcare professionals worldwide. In England, there are approximately 100,000 HAI's per year which lead to approximately 5,000 deaths and have a significant financial impact on the NHS – an institution already under enormous strain. Approximately 25% of these occur in ICU patients and arise as a complication of critical illness. Hospital acquired infections in the critically ill population have been the focus of much attention in recent years, in particular, ventilator associated pneumonia.

Up to 30% of ICU patients develop a ventilator associated pneumonia, increasing length of stay, morbidity, mortality and cost. VAP is a nosocomial pneumonia that develops more than 48 hours after tracheal intubation and mechanical ventilation (8). VAP occurs because potentially pathogenic bacteria from the sinuses, oropharynx, subglottis and gastrointestinal tract gain access to the bronchial tree. This is facilitated by the presence of the tracheal tube, which allows microaspiration of secretions and biofilm formation, by-passing the body's natural defences (9). The elegant study by Garrouste-Orgeas *et al* examines the relative contribution of gastric and oropharyngeal colonisation and the role of these organisms in the development of VAP (227).

Although there is no doubt about the importance of VAP and its sequelae, it is a diagnosis that exists without a clear consensus definition. It is based on a combination of clinical, radiological and microbiological criteria but no combination of these has, thus far, been felt to offer enough diagnostic accuracy or confidence to justify their use in its definition. The lack of a universally accepted definition is a major obstacle in the diagnosis of VAP. It also hampers efforts to

monitor incidence, make meaningful comparisons of studies into prevention and in examining the implementation of these techniques.

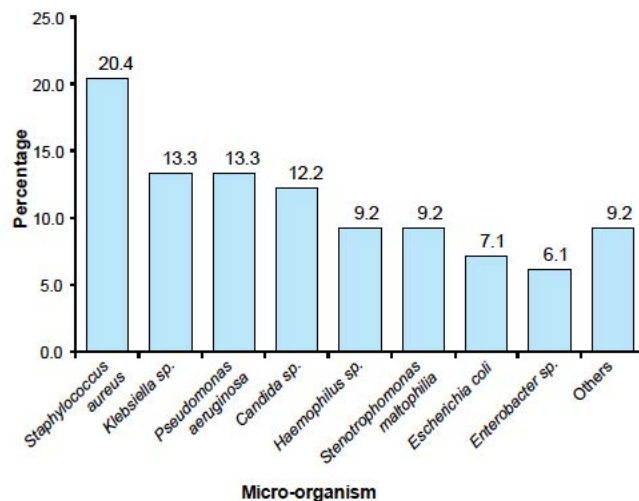
Khan *et al* make an interesting observation about the term itself, “ventilator associated pneumonia” (228). The term suggests a casual rather than *causal* association between invasive ventilation and VAP. The authors argue that VAP conveys an almost incidental relationship between invasive ventilation and the development of pneumonia. In fact, ventilation is really the “driving force” behind the pathology and a more accurate and appropriate term would perhaps be “ventilator induced pneumonia”.

The vast majority of cases of VAP are bacterial in origin, with approximately 60% of organisms being Gram-negative. Fungal and viral VAP can occur but their relevance and pathogenesis are unclear (22).

The organisms commonly associated with VAP are detailed in table 4.1 which is taken from the 2002 publication by Chastre and Fagon (22). The organisms were isolated by bronchoscopic techniques in 24 international studies over the period 1989-2000. These data may give the impression that VAP is caused by a single organism, when in fact it may be polymicrobial.

There is a paucity of data from the UK regarding national VAP rates and causative organisms. Data from Scottish ICUs is presented in the Intensive Care Unit Associated Infection National Surveillance Programme Pilot Report (2011) from NHS Scotland. In this document 117

episodes of HAP were examined, 91.5% of which were VAP. The distribution of organisms is shown in figure 4.1.



**Figure 4.1 Distribution of organisms isolated from cases of pneumonia from Scottish ICUs (229)**

There are some parallels with the international data shown in table 4.1, with *Staphylococcus aureus* and *Pseudomonas aeruginosa* being the predominant species in both series. Interestingly, *Candida* species appear to be much more prominent in the Scottish data.

An important factor when considering the likely causative organism or organisms in VAP is the duration of mechanical ventilation. An episode of VAP that develops within four days of the commencement of invasive ventilation is likely to be caused by a community acquired organism, for example, *Haemophilus* species, *Streptococcus pneumoniae* or methicillin sensitive *Staphylococcus aureus*. Episodes of VAP developing after this time are more likely to be caused by multi-drug resistant species such as *Pseudomonas aeruginosa*, *Acinetobacter* species and methicillin resistant *S. aureus* (14). However, a confounding factor may be recent

contact with the healthcare system or environment, for example, regular attendance at a haemodialysis unit or being resident in a nursing home. These patients are more likely to develop infections with multidrug resistant bacteria (11).

Pathogen	Gram Stain	Frequency (%)
<i>Pseudomonas aeruginosa</i>	Negative	24.4
<i>Acinetobacter</i> species	Negative	7.9
<i>Stenotrophomonas maltophilia</i>	Negative	1.7
Enterobacteriaceae*	Negative	14.1
<i>Haemophilus</i> species	Negative	9.8
<i>Staphylococcus aureus</i> †	Positive	20.4
<i>Streptococcus</i> species	Positive	8.0
<i>Streptococcus pneumoniae</i>	Positive	4.1
Coagulase-negative staphylococci	Positive	1.4
<i>Neisseria</i> species	Negative	2.6
Anaerobes	n/a	0.9
Fungi	n/a	0.9
Others (<1% each) ‡	n/a	3.8

\* Distribution when specified - *Klebsiella* 15.6%; *E. coli* 24.1%; *Proteus* 22.3%; *Enterobacter* 18.8%; *Serratia* 12.1%; *Citrobacter* 5.0%; *Hafnia alvei* 2.1%

† Distribution when specified - methicillin-resistant *S. aureus* 55.7%; methicillin-sensitive *S. aureus* 44.3%

‡ Including *Corynebacterium*, *Moraxella*, and *Enterococcus*

**Table 4.1 Distribution of organisms isolated from cases of ventilator associated pneumonia by bronchoscopic techniques – International data (22)**

The organisms associated with VAP on a given ICU will also depend on the case mix and nature of admissions, the hospital itself and patient comorbidities (22). As part of good institutional antibiotic stewardship, it is essential to monitor the profile of organisms associated with VAP to ensure that the most appropriate empirical antibiotic therapy is used.

As described above, the bacteria responsible for the development of VAP are predominantly Gram-negative, and with growing concerns about the rise of multidrug-resistant strains, we must try to move away from reliance on antimicrobials for the treatment of VAP and continue to explore non-antibiotic strategies in its prevention.

Prevention of VAP is a significant part of ICU “housekeeping” and its importance is highlighted by the guidance from the National Institute for Health and Clinical Excellence. Although much emphasis is placed on this, many studies looking at strategies and interventions to reduce VAP incidence fail to demonstrate convincing clinical benefit in terms of duration of mechanical ventilation, length of ICU admission or mortality. This is undoubtedly, at least in part, linked to the difficulties of accurate diagnosis discussed before.

VAP prevention strategies focus on three areas – reducing colonisation of the aerodigestive tract with potentially pathogenic organisms, prevention of aspiration into the bronchial tree and minimisation of the duration of mechanical ventilation.

With the focus on the microbiological aspects of VAP, it is easy to overlook the deleterious effect of invasive ventilation on the capillary endothelium, epithelium and basement membrane and the ensuing inflammatory response which is inextricably linked to the development of

pneumonia. However, in terms of modifiable risk factors, manipulation of the microbiological milieu of the aerodigestive tract is a strategy that may yield more immediate results, hence the consideration of probiotic therapy in this quest.

Although the mechanisms underlying the putative protective effects of probiotics in critically ill patients remain to be elucidated, there is evidence that probiotics could inhibit colonisation of pathogenic bacteria and enhance immunity. It is clear that there are multiple mechanisms by which different probiotic bacteria may exert their effects and these effects vary with the strain and population studied (230).

The Cochrane systematic review, Probiotics for preventing ventilator-associated pneumonia, by Bo *et al* found that probiotics significantly reduced the incidence of VAP (184). In the per-protocol analysis of probiotics versus control and their effect on incidence of VAP eight relevant trials were analysed. There was a significant difference between various probiotic treatments and their respective control groups for the incidence of VAP ( $p=0.02$ , OR 0.70, 95% CI 0.52-0.95), albeit from evidence graded as low quality.

Interestingly, despite the range of beneficial effects which appear to be offered by probiotics in individual studies, the systematic review did not demonstrate any additional benefit in terms of the other outcomes measured - ICU mortality, in-hospital mortality, incidence of diarrhoea, length of ICU stay, duration of mechanical ventilation or antibiotic use.

As the ICU population is relatively immunocompromised, it was felt that these patients may be vulnerable to developing probiotic-related diseases or complications, in particular diarrhoea.

There were no reports of any nosocomial probiotic infections in the included studies (184). In making this observation, one must be cognisant of the fact that outcomes such as adverse events, duration of mechanical ventilation and antibiotic use, were underreported in many of the studies, making it difficult to draw firm conclusions even from the Cochrane review.

Bo *et al*'s main findings are in agreement with the previous meta-analyses by Siempos *et al* (186) and Petrof *et al* (195), but contradicted the results of the meta-analysis by Gu *et al* (196). There were several important differences between these meta-analyses which were highlighted and discussed in section 1.5.5, all of which lends weight to the argument for an adequately powered and methodologically sound study of the use of probiotics in the context of VAP prevention.

The existing evidence for the beneficial effects of probiotics in a range of clinical situations, coupled with the lack of a consistent message from the studies on probiotic prevention of VAP, lead our group to discuss the use of a *single* probiotic species in a feasibility study in the ICU population.

*Escherichia coli* Nissle 1917 was selected as it had already been studied extensively in other contexts and had an excellent safety record. In addition to its immunomodulatory effects, *E. coli* Nissle is known to possess strain-specific characteristics which enable it to readily colonise the gut and inhibit colonisation by other Gram-negative organisms, specifically *Enterobacteriaceae*. We suggested that administration of *E. coli* Nissle to invasively ventilated ICU patients had the potential to reduce or eliminate gastric colonisation by pathogenic Gram-negative bacteria and theoretically reduce the incidence of VAP in this population. We



proposed that twice daily nasogastric administration of 5 ml or 15 ml of *E. coli* Nissle suspension to invasively ventilated ICU patients would lead to successful gastric colonisation by this species, and significantly reduce ICU acquired gastric colonisation by pathogenic Gram-negative bacteria compared to non-treatment controls.

The group had previously demonstrated successful bacterial culture from 20 gastric aspirate specimens (unpublished data). Eleven patients were included in this group, with a median duration of mechanical ventilation of 7.5 days. These samples were collected from 11 patients, 10 of whom had been treated with broad-spectrum antibiotics. Bacterial colonisation was demonstrated in 95% of specimens with 50% having a heavy growth of Gram-negative bacteria, and 45% having a moderate growth of Gram-positive bacteria. This demonstrated that culture of gastric aspirates was possible with standard microbiological techniques in our laboratory and was an acceptable method of demonstrating gastric colonisation in a feasibility study.

Based on the historical admission data from our ICU as detailed in section 2.4, we anticipated recruiting 3 patients per month into the study. We failed to reach our recruitment target by the end of 12 months and due to time constraints and the logistical difficulties of obtaining the probiotic via a third-party importer, it was decided by the group to terminate the study at this point.

The likely duration of mechanical ventilation (>48 hours or not) was determined by the treating consultant of the day. Of the 27 patients recruited, three were ventilated for less than 48 hours – due to extubation earlier than anticipated, rather than death. It is recognised that intensivist

prediction of duration of mechanical ventilation is unreliable, particularly in the early part of the admission and this was a drawback of the study (220).

None of the patients recruited to this study was a planned admission. Planned admission of an invasively ventilated patient is uncommon in our ICU. Therefore, all of the patients in the study were unplanned or emergency admissions. This posed a degree of difficulty as patients had to be recruited within 24 hours of intubation, and hence we were unable to recruit some admissions which occurred out of hours or at weekends.

During the PECaN-ED study, one patient (study ID 11, 15ml ECN group) was also enrolled into the treatment arm of another study looking at weaning from mechanical ventilation. This resulted in an earlier than anticipated extubation. It is difficult to determine if this would have had any implication for the results obtained, however, it would be an important consideration for co-enrolment in any future studies.

As with any trial involving ICU patients, consent was ideally obtained from a personal legal representative. Again, due to the timing of unplanned or emergency admissions and the necessity to recruit within 24 hours of intubation, we did encounter some difficulties in obtaining consent from personal legal representatives in a timely fashion.

Although consent was declined in some instances, this was in a minority of cases and, in general, the study was well received by the personal legal representatives we approached. Although it was clearly explained that the use of probiotics in ICU patients was a relatively novel technique, the general perception appeared to be that this was a benign intervention, with

many people making reference to commercially available probiotics and expressing a genuine interest in the proposed benefits and potential mechanism of action in ICU patients.

It is important to acknowledge the contribution of the ICU nursing staff both in terms of probiotic administration and sample collection. Training on the study was delivered to assist the nurses in this role. As an investigational medicinal product (IMP), the probiotic suspension was kept in a locked refrigerator, administered twice daily and required a two person check for administration. This did impose to some extent on the ICU nurse workload and their help in administration was greatly appreciated.

Both administration of the probiotic suspension and sample collection had to be done with a great deal of care due to the possibility of cross contamination. The probiotic was presented as a concentrated suspension of bacteria which, if not handled carefully, could have contaminated other research or clinical samples.

Obtaining gastric aspirate samples from nasogastric tubes at times proved difficult. Although gastric aspirates are measured routinely as part of ICU care – to determine if the patient is absorbing enteral feed – they are not always readily obtained. If it was not possible to aspirate gastric contents freely from the nasogastric tube, a number of techniques were tried in order to facilitate this. These techniques included waiting for 20-30 minutes and re-attempting aspiration, tilting the patient to the left and/or right-hand side and re-attempting aspiration, or instilling 10-20 millilitres of normal saline and then re-attempting aspiration. If the sample was obtained after instillation of normal saline, this was taken into account when the

microbiological processing took place. Despite these difficulties, 180 out of 186 planned samples were obtained.

Prior to the discovery of *Helicobacter pylori* in 1982, the stomach had been regarded as a “sterile” environment, inhospitable to bacteria due to acid production, peristaltic activity, thick mucus layer and the presence of bile acids from the small intestine. The difficulties in obtaining samples and the limited techniques available for analysis also hampered study of the gastric microbiome. However, the discovery that acid-resistant strains of bacteria were present in the stomach, together with the advent of a range of culture-independent molecular techniques based on 16S rRNA gene sequencing, has led to renewed interest in the gastric microbiota in health and disease (231).

In recent years molecular techniques have been used to analyse the gastric mucosa and microbiota of small groups of healthy volunteers. The microbiota is obviously affected by dietary intake, medication, mucosal inflammation and *H. pylori* effect but, at a phyla and genera level, appears to be surprisingly similar between races (232–235).

With regard to the gastric microbiota in the ICU population, factors of particular relevance are the use of broad-spectrum antibiotics and gastric acid suppression. Gastric acid suppression with either PPIs or H2RAs can significantly alter the profile of the gastric microbiota. Bacterial overgrowth in the stomach will occur when the gastric pH>3.8 (236); anecdotally, many of the gastric aspirates in the ICU population are approximately pH 6.0. Use of antibiotics will also cause changes in the gastric microbiota – for example, treatment with a third-generation

cephalosporin has been shown to cause a significant reduction in the number of *Lactobacilli* and overgrowth of *Enterococci* (237).

Although molecular techniques are becoming the norm in the exploration of the microbiological profiles of various physiological environments, the stomach of the ventilated ICU patient remains a relatively uncharted territory, although some work has been done on the intestinal bacterial composition of these patients (238).

The technique used previously to investigate gastric colonisation in patients relied on traditional culture-based methods. These have been reproduced in a number of studies; the assumption being that the presence of an organism on gastric culture signifies colonisation (104,128,171,189). However, as over 80% of microorganisms are non-culturable, the identification of bacterial species by conventional methods provides an incomplete and not unbiased view of the gastric microbiota. Furthermore, the study of gastric aspirate fluid alone does not allow us to consider the bacterial colonisation of the gastric mucosa. This not only overlooks the *presence* of specific organisms, but fails to take into account bacterial-host cross-talk (231).

The PECaN-ED study was carried out prior to the current Intensive Care Society recommendations on the use of stress ulcer prophylaxis as part of the ventilator care bundle (9) and the widespread use of both PPIs and H2RAs is clearly shown in the individual patient timelines in appendix III. Although previously considered a relatively innocuous therapy, the impact of acid suppression may be more far-reaching than first anticipated. While detailed discussion is outside the scope of this thesis, it is still important to mention three relevant

aspects of this: There are acid-producing cells throughout the upper aerodigestive tract in humans, the impact of acid suppression on which is unclear; PPIs will have an effect on naturally occurring gut bacteria which possess the  $H^+/K^+$ -ATPase pump; and the gastric microbiota will be altered as a result of the change in pH (236).

Colonisation of the airway is an essential step in the development of a VAP as a result of microaspiration of gastric or oropharyngeal colonised with potentially pathogenic organisms (32). The term colonisation is widely used in related literature but is infrequently and variably defined. The trial by Garrouste-Orgea *et al* uses precise definitions of colonisation and infection: Colonisation at the time of ICU admission was defined as the isolation of at least one microorganism from the oropharynx and/or stomach at a concentration of  $\geq 10^2$  CFU ml<sup>-1</sup> within the first 48 hours of admission. Colonisation was considered to be ICU-acquired only when there was no colonisation on admission and when the same potentially pathogenic microorganism at a concentration of  $\geq 10^2$  CFU ml<sup>-1</sup> was detected from at least two consecutive samples from the same site (227). In contrast, Morrow *et al* used presence of an organism at a single time point to define colonisation (104).

In the trial by Garrouste-Orgea *et al* the diagnosis of VAP was made using the association of clinico-radiological criteria and a positive quantitative culture  $\geq 10^3$  CFU ml<sup>-1</sup> (protected specimen from plugged catheter or telescopic brush catheter). When protected samples were positive but below the  $10^3$  CFU ml<sup>-1</sup> threshold, these episodes were diagnosed as distal bronchitis (227).

Using culture-based techniques for determination of gastric colonisation, we demonstrated gastric colonisation with *E. coli* Nissle in those patients whom had received the IMP. The technique used does have certain drawbacks, primarily the assumption that the presence of *E. coli* Nissle, without quantification, in two consecutive gastric aspirate samples represents colonisation. This may only represent a transient population of *E. coli* Nissle in the stomach or that the stomach is effectively acting as a “sump” of *E. coli* Nissle which is simply being sampled periodically. It also overlooks the effect at a mucosal level and gives no indication of bacterial-mucosal interaction or potential mucosal colonisation.

We are also, of course, making the assumption that true colonisation is actually necessary for the probiotic organism to exert a beneficial effect - some benefit may come simply from the presence of the probiotic organism in the stomach due to regular administration via the nasogastric tube.

Another potential drawback of this sampling technique is the risk of biofilm formation on the internal surface of the nasogastric tube. This poses the risk that sampling and administering through the same lumen may lead to false positive results. The counter-argument to this is that study patients were all receiving enteral nutrition, and the continuous delivery of feed via the nasogastric tube should mitigate against this.

It is, nevertheless, an interesting achievement that we were able to demonstrate the presence of culturable *E. coli* Nissle in an, albeit acid-suppressed, non-native environment with a high frequency of broad-spectrum antibiotic use.

In the analysis of gastric aspirates, the predominant species was identified at baseline and after 48 hours. The proportion of Gram-negative organisms remained relatively constant in the post 48-hour control and 5ml ECN groups (26.7% & 22.0% vs 22.2%). However, the proportion of Gram-negative organisms decreased to 4.3% in the 15ml ECN group. A statistically significant difference in the proportion of Gram-negatives was identified between the post 48-hour 15ml ECN and control groups ( $p<0.01$ ), and post 48-hour 15ml ECN and 5ml ECN groups ( $p=0.01$ ). The overall proportion of potential pathogens in the gastric aspirates, both Gram-positive and Gram-Negative decreased to 15.2% in the post 48-hour 15ml ECN group, which represented a significant difference between the 15ml ECN group and baseline ( $p<0.01$ ).

This appears to show that, after 48 hours, in the higher dose group *E. coli* Nissle was becoming the predominant species in the gastric aspirate samples, both in terms of Gram-negatives and overall species. The reasons for this may include out-competing other bacteria or a direct effect on other bacteria such as bacteriocin production - the microcins M and H 47 produced by *E. coli* Nissle could help it compete against bacteria that utilise catecholate siderophores such as the Enterobacteriaceae (239). *Escherichia coli* Nissle may also out-compete pathogenic bacteria for essential nutrients (240).

In the analysis of the OP aspirates, there was no significant difference between the proportion of pathogenic Gram-negatives in the control, 5ml ECN or 15 ml ECN groups compared to baseline ( $p=0.99$ ,  $p=0.28$  and  $p=0.85$  respectively). At the time of this study, chlorhexidine gel was still being routinely used orally as part of the ICU ventilator care bundle. This is no longer a recommendation in the general ICU. Although samples OP samples were collected prior to administration of the chlorhexidine gel, this could still have had a bearing on the results.



The results for the post 48-hour tracheal aspirates showed that potential Gram-negative and Gram-positive pathogens persisted through the three groups but, interestingly, there was a higher proportion of potentially pathogenic Gram-negative organisms in the 15ml ECN group (28.9%) compared to baseline (14.8%) and compared to the 5ml ECN (9.4%) and control groups (19.4%). There was no statistically significant difference between the control, 5ml ECN or 15ml ECN and baseline, however, this relative increase in potential Gram-negative pathogens did occur despite a significant reduction in the Gram-negatives in the gastric aspirates. It does emphasise that we have an over-simplistic view of the mechanisms at play in the development of VAP. *Escherichia coli* Nissle was detected in two tracheal aspirates from the treatment groups, which may represent the expected passive aspiration of gastric contents into the bronchial tree.

Commercially, *E. coli* Nissle is available as lyophilised bacteria in an enteric coating, with each capsule containing  $2.5\text{--}25 \times 10^9$  CFU. The recommended adult dose is 2 capsules per day (up to a maximum of 4 capsules per day) for chronic constipation or maintenance of remission in ulcerative colitis. Each millilitre of the suspension contains  $1 \times 10^8$  CFU, therefore the doses administered in this study were  $5 \times 10^8$  *b.d.* or  $1.5 \times 10^9$  *b.d.*

Using these doses, we were unable to demonstrate a significant difference in the number of patients colonised or the time to colonisation. As no adverse outcomes were demonstrated, a higher dose could be considered for use in future studies.

The study was not powered to detect a reduction in the incidence of VAP, however, six patients were diagnosed as having microbiologically confirmed VAP, which gives a VAP incidence for

the study population of 25%. In five of the six patients who developed VAP, the causative organism was a Gram-negative species.

Interestingly, three of the VAP patients (study ID 13, 26, 28) were from the 15ml ECN study group. Of the other three VAP patients, two were from the control group (study ID 18, 20) and one was from the 5ml ECN group (study ID 6) (table 3.10). The two patients (20, 26) who developed an *E. coli* VAP were negative for *E. coli* Nissle in all surveillance cultures.

Patient 13 (from the 15ml ECN group) had a single tracheal aspirate in the study samples which was positive for *E. coli* Nissle. *Escherichia coli* was not cultured in any of the clinical tracheal aspirates.

Patient 6 (from the 5ml ECN group) also had a single tracheal aspirate in the study samples which was positive for *E. coli* Nissle. Again, *E. coli* was not demonstrated in any of the clinical tracheal aspirates.

## **4.2 Limitations**

*Escherichia coli* was identified in a number of clinical samples, as discussed at the end of section 4.1. In only one case did this possibly represent an *E. coli* Nissle contaminant which had clinical significance. To avoid the possibility of *E. coli* Nissle inadvertently being treated as a pathogen (false positive) in clinical samples, it should be specifically tested for prior to antibiotic treatment.

By definition, patients enrolled into the study were receiving enteral nutrition. However, this is not without interruption, due to factors such as confirmation or re-confirmation of nasogastric tube position, interruptions to feeding for patient transfer or alteration of enteral feed administration rate due to patient specific factors such as problems with absorption. These factors were not recorded as part of the study protocol and could conceivably have had an effect on the host-probiotic interaction.

A number of the study patients were already admitted to hospital prior to coming to ICU. Changes that may have already taken place in their gut flora due to illness, antibiotic exposure or acid suppression will not have been accounted for in this study. This is a further argument in favour of trials using trauma patients as the population studied, as they will generally be admitted directly from the community avoiding prior hospital admission as a potential confounding factor.

### **4.3 Future work**

It is recognised that ICU admissions due to trauma are an independent risk factor for the development of VAP (20). In the U.S. the National Healthcare Safety Network (NHSN) data show that VAP rates for trauma patients are consistently two to three times higher than the general ICU population. The International Nosocomial Infection Control Consortium (INICC) has shown similar results from the developing world. The INICC report from 2004–2009 showed a VAP rate of 40.0 per 1000 ventilator days in trauma patients, with a corresponding rate of 15.8 per 1000 ventilator days in ICU patients overall. This was also demonstrated in the EU-VAP study with trauma being associated with a higher risk of developing VAP (OR 2.89, 95% CI 2.26–3.69) (241). As this is a less heterogenous group of patients than the general ICU

population, it may be an appropriate population on which to focus probiotic studies in the future. The Cochrane Review by Bo *et al* also highlighted this saying, “there may be a strong rationale for the use of probiotics in trauma patients. Studies in this group of patients showed a significant reduction in the incidence of VAP as well as improved survival”. In addition, the severity of injury may predict a more prolonged period of invasive ventilation.

There is conflicting opinion regarding the attributable mortality due to VAP. Some studies suggest that the attributable mortality is low and that this should be taken into account when using mortality as an end point or when using superiority studies. Outcome measures such as duration of ICU stay, hospital stay and mechanical ventilation would appear to be more relevant and more consistently influenced by VAP (28).

Work has been carried out examining the concentration of antibiotics in gastric secretions and gastric mucosa. Although this has largely been in relation to the antibiotics used for the eradication of *H. pylori*, and also the effect of omeprazole on these, some interesting observations have been made. For example, metronidazole and erythromycin are secreted across the gastric mucosa but ampicillin is not; omeprazole decreases the intragastric concentrations of metronidazole but increases the intragastric concentrations of amoxicillin (242,243). The widespread use of broad-spectrum antibiotics in ICU patients may have an incidental effect on probiotics administered via the nasogastric route, yet this is an area that requires further investigation.

Some simple interventions may aid future study and help with the difficulties of gastric aspirate sampling. The probiotic could be presented as a coloured solution to highlight the risk of

contamination and make handling easier. Use of a double-barrelled nasogastric tube would allow one port for feeding and administration of probiotic and a dedicated port for sampling. This may make sampling more straightforward and would also reduce the chance of contamination of the aspirate by any biofilm formation on the inside of the nasogastric tube.

In our study, *Escherichia coli* Nissle was administered at the lower end of its dose range and a tendency toward a shorter time to colonisation in the higher dose 15 ml ECN group was demonstrated. Further studies using different dose ranges may therefore be warranted.

The methods used to date for determining gastric colonisation are culture-based. In our study we used two consecutive gastric aspirate samples positive for *E. coli* Nissle to signify colonisation. Previous studies have simply used the presence of the probiotic in question in the gastric aspirate as evidence of gastric colonisation. Undoubtedly this technique could be improved, and much could be gained by the use of molecular techniques to obtain a detailed profile, over the period of ventilation, of the gastric microbiome in the ICU patient.

With the advent of culture-independent metagenomic techniques, such as 16S rDNA sequence analysis, we have the ability to obtain much more detailed information about the gut microbiota in critical illness and the impact of probiotic administration. The probiotic studies to date have relied upon culture-based techniques to investigate a complex and dynamic microbial community. As these techniques are now more mainstream and readily available, consideration should be given to their use in future probiotic studies. Current data suggest that, in health, the microbiome of the stomach is dominated by *Prevotella*, *Streptococcus*, *Veillonella*, *Rothia* and *Haemophilus* species. In the *H. pylori*-infected stomach and in gastric

cancer there is a change in this profile with an increase in *Firmicutes*, *Streptococcus* and *Prevotella* species. Gastric acid suppression and gastric atrophy enable the survival and proliferation of microbes that are normally eradicated by gastric acid.

There is also still much to be learned not only about the relationship between the gut microbiota and the host, with the evolving concept of a mutualistic rather than commensal relationship – at the centre of which is the manner in which gut bacteria communicate with the host's immune system and participate in a variety of metabolic processes of mutual benefit to the host and the microbe (244).

The human, animal and *in vitro* studies of probiotics carried out to date exhibit a high level of heterogeneity in the conditions targeted, models used, and probiotics tested. Furthermore, it is likely that we are adopting a simplistic view of the mechanisms of action of the various probiotic species, bearing in mind that specific effects may be strain-related.

Even within the studies which appear to demonstrate the beneficial effects of probiotics, there is considerable heterogeneity with regard to probiotic species used and dosage, route and timing of administration. In future research careful consideration should be given to these factors, as well as greater measures to assess the safety of probiotics.

As discussed in section 1.3.1, ventilator care bundles are routinely employed in ICUs worldwide and yet adherence to these and variation in implementation have not been accounted for in many probiotic studies to date. This could certainly have a bearing on the overall results

in terms of VAP prevention. Any further studies should include ventilator care bundles as part of the methodology.

Further studies of probiotics in ICU patients should also give consideration to examining their immune modulatory effects. *Escherichia coli* Nissle has previously been shown in other study populations to decrease pro-inflammatory cytokines (IL-2, IL-5, IL-6, TNF $\alpha$ , IFN $\gamma$ ) and increase IL-10 levels and defensin synthesis. These effects on the host may be relevant to their mechanism of action and warrant study in more detail.

Relatively broad exclusion criteria were applied to this study as it involved the novel use of a bacterium in a critically ill population. These are in keeping with the criteria employed in the study of other probiotics. Although no adverse events relating to the probiotic were encountered, in my opinion, more extensive testing in this population would be required before relaxing these criteria.

#### **4.4 Conclusion**

Concerns are mounting about multidrug-resistant Gram-negative bacteria and in particular the emergence of *Enterobacteriaceae* with resistance to carbapenems. New antimicrobial agents with which to tackle resistant bacteria are in limited supply. The development of new antibacterial agents will need to be sustained long term to combat the evolution of resistant strains. In the absence of universally effective treatments, preventative strategies such as probiotics should be explored.

The human, animal and *in vitro* studies of probiotics carried out to date exhibit a high degree of heterogeneity in the conditions targeted, models used, and probiotics tested. These studies are likely to reflect an over-simplistic view of the mechanisms of action of probiotic species, as any beneficial effects are likely to occur through a variety of mechanisms with different strains having specific effects.

These studies have many drawbacks including differences in patient groups and bacteria administered; clinical and statistical heterogeneity; study limitations and small sample sizes. Hence, there is insufficient evidence from which to draw firm conclusions on the efficacy and safety of routine use of probiotics for the prevention of VAP in ICU patients. Furthermore, the study of probiotics may have suffered to some extent from the rather extravagant and unsubstantiated claims made to promote them commercially. The pseudoscience surrounding them may have detracted from their potential use as a scientifically-based, therapeutic option. In this study we tested a single probiotic species, *E. coli* Nissle, which has a clear rationale for pathogen displacement. We demonstrated gastric colonisation by this bacterium in a feasible and safe way in selected group of ICU patients. A significant reduction in the burden of pathogenic Gram-negative bacteria in gastric aspirates after 48 hours was demonstrated in the 15 ml *b.d.* treatment group.

We are still far from fully understanding the probiotic-host interaction but given the potential benefits that probiotic bacteria have to offer, further study is warranted. Careful consideration should be given to appropriately powered studies addressing the questions: which probiotic, by what route, in which dose and at what time.



In an interesting twist, *E. coli* Nissle was first isolated in the pre-antibiotic era and used for its ability to combat infection. Its use in this area waned with the advent of antibiotics. With the increased threat of antibiotic resistance, we are once again turning towards Alfred Nissle's original concept.

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# **Appendix I**

## **Approval Letters**

















## **Appendix II**

### **Evidence of Funding**





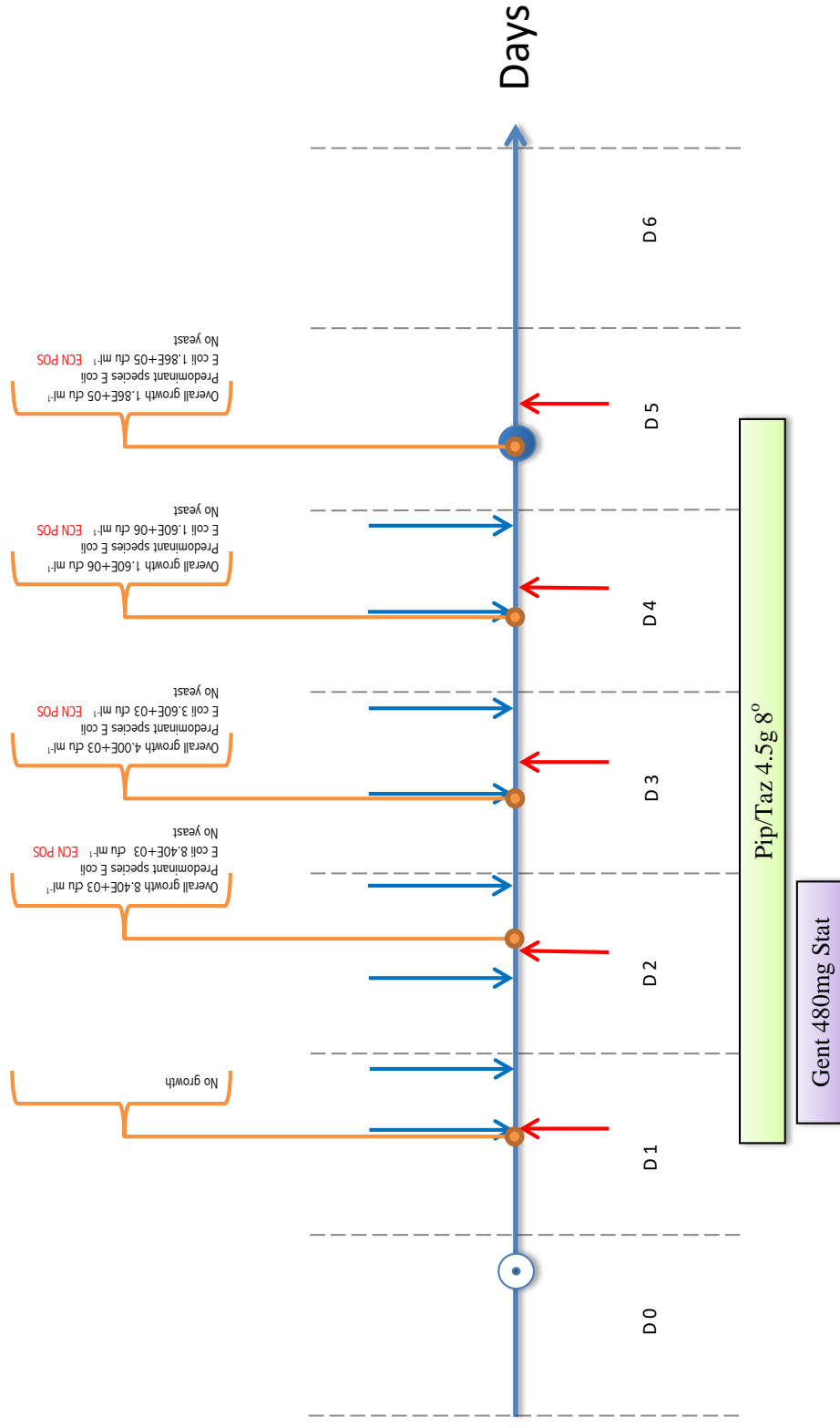


# **Appendix III**

## **Individual Patient Timelines**

Clinical vignette: Male 57 yrs  
Fall & Decreased GCS. Aspiration pneumonia  
PMHx: Sarcoidosis, Hypertension, COPD, Home O<sub>2</sub>

## Timeline Patient 01 - 15ml ECN BD

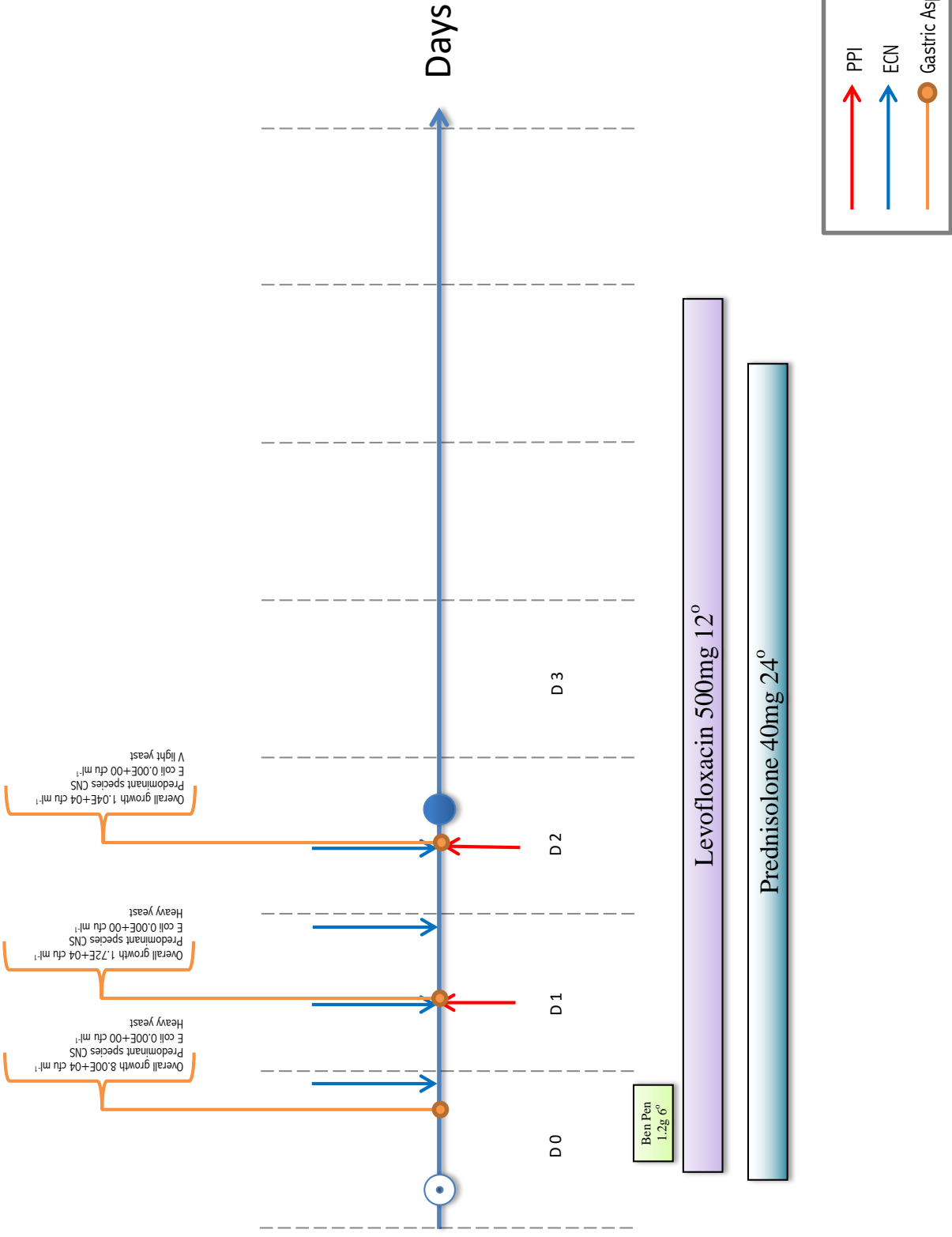


Legend:

- PPI (Red arrow)
- ECN (Blue arrow)
- Gastric Aspirate (Orange dot)
- Intubation (Blue circle with dot)
- Extubation (Blue circle)

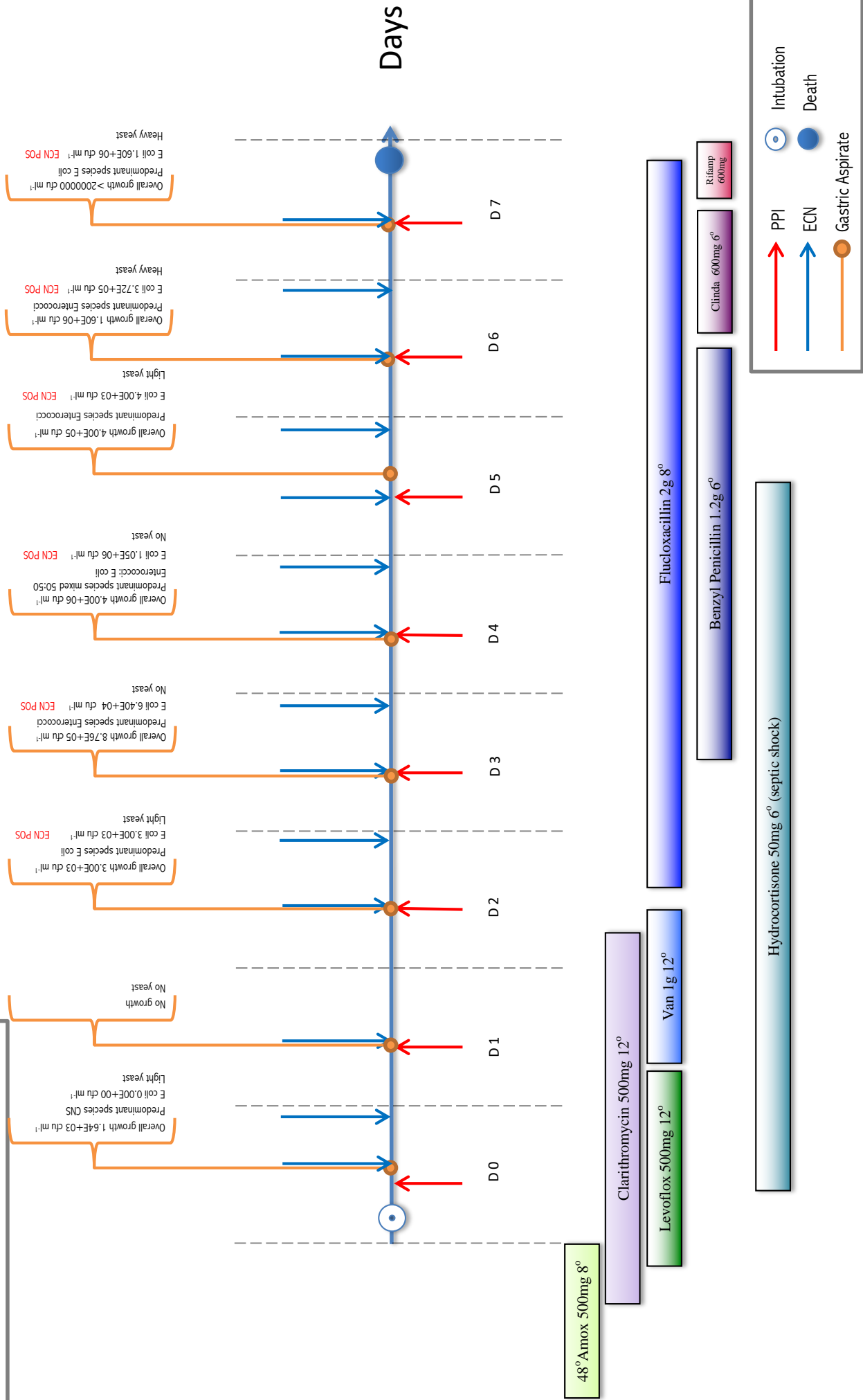
Clinical vignette: Female 57 yrs  
Infective Exacerbation COPD  
PMHx: Hypertension, Smoker

## Timeline Patient 02 – 5ml ECN BD



# Timeline Patient 03 - 15ml ECN BD

Clinical vignette: Female 41 yrs  
Severe Influenza A (H<sub>1</sub>N<sub>1</sub>) pneumonia, bacterial endocarditis, septic shock.  
PMHx: Smoker, Alcohol excess

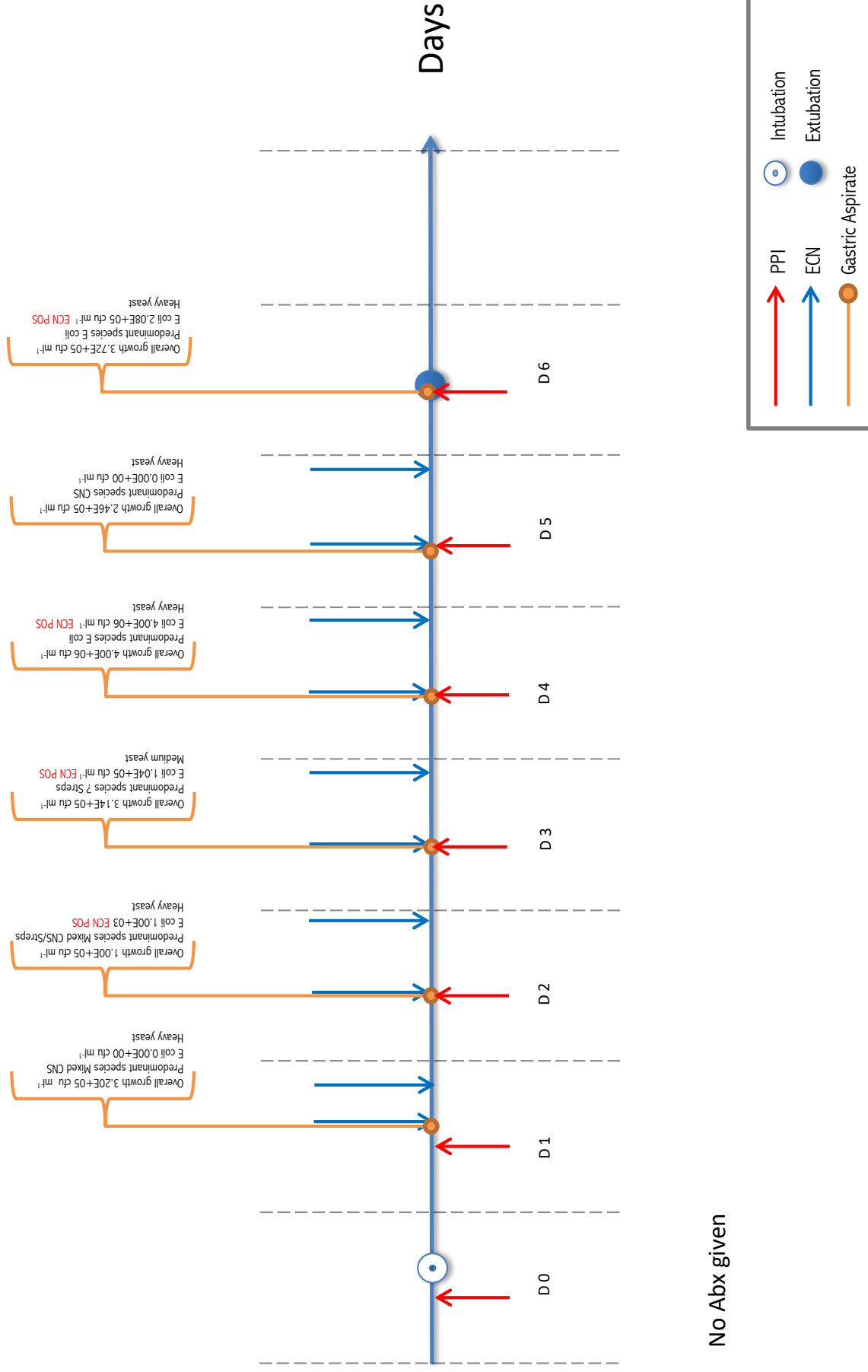


Clinical vignette: Female 43 yrs

Bilat PE.

PMHx: Hypertension, Smoker, Alcohol excess

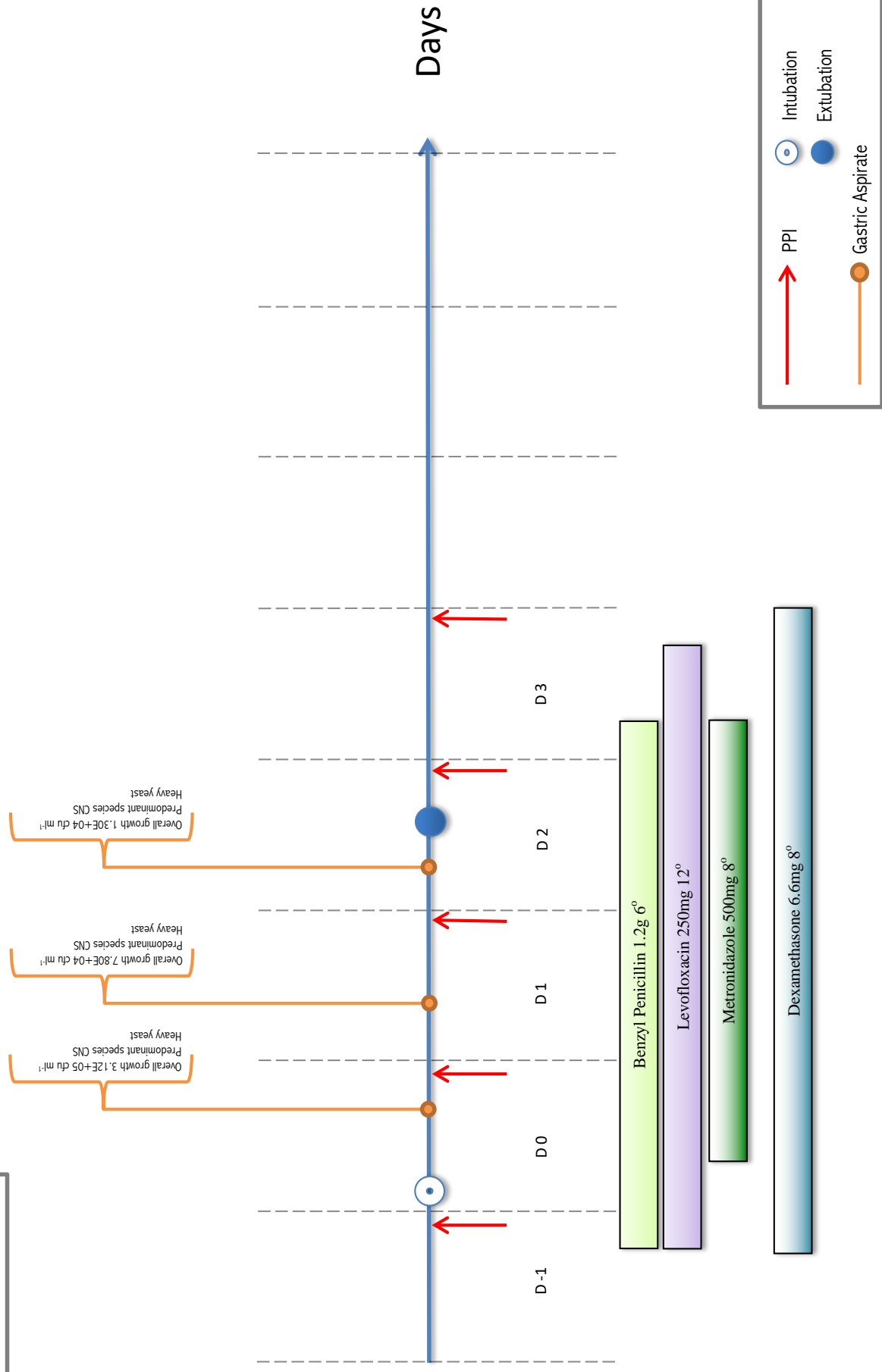
## Timeline Patient 04 - 15ml ECN BD



No Abx given

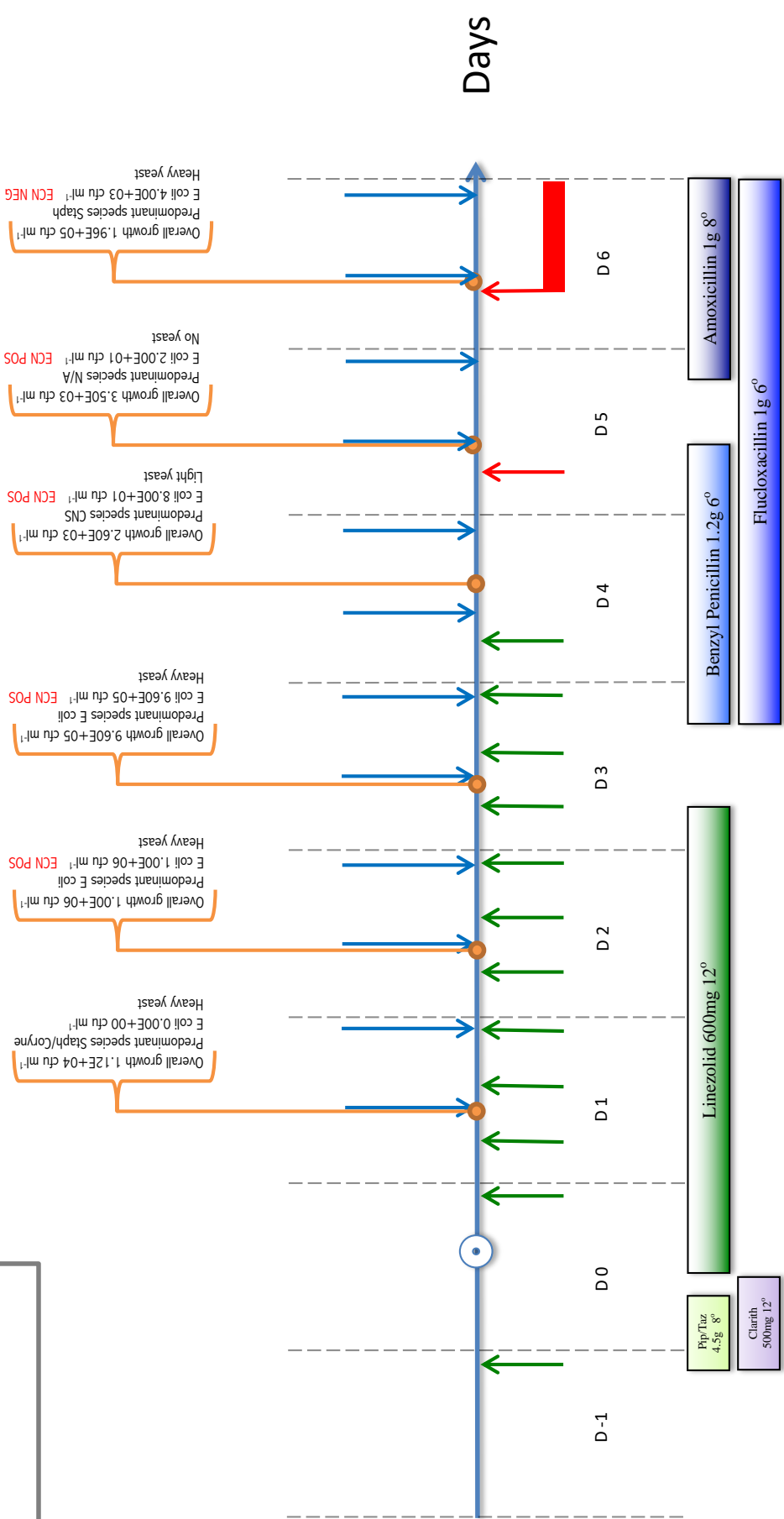
Clinical vignette: Male 82 yrs  
Type II respiratory failure. CAP. Pharyngeal  
oedema, AKI  
PMHx: Hypertension, CKD

## Timeline Patient 05 – Control



Timeline Patient 06 - 5ml ECN BD (a)

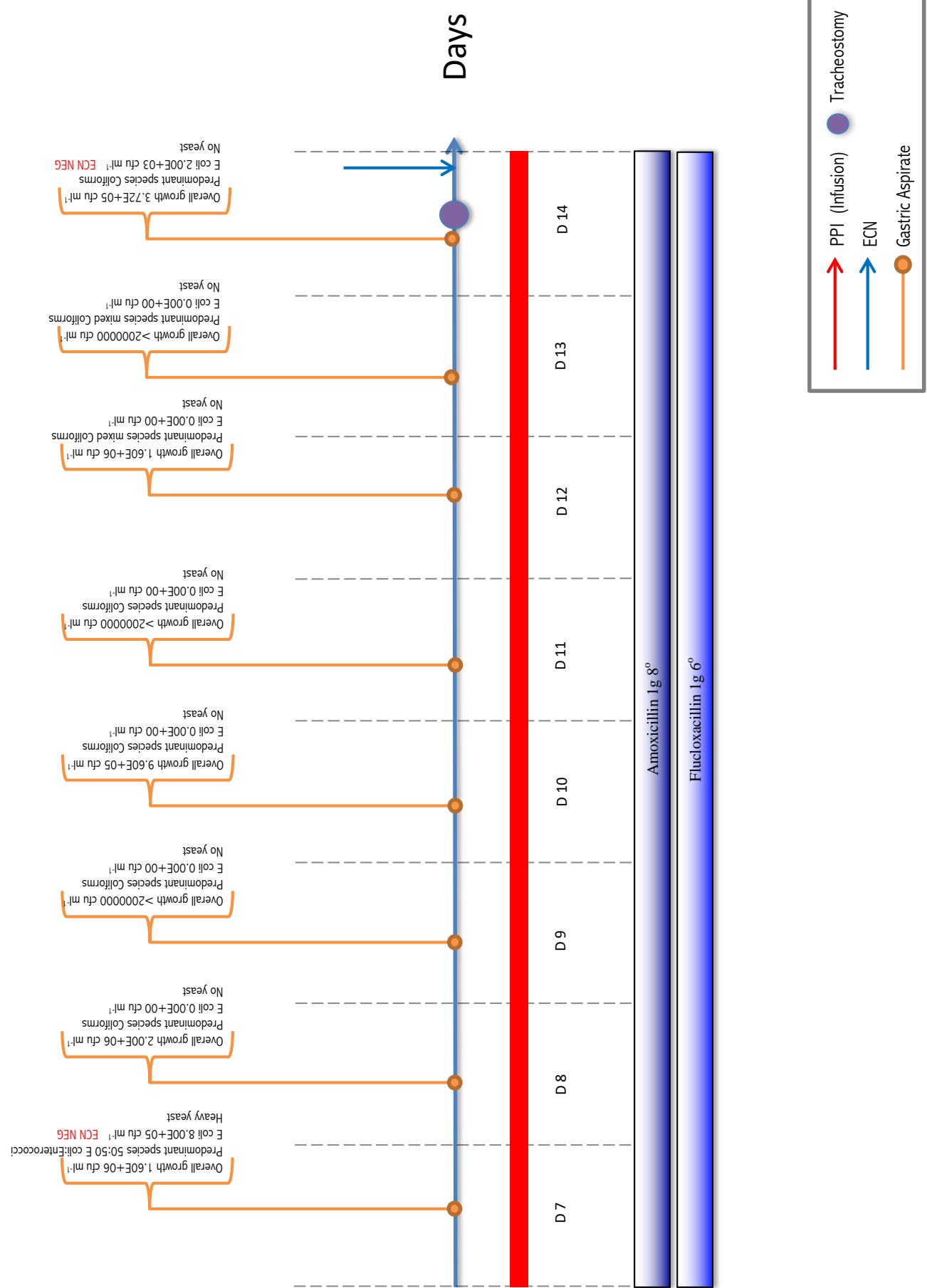
Clinical vignette: Male 68 yrs  
Left Broncho-pleural fistula  
D8 Bleeding from DU -> Laparotomy (ECN paused)  
D15 Ischaemic perforation small bowel.  
PMHx: Smoker



Legend:

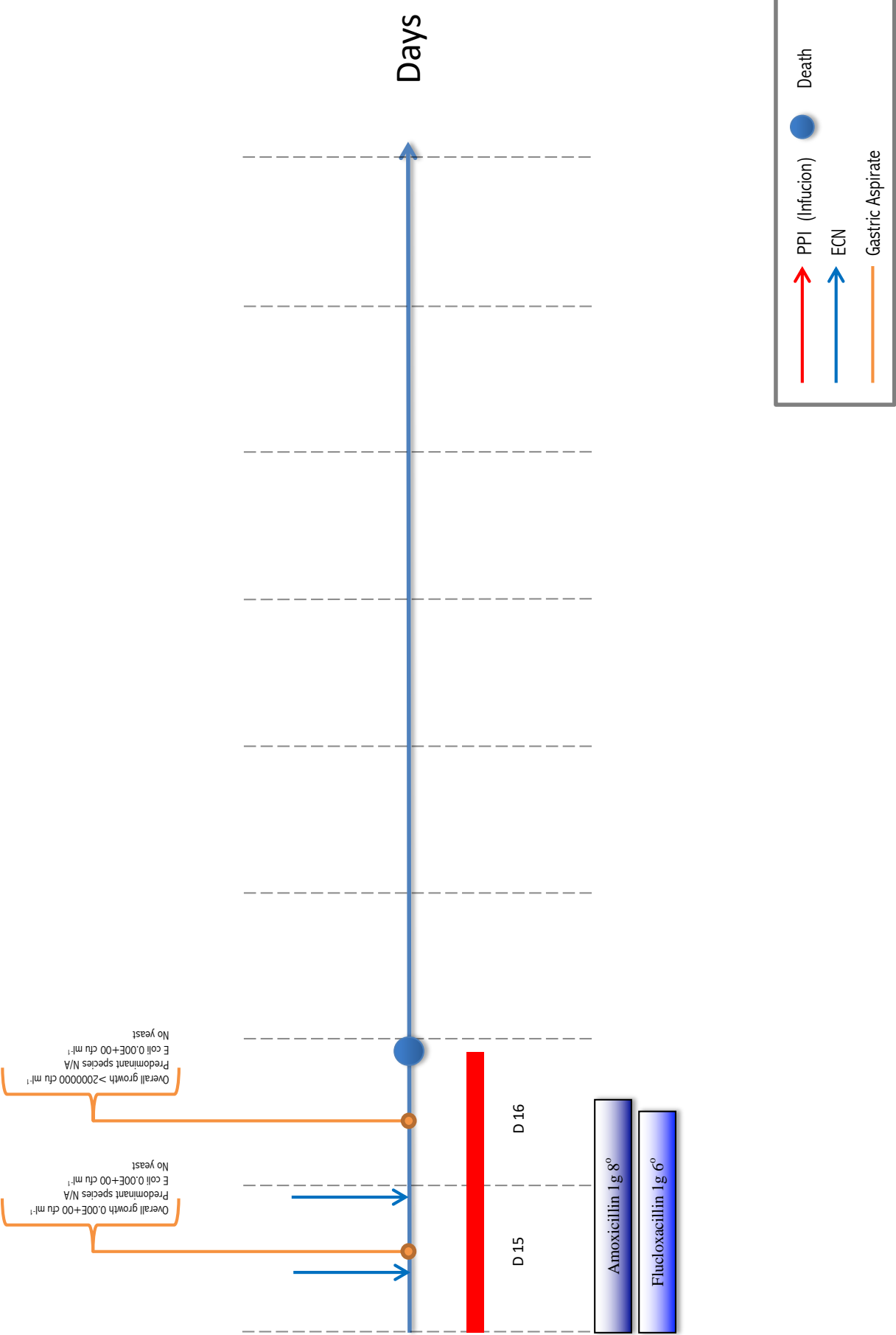
- PPI (Infusion) (Red arrow)
- H<sub>2</sub>RA (Green arrow)
- ECN (Blue arrow)
- Gastric Aspiration (Orange arrow)
- Intubation (Blue circle with dot)

Timeline Patient 06 - 5ml ECN BD (b)



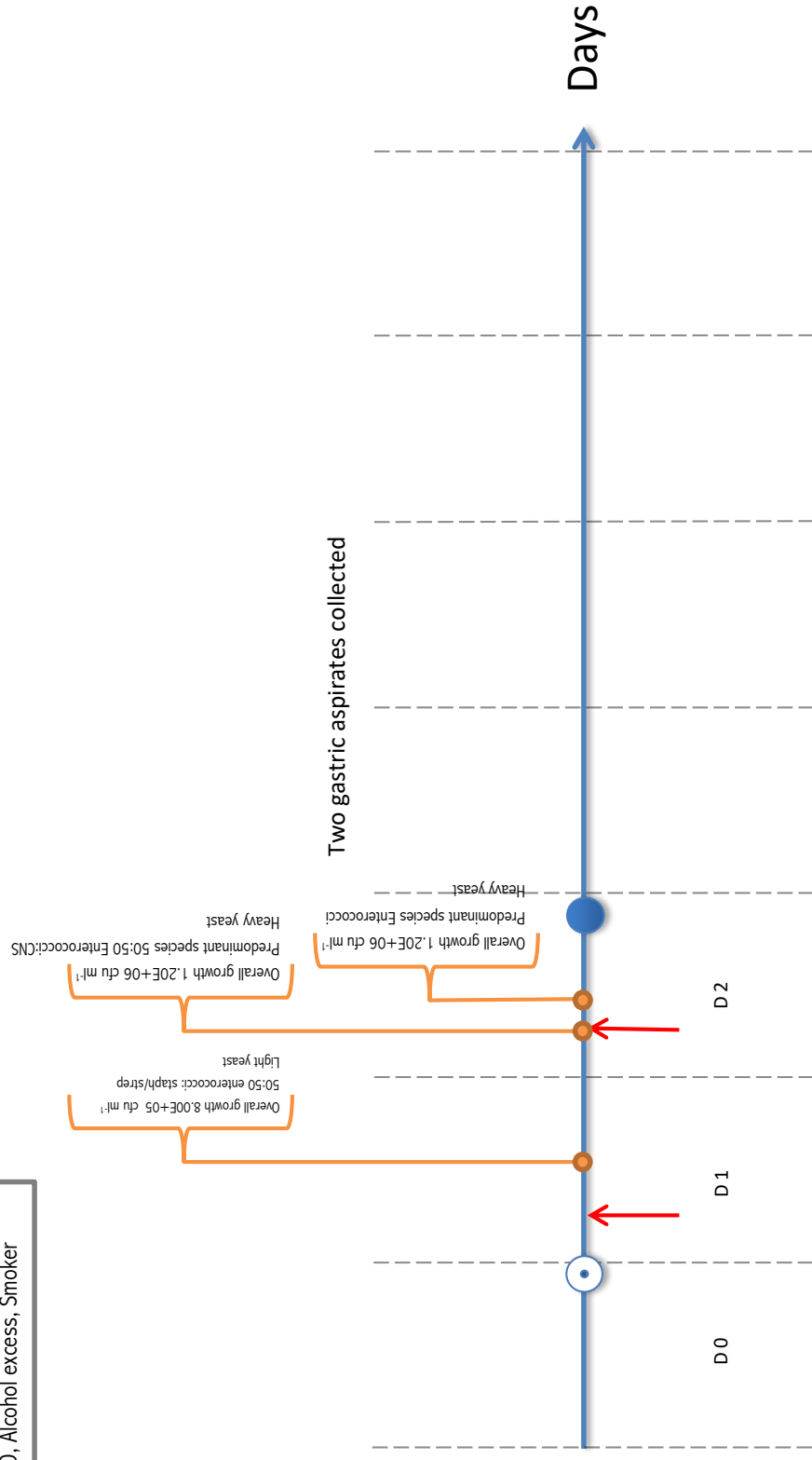


Timeline Patient 06 - 5ml ECN BD (c)



Clinical vignette: Male 74yrs  
OOHCA ? ACS  
PMHx: Type II DM, CKD, Alcohol excess, Smoker

## Timeline Patient 07 – Control

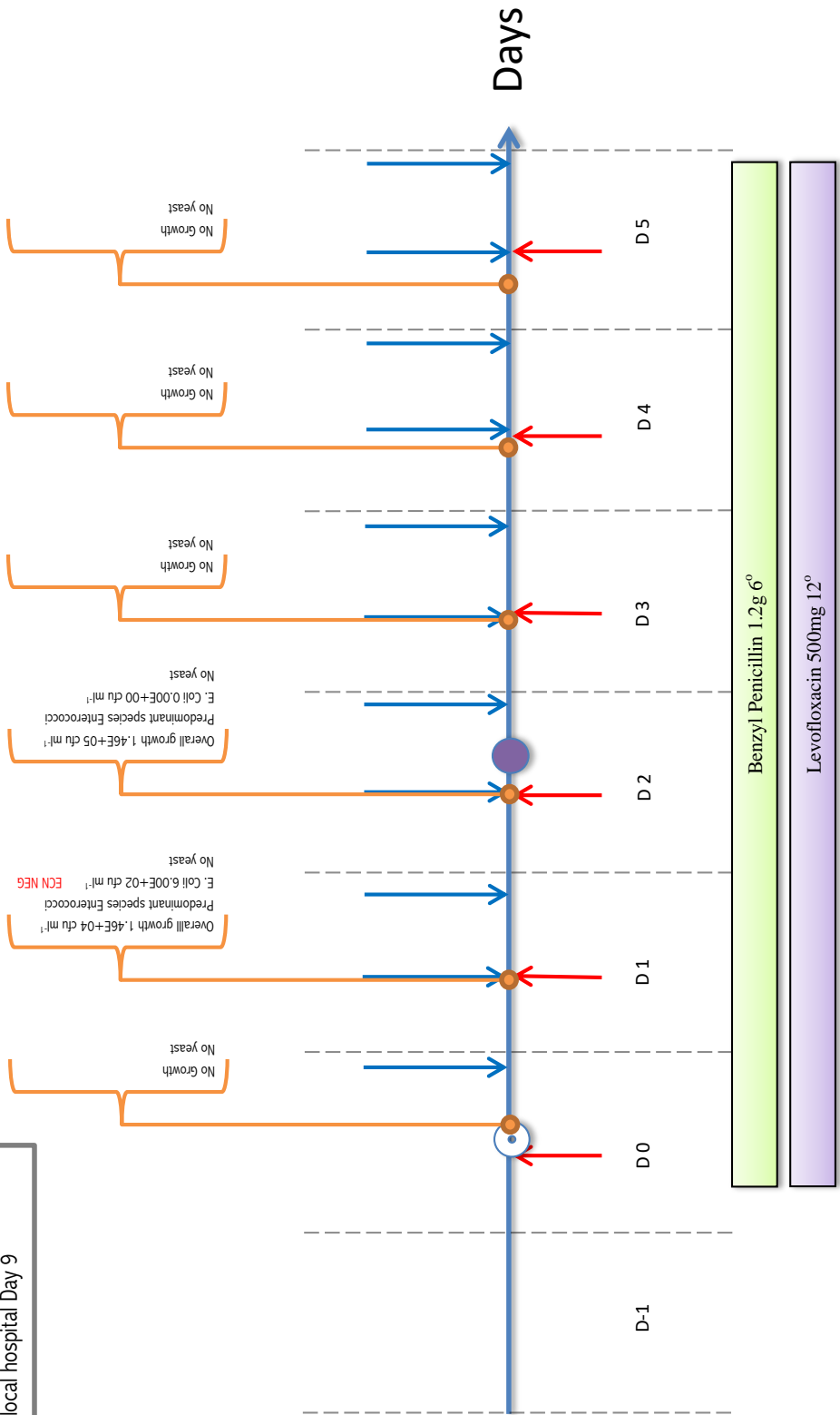


No Abx given



# Timeline Patient 08 - 5ml ECN BD (a)

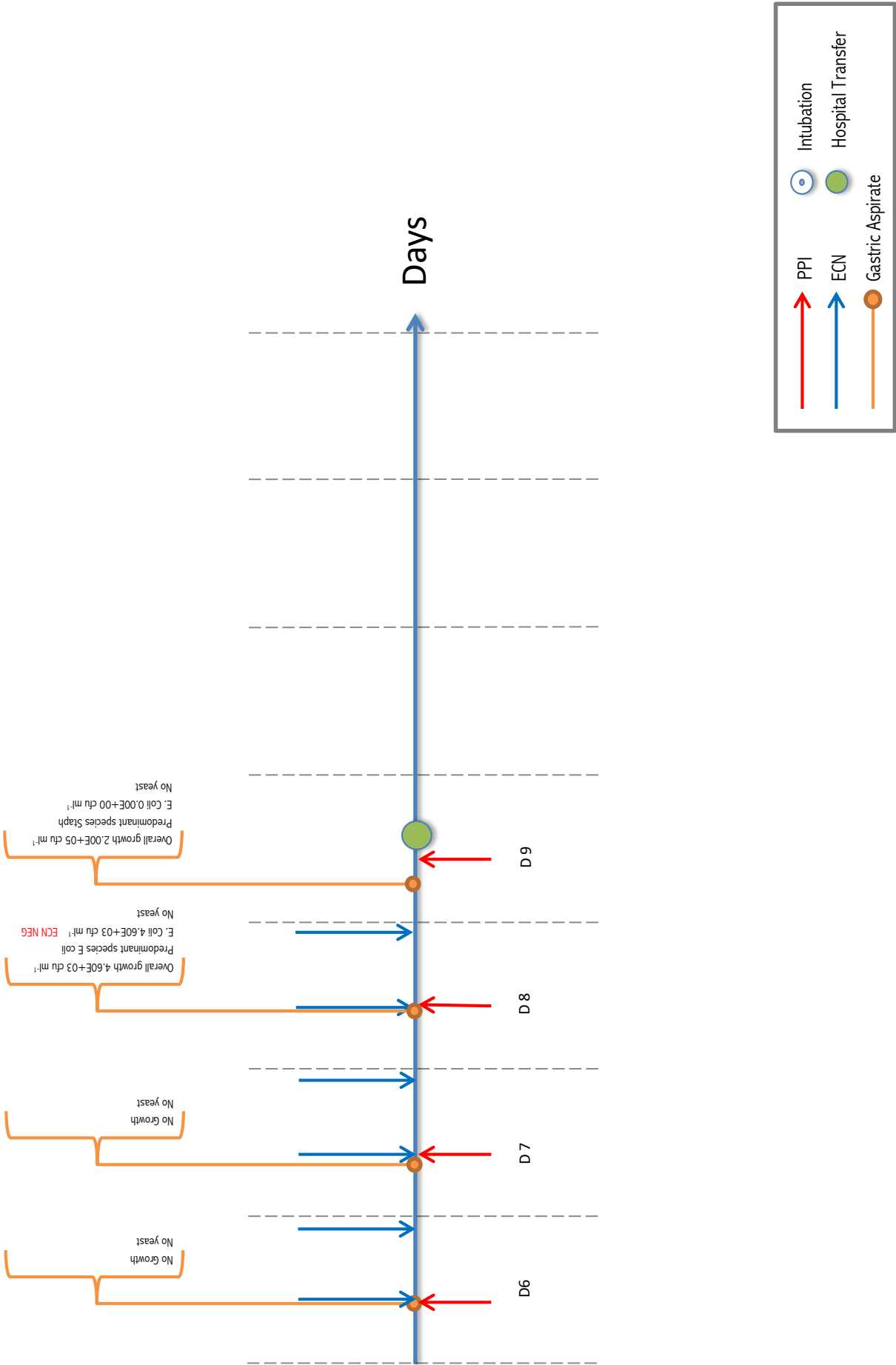
Clinical vignette: Male 03/01/1951  
Guillain-Barré Syndrome (IVG), Community acquired pneumonia  
PMHx: Asthma, Hypertension, Smoker  
Non-clinical transfer to local hospital Day 9



Legend:

- PPI (red arrow)
- ECN (blue arrow)
- Gastric Aspirate (orange circle)
- Intubation (blue circle with a dot)
- Tracheostomy (blue circle)

Timeline Patient 08 - 5ml ECN BD (b)



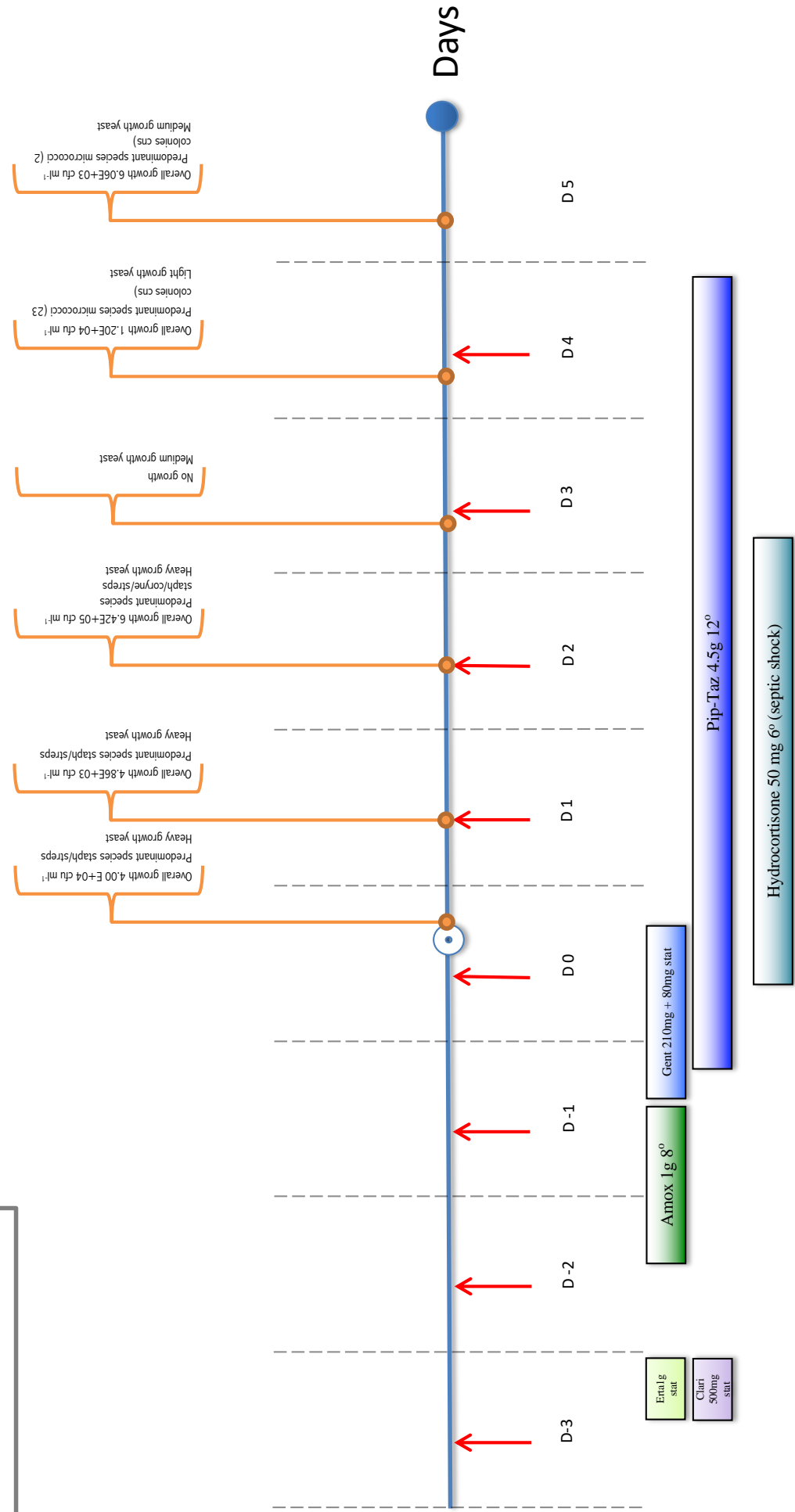
Clinical vignette: Female 31yrs

E.coli urosepsis

PMHx: Type I DM, Etoh excess, Vit D deficiency,

Smoker

## Timeline Patient 09 – Control



↑ PPI

↑ ECN

● Gastric Aspirate

○ Intubation

● Extubation

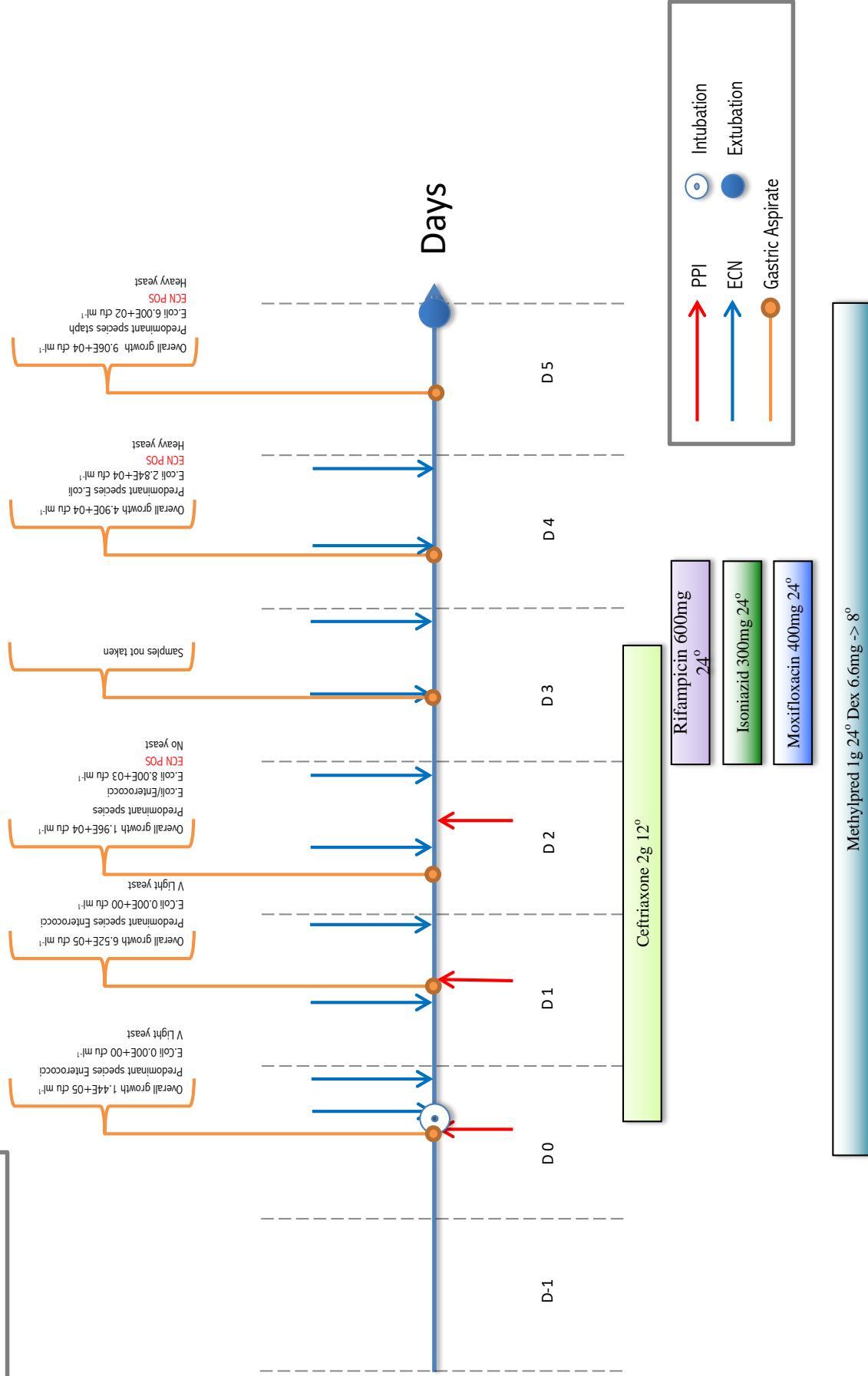
Clinical vignette: Female 31yrs

Seizures ? cause

PMHx: Ablation for paroxysmal atrial tachycardia,

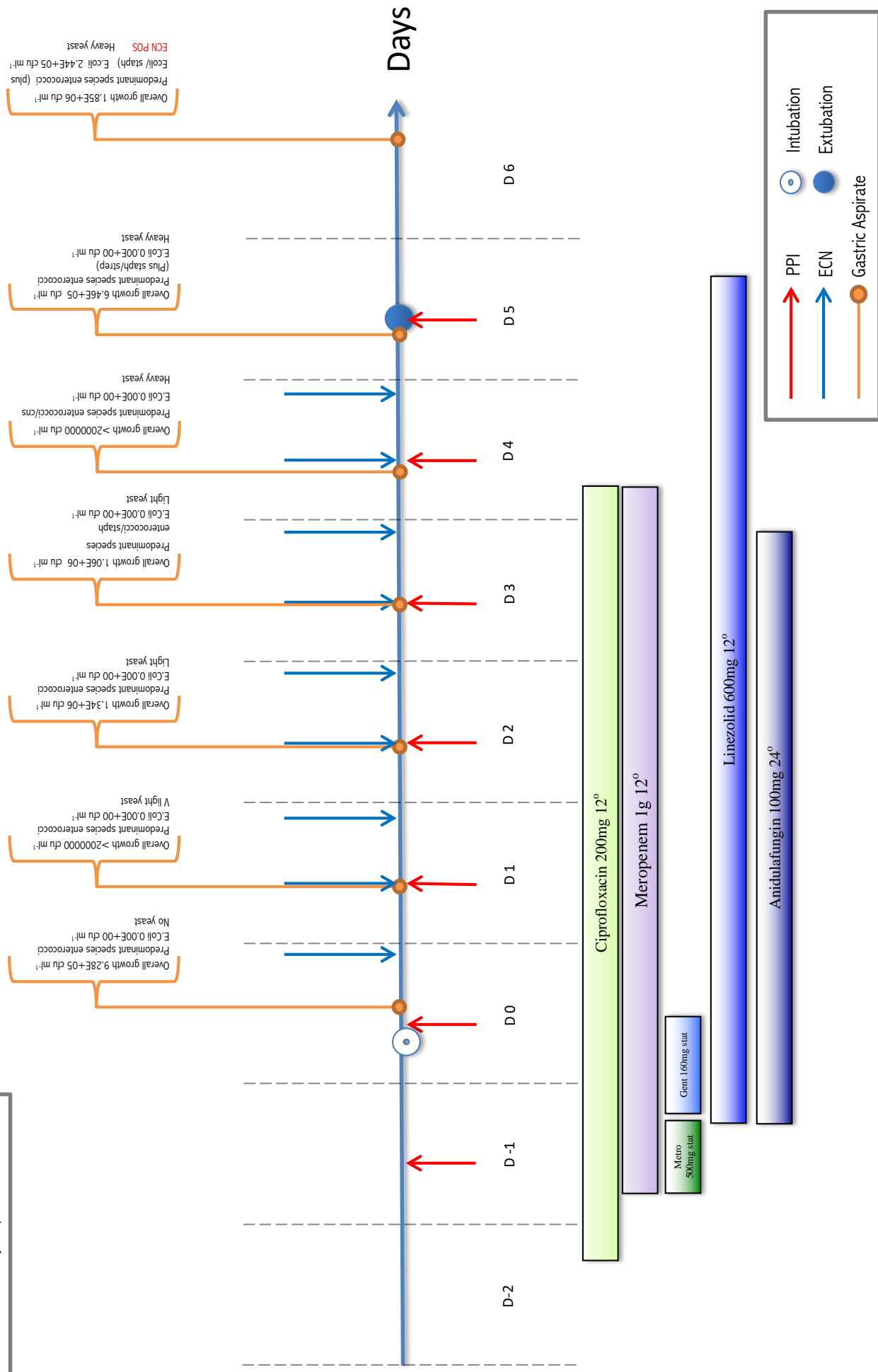
Smoker

## Timeline Patient 10 - 5ml ECN BD



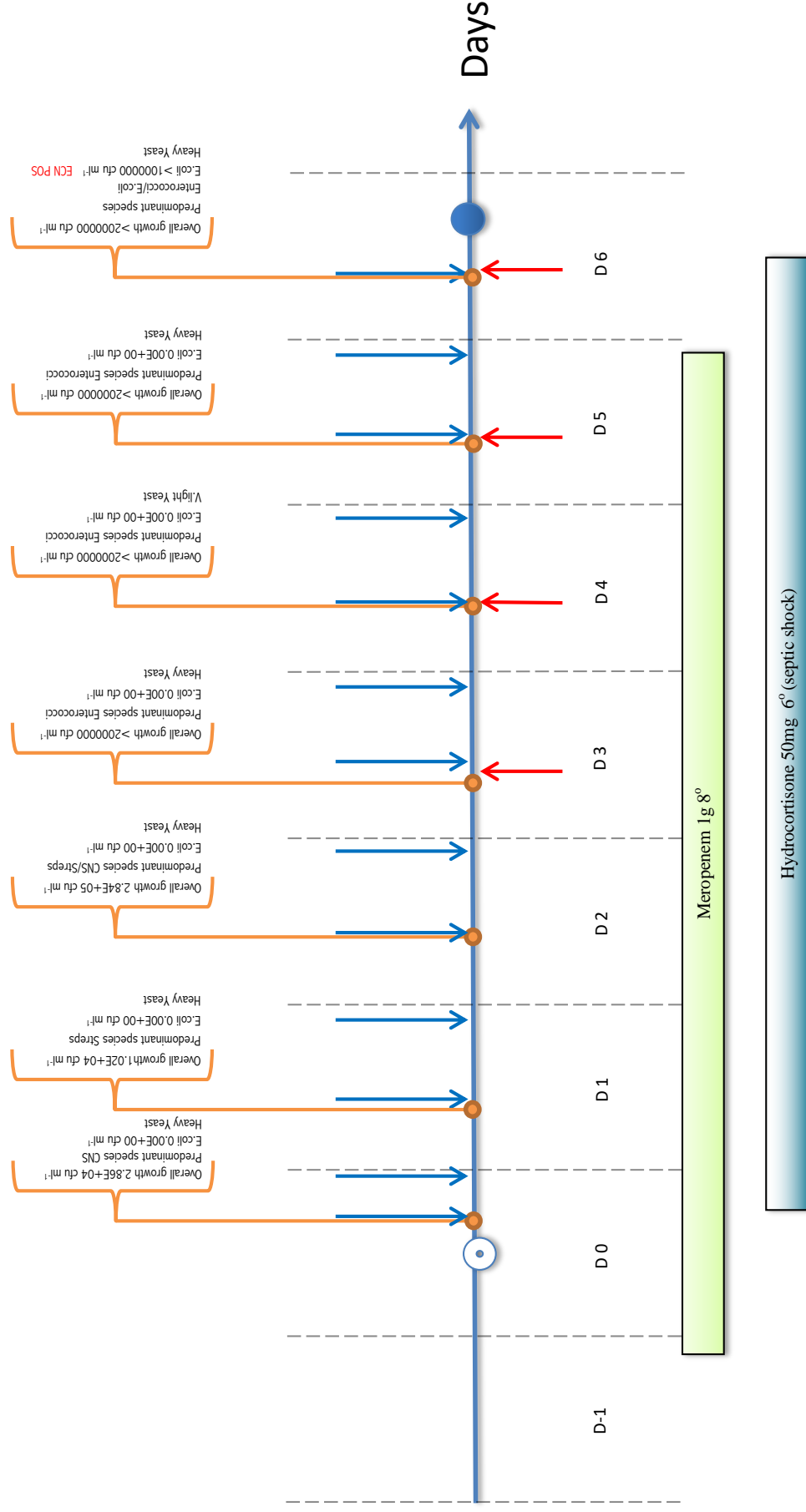
Clinical vignette: Male 70yrs  
Septic shock ? cause  
PMHx: AF, Type II DM, Hypertension, ESRF on HD  
(Recruited into BREATHE Study D5)

## Timeline Patient 11 – 15ml ECN BD



Clinical vignette: Female 87yrs  
Influenza A (H<sub>1</sub>N<sub>1</sub>) pneumonia  
PMHx: Type II DM, CKD, IHD

## Timeline Patient 12 – 5ml ECN BD

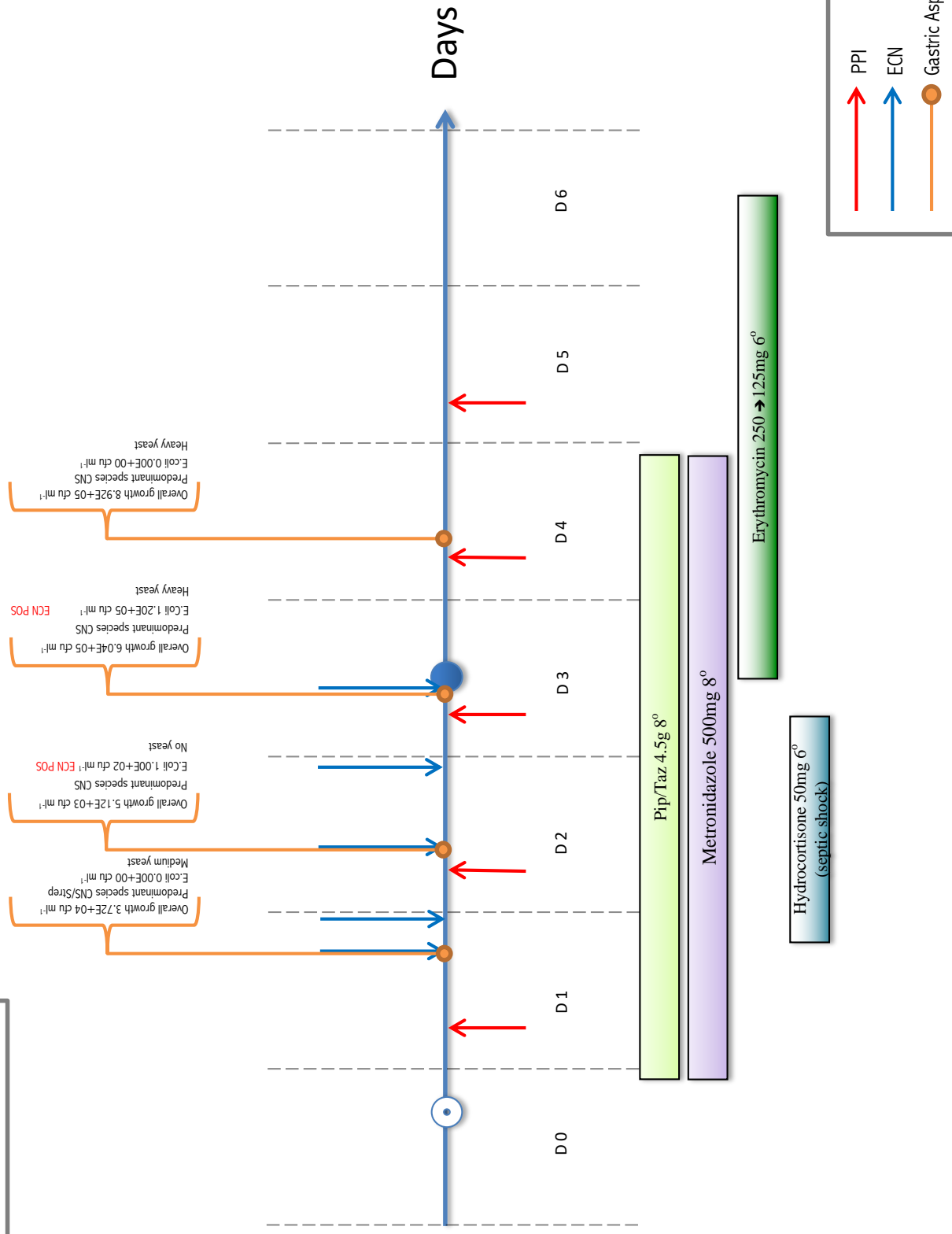


Intubation  
Death  
PPI  
ECN  
Gastric Aspirate



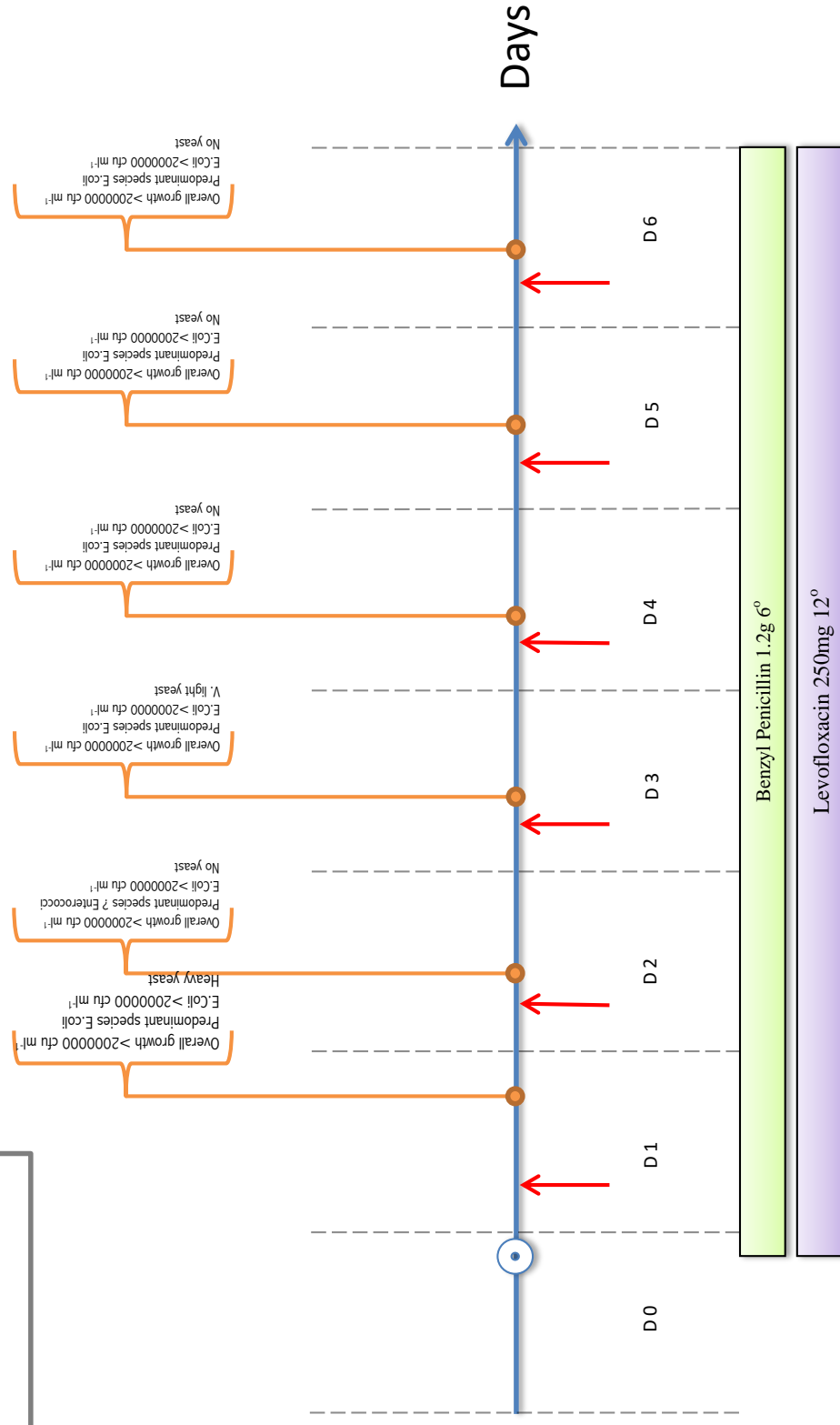
Clinical vignette: Male 62yrs  
Laparotomy and colectomy (ischaemic gut)  
Septic shock  
PMHx: Hypertension, IHD, Smoker, Schizophrenia

## Timeline Patient 13 - 15ml ECN BD

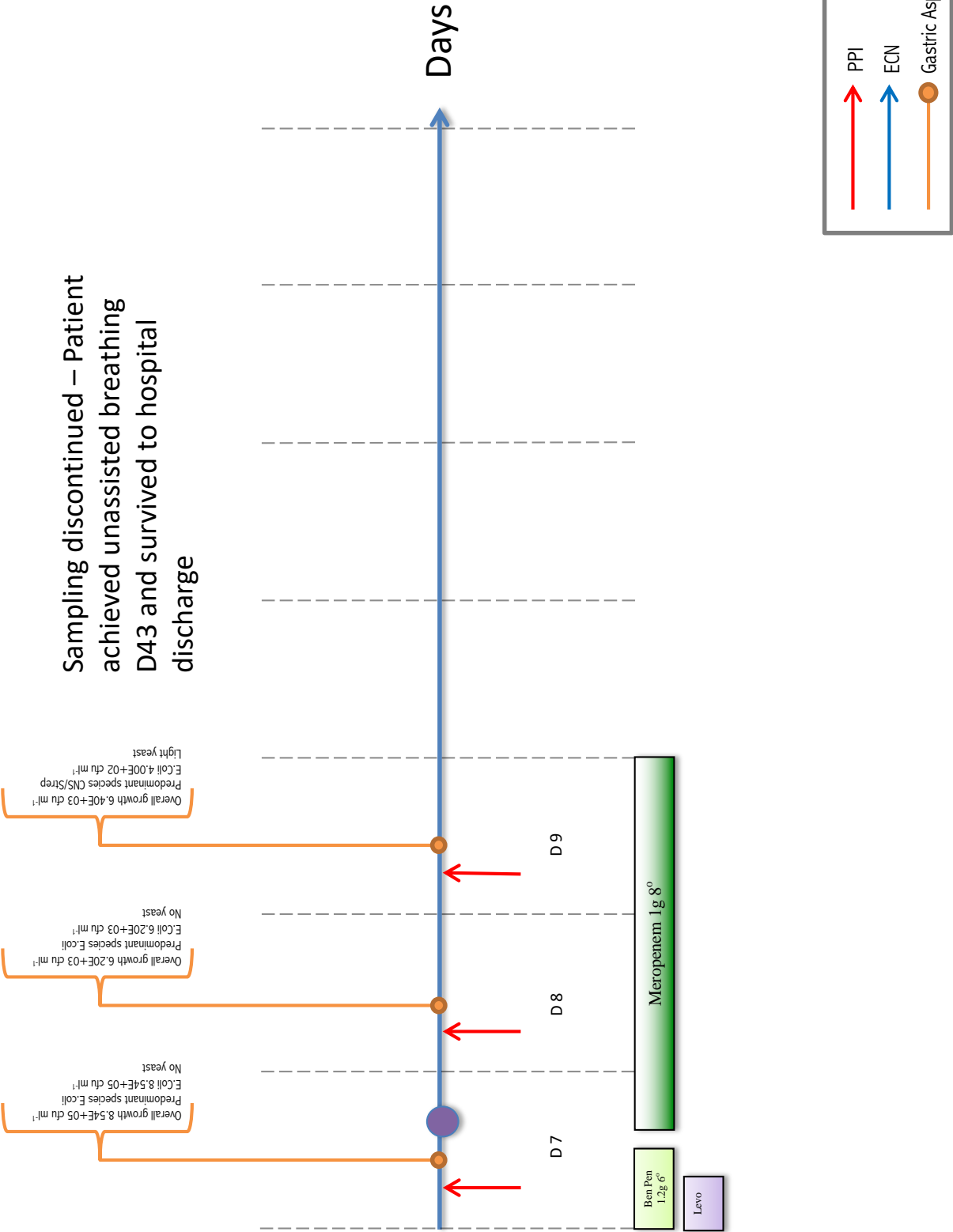


Clinical vignette: Female 80yrs  
CAP, Heart failure  
PMHx: Hypercholesterolaemia  
ESBL — sampling discontinued D9

## Timeline Patient 14 – Control (a)

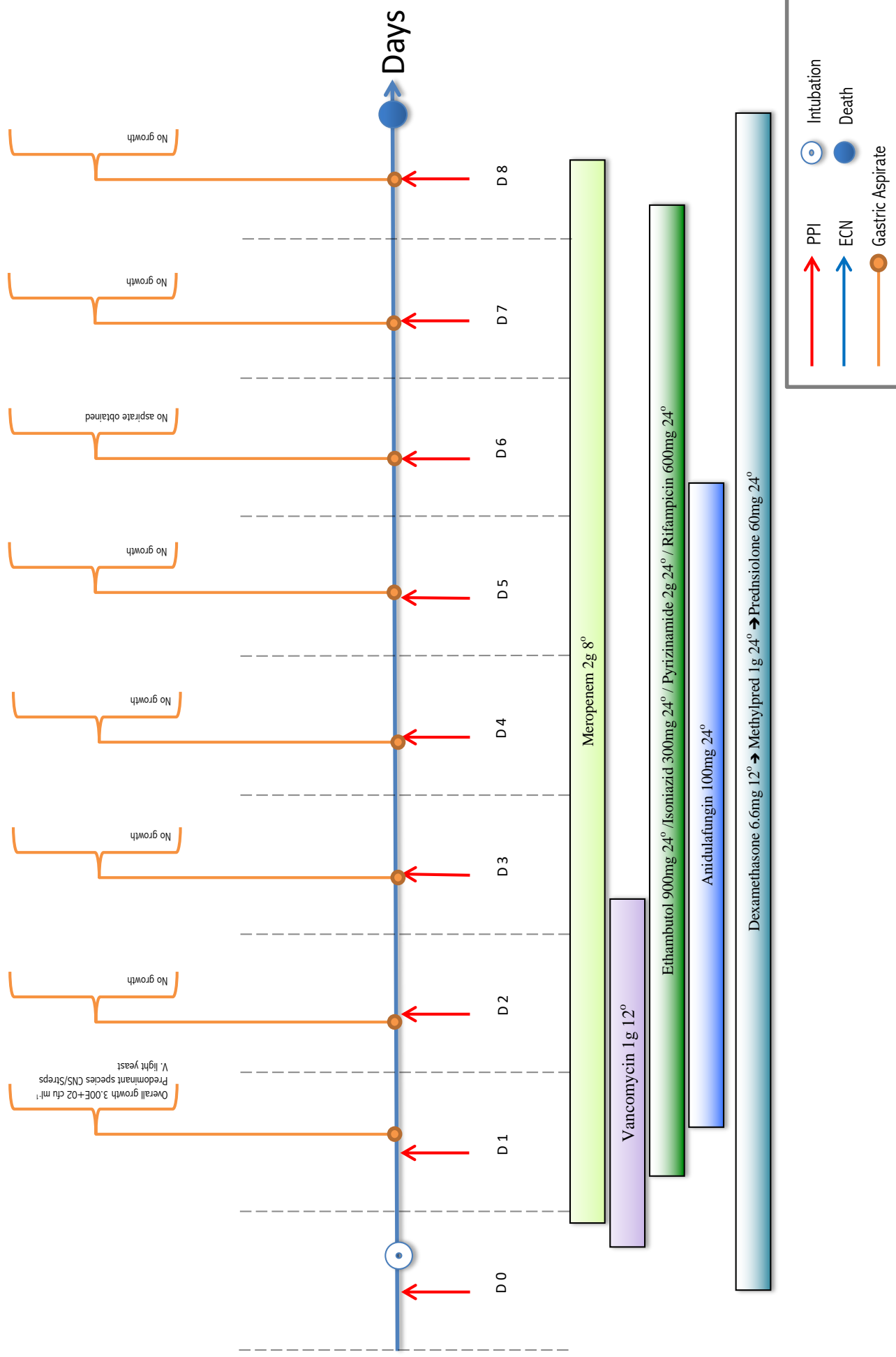


Timeline Patient 14 – Control (b)



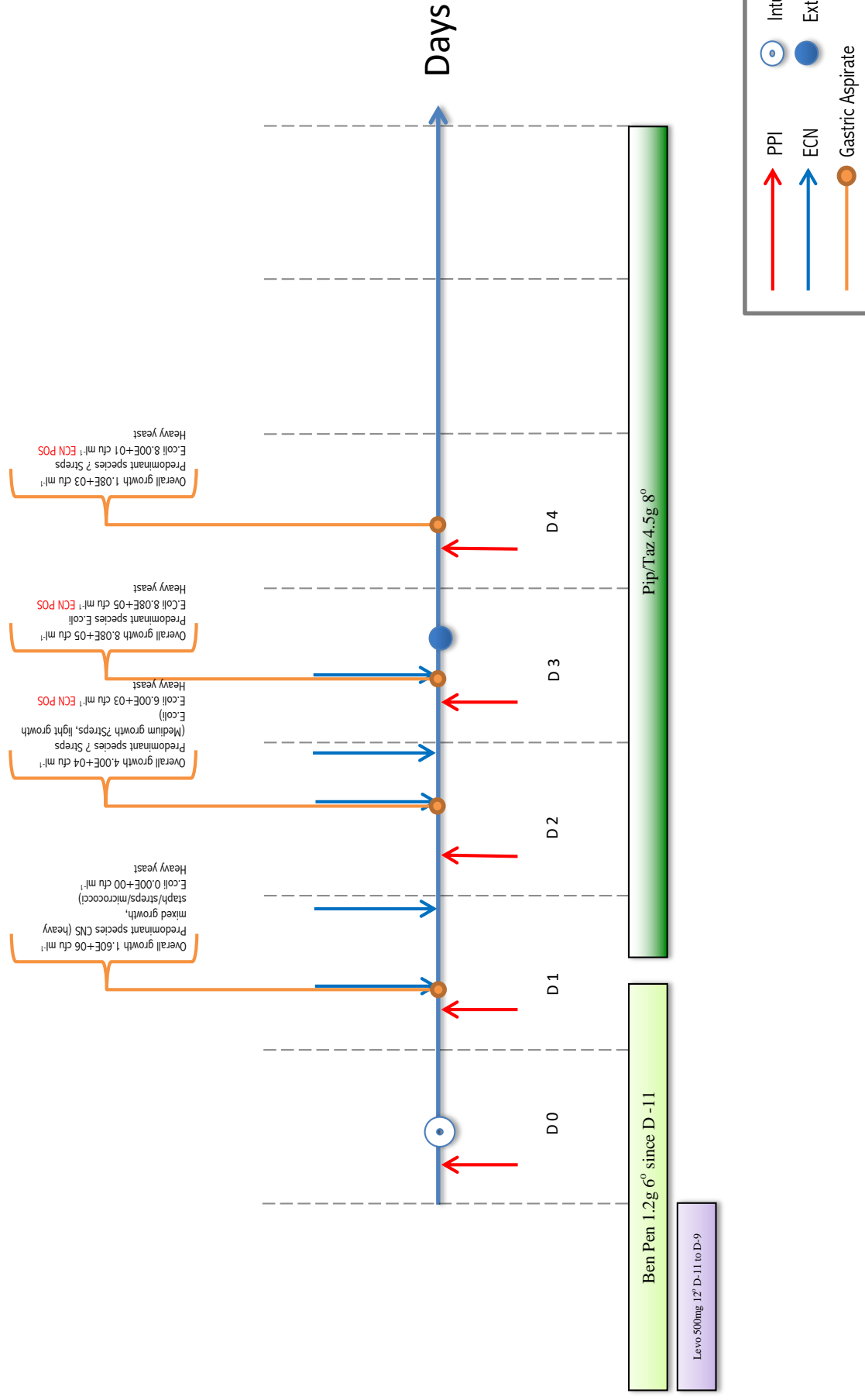
Clinical vignette: Female 66yrs  
Decreased GCS ? cause, aspiration pneumonia  
PMHx: Type II DM, Hypothyroidism

## Timeline Patient 15 – Control



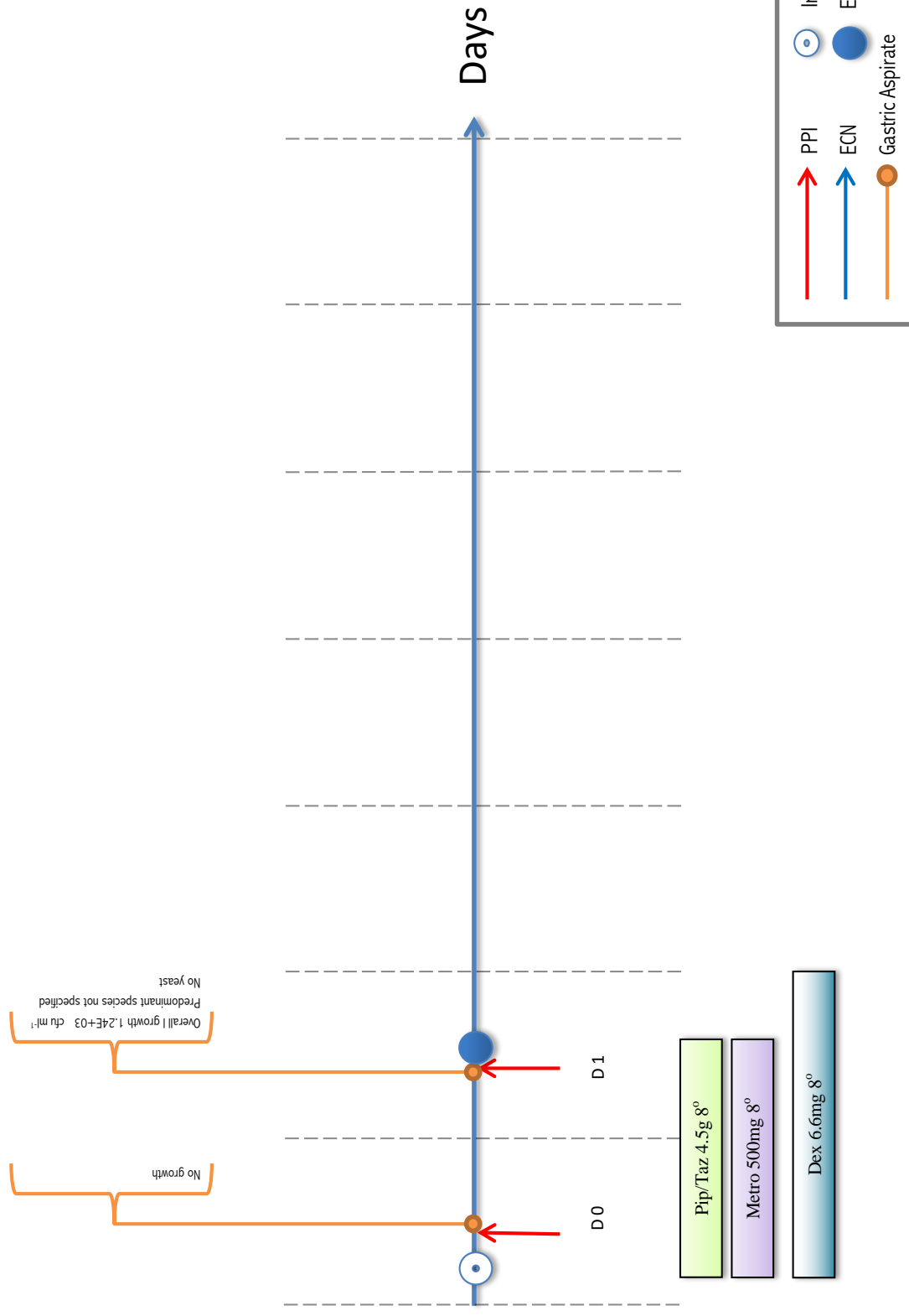
Clinical vignette: Female 79yrs  
CAP, Type II respiratory failure, Fast AF  
PMHx: Smoker

## Timeline Patient 16 - 5ml ECN BD



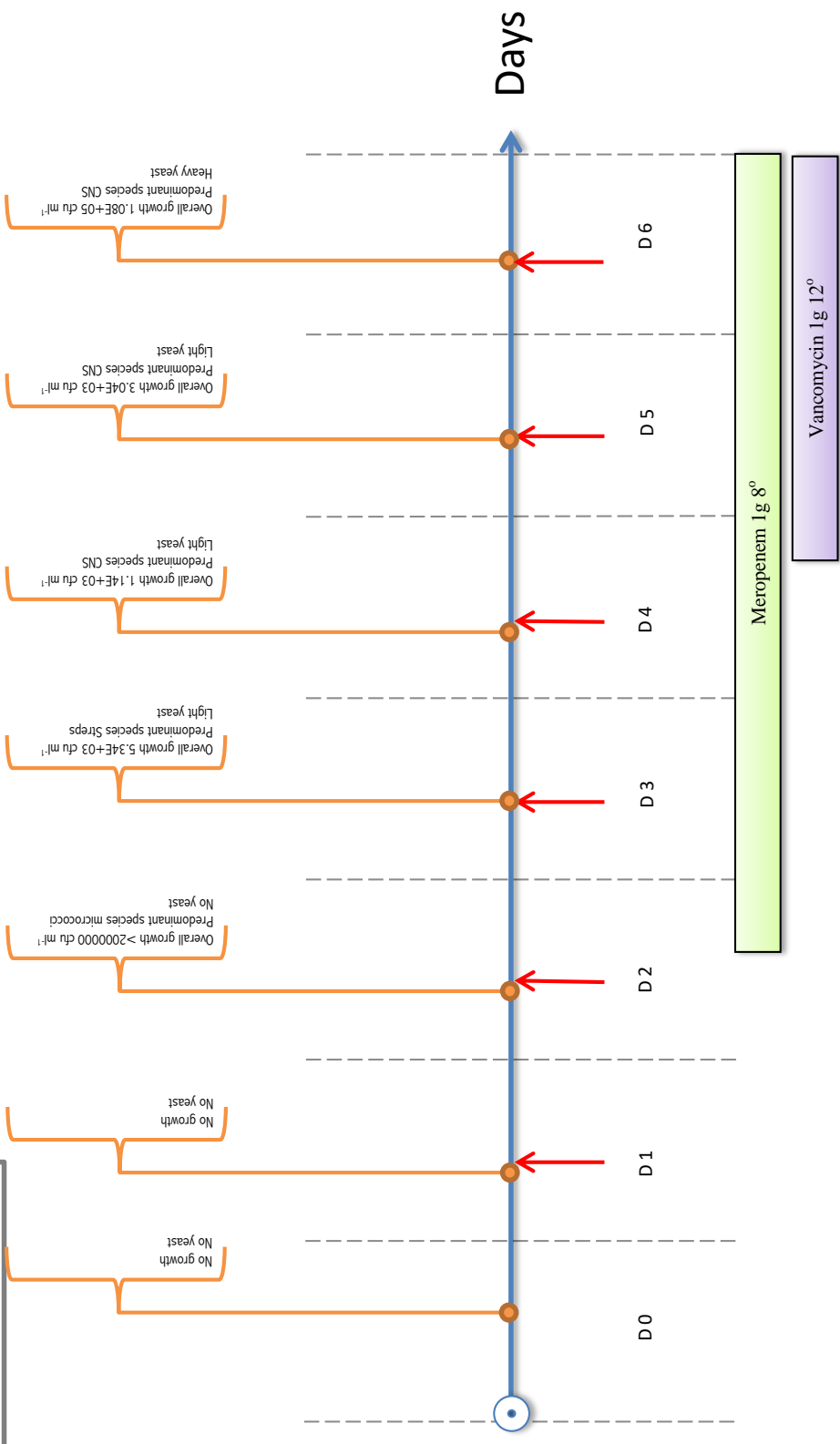
Clinical vignette: Female 50yrs  
Supraglottitis  
PMHx: Type II DM, Smoker

## Timeline Patient 17 – Control



# Timeline Patient 18 – Control (a)

Clinical vignette: Female 68yrs  
In hospital cardiac arrest  
PMHx: IHD, Type II DM, Hypertension, EtOH excess,  
Smoker



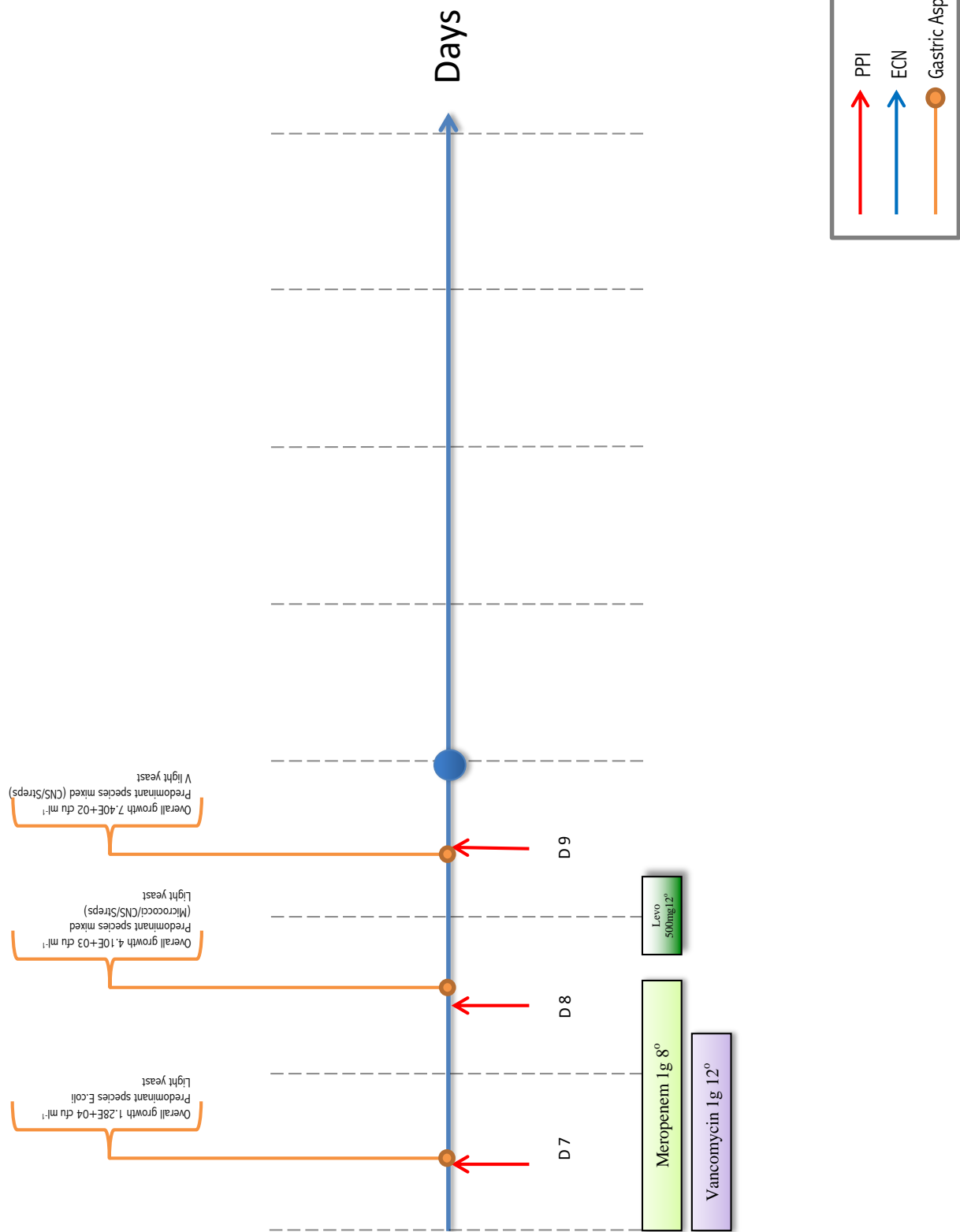
PPI

ECN

Gastric Aspirate

Intubation

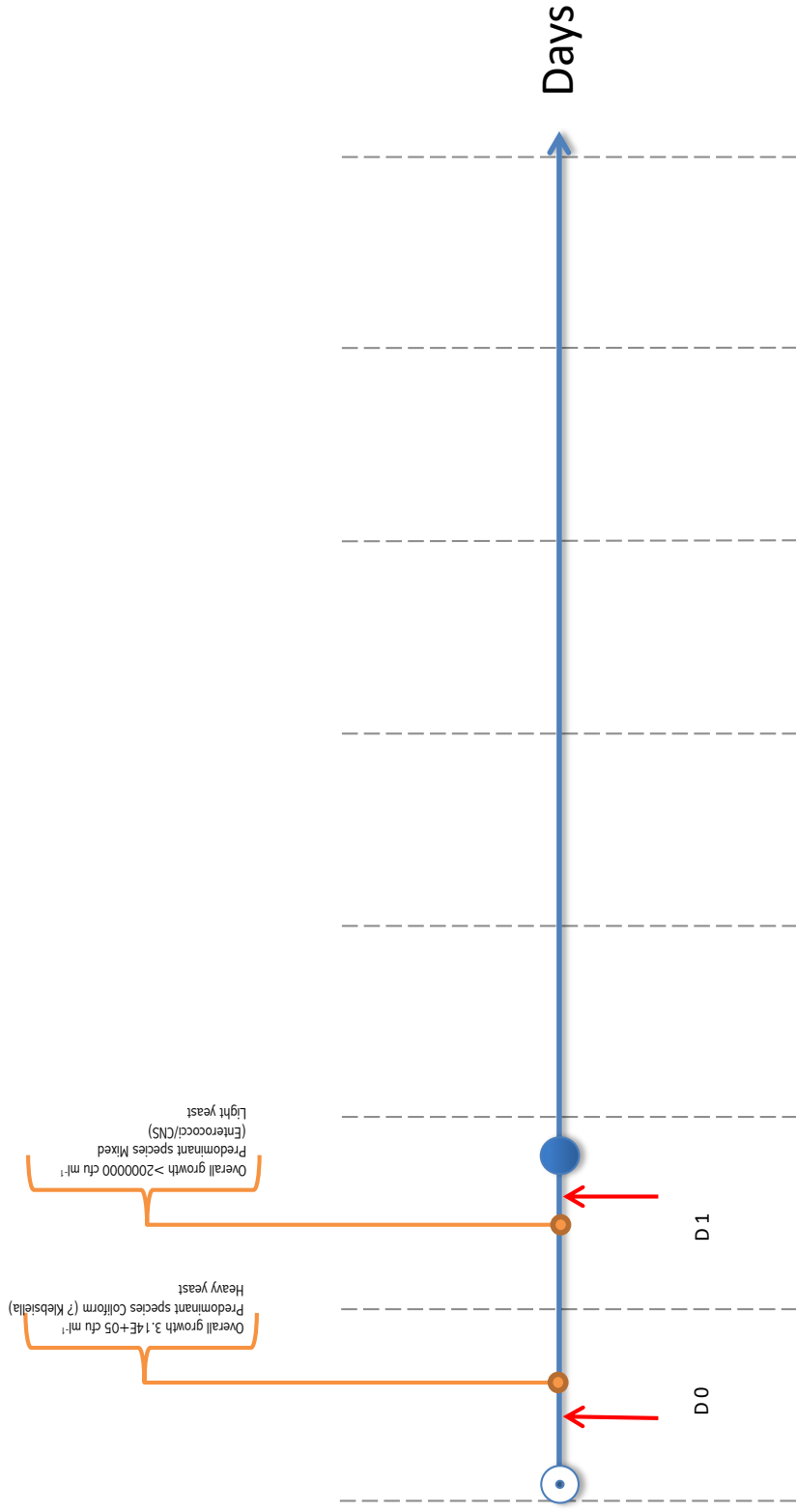
Timeline Patient 18 – Control (b)





Clinical vignette: Male 74yrs  
In hospital cardiac arrest (post AKA)  
PMHx: IHD, AF, Type II DM

## Timeline Patient 19 – Control

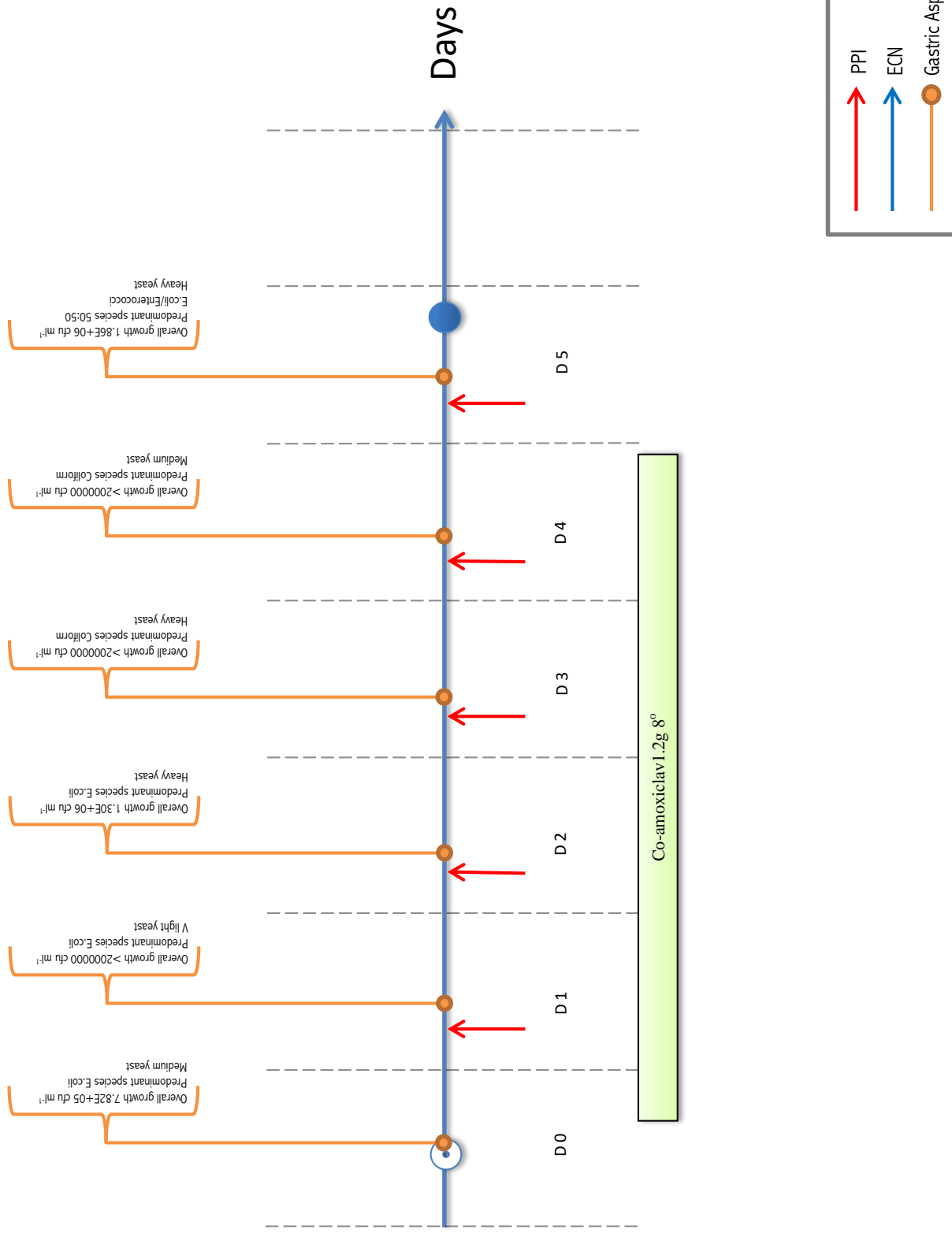


No Abx given



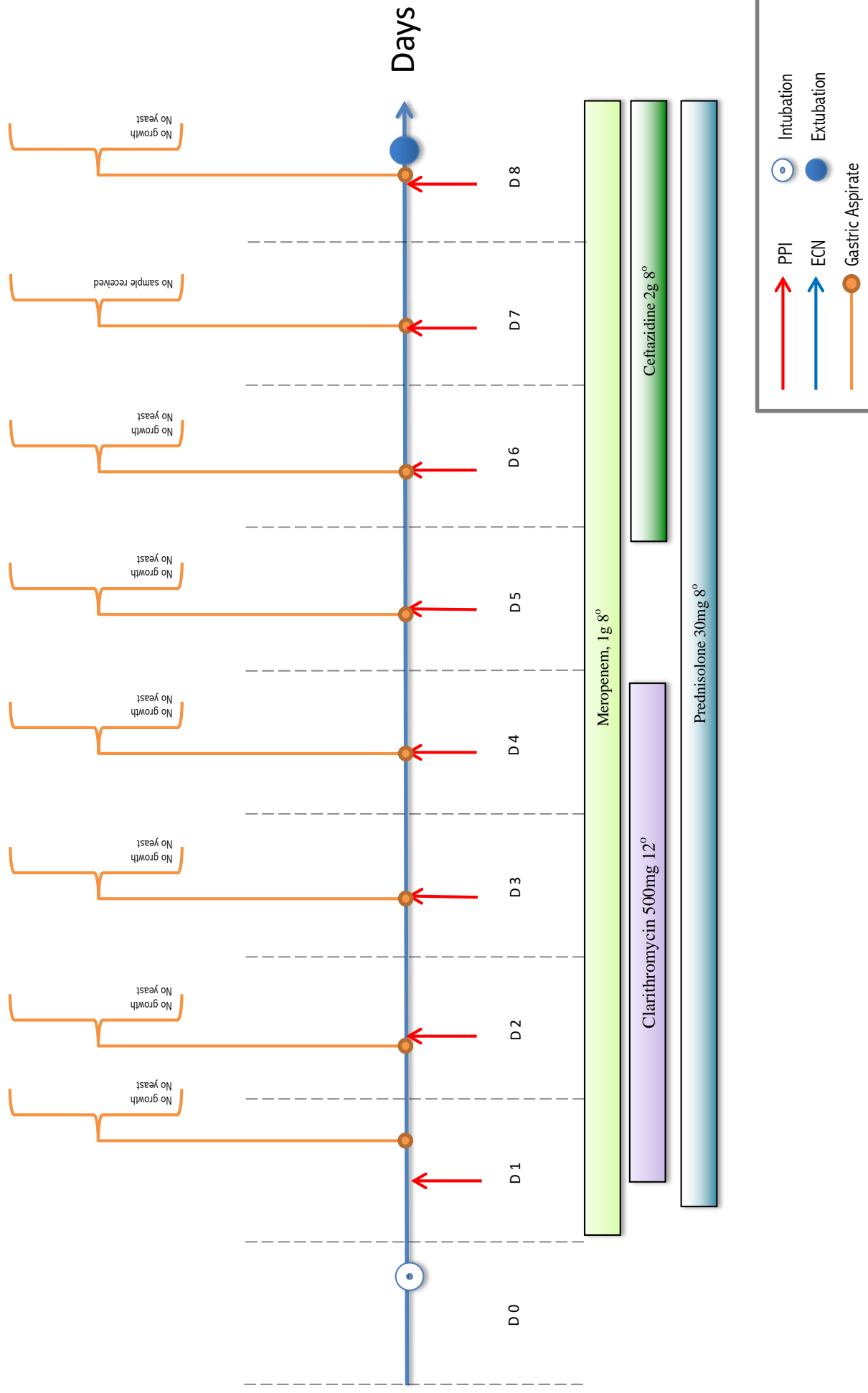
Clinical vignette: Male 89yrs  
In hospital cardiac arrest 2<sup>ary</sup> to hypoxia, # C<sub>1</sub>/C<sub>2</sub>  
PMHx: Hypothyroidism, AF

## Timeline Patient 20 – Control



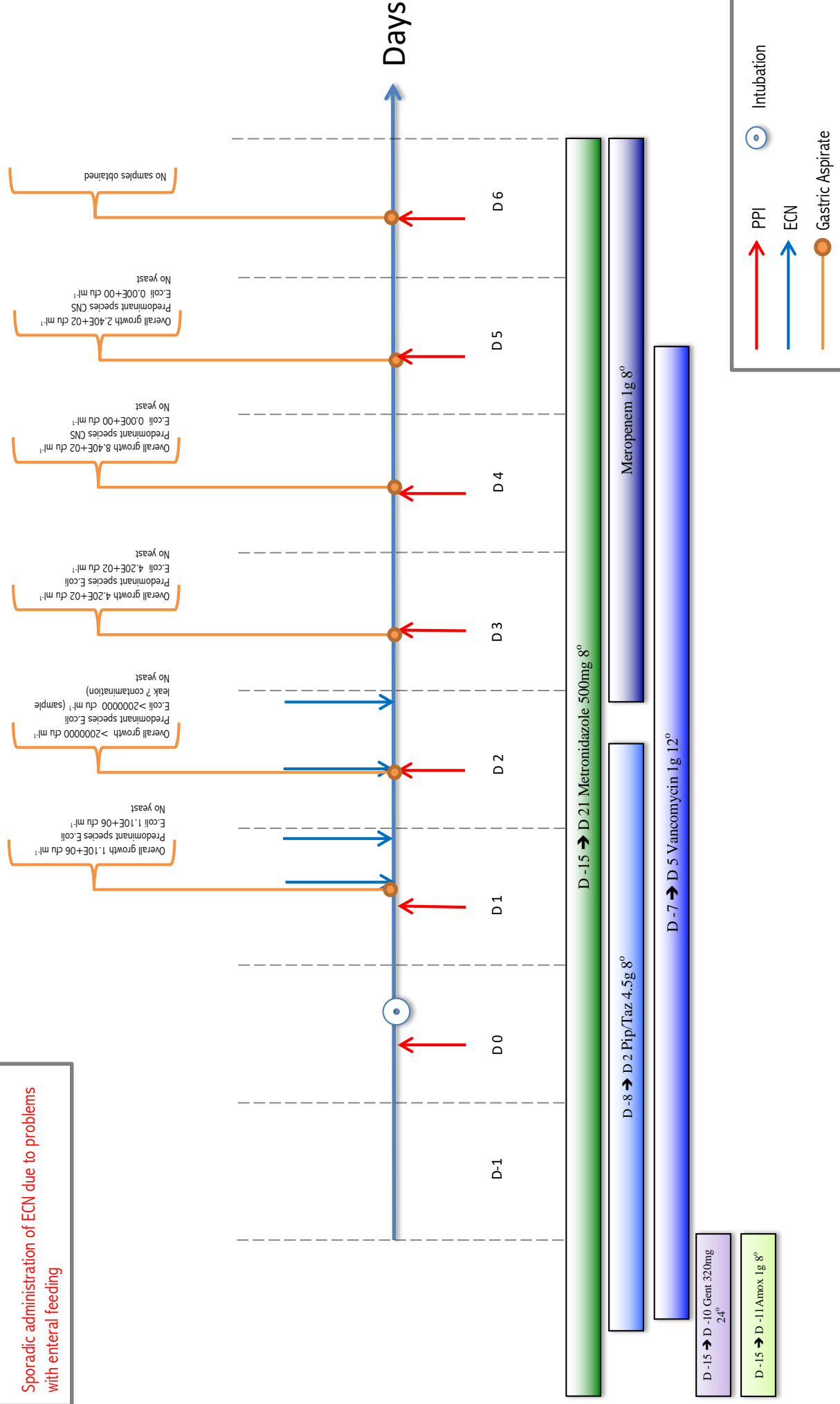
Clinical vignette: Female 23yrs  
Bilateral pneumonia, ARDS  
PMHx: Asthma, Smoker, previous ARDS

## Timeline Patient 21 – Control

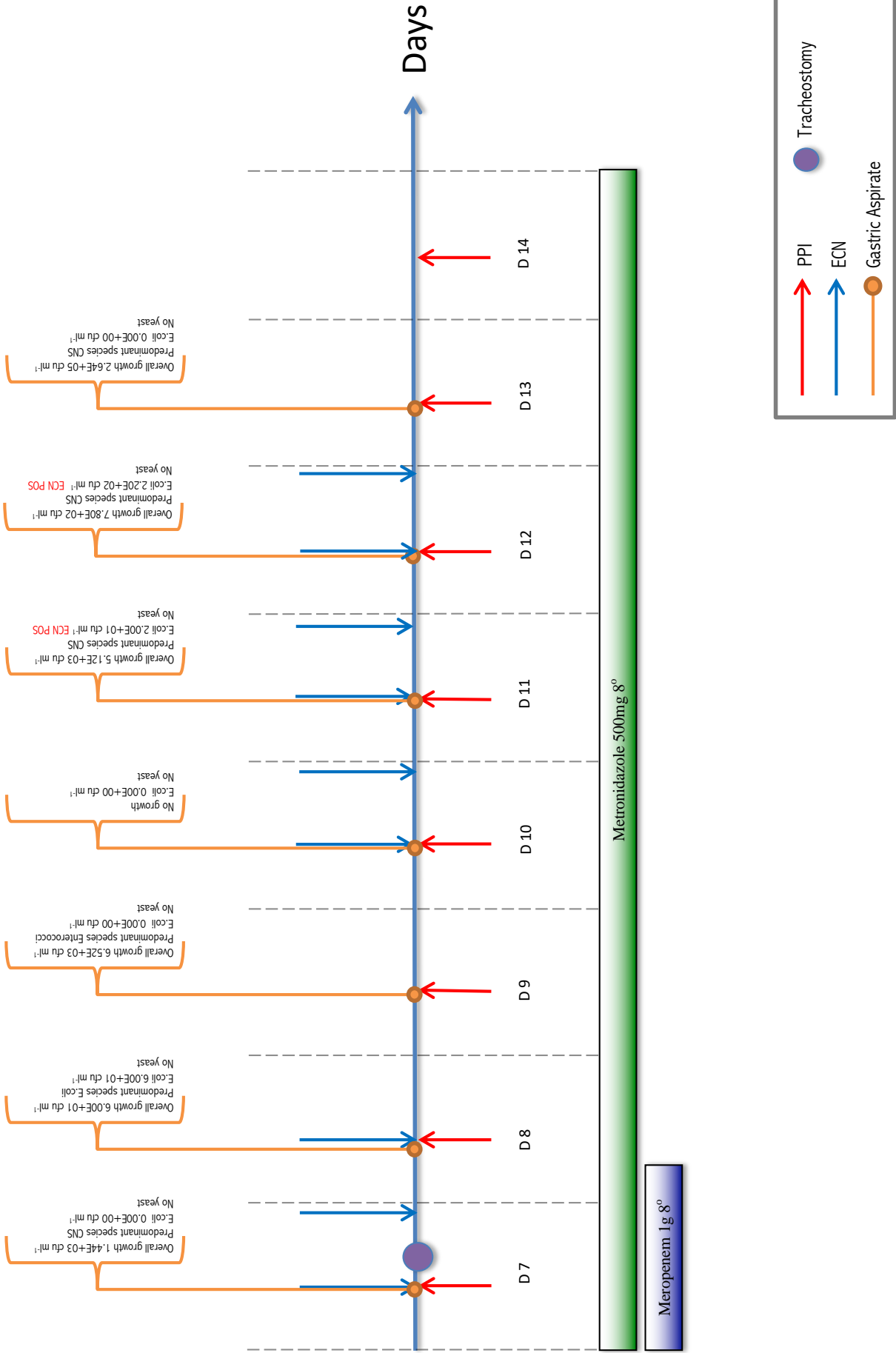


Clinical vignette: Male 41 yrs  
Laparotomy & bowel resection for SMV thrombosis  
PMHx: Alcohol excess, Smoker  
**Sporadic administration of ECN due to problems with enteral feeding**

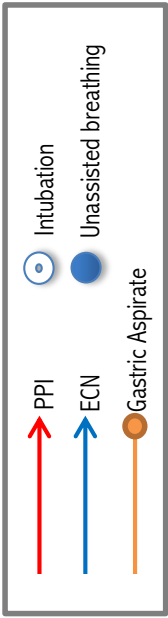
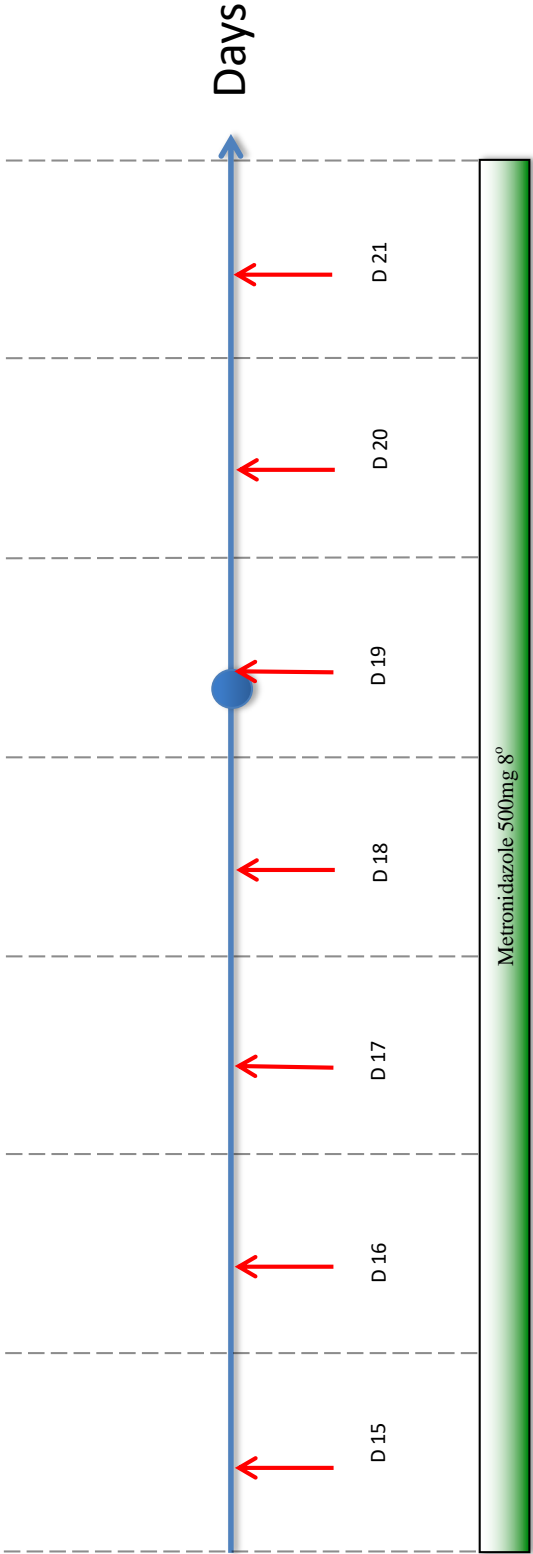
## Timeline Patient 22 - 5ml ECN BD (a)



### Timeline Patient 22 - 5ml ECN BD (b)

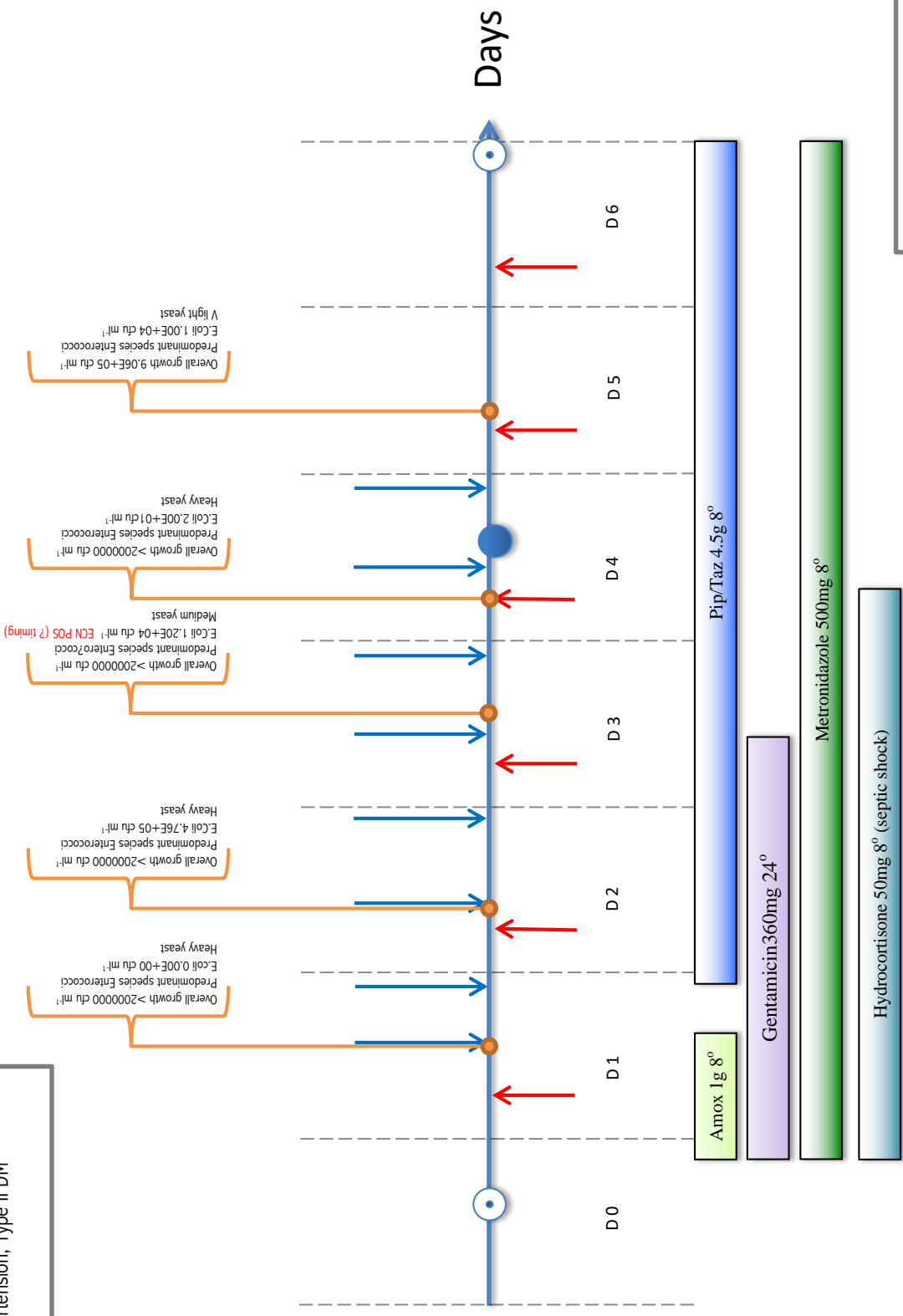


Timeline Patient 22 - 5ml ECN BD (c)



Timeline Patient 23 - 5ml ECN BD (a)

Clinical vignette: Female 71yrs  
Septic shock 2<sup>nd</sup> to faecal peritonitis (perforated sigmoid diverticulum)  
PMHx: IHD, CVA, Hypertension, Type II DM  
(Reintubated D6)



PPI

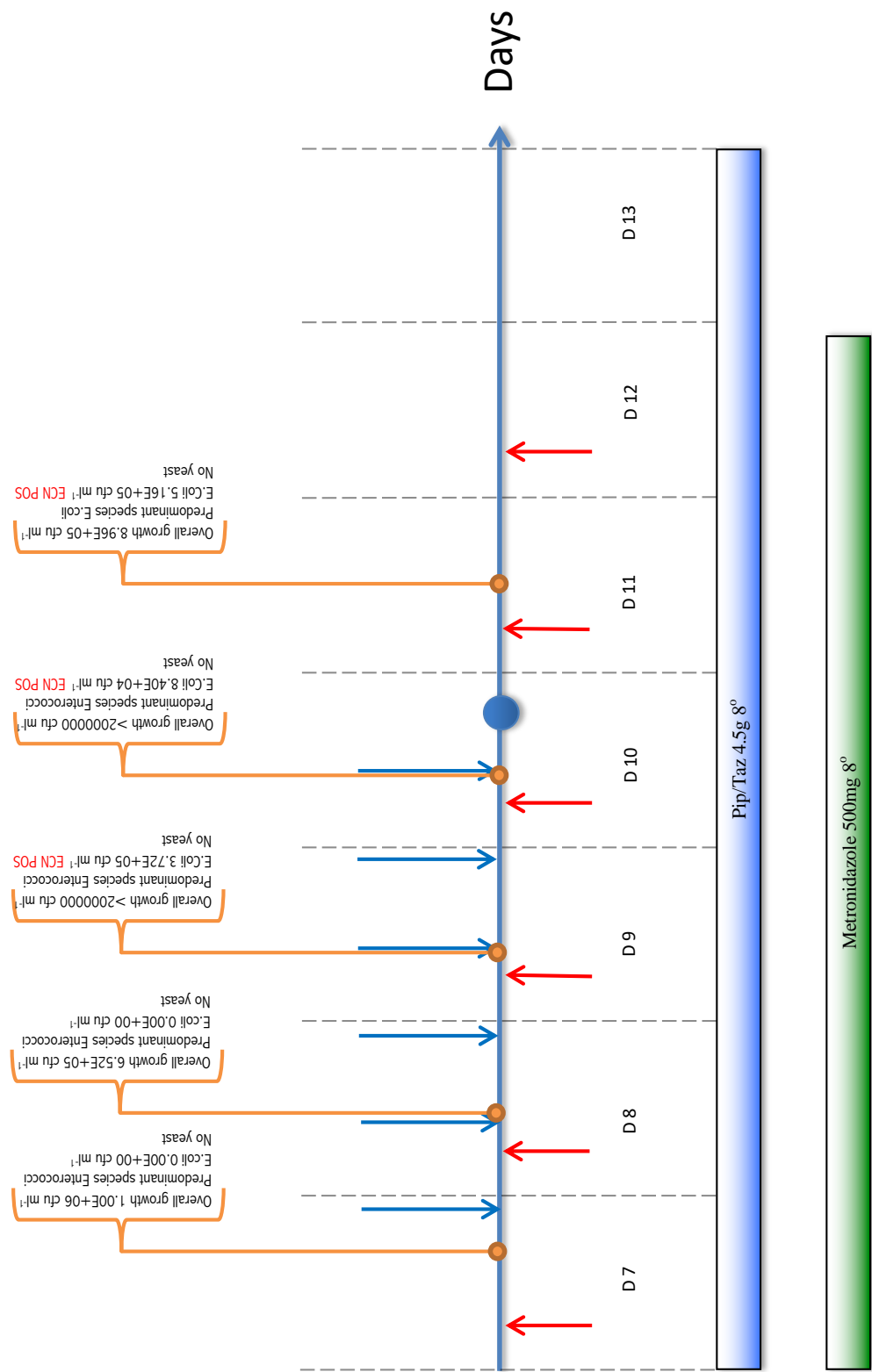
ECN

Gastric Aspiration

Intubation

Extubation

Timeline Patient 23 - 5ml ECN BD (b)



PPI

ECN

Gastric Aspirate

Intubation

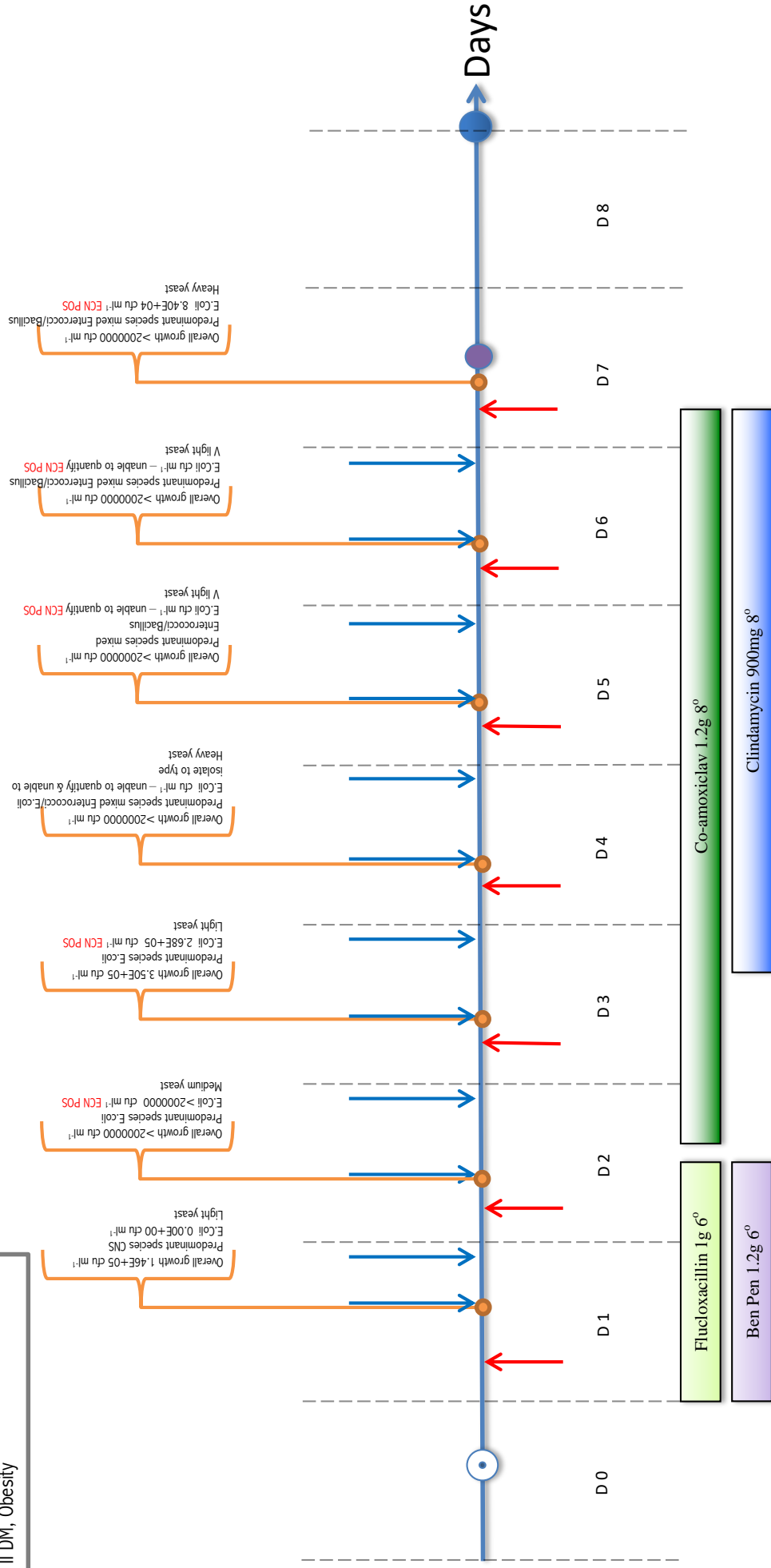
Extubation

Metronidazole 500mg 8<sup>o</sup>



# Timeline Patient 24 – 15ml ECN BD

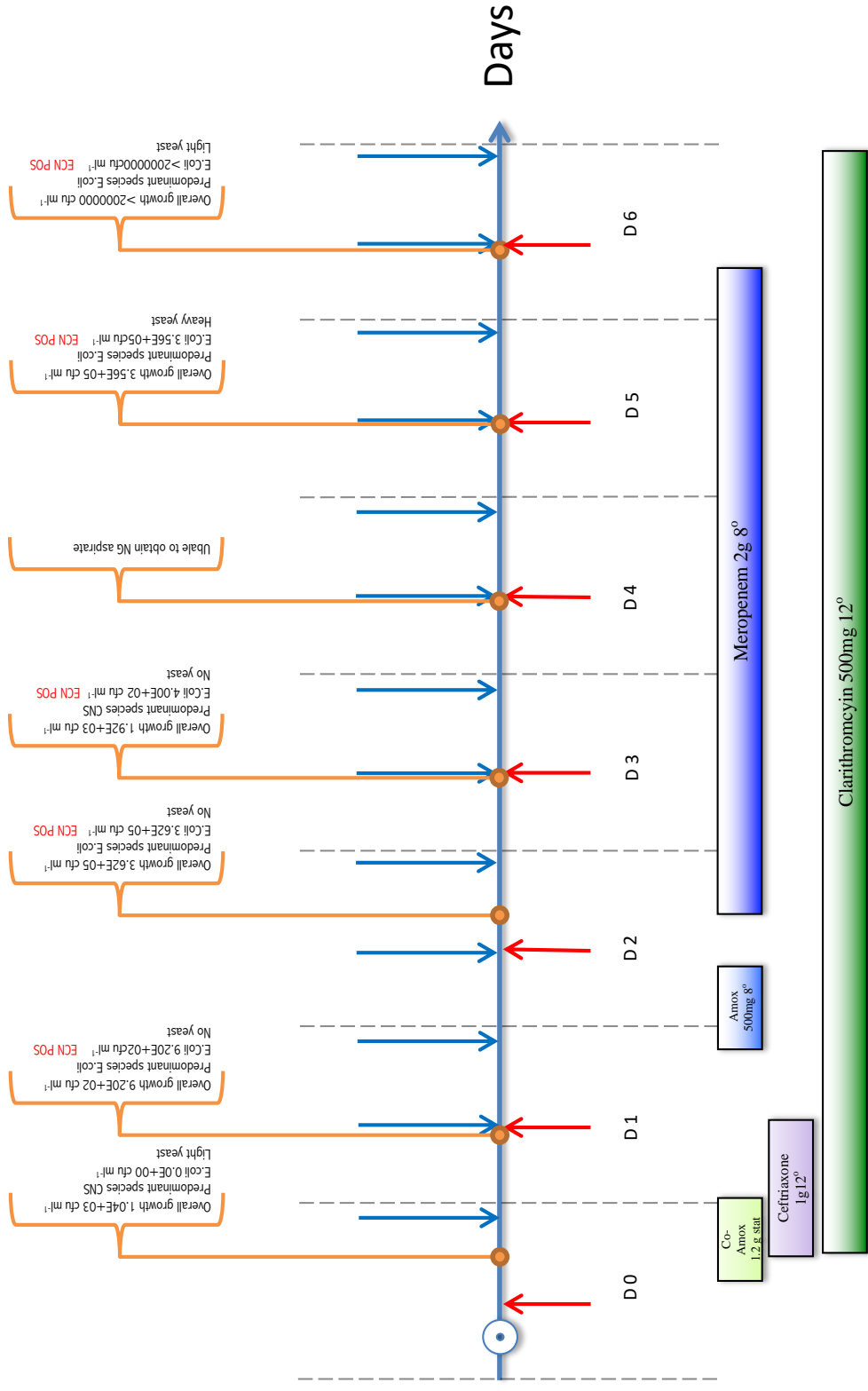
Clinical vignette: Male 75yrs  
OOH Cardiac Arrest, Cellulitis  
PMHx: Type II DM, Obesity



- Intubation
- Tracheostomy
- Death
- PPI
- ECN
- Gastric Aspiration

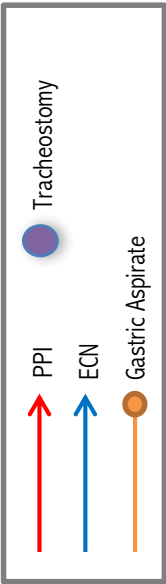
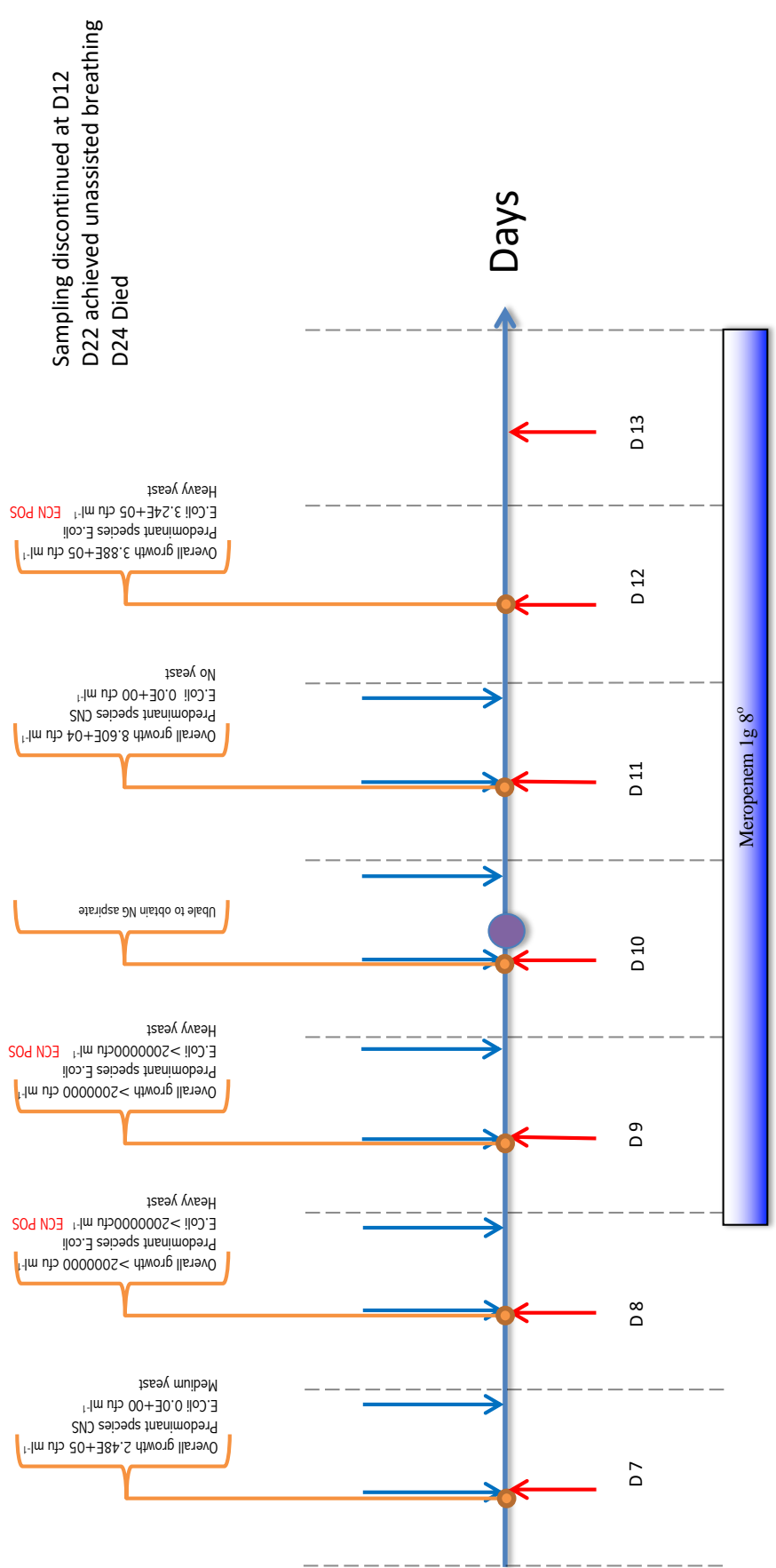
Clinical vignette: Female 69yrs  
 Infective exacerbation COPD  
 PMHx: COPD, Smoker, Diverticular disease

## Timeline Patient 26 - 15ml ECN BD (a)



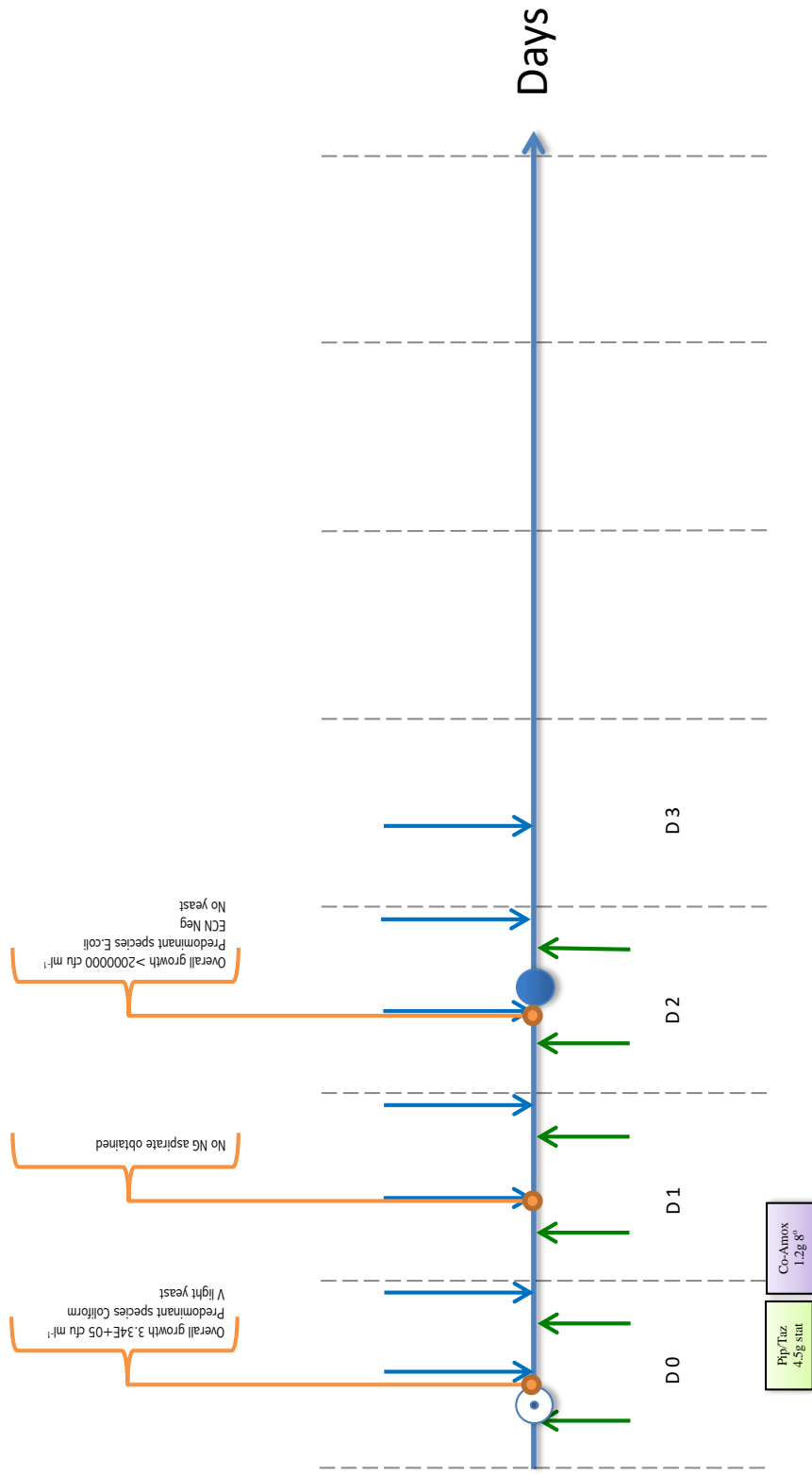
PPI  
 ECN  
 Gastric Aspirate  
 Intubation

Timeline Patient 26 - 15ml ECN BD (b)



# Timeline Patient 27 - 15ml ECN BD

Clinical vignette: Male 63 yrs  
Acute pulmonary oedema 2<sup>ary</sup> to ACS, AKI  
PMHx: IHD, CKD, Type II DM, AF, Hypertension, CCF

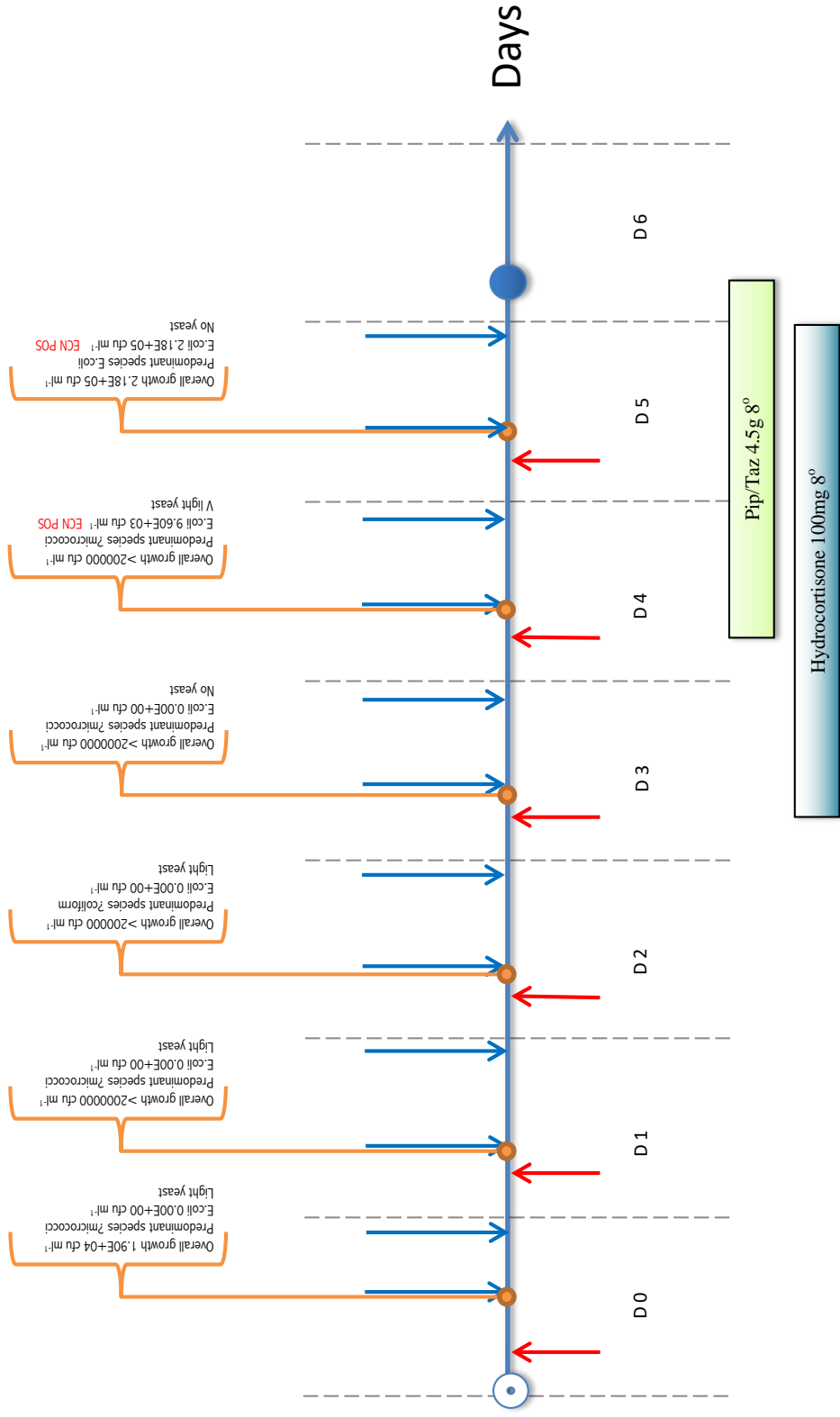


Clinical vignette: Male 70yrs

VF arrest

PMHx: IHD (CABG), Hypertension, Asthma, Smoker

## Timeline Patient 28 - 15ml ECN BD



Legend:

- PPI (Red arrow)
- ECN (Blue arrow)
- Gastric Aspiration (Orange dot)
- Intubation (Blue circle with dot)
- Death (Blue circle)

## **Appendix IV**

### **Probiotic *Escherichia coli* Nissle 1917 (Mutaflor®) Product Information and Summary of Medicinal Product Characteristics (SMPC)**













































## **Appendix V**

### **Publications and Presentations arising from this thesis**

The original concept for this work was presented at the Intensive Care Society Research Prioritisation Exercise in London on the 6<sup>th</sup> July 2011.









































