Enteral Vancomycin to Control Severe Methicillin-Resistant Staphylococcus aureus Infections in the Intensive Care Unit

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

Luciano Silvestri

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ABSTRACT

Background

Oropharyngeal chlorhexidine and mupirocin have been used in the intensive care unit (ICU) to control lower respiratory tract infection, including methicillin-resistant *Staphylococcus aureus* (MRSA) infection. The use of enteral vancomycin to control MRSA carriage of the oropharynx and the gut and to prevent MRSA infection of the lower airways and the bloodstream is a promising manoeuvre.

Aims of the research

1. To assess the effectiveness of oropharyngeal mupirocin and chlorhexidine to control severe ICU infection, including MRSA infection;
2. To assess the effectiveness of enteral vancomycin to control MRSA infection in the ICU, and to assess the safety of the manoeuvre, i.e. emergence of vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant enterococci (VRE);
3. To undertake a cohort study in the ICU in order to evaluate the impact of topical oropharyngeal vancomycin on MRSA infection and on the emergence of VISA and VRE.

Design of the research

Systematic review and meta-analysis of randomised studies for point 1; systematic review and meta-analysis of randomised and non-randomised studies for point 2; observational retrospective study for point 3.

Results

The systematic review and meta-analysis showed that mupirocin and chlorhexidine reduced lower respiratory tract infection, but did not demonstrate any impact on MRSA infection and mortality in critically ill ICU patients.

The systematic review and meta-analysis of the effectiveness of enteral vancomycin demonstrated a reduction in overall infection rates (Odds Ratio [OR] 0.35, 95% confidence interval [CI] 0.24-0.50, p=0.00199), *Staphylococcus aureus* carriage (OR 0.03, 95% CI 0.01-0.17, p<0.001) and infection (OR 0.21, 95% CI 0.15-0.32, p<0.001). MRSA carriage, MRSA infection, and mortality were reduced by enteral vancomycin (OR 0.15, 95% CI 0.09-0.25, p<0.001, OR 0.24, 95% CI 0.12-0.50, p< 0.001, and 95% CI 0.43-0.79, p<0.001, respectively). VISA and VRE were not a clinical problem.

The 16-year retrospective study showed that, in patients receiving oropharyngeal vancomycin, MRSA secondary endogenous infections were significantly reduced compared with patients who did not receive enteral vancomycin (OR 0.26 95% CI 0.1-0.69, p=0.007). VISA and VRE were not demonstrated.

Conclusions

Oropharyngeal mupirocin and chlorhexidine did not demonstrate any effect on MRSA infection and mortality in systematic review. The use of enteral vancomycin may be an effective strategy to reduce MRSA carriage and infection in ICU patient. The manoeuvre is safe in terms of emergence of VISA and VRE.

ABBREVIATIONS*

AGNB	aerobic Gram-negative bacilli
BAL	bronchoalveolar lavage
BSI	bloodstream infection
CA-MRSA	community-acquired methicillin-resistant *Staphylococcus aureus*
CFU	colonies forming unit
Chx	chlorhexidine
CI	confidence interval
CLSI	clinical and laboratory standard institute
CNS	coagulase-negative staphylococci
COPD	chronic obstructive pulmonary disease
CDC	centers for disease control and prevention
CPIS	clinical pulmonary infection score
DNA	deoxyribonucleic acid
EPIC	European prevalence of Infection
EUCAST	European committee on antimicrobial susceptibility testing
H-VISA	heteroresistant vancomycin-intermediate *Staphylococcus aureus*
ICU	intensive care unit
IPI	intrinsic pathogenicity index
IQR	interquartile range
L	litre
LRTI	lower respiratory tract infection
MIC	minimum inhibitory concentration
ml	millilitre

MRSA	methicillin-resistant *Staphylococcus aureus*
MSSA	methicillin-sensitive *Staphylococcus aureus*
Mup	mupirocin
NA	not available
NE	not evaluable
NNT	number needed to treat
NOS	Newcastle-Ottawa quality assessment scale
OR	odds ratio
PBP	penicillin-binding protein
PE	primary endogenous
PRISM	preferred reporting items for systematic review and meta-analysis
PICOS	participants, intervention, comparisons, outcomes, study design
PICU	paediatric intensive care unit
PPM	potentially pathogenic microorganism
PSB	protected specimen brush
RCT	randomised controlled trial
RR	relative risk
SAPS	simplified acute physiology score
SDD	selective decontamination of the digestive tract
SE	secondary endogenous
SOD	selective oropharyngeal decontamination
SSC	staphylococcal cassette chromosome
SSI	surgical site infection
STROBE	strengthening the reporting of observational studies in epidemiology
VISA	vancomycin-intermediate *Staphylococcus aureus*

VRSA	vancomycin-resistant *Staphylococcus aureus*
UTI	urinary tract infection
VAP	ventilator-associated pneumonia
VRE	vancomycin-resistant enterococci

*Additional abbreviations are included in tables' and figures' legends

1 INTRODUCTION

1.1 *Staphylococcus aureus* and resistance to methicillin

Staphylococci were first described and classified by the Scottish surgeon Sir Alexander Ogston in 1882 [1]. He named these bacteria using the Greek words "*staphylos*" ("grape") and "*kokkos*" ("berry" or "seed"). The name *Staphylococcus aureus* was coined in 1884 by the German physician Friedrich Rosenbach [2]. He distinguished some colonies of staphylococci into "*albus*" and "*aurum*", the Latin words indicating "white" and "gold". The main characteristic of *S. aureus* as human pathogen is its versatility and adaptability to the newer antibiotics.

An effective therapy for *S. aureus* infections was available in 1928 when penicillin was discovered by Alexander Fleming. Unfortunately, *S. aureus* quickly developed penicillin resistance due to the acquisition of genes producing β-lactamases [3]. Within few years *S. aureus* became able to destroy penicillin by producing a specific enzyme called "penicillinase" which was encoded by a plasmid, and which quickly spread among *S. aureus* strains [4].

A modified penicillin, methicillin, was introduced in 1959. Methicillin was designed to resist the destructive action of the staphylococcal penicillinase. Within two years, methicillin-resistant *S. aureus* (MRSA) strains were reported [5]. Methicillin resistance was not due to a hydrolysing enzyme, but to a sophisticated mechanism. Methicillin exerts its action by blocking the proteins called penicillin-binding proteins (PBPs), which are responsible for the construction and maintenance of the bacterial cell wall. *S. aureus* resistant strains acquired a new protein, called PBP2a, which was not blocked by methicillin and could replace the other PBPs, thus allowing the survival of *S. aureus* in the presence of methicillin. PBP2a is encoded by the gene *mecA*. *mecA* resides in a chromosome as a mobile genetic element called

the "staphylococcal cassette chromosome (SCC) *mec"*. SCC*mec* is flanked by chromosome recombinase genes (*ccrA*, *ccrB* or *ccrC*), which allow intra- and interspecies transmission of the SCC*mec*. The initial reservoir of SCC*mec* is unclear, but may have been a coagulase-negative staphylococcal species. The presence of PBP2a means MRSA is resistant not only to methicillin but also to all β-lactam antibiotics, including synthetic penicillins, cephalosporins and carbapenems.

MRSA is worldwide spread in hospitals, with prevalence reaching rates of 25-50% in Americas, Australia and Southern Europe [6]. Rates of MRSA among *S. aureus* in Spanish intensive care units (ICUs) from 1994 to 2008 shows similar patterns (≈25%) in the first and last years with oscillations ranging from 13% in 1997 to 42.3% in 2006 [7]. In addition to intra-ICU transmission dynamics of MRSA, influenced by colonisation of ICU healthcare workers [8], it should be taken into account of MRSA imported cases in the ICU [9], with community-acquired MRSA (CA-MRSA) genotypes as emerging cause of carriage among patients admitted in adult ICUs [10].

MRSA may be multi-drug resistant. The resistance is not only to β-lactam antibiotics, but also to other different antibiotic classes, such as tetracyclines, fluoroquinolones, macrolides, lincosamides, and aminoglycosides [11]. Intermediate resistance to vancomycin (VISA, vancomycin-intermediate *S. aureus*) or full resistance (VRSA, vancomycin-resistant *S. aureus*) have emerged [12]. The former appeared in Japan and subsequently in USA and Europe, and was designated as having minimum inhibitory concentrations (MICs) of 8-16 mg/L [13]. Among VISA strains, 90% are heterogeneous vancomycin-intermediate (heteroresistant; H-VISA); they are methicillin-resistant but contain a low number of VISA, which may increase

after vancomycin exposure [14]. A limited number of isolates of VRSA have been identified to date.

In addition to VISA and VRSA, a number of strains have ‘‘decreased susceptibility’’ to vancomycin. Some high-inoculum staphylococcal infections as bacteraemia, endocarditis and osteomyelitis have been associated with heteroresistance [15]. Vancomycin heteroresistance has been linked to strains susceptible to vancomycin but with high MIC values within the susceptibility category [16]. These strains are still considered susceptible by the sensitivity tests, but high concentration of vancomycin, close to the ‘‘intermediate’’ level, are required to inhibit them (MIC 2 mg/L). Vancomycin MICs of 1.5-2.0 mg/L are considered an independent predictor of poor response to vancomycin therapy for MRSA infection, even when vancomycin trough levels >15 mg/L are achieved [17].

The decreased susceptibility makes serious infections poorly treated by vancomycin. This may be especially true for MRSA pneumonia, due to a difficult penetration of vancomycin in the alveolar lining fluid [18]. It has been suggested that strains with vancomycin MIC of 1-2 mg/L should be considered H-VISA or VISA [19] since even the new Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoint for vancomycin (≤2 mg/L) may fail to precisely differentiate potential responders to vancomycin therapy. This suggested that the breakpoint value should be lowered to 1 or 0.5 mg/L [19]. Additionally, resistance to the newest antibiotics licensed to treat MRSA infections, such as linezolid and daptomycin, has emerged [20, 21]. Therefore, there are two main problems with MRSA: the therapeutic options to treat MRSA infections are limited and, in addition, MRSA has also a propensity to acquire resistance to the newest antibiotics.

1.2 Epidemiology of MRSA

S. aureus is a human pathogen causing a multitude of infections, not only in the hospital, but also in the community [22]. *S. aureus* infections range from skin infections, such as furunculosis and impetigo, to severe infections such as bloodstream infections, nosocomial pneumonia, surgical wound infection and prosthetic implant infection [23-25].

Data from the US National Nosocomial Infections Surveillance System found that, in 2003, 60% of *S. aureus* in the ICU were MRSA [26]. In this setting, MRSA causes mainly bloodstream infection and lower respiratory tract infection [27].

Similarly, the one-day European prevalence of infection (EPIC) study in the ICU [28] has shown that *S. aureus* is the second most common microorganism isolated in culture-positive infected patients (16%) after *Pseudomonas aeruginosa* [29]. Among *S. aureus*, methicillin resistance ranges from 6.2% in countries with low resistance (e.g. Denmark, Finland, Netherlands, Norway, and Sweden) to 50.4% in countries with high antimicrobial resistance (e.g. Greece, Israel, Italy, Malta, Portugal, Spain, and Turkey), accounting for 8% of all isolates.

However, in the last years, several countries with a high proportion of MRSA, including the UK, Italy, France and Spain, reported a significant decrease. In UK the percentage of MRSA in bloodstream infection decreased from 31% in 2007 to 19% in 2009, perhaps related to national actions such as the mandatory reporting of bacteraemia or the use of decolonisation protocols [30, 31]. Nevertheless, the map of MRSA prevalence in Europe still shows a remarkable distinction between the rates in Southern and Western Europe and those of Northern Europe.

The different distribution of MRSA among countries and hospitals may be due to several factors [32, 33]:

- Different national guidelines for prevention of MRSA dissemination and infection;
- Absence of reporting outbreaks at national or regional level;
- Lack of monitoring of readmission in the hospital of known MRSA carriers;
- Different hygiene precautions/isolation, recommendations for MRSA screening, and barrier precautions to prevent re-colonisation;
- Different decolonisation strategies and prescriptions;
- Lack of guidelines on restriction of antibiotic use;
- Inadequate level of education of personnel;
- Different guidelines for care or social interactions with MRSA positive patients after discharge from the hospital also in relation to decolonisation schedules.

1.3 **Pathogenesis of ICU infection**

1.3.1 Definitions

Carriage is defined as the patient's state where the same strain of potentially pathogenic microorganism (PPM) is isolated from two consecutive surveillance samples of saliva, gastric fluid or faeces, in any concentration, over a period of at least one week [34, 35].

High-grade carriage or overgrowth is defined as the presence of $\geq$ 2+ or $\geq 10^5$ colonies forming units (CFU) per millilitre of saliva, or per gram of faeces, or per millilitre of other digestive tract secretions [34, 35].

Colonisation should be distinguished from carriage. Colonisation is defined as the presence of a microorganism in an internal organ that is normally sterile (e.g. lower airways, bladder), without inflammatory host response [34, 35]. Samples of lower airway secretions, wound fluid, and urine generally yield $<10^5$ CFU of PPMs per millilitre of diagnostic sample. In general, only a few leukocytes are present in colonised internal organs on a semiquantitative scale of + = few, ++ = moderate, and +++ = many leukocytes [34, 35].

Carriage and colonisation are two different stages of the pathogenesis of endogenous infection in ICU patients. The first stage is practically always the oropharyngeal and gastrointestinal carrier state followed by overgrowth. Once the PPM is present in overgrowth concentrations it migrates into the sterile internal organs in order to colonise the lower airways and the bladder [Figure 1.1]. Unfortunately, the term colonisation is often used to cover both stages of carriage and colonisation.

Infection is a microbiologically proven clinical diagnosis of inflammation, local and/or generalized. This includes not only clinical signs but also the presence of a moderate number of leukocytes, and of $\geq 10^5$ CFU per millilitre of diagnostic samples obtained from an internal organ, or the isolation of the microorganism from blood, cerebrospinal fluid, pleural or peritoneal fluid [34, 35]. Criteria for the diagnosis of lower respiratory tract infection remain controversial. The association between radiological, clinical and laboratory findings are commonly required. The diagnosis of microbiologically confirmed lower respiratory tract infection relies on the following criteria [36]:

- presence of new or progressive infiltrate on chest X-ray, and
- fever $\geq 38.3°$ C, and

- leucocytosis (white blood cells > 12,000/ml) or leucopenia (white blood cells < 4,000/ml), and
- purulent tracheal aspirate, and
- tracheal aspirate yielding $\geq 10^5$ CFU/ml, or protected specimen brush (PSB) yielding $\geq 10^3$ CFU/ml, or bronchoalveolar lavage (BAL) yielding $\geq 10^4$ CFU/ml.

In case of the first four criteria are fulfilled, but tracheal aspirate, or PSB, or BAL are sterile, the diagnosis is only clinical. Ventilator-associated pneumonia (VAP) is a lower respiratory tract infection that develops 48 hours or more after intubation with an endotracheal tube or tracheostomy tube, and was not present before intubation [37]. The absence of lung infiltrates on chest X-ray defines the diagnosis of tracheobronchitis [36, 38]. The diagnosis of urinary tract infection rests on a freshly voided catheter urine specimen containing $\geq 10^5$ bacteria or yeasts per millilitre of urine and ≥ 5 white blood cells per high power field [36].

Diagnostic samples are samples from internal organs that are normally sterile, such as the lower airways, bladder, and blood. They are obtained when clinically indicated and allow the diagnosis of colonisation and infection [34, 35].

Surveillance samples are samples from body sites where PPMs are carried, such as the digestive tract. A set of surveillance samples consists of throat and rectal swabs taken on admission of the patient to the ICU and twice weekly, thereafter. The purpose of surveillance samples is the determination of the microbiological endpoint of the level of carriage of PPMs. They are not useful for diagnosing infection of internal organs, as diagnostic samples are required for this purpose [34, 35].

Data obtained from surveillance samples and diagnostic samples allow us to calculate the intrinsic pathogenicity index (IPI) of microorganisms, including *S.*

aureus (Table 1.1). IPI is defined as the ratio between the number of patients infected by a microorganism and the number of carriers of the same microorganism in throat and rectum [35, 36]. The range of this index is from 0 to 1. In accordance with IPI microorganisms can be divided into three categories: low pathogenic microorganisms, potentially pathogenic microorganisms (PPMs), and highly pathogenic microorganisms. Low pathogens have an IPI of 0.01 and rarely cause infections when carried by an individual. PPMs may cause infection when the defence mechanisms are reduced and they have an IPI of about 0.1. MRSA is classified as an "abnormal" PPM as its typical feature is an IPI between 0.1 and 0.3 [40]. The classification is useful in ICU practice because it is best adapted to the ICU flora, and integrates several concepts of clinical epidemiology, such as the distinction between "normal" and "abnormal" flora rather than the distinction of microorganisms into "community" and "hospital".

Table 1.1 Classification of microorganisms based on their intrinsic pathogenicity

Microorganisms	Intrinsic Pathogenicity	Flora	IPI
1.- **Indigenous flora** **Oropharynx**: Peptostreptococci, *Streptococcus viridans, Veilonella spp.* **Gut**: *Bacteroides* spp, *Clostridium spp*, *Enterococci* **Skin**: CNS, *Propionibacterium acnes* Vagina: Peptostreptococci, *Bacteroides, Lactobacillus* spp.	**Low**	**Normal**	**≤0.01**
2.- **Community micro-organisms** **Oropharynx**: *Streptococcus pneumoniae, Haemophilus influenzae, Branhamella catarrhalis, methicillin-sensitive Staphylococcus aureus, Candida spp.* **Gut**: *Escherichia coli, methicillin-sensitive Staphylococcus aureus, Candida spp.*	**Potential**		**≤0.1**
3.- **Hospital micro-organisms** *Klebsiella, Proteus, Morganella, Enterobacter, Citrobacter, Serratia, Pseudomonas spp, Acinetobacter spp, Stenotropomonas maltophilia, Burkolderia cepacia, methicillin-resistant Staphylococcus aureus*	**Potential**	**Abnormal**	**≤0.1**
4.- **Epidemic micro-organisms** *Neisseria meningitidis. Salmonella spp*	**High**		**Around 1**

IPI, intrinsic pathogenicity index; spp, species; CNS, coagulase-negative staphylococci

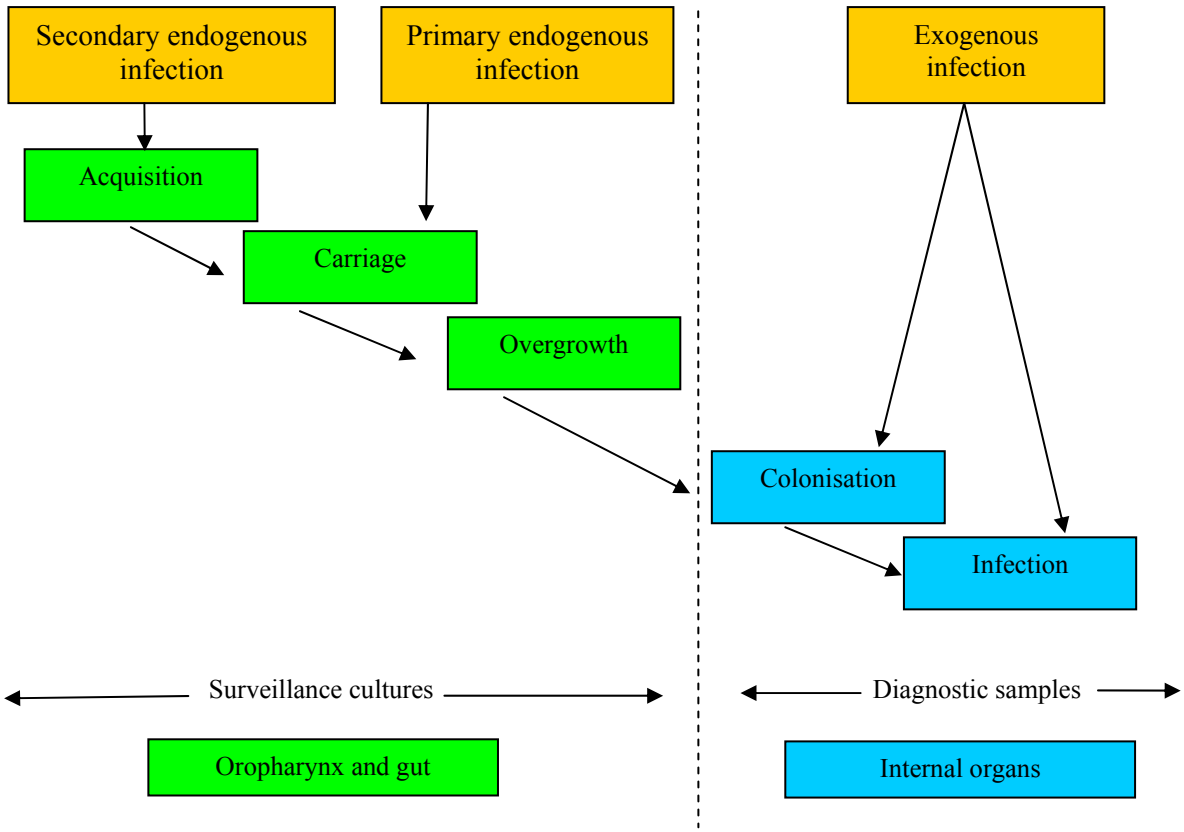

Figure 1.1 The slippery slope of the pathogenesis of endogenous and exogenous infection due to PPMs in the ICU

Knowledge of the carrier state allows the distinction between three types of infection in the ICU (Table 1.2, Figures 1.1 and 1.2) [35]:

1. Primary endogenous infections are the most frequent infection in the ICU (50%-85%) [41, 42]. These infections are caused by normal or abnormal PPMs imported into the ICU by the patient's admission flora. These episodes of infections in general occur early, during the first week of ICU stay. Adequate parenteral antibiotics given immediately on ICU admission may reduce the incidence of primary endogenous infection [43, 44].

Table 1.2 Classification of ICU infections according with the criterion of carriage

Type of infection	PPM	Timing	Frequency
Primary endogenous	Normal and abnormal	Within 1 week of ICU stay	55%
Secondary endogenous	Abnormal	After 1 week of ICU stay	30%
Exogenous	Abnormal	Any time during ICU stay	15%

PPM, potentially pathogenic microorganism; ICU, intensive care unit.

2. Secondary endogenous infections are caused by abnormal PPMs, including aerobic Gram-negative bacteria (AGNB) and MRSA, not carried in throat and/or gut at the time of admission to the ICU, but acquired during ICU-treatment prior to the infection. The incidence of this infection is about 30% of all ICU infections [35, 41, 42]. Secondary endogenous infection usually occurs after 1 week of stay on the ICU. The PPMs are firstly acquired in the oropharynx and/or gut and subsequently carriage and overgrowth may develop. Topical application of antimicrobials has been shown to control secondary endogenous infections together with high levels of hygiene [44].

3. Exogenous infections are approximately 10-15% of all ICU infections and are caused by abnormal hospital PPMs introduced into the patient's internal organ from the environment, either animate or inanimate [44]. Bacteria are transferred directly into an internal organ, without previous carriage, and surveillance samples are negative for PPMs that readily appear in diagnostic samples. This infection may occur at any time during ICU stay. High levels of hygiene are required to control these infections.

According to this criterion of carriage, only secondary endogenous and exogenous infections are true ICU-acquired infections, whilst primary endogenous infections are considered to be imported infections.

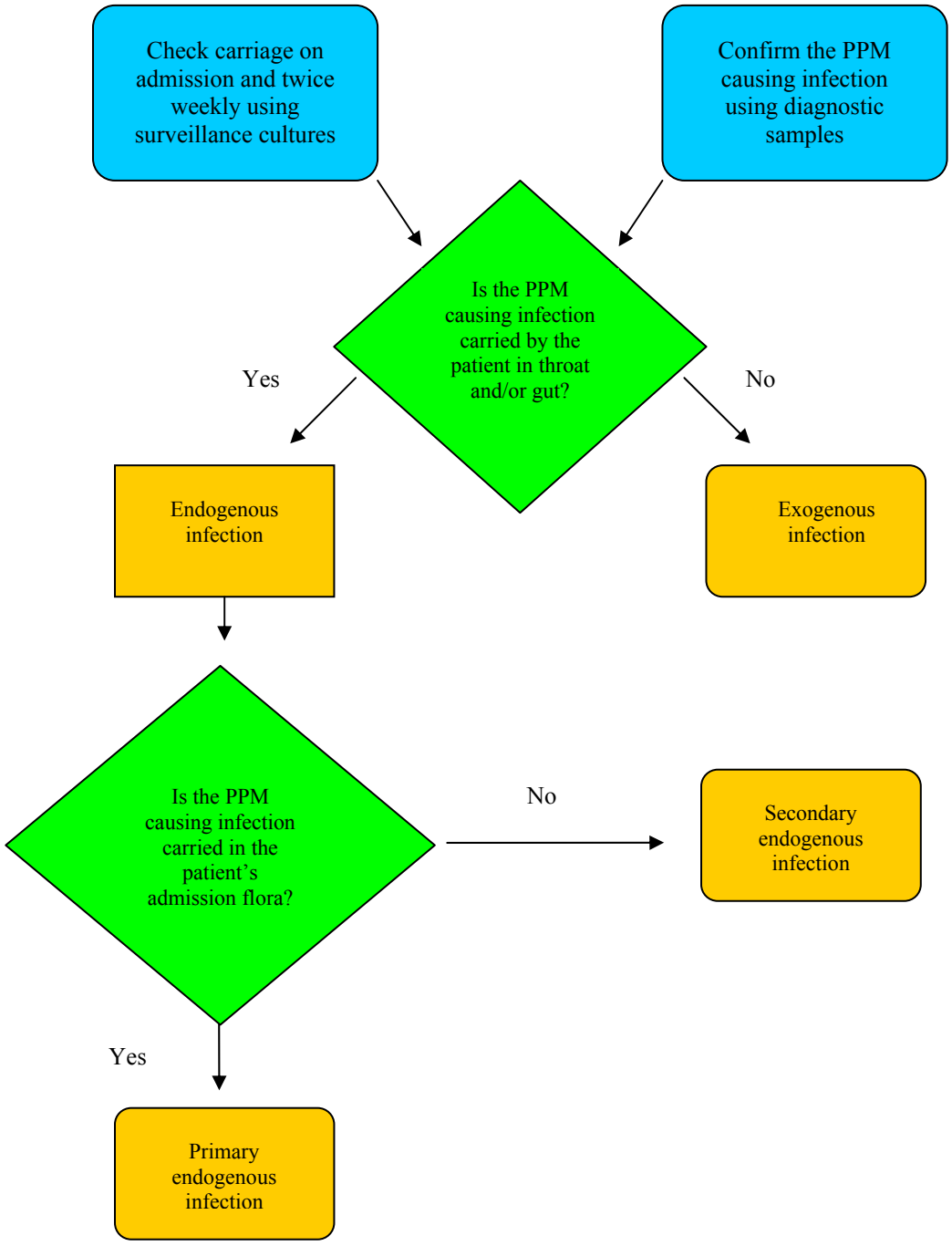

Figure 1.2 Flowchart for classifying ICU infections using the carrier state criterion
PPM, potentially pathogenic microorganism

1.3.2 The microbiota of critically ill patients

In recent years, the use of new laboratory techniques has opened up a new area of research, allowing investigation of the complex population of microorganisms (not cultivable under conventional methods) present in the gut of the critically ill: the microbiota and the microbiome. The microbiota is an ecological community of commensal, symbiotic and pathogenic microorganisms, including bacteria, archaea, protists, fungi and viruses. The microbiome describes their collective genome [45]. In literature, the distinction in terminology is sometimes ambiguous as microbiome is used interchangeably with microbiota.

The intestinal microbiota in healthy adults is dominated by four phyla: *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%) and *Actinobacteria* (3%) [46]. *Firmicutes* are Gram-positive aerobic and anaerobic bacteria, including *Staphylococci*, *Streptococci*, *Enterococci*, *Bacilli*, and *Clostridia*. *Bacteroidetes* are Gram-negative bacteria including *Bacteroides* species. *Proteobacteria* are the major phylum of Gram-negative bacteria, including aerobic and anaerobic Gram-negative bacteria: potentially pathogenic aerobic Gram-negative bacilli belong to this phylum. In general, the *Firmicutes* to *Bacteroidetes* ratio is important in human microbiota composition. It evolves during different stages of age being 0.4 in infants, 10.9 in adults, and 0.6 in elderly individuals [47]. Within the microbiota exists a hierarchy of dominant anaerobic bacteria of the genera *Bacteroides*, *Aubacterium*, *Bifidobacterium*, *Peptostreptococcus*, *Ruminococcus*, *Clostridium* and *Propionibacterium*, and a subdominant bacteria of Enterobacteriaceae family, in particular *E. coli*, and the genera *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Fusobacterium*, and *Methanobrevibacter* [48].

Dysbiosis of the gut microbiota, i.e. a condition in which normal composition of microbiota is disrupted and is detrimental to the host, has been associated with different diseases such as diabetes, obesity, inflammatory bowel disease, and rheumatoid arthritis [49]. In healthy subjects intestinal microbiota protects against the invasion of low pathogenic microorganisms (e.g. *Enterococci*), potentially pathogenic microorganisms (e.g. AGNB), and *C. difficile*.

In critically ill patients the severity of the underlying disease, nutrients deprivation, the use of vasoactive agents, opioids, stress ulcer prophylaxis, and other drugs including antibiotics, have been shown to impact the gut microbiota [50]. However, it is not yet clear which of these factors are more important in contributing to dysbiosis [50]. Antibiotic treatment may cause a disruption of normal microbiota allowing for the carriage and overgrowth of antibiotic-resistant PPMs and opportunistic microorganisms [51]. In general, the microbiota of the critically ill shows a loss of microbial diversity and richness, and demonstrates a tendency for a single taxon, usually PPMs, to dominate a given microbiota, and a loss of site specificity, i.e. carriage with the same microorganism at multiple sites [52].

Therefore, restoring the microbiota may have a potential to reduce infection, but, at the present time, information on the dynamics of the microbiota in the critically ill is limited. Section 1.4 will provide current evidence of the impact of selective decontamination of the digestive tract on gut microbiota.

1.3.2.1 Carriage, overgrowth and infection due to PPMs.

Microorganisms carried by healthy individuals usually belong to the normal flora (Table 1.1). They are mainly anaerobes and aerobes of the indigenous flora, together

with PPMs, such as *Streptococcus pneumoniae,* methicillin-sensitive *S. aureus* (MSSA), *Haemophilus influenzae, Branhamella catarrhalis, Escherichia coli* and *Candida albicans*. *E. coli* is considered normal flora in the intestinal tract, but its presence in the oropharyngeal cavity is abnormal. *S. aureus* is part of the flora of the oropharynx, anterior nares, and the intestinal tract in 20-40% of healthy individuals and is considered normal flora [34]. Abnormal flora is uncommon in healthy people, and may be only transiently carried, and includes AGNB, such as *Klebsiella, Proteus, Morganella, Enterobacter, Citrobacter, Serratia, Acinetobacter,* and *Pseudomonas* species, and MRSA [34, 53]. About 30% of patients with an underlying chronic disease, such as diabetes, chronic obstructive pulmonary disease (COPD), end-stage renal failure receiving haemodialysis, may demonstrate abnormal flora in their oropharynx and gut [34]. Moreover, previously healthy patients admitted to the ICU and requiring long-term ventilatory support due to an acute insult, such as (surgical) trauma, pancreatitis, or acute liver failure, may become carriers of abnormal flora in their digestive tract. Therefore the critical illness is the most important factor in the conversion of normal to abnormal carrier state and from low- to high-grade carriage [34, 44].

The anterior nares are the main ecological niche for *S. aureus*, including MRSA. About 20% of individuals are nasal carriers of *S. aureus*, and 30% are transiently carriers [54]. However, *S. aureus* may be carried in other different sites such as axillae, groin, oropharynx and gastrointestinal tract, also in overgrowth concentration. Oropharyngeal carriage in overgrowth concentration is a reservoir from which *S. aureus* may migrate through aspiration into the tracheobronchial tree causing colonisation and infection of the lower airways. Similarly, gut carriage and overgrowth favour migration into the urinary tract and translocation into the

bloodstream, causing urinary tract and bloodstream infection, respectively (Figure 1.3). Subjects with *S. aureus* carriage generally develop infection with their colonising strain [55]. In a study on bacteraemia, blood isolates of *S. aureus* were related to nasal isolates in 82% of patients [56].

During mechanical ventilation, micro aspiration of the oropharyngeal content, and subsequent endogenous colonisation of the lower airways is the main mechanism of lower respiratory tract infection, and, in particular, of ventilator associated pneumonia (Figure 1.1, Figure 1.3). Additionally, MRSA may be introduced directly into the lower airways from an external source, either animate or inanimate (i.e. exogenous colonisation). The host's mechanical, cellular and humoral defences are available in the oropharynx and the lower airways to prevent pneumonia.

Risk factors for MRSA carriage and infection on admission to the hospital or the ICU are previous MRSA infection and/or carriage, previous nursing home admission, prior hospital or ICU admission, prior antibiotics, presence of skin lesions or pressure ulcers, comorbidities such as heart failure, COPD, renal failure and diabetes [57].

The basis for *S. aureus* carriage is complex and not clearly understood, but appears to involve the host's contact with *S. aureus*, and the ability of *S. aureus* of adhering to host cells and to escape the immune response [54]. *S. aureus* has an extensive body of virulence factors, which are involved in infection. Numerous surface proteins bind to collagen, fibronectin and fibrinogen, and may explain the ability of *S. aureus* to cause vascular, bone and joint, and prosthetic-device infections. Once *S. aureus* adheres to tissue or devices, it is able to grow and live forming biofilms, or to survive into the endothelial cells in order to escape host defences and antibiotics [58]. Additionally, *S. aureus* generates an anti-phagocytic microcapsule, is able to

bind the Fc portion of immunoglobulins, secretes a protein which inhibits the chemotaxis, and produces leukocidines which destroy leukocytes [54]. *S. aureus* produces numerous enzymes, proteases, lipases, elastases, that allow it to invade and destroy host tissues. *S. aureus* is able to determine septic shock by interacting with the immune system and the coagulation pathway [59]. Some strain produces exfoliative toxins capable of causing scalded skin syndrome or bullous impetigo. In summary, *S. aureus* has many mechanisms to produce disease and to escape host defences. Strains of *S. aureus*, including MRSA, are different to each other due to a different distribution and presence of virulence factors.

Although there is a level of uncertainty about whether MRSA is more virulent than MSSA, invasive MRSA infections are associated with higher cost and limited treatment options. A meta-analysis and epidemiological studies demonstrated an increased morbidity and mortality from nosocomial MRSA infections, such as pneumonia, bloodstream infections, and surgical-site infections, compared with those from MSSA [60-65]. Other studies did not demonstrate this correlation [66-68]. A recent systematic review evaluated the impact of methicillin resistance on mortality in *S. aureus* VAP [69]. The crude in-hospital and ICU-mortality was higher in patients with VAP due to MRSA as opposed to MSSA (OR 1.79, 05% CI 1.21-2.65 and OR 2.49, 95% CO 1.54-4.06, respectively).

1.4 Prevention of ICU infections

This section briefly illustrates the evidence of the literature behind the manoeuvres used to prevent ICU infection with a particular attention to the use of enteral

antimicrobials to control carriage, overgrowth and infection due to PPMs. Figure 1.3 shows the pathogenesis of ICU infections and the preventative manoeuvres.

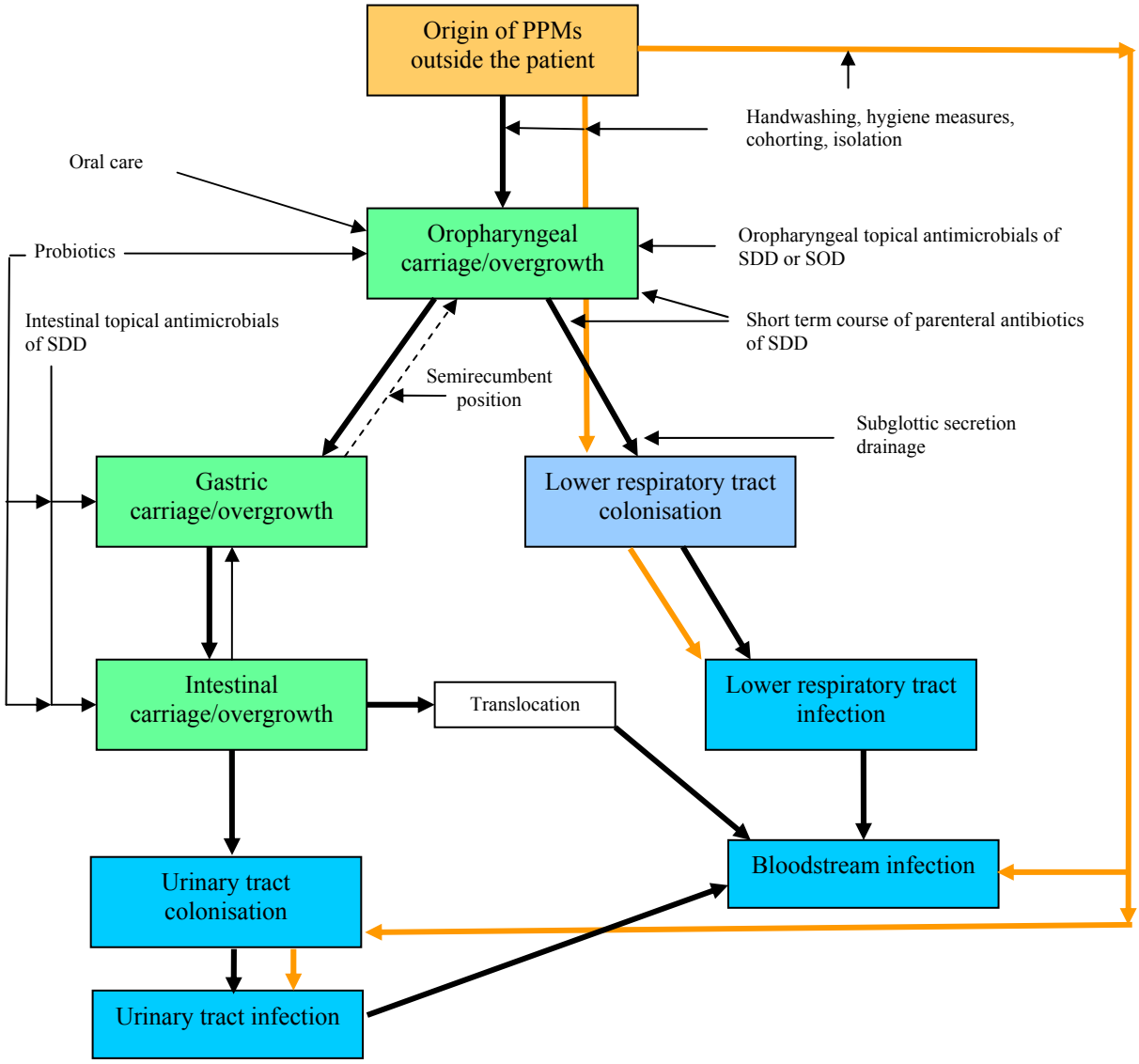

Figure 1.3 Summary of the pathogenesis of ICU infections due to potentially pathogenic microorganisms and their preventative measures.
PPMs, potentially pathogenic microorganisms; SDD, selective decontamination of the digestive tract; SOD, selective oropharyngeal decontamination. Black arrows indicate endogenous infections. Orange arrows indicate exogenous infections.

1.4.1 Hygiene measures

Handwashing is a fundamental manoeuvre for the prevention of infection in the ICU. It is a well recognized, simple, and low-cost prevention strategy that reduces the risk of healthcare workers transmitting PPMs from one patient to another and, thus, reducing ICU-acquired infection. Therefore, handwashing and other hygiene manoeuvres can prevent secondary endogenous and exogenous infection. Handwashing does not affect primary endogenous infections caused by PPMs already present in throat and gut of the patient on ICU admission [37, 70].

1.4.2 Probiotics

Probiotics are "living microorganisms that (when ingested) have a beneficial effect in the prevention and treatment of specific pathologic conditions" [71]. They may prevent ICU infections by reducing carriage and overgrowth of PPMs in the oropharynx and gut. Additionally, probiotics exert their beneficial effect by enhancing gut barrier function, maintaining a normal intestinal microbiota, synthesizing antibacterial substances, and stimulating local immunity [72]. Two recent meta-analyses showed that probiotics reduced the incidence of lower respiratory tract infection in mechanically ventilated patients, although an impact on mortality was not demonstrated [73, 74]. However, these results are not robust do to several limitations of meta-analyses. Large sample size and high-quality RCTs are required to assess the effect of probiotics in preventing lower respiratory tract infection, and in reducing mortality in ventilated ICU patients.

1.4.3 Semirecumbent position

The guidelines of the American Thoracic Society, the Infectious Diseases Society of America, and the Centers for Disease Control and Prevention (CDC), have recommended semi-recumbent positioning for the prevention of VAP in mechanically ventilated patients (i.e. head of bed elevation to 30°-45°) [75, 76]. Supine positioning, gastric tubes and stomach contents lead to the reflux of gastric contents and aspiration, causing VAP. Semi-recumbent position may avoid these events and may reduce VAP. Using radioactively labelled enteral feeding, the risk of gastro-oesophageal reflux and aspiration was lower among patients in a semi-recumbent position than those in a supine position [77, 78].

A recent systematic review showed that semirecumbent position of 30° to 60° significantly reduced the risk of clinically suspected VAP compared to supine position (8 trials, 759 participants, relative risk [RR] 0.36, 95% CI 0.25-0.50) [79]. However, semirecumbent position did not impact microbiologically confirmed VAP, mortality, length of ICU stay, duration of mechanical ventilation, and antibiotic use. Adequately powered RCTs are needed to assess whether the semi-recumbent position is superior to the supine position regarding patient-important outcomes, and which degree of head-of bed elevation is optimal to balance the benefits with the potential risks, e.g. thromboembolism, pressure sores or haemodynamic instability.

1.4.4 Subglottic secretion drainage

Current guidelines recommend the use of endotracheal tubes with subglottic secretion drainage to prevent ventilator-associated pneumonia. In a recent meta-

analysis including 17 trials and 3,369 patients, subglottic secretion drainage was associated with lower ventilator-associated pneumonia rates (RR, 0.58; 95% CI, 0.51-0.67), but there were no significant differences between groups in duration of mechanical ventilation, ICU length of stay, ventilator-associated events, and mortality (RR, 0.93; 95% CI, 0.84-1.03) [80]. Further studies are required to demonstrate the benefits of subglottic secretion drainage on mortality.

1.4.5 Oral care

Oral care with chlorhexidine to prevent lower respiratory tract infection in ICU patients has been reported by scientific societies. However, the evidence that chlorhexidine is effective and safe seems less robust than previously supposed. Many meta-analyses of RCTs have reported lower VAP rates, but in blinded studies the impact was absent [81]. Additionally, chlorhexidine has never been shown to reduce mortality, and, even, some meta-analyses have demonstrated an increased mortality [83]. The first part of this thesis will focus on the efficacy of oral chlorhexidine (chapter 5) and topical mupirocin (chapter 4) to control infection in ICU patients.

1.4.6 Selective decontamination of the digestive tract

To control carriage and overgrowth of PPMs in the oropharynx and gut of critically ill ICU patients a regimen of antimicrobial prophylaxis using parenteral and enteral antimicrobials was designed in the early eighties of the previous century. The aim of the manoeuvre was to eradicate carriage of normal and abnormal flora, and to prevent endogenous and exogenous infections due these microorganims. This

regimen was termed selective decontamination of the digestive tract or SDD. Its full protocol is based on four pillars [44, 84]:

1. parenteral antibiotics given immediately on admission for 4 days to control primary endogenous infections due to PPMs already present in the patient's admission flora. Healthy patients with normal flora can be treated with cefotaxime 80-100 mg/kg/day. Patients with a chronic underlying disease or patients transferred from other ICUs or general wards may carry both normal and abnormal flora in throat and gut, and may require an antipseudomonal cephalosporin or a glycopeptide if *P. aeruginosa* or MRSA carriage are expected, respectively.
2. enteral non-absorbable antimicrobials, i.e. polymyxin E, tobramycin and amphotericin B (PTA) to control secondary carriage/overgrowth and subsequent secondary endogenous infections due to PPMs acquired during ICU stay. Half a gram of gel or paste containing 2% PTA is applied to the oropharyngeal mucosa four times a day. Additionally, 10 ml of a suspension containing 100 mg of polymyxin E, 80 mg of tobramycin and 500 mg of amphotericin B is administered into the gut through the nasogastric tube four times a day. In case of MRSA endemicity in the ICU or MRSA carriage, half a gram of a 4% vancomycin gel/paste in the oropharynx and/or 500 mg of vancomycin solution in the intestine can be added four times a day to the classical PTA regimen to eradicate MRSA carriage and to prevent MRSA infections.
3. high standards of hygiene are required to control secondary endogenous and exogenous infections due to transmission of ICU-associated microorganisms.

4. surveillance cultures of throat and rectum on admission and, afterwards, twice weekly are needed to monitor the efficacy of SDD, and to detect the emergence of resistance at early stage.

The combination of polymyxin and tobramycin was chosen because it covers the majority of abnormal AGNB, including *Pseudomonas* species, and it is synergic in vitro. The use of a polyene, such as amphotericin B or nystatin, eradicates fungal overgrowth [44]. The use of the parenteral antibiotic cefotaxime was chosen because its spectrum included both normal and most abnormal PPMs, and its pharmacokinetic properties included a high excretion in saliva and bile, associated with eradication of overgrowth [44].

A modified regimen of SDD, called oropharyngeal selective decontamination (SOD), was introduced. SOD includes the oropharyngeal component without intestinal and parenteral antimicrobials.

There have been 71 RCTs of SDD and more than 15 meta-analyses of RCTs over a research period of more than 30 years. The most robust meta-analyses showed that SDD significantly reduced lower airway infection by 72% (odds ratio [OR] 0.28, 95% CI, 0.20-0.38), bloodstream infection by 27% (OR 0.73, 95% CI, 0.59-0.90), and mortality by 18% (OR, 0.82, 95% CI, 0.72-0.93) to 29% (OR, 0.71, 95% CI, 0.61-0.82) [44, 83, 85-87]. A recent meta-analysis demonstrated that SDD was superior to SOD in reducing mortality [88]. This difference in effectiveness may be related to the intestinal and parenteral components which are not part of the SOD regimen [88]. Emergence of resistance was not demonstrated in meta-analyses and large RCTs of SDD/SOD [89, 90].

Recent studies analysed the impact of SDD on gut microbiota. These studies showed that, compared with healthy subjects, the microbiota of ICU patients receiving SDD

was characterized by lower microbial diversity, lower levels of *E. coli* and anaerobic Gram-positive butyrate-producing bacteria (e.g. *Faecalibacterium prausnitzii*), and increased abundance of *Bacteroidetes* and enterococci [91-93].

A common effect of SDD is a relative increase of enterococci in the gut [90]. This can be explained by the intrinsic resistance of enterococci to the SDD antimicrobials. Enterococci have been considered low pathogenic microorganisms as usually, in case of infection, lead to a relative low inflammatory state. However, these microorganisms may be a concern in ICU due to emerging antibiotic resistance (e.g. VRE) and increasing virulence. Previous studies have shown that SDD therapy can select for intestinal colonisation by enterococci [91, 94]. A recent meta-analysis of van der Bij et al [95], determined the antibiotic resistance rate of Gram-positive cocci in blood and respiratory specimens in 42 Dutch ICUs in the period from 2008 to 2013, indicating that SDD was not associated with an increase of antibiotic resistance in Gram-positive cocci, including enterococci. Additionally, a meta-analysis of the microbiology of SDD did not demonstrate any increase in Gram-positive infections [96].

There are scarce data regarding the re-colonisation of the gut and infection rates upon ICU discharge and cessation of SDD. After discontinuation of SDD/SOD patients may acquire carriage with typical hospital pathogens or suppressed carriage of these bacteria may emerge. Although incidences of hospital acquired infections tend to be higher in patients that had received SDD or SOD during ICU-stay, it seems unlikely that these infections have an effect on hospital mortality [97].

As the traditional SDD regimen does not target MRSA, adjustment of the SDD antimicrobials by adding vancomycin have been used to control MRSA carriage and overgrowth in ICUs with MRSA endemicity. The enteral administration of 2 gr/day

of vancomycin obtains faecal vancomycin levels between 3,000 and 24,000 μg per gram of faeces, whilst after 2 gr/day of parenteral vancomycin faecal vancomycin levels are between 6 and 11 μg per gram of faeces [98, 99]. High levels of faecal vancomycin may explain why VRE is absent when using enteral vancomycin. On the contrary, non-lethal, low concentrations of vancomycin in the gut after parenteral administration may promote the selection of VRE.

Although data on the impact of enteral vancomycin on gut microbiota in ICU patients are very limited, changes of the gut microbiota in these patients should be acknowledged. In non-ICU patients, oral vancomycin treatment decreases the richness and diversity of human microbiota, reduces the level of *Bacteroidetes* phylum, whilst *Proteobacteria* and *Fusobacteria* phyla increase. Additionally, among *Proteobacteria*, abnormal AGNB increase [100]. Additionally, a disadvantage of this approach might be the increased selective pressure for VRE and VISA in the ICU.

The main issue of this thesis is the effectiveness of enteral vancomycin to control MRSA infection in ICU patients. The effect of enteral vancomycin on the emergence of resistant strains, i.e. VISA and VRE, will be assessed, and the potential impact on gut microbiota will be discussed.

2 AIMS OF THE THESIS

2.1 Background

Traditionally, the control of MRSA infection has been focused on the prevention of transmission between patients via the hands of healthcare workers, such as handwashing, patient's isolation or cohorting, use of sterile equipments, hygiene, and cleanliness of the environment [101, 102]. However, it has been repeatedly shown that a large proportion of MRSA infections in the ICU originate from the patient's own flora (i.e. endogenous infections) [103, 104], as critical illness promotes abnormal carriage in throat and gut of abnormal potential pathogens, including MRSA [105]. Moreover, MRSA carriage in the oropharynx and gut has been shown to be an independent risk factor for overgrowth and subsequent endogenous MRSA infection of the lower respiratory tract and the bloodstream.

Both oropharyngeal chlorhexidine and nasal mupirocin have been used to control oropharyngeal overgrowth of MRSA [104, 106, 107]. However, these manoeuvres are expected to impact only lower respiratory tract infection. The use of enteral vancomycin, including oropharyngeal and intestinal administration, to control MRSA carriage of the oropharynx and gut, and to prevent severe MRSA infection of the lower airways and the bloodstream is uncommon [108].

2.2 Aims of the thesis

The aims of the thesis are:

- To assess the effectiveness of oropharyngeal mupirocin and chlorhexidine to control severe ICU infections, including MRSA infection;

- To assess the effectiveness of enteral vancomycin to control MRSA infections in ICU patients and to evaluate the safety of the manoeuvre in terms of VISA and VRE emergence;

- To evaluate the impact of oropharyngeal vancomycin on MRSA infection in mechanically ventilated ICU patients.

3 MATERIALS AND METHODS

3.1 Structure of the research

The research project is divided into two research sections:

1. The first part focuses on the effectiveness of some manoeuvres applied to the oral cavity to control severe ICU infections, including MRSA infections.

2. The second part explores:

   - The effectiveness of enteral vancomycin to control severe ICU infection, including MRSA infection;
   - The safety of enteral vancomycin;
   - The impact of enteral vancomycin on MRSA infections in a cohort of mechanically ventilated ICU patients.

3.2 Details of the research

The main details of the research project are depicted in Figure 3.1, and are reported in the following summary. The research is divided into four chapters:

1. Chapter 4: systematic review and meta-analysis of RCTs to assess the effectiveness of topical mupirocin to control severe ICU infections, including infections due to both MSSA and MRSA, and to evaluate the impact on mortality.
2. Chapter 5: systematic review and meta-analysis to assess the effectiveness of oropharyngeal chlorhexidine to control severe infection in critically ill patients, including MRSA infection, and to evaluate the impact on mortality.

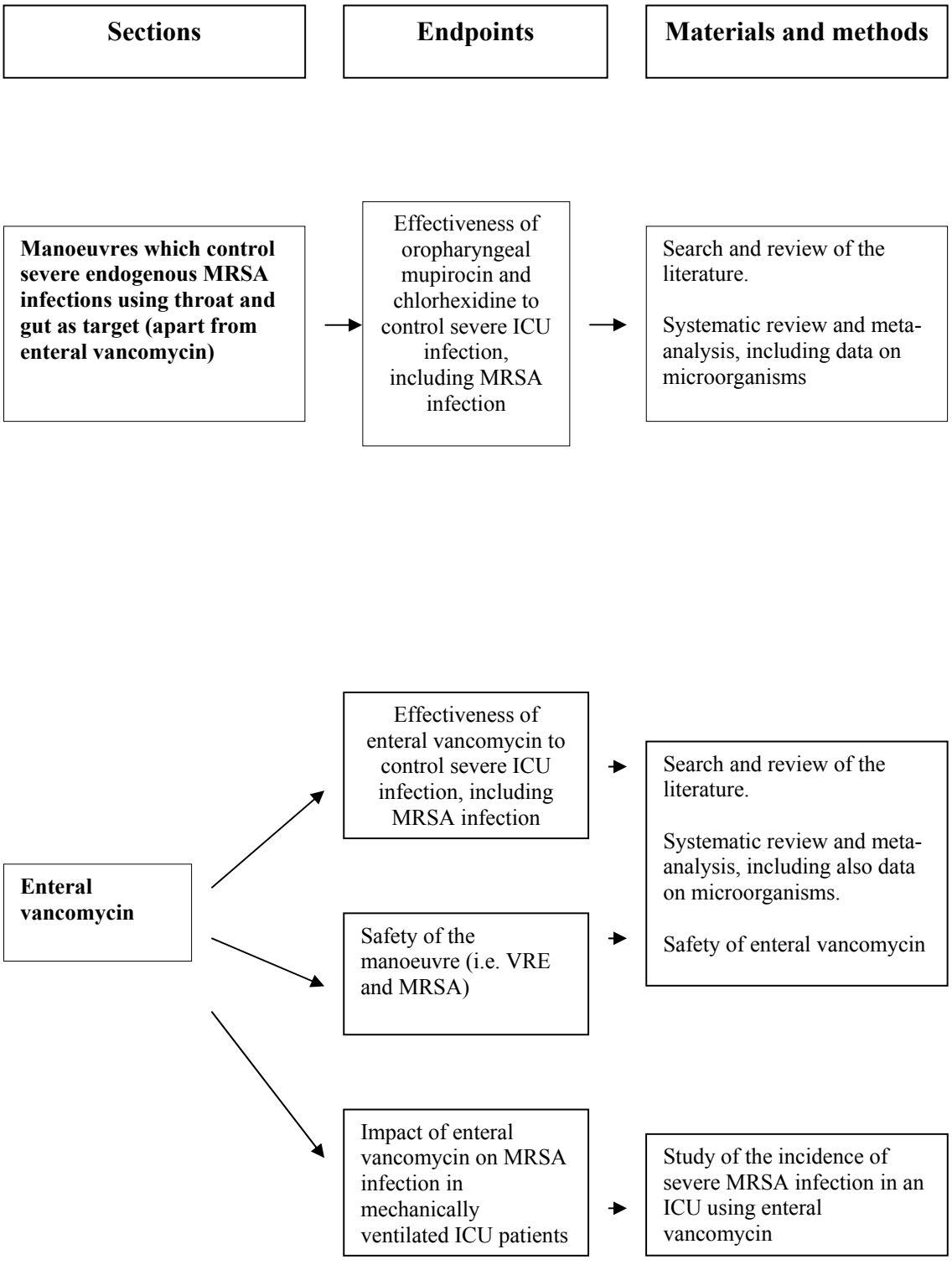

Figure 3.1 Diagram flow of the research project

3. Chapter 6: systematic review and meta-analysis to assess the effectiveness of enteral vancomycin to control MRSA carriage and infection. The emergence of VISA and VRE is evaluated.
4. Chapter 7: retrospective, 16-year, cohort study in ICU patients receiving enteral vancomycin. The endpoint is the incidence of MRSA severe infection.

For each chapter a separate section named “materials and methods” or “patients and methods” will be provided. Additionally, for each chapter a separate section entitled “discussion” will comment the results in the context of the evidence and the literature.

The main structure of the four chapters is described in the following points 3.3 and 3.4.

3.3 Systematic review and meta-analysis

In accordance with the PRISMA (**P**referred **R**eporting **I**tems for **S**ystematic Review and **M**eta-**a**nalysis) guidelines [109], chapters 4, 5, and 6 include specific sections labelled introduction, materials and methods, results, discussion, and conclusion. Each section will be differently expanded according to the endpoints of the systematic review. In general:

- The introduction describes the rationale of the review and what is already known, and delineates all questions being addressed with reference to **p**articipants, **i**nterventions, **c**omparisons, **o**utcomes and **s**tudy design (PICOS) [109].

- The materials and methods section specifies the eligibility criteria for each included study, the sources of information (e.g. databases), the search strategy, the study selection, the inclusion and exclusion criteria, the data collection process, the assessment of the quality of study using the Jadad score [110] or the risk of bias tool of the Cochrane collaboration [111] for RCTs, and the Newcastle-Ottawa scale for non-randomised studies [112], the summary measures (i.e. odds ratio with 95% confidence interval using the random effects model), and the synthesis of the results, including the evaluation of the heterogeneity (Cochran Q statistics and I^2) [113]. A funnel plot is created (if appropriate) for the main outcome to explore the presence of a publication bias. Computations are performed using the EasyMA software (M. Cucherat, Lyon, France) [114].
- The result section gives details on the number of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage. A flow diagram of the research is provided. Results of different outcomes are presented in detail, including confidence intervals, measures of heterogeneity, and if necessary, the forest plot of meta-analysis. Results of additional analyses, e.g. subgroup analyses, are included.
- The discussion section examines the main findings in the context of the evidence and the literature, presents details on the study limitations, and, if applicable, suggests implications for a future research.
- The conclusion section provides a summary of the results.

3.4 Observational study

Chapter 7 includes a retrospective, cohort study in intensive care unit patients receiving long-term mechanical ventilation and enteral vancomycin with the endpoint of MRSA infections.

The study adheres to the STROBE (**St**rengthening the **R**eporting of **Ob**servational studies in **E**pidemiology) statement [115]. The study includes the following sections: introduction, patients and methods, results, discussion, and conclusions. Details for each section are included in chapter 7. In general:

- The introduction section explains the rationale, the background, and the objectives of the study.
- The patients and methods section presents the key elements of the study design, describes the setting, the period of recruitment, the method of data collection, the participants (including the eligibility criteria), the microbiological methods, and the statistics.
- The results section gives details on participants, descriptive data (demographic and clinical data), and outcome data.
- The discussion summarises the key results, discusses limitations of the study, and gives the interpretation of the results.
- The conclusion provides a summary of the results.

4 EFFECTIVENESS OF MUPIROCIN TO CONTROL INFECTION IN ICU PATIENTS.

A SYSTEMATIC REVIEW AND META-ANALYSIS

4.1 Introduction

Digestive tract carriage and subsequent colonisation of internal organs are important steps in the pathogenesis of *S. aureus* infection, including MRSA infection. Approximately 30% of the population carries *S. aureus* in the oropharyngeal cavity, nares and the digestive tract. A large proportion of *S. aureus* infections originate from the patient's own flora [56, 116]. Carriage of *S. aureus* in overgrowth concentration has been considered a risk factor for endogenous infection, both primary and secondary, in patients undergoing surgery, in patients on dialysis, in those with liver cirrhosis, and in ICU patients [22, 57].

Mupirocin is a pseudomonic acid naturally produced by *Pseudomonas fluorescens*. It inhibits bacterial protein synthesis by reversibly binding to bacterial isoleucyl-tRNA-synthetase. It has excellent *in vitro* activity against staphylococci and the majority of streptococci, but has less activity against other Gram-positive and most Gram-negative bacteria. Used as topical ointment, mupirocin is effective against both MSSA and MRSA. The first trial in volunteers was undertaken in 1986. It demonstrated the ability of mupirocin applied into the anterior nares to eliminate staphylococcal nasal carriage [117]. Subsequently, several studies conducted in different populations showed that mupirocin was effective in the short term in eradicating nasal carriage of *S. aureus* when compared with placebo [118]. Mupirocin ointment has been used to eradicate staphylococcal carriage and to prevent infections, to control infections in surgical, cardiac and orthopaedic patients, in non-surgical patients and in haemodialysis patients [107, 119-123]. However, its efficacy on MRSA infection in critically ill ICU patients has not yet been assessed in a systematic review.

We undertook a systematic review and meta-analysis to assess the efficacy of topical mupirocin to control infection, including lower respiratory tract infection due to both MSSA and MRSA, and to evaluate the impact on mortality in critically ill ICU patients.

4.2 Materials and methods

4.2.1 Search strategy

The systematic review was performed in accordance with PRISMA guidelines [109]. PubMed, Embase and the Cochrane Register of Controlled Trials for RCTs were searched with no language restriction. Search terms were mupirocin, decontamination, decolonisation, nasal, intensive care unit. Previous published meta-analyses were searched and references were crosschecked. Two investigators independently performed the search and screened titles and abstracts for relevant clinical trials published from 1983 to April 2016. Finally, RCTs were analyzed based on the full text using a standardized data extraction form.

4.2.2 Selection criteria

Inclusion and exclusion criteria were decided before reviewing abstracts and articles. All RCTs including usable information on MSSA and/or MRSA infection were included. RCTs were in critically ill ICU patients receiving topical mupirocin in the test group; the control group received placebo or different oral care products. RCTs in neutropenic and bone marrow transplant patients were excluded.

4.2.3 Data extraction

Two investigators independently retrieved and compared the sets of data from each clinical trial. Any disagreement was resolved by discussion. The following data were sought: first author, publication year, population included, description of test and control arms, randomization and allocation procedures, blinding, handling of dropouts and withdrawals, number of patients included, number of patients with infection, number of patients with lower respiratory tract infection due to MSSA and MRSA, and overall mortality.

4.2.4 Quality assessment

The quality of RCTs was assessed using the Jadad score [110]. The studies were considered of low quality if the score was ≤ 2 and of high quality if the score was ≥ 3.

4.2.5 Endpoints and statistical analysis

The primary endpoints were infection and lower respiratory tract infection due to MSSA and MRSA. The secondary endpoint was mortality. A subgroup analysis was performed in high quality *vs*. low quality studies, and in blinded *vs*. non-blinded studies.

Results were presented as odds ratio (OR) with 95% confidence interval (CI) using the random effects model. The Cochran *Q* statistic for heterogeneity was used both for the outcome measures and throughout subgroup analyses; heterogeneity was significant if the *p* value was <0.10. Additionally, I^2 was evaluated using the formula 100% x (*Q*-df)/*Q*, where *Q* is Cochran's *Q* statistics and *df* is the degree of freedom (number of studies- 1). Negative values of I^2 are equal to 0%; an I^2 of 0% indicates

no observed heterogeneity, whilst < 30% indicates mild heterogeneity, 30-50% moderate, and > 50% severe. Significant heterogeneity was predefined as an I^2 greater than 50% [113]. We explored the funnel plot to estimate potential publication bias. Computations were performed using the EasyMA software (M. Cucherat, Lyon, France) [114].

4.3 Results

4.3.1 Search findings and description of studies

The search strategy yielded 240 publications. After the exclusion of duplicates and irrelevant publications, we considered 23 RCTs for more detailed evaluation. The subsequent screening yielded six randomised controlled trials undertaken in the intensive care unit, and assessed for eligibility. Two RCTs were not included: one, including 74.256 patients, because data on infection were reported as number of infection per 1000 attributable days [124], the second due to unclear data on infection [125]. A final sample of 4 RCTs, including 1024 patients (521 mupirocin, 503 control), was the basis for the systematic review and meta-analysis [126-129] (Figure 4.1).

Tables 4.1 and 4.2 describe the main characteristics and data on outcomes extracted from the 4 RCTs published from 1999 to 2011. Two studies were Italian [126, 127] and two were French [128, 129]. One RCT was published in Italian [126]. All RCTs were performed in medical or surgical ICUs. One study used 2 millilitres of mupirocin ointment three times a day compared with placebo [126]. In the other Italian study [127] the test group received 0.2 millilitres of a 2% mupirocin ointment four times a day into the nostrils; the placebo group received 0.2 millilitres of a

placebo ointment which was indistinguishable from mupirocin ointment. In a multicenter French study [128] including three test arms, only the arm using 2% mupirocin was compared with the control group which received a placebo. In the second French study [129] only MRSA carriers were treated with nasal mupirocin and were compared with the control group receiving standard care. In three studies mupirocin was administered three times a day [126, 128, 129], and in one study four times a day [127]. The site of administration was the anterior nares in all studies, and in one [127] was combined with the oropharynx. In one study [127] both test and control patients received also oropharyngeal and intestinal SDD, containing polymyxin E, tobramycin and amphotericin B. Three studies were performed in mechanically ventilated subjects [126-128], and one study in ICU patients admitted for more than 48 hours [129].

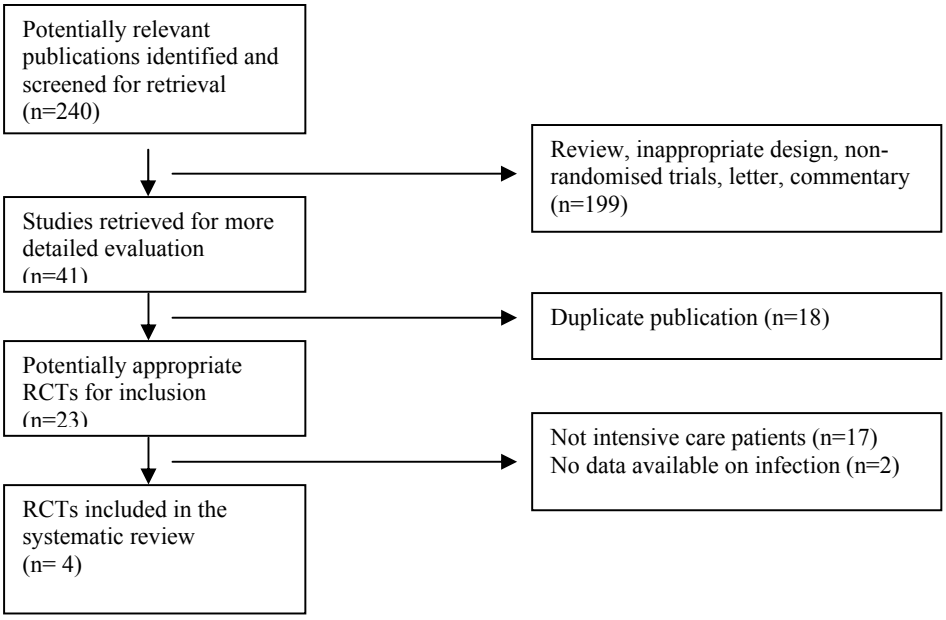

Figure 4.1 Diagram flow of the research strategy

Table 4.1 Characteristics of RCTs on mupirocin

First Author	Year	Patients		Intervention		Population	Randomization	Allocation	Blinding	Jadad score
		Mup	C	Mup	C					
Di Filippo [126]	1999	24	24	2 ml mupirocin ointment into the nostrils 3 times a day for 3 days	Placebo same frequency	Medical and surgical ICU	List of random numbers	Unclear	Only personnel was blinded	0
Nardi [127]	2000	119	104	0.2 ml of a 2% mupirocin ointment into the nostrils 4 times a day, plus SDD containing 2% mupirocin in the oropharyngeal paste	0.2 ml of a placebo ointment into the nostrils same frequency, plus SDD without mupirocin	Medical, surgical and trauma ICU	Computer generated list	Unclear	Double bind	2
Camus [128]	2005	130	126	2% mupirocin into the anterior nares 3 times a day	Placebo same frequency	Medical ICU of 3 hospitals	Computer generated list	Not stated	Double-blind	3
Camus [129]	2011	248	249	2% mupirocin into the anterior nares 3 times a day for 5 days	Standard care	Medical ICU of 2 university hospitals	Computer generated list	Unclear	Not blinded	0

Mup, mupirocin; C, control; SDD, selective digestive tract decontamination (polymyxin E, tobramycin, amphotericin B); ICU, intensive care unit; ml, millilitres.

Table 4.2 Summary of data retrieved from RCTs on mupirocin

First Author	Patients enrolled		LRTI		Bloodstream infection		Urinary tract infection		Mortality		MSSA LRTI		MRSA LRTI	
	Mup	C	Mup	C	Mup	C	Mup	C	Mup	C	Mup	C	Mup	C
Di Filippo [126]	24	24	3	5							0	3	1	1
Nardi [127]	119	104	9	20					25	26	0	2	1	7
Camus [128]	130	126	14	20	8	14	27	24	36	41				
Camus [129]	248	249							49	52				
Total	**521**	**503**	**26**	**45**	**8**	**14**	**27**	**24**	**110**	**119**	**0**	**5**	**2**	**8**

RCTs, randomised controlled trials; Mup, mupirocin; C, control; LRTI, lower respiratory tract infection; MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*

Data on the type of microorganism causing bloodstream infection were reported by three studies [126-128]. Data on infections due to *S. aureus* were obtainable from three studies [126, 127, 129]. A French study [128] reported details on episodes of infection only, and details on *S. aureus* infection were not given. Mortality data were available from three studies [127-129].

The quality assessment for all trials using the Jadad score showed a median of 2; only one RCT was classified as high-quality study [128]. Two studies were double blinded [127, 128], in one study [126] only the personnel was blinded, and one study was not blinded [129]. The randomization procedure was adequate in all studies, but the allocation procedure was at high risk of bias in all studies. We did not explore the funnel plot asymmetry for publication bias due to the small number of included studies.

4.3.2 Infection

Three studies [126-128], including 527 patients (273 mupirocin, 254 control), were the basis for the meta-analysis of overall lower respiratory tract infection (LRTI) (Figure 4.2). There were 26 patients (9.5%) with lower respiratory tract infection in the mupirocin group, and 45 (17.7%) in the control group demonstrating a significant reduction of lower respiratory tract infection (OR 0.49, 95% CI 0.29-0.83, p=0.008). The test for heterogeneity was not significant (χ^2=1.2161, p=0.54, I^2=0%).

One trial [128] reported data on urinary tract infection and bloodstream infection. Mupirocin did not impact any of these infections (OR 1.11, 95% CI 0.60-2.06, and OR 0.53, 95% CI 0.21-1.30, respectively).

Results from two RCTs including 271 patients (143 test, 128 control) were available for the analysis of MRSA lower respiratory tract infection [126, 127]. There were two (1.4%) and 8 (6.2%) patients with MRSA lower respiratory tract infection, indicating a lack of effect of mupirocin (OR 0.28, 95% CI 0.04-2.16, p=0.22). The test for heterogeneity was not significant (χ^2=1, p=0.32, I^2= 0%) (Figure 4.3). The incidence of methicillin-sensitive *S. aureus* lower respiratory tract infection was reported by 2 RCTs [126, 127], and no impact of mupirocin was found (OR 0.08, 95% CI 0.00-1.48, p=0.09).

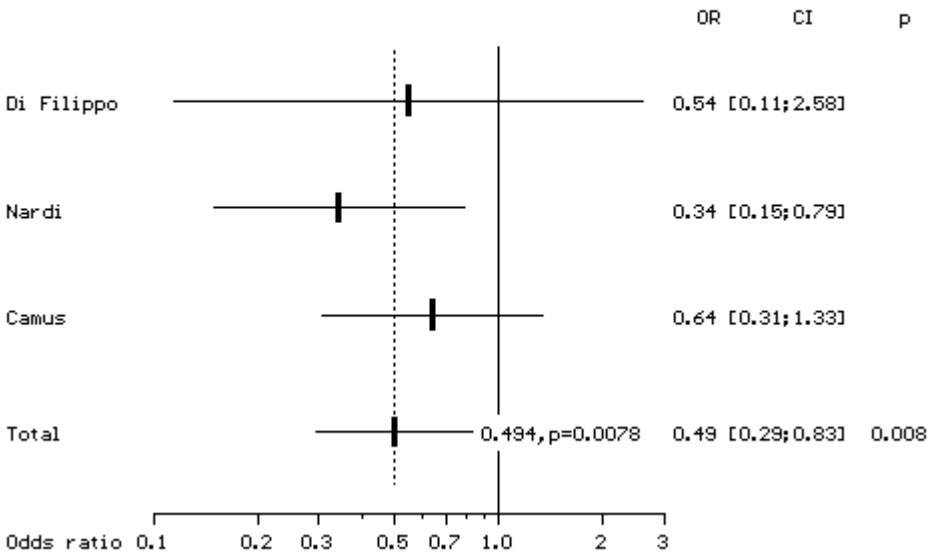

Figure 4.2 Forrest plot of the impact of mupirocin on LRTI
OR, odds ratio; CI, 95% confidence interval. OR < 1 favours treatment; OR > 1 favours controls.

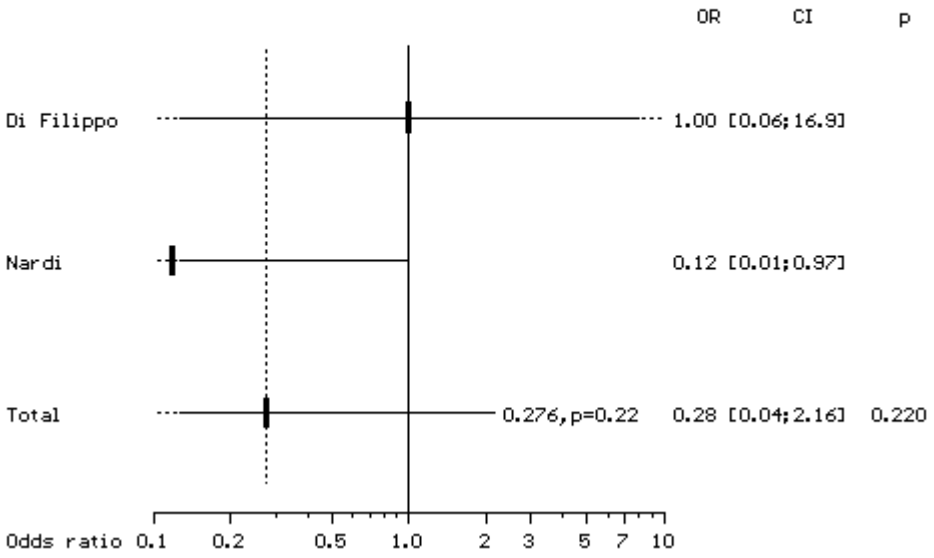

Figure 4.3 Forrest plot of the impact of mupirocin on MRSA LRTI
OR, odds ratio; CI, 95% confidence interval. OR < 1 favours treatment; OR > 1 favours controls.

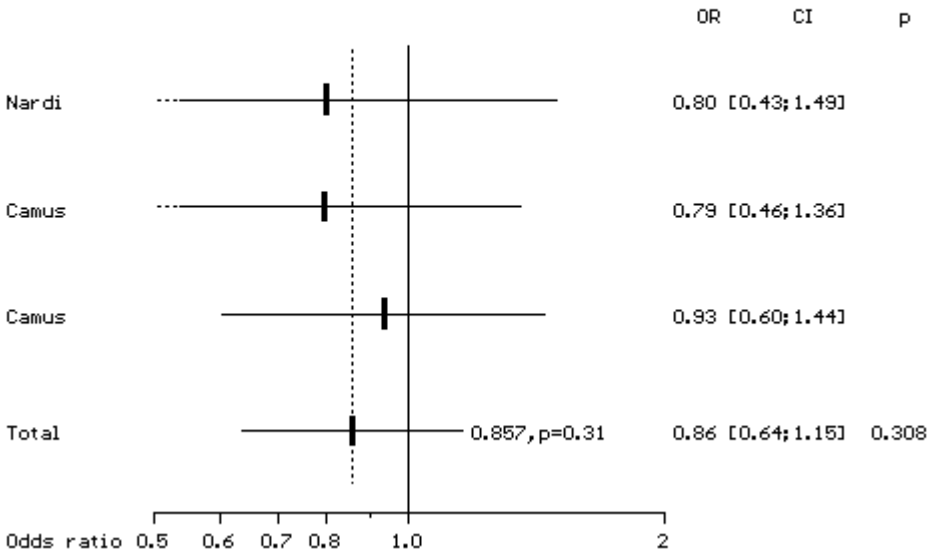

Figure 4.4 Forrest plot of the impact of mupirocin on mortality
OR, odds ratio; CI, 95% confidence interval; OR < 1 favours treatment; OR > 1 favours controls.

4.3.3 Mortality

The analysis of mortality was based on 3 RCTs and 976 patients (497 test, 479 control) [127-129]. There were 110 (22.1%) and 119 (24.8%) deaths in test and control group, respectively. Mupirocin did not significantly reduce mortality (OR 0.86, 95% CI 0.64-1.15, p=0.31). The test for heterogeneity was negative (χ^2=0.2729, p=0.87, I^2=0%) (Figure 4.4)

4.3.4 Subgroup analysis

The subgroup analysis for lower respiratory tract infection is reported in Table 4.3. In double-blinded [127, 128] and in low-quality RCTs (126, 128) mupirocin reduced overall lower respiratory tract infection (OR 0.49, 95% CI 0.27-0.89, p=0.02 and OR 0.49 95% CI 0.29-0.83, p=0.007, respectively). No effect on other variables has been shown.

The study by Nardi et al [127] was the only positive study for overall and MRSA lower respiratory tract infection: Additionally, in this study both test and control arms received the antibiotic policy of SDD. A post-hoc analysis of RCTs after the exclusion of this study, did not demonstrate any significant impact of mupirocin on LRTI (OR 0.62, 95% CI 0.32-1.21, p= 0.16) and other outcome measures.

Table 4.3 Subgroup analysis of the endpoint of lower respiratory tract infection

Subgroups	N° RCTs	N° Patients		N° events		OR (95% CI)	*p*
		Mup	C	Mup	C		
Blinding							
- double-blinded	2	249	230	23	40	0.49 (0.27-0.89)	0.02
- not blinded	1	24	25	3	5	0.54 (0.11-2.58)	0.5
Quality							
- low quality	3	273	254	26	45	0.49 (0.29-0.83)	0.007
- high quality	-	-	-	-	-	-	-

RCTs, randomised controlled trials; Mup, mupirocin; C, control; OR, odds ratio; CI, confidence interval. NE, not evaluable The I^2 test for heterogeneity was not significant in all comparisons.

4.4 Discussion

Two main findings emerge from this systematic review of only RCTs:

1. Mupirocin reduces lower respiratory tract infection, but not mortality;

2. There was not any evidence of the effect of mupirocin on MSSA and MRSA lower respiratory tract infection.

This is the first analysis of the effectiveness of topical mupirocin to prevent lower respiratory tract infection and MRSA infections in ICU patients. A subgroup analysis showed that the effect on lower respiratory tract infection has been found in only low-quality studies. Additionally, the analysis after the exclusion of the only positive study on overall and MRSA lower respiratory tract infection, did not demonstrate any significant effect of mupirocin [127].

Our findings that mupirocin reduced overall lower respiratory tract infection in critically ill patients is consistent with previous observations and meta-analyses in non-ICU patients. In a meta-analysis of 4 studies in surgical patients, van Rijen et al. [120] showed that among 686 mupirocin-treated surgical patients with *S. aureus* nasal carriage, there were 25 *S. aureus* infections (3.6%), compared with 46 (6.7%) in the controls (relative risk [RR] 0.55, 95% CI 0.34–0.89; p=0.02). They concluded that prophylactic intranasal mupirocin significantly reduced the rate of post-operative *S. aureus* infections among surgical patients who were *S. aureus* carriers. In surgical patients who were not carrying *S. aureus*, there was no effect of treatment, with a slightly higher infection rate noted in the treated group (RR 1.09, 95% CI 0.52–2.28). Nine RCTs, involving 3396 participants, were included in a meta-analysis of the same group on the prevention of *S. aureus* infections in nasal carriers [106]. Patient populations varied and several types of nosocomial *S. aureus* infection were

described including bacteraemia, exit-site infections, peritonitis, respiratory tract infections, skin infections, surgical site infections, and urinary tract infections. After pooling eight studies that compared mupirocin with placebo or no treatment, there was a statistically significant reduction in the rate of *S. aureus* infection associated with intranasal mupirocin (RR 0.55, 95% CI 0.43 to 0.70). The infection rate caused by microorganisms other than *S. aureus* was significantly higher in patients treated with mupirocin compared with control patients (RR 1.38, 95% CI 1.12-1.72). The surgical sub-group of patients showed a significant reduction in nosocomial *S. aureus* infection with mupirocin (RR 0.56, 95% CI 0.34-0.91), but failed to extend to surgical site infection (RR 0.63, 95% CI 0.38-1.04).

Another systematic review analysed the different manoeuvres to control healthcare-associated MRSA infection [130]. Among them, 11 RCTs, 23 comparative studies and one prospective cohort study evaluated the effectiveness of mupirocin-based nasal decontamination regimens for the prevention of *S. aureus* infections. Six out of 7 studies included in the review were carried on in ICU setting and demonstrated a successful effect of mupirocin treatment. However, all these studies were not randomised trials and included observational, historical, and before-after studies.

A recent meta-analysis assessing the relationship between nasal carriage of *S. aureus* and the development of osteo-articular infection showed increased risk of surgical site infection in case of nasal carriage of *S. aureus* (OR 5.92, 95% CI 1.15-30.39, $p = 0.033$) [122]. The review confirmed that nasal carriage of *S. aureus* is a major risk factor for surgical site infection. The efficacy of eradication could not be demonstrated for orthopaedic surgery as samples were too small. Very large numbers of patients would be needed to confirm intranasal mupirocin efficacy in reducing surgical site infection with statistical significance. It is estimated that about 14.000

patients with a baseline surgical site infection rate of 5% would be needed to demonstrate a 20% reduction in the SSI rate [131].

This meta-analysis showed that mupirocin did not reduce MSSA and MRSA lower respiratory tract infections. However, only two studies, including 271 patients, reported data on these infections. The sample size and the number of patients with MRSA or MSSA infection were too small to draw robust conclusions.

Although not included in this systematic review and meta-analysis, a large cluster-randomised multicenter trial in 74 adults ICUs, including 74,256 patients, was carried on to explore the effectiveness nasal mupirocin and daily bathing with chlorhexidine to all patients known to have MRSA carriage or infection (targeted decolonisation) compared with universal decolonisation in all patients and no decolonisation (only screening and isolation) to prevent ICU infection [128]. Unfortunately, the unit of randomization was the hospital, not the patient. The authors concluded that universal decolonisation in all patients was more effective than targeted decolonisation or no decolonisation in reducing MRSA isolates and bloodstream infection from any pathogen, but not MRSA bloodstream infection.

In this meta-analysis mupirocin has been shown to control lower respiratory tract infection in ICU patients, but this effect was not associated with a mortality reduction. First, the small sample size of 976 patients may explain the lack of any effect on mortality. Second, mupirocin does not affect the intestinal overgrowth of *S. aureus*, and, therefore, does not prevent bloodstream infection [57] which is associated with an increased mortality [60]. MRSA LRTI is also associated with an increase in mortality [68]. The lack on any significant effect on MRSA LRTI and bacteraemia may explain why mortality is not affected by mupirocin. Finally, an inherent limitation of mupirocin is the control of exogenous infections. The source of

PPMs causing this infection is external to the patient (e.g. tracheostomy care, devices).

Development of resistance is an important issue during mupirocin use. Mupirocin resistance occurs in two phenotypes: low-level mupirocin resistance (MIC between 8 and 64 mg/L) and high-level mupirocin resistance (MIC ≥512 mg/L). High-level resistance is mediated by plasmids carrying the mupA gene, while low-level resistance occurs through point mutations in the isoleucyl-tRNA synthetase gene. An increase in mupirocin resistance in response to increasing use has been reported in practice [132-135]. A recent simulation of the transmissibility of the mupirocin-resistant and mupirocin-sensitive MRSA strain demonstrated that, although the transmission probability of a mupirocin-sensitive MRSA is higher than a mupirocin-resistant strain, in the long term the prevalence of a mupirocin-resistant strain will increase with the universal use of mupirocin [136].

This systematic review has some limitations. First, after the exclusion of the only positive study on overall lower respiratory tract infection [127], the impact of mupirocin on this outcome was not significant. Therefore, results on overall lower respiratory tract infection should be interpreted with an appropriate degree of caution. Second, a clinical heterogeneity was present due to different sites of administration of mupirocin, different definitions of lower respiratory tract infection, different treatment of the control patients (placebo, standard care), the use of SDD antimicrobials in both test and control patients in one study [127], different types of patients (medical, surgical, and trauma patients), and the use of mupirocin only in MRSA carriers in one study [129]. Third, the quality of studies included in the review was low. Low quality studies and non-blinded studies are prone to possible increased risk of bias. A subgroup analysis has been performed on these variables.

Fourth, this review includes a small number of studies. Small meta-analyses (i.e. less than 200 outcome events) may be mainly useful for generating hypotheses for further research [137]. Therefore, the results of this systematic review and meta-analysis should be cautiously interpreted.

4.5 Conclusion

This systematic review showed that mupirocin, although reduces lower respiratory tract infection, does not reduce mortality and does not impact MSSA and MRSA LRTI. The results should be interpreted with caution due to several important limitations. Future RCTs of topical mupirocin to prevent lower respiratory tract infection in ICU patients are warranted.

5 EFFECTIVENESS OF ORAL CHLORHEXIDINE ON INFECTION, MICROORGANISMS AND MORTALITY IN ICU PATIENTS. A SYSTEMATIC REVIEW AND META-ANALYSIS

5.1 Introduction

Lower respiratory tract infection and bloodstream infection are the most common serious infections developing in critically ill patients requiring treatment on the ICU [75, 138]. Hospital-acquired lower respiratory tract infection accounts for up to 25% of all ICU infections; VAP occurs in 9-27% of all intubated patients [75]. Lower respiratory tract infection prolongs the length of ICU treatment and hospital stay [75], increases costs [139] and mortality [140].The incidence of BSI is between 5 and 19 per 1,000 patient days, and is associated with increased mortality and costs [24, 138]. Therefore, the prevention of these infections is a high priority for infection control in the ICU.

Oropharyngeal carriage of potential pathogens in overgrowth concentrations is the first essential step in the pathogenesis of lower respiratory tract infection in critically ill patients [44]. There is a qualitative and quantitative relationship between surveillance cultures of the throat and the diagnostic samples of lower airway secretions. As soon as the potential pathogen reaches overgrowth concentration in the oropharynx the lower airway secretions become positive for the same potential pathogen. This leads to colonisation and subsequent infection of the lower respiratory tract [141, 142].

The main sources of BSI are internal organs (i.e. lung and bladder) and the gut [87, 143], apart from catheter-related BSI. Microorganisms causing infection of the lower airways or the bladder may be responsible for blood invasion [143-145]. Moreover, gut overgrowth promotes translocation of bacteria into the systemic circulation [56, 146]. Finally, a substantial number of catheter-related BSI may be due to skin microorganisms [143].

There are approximately 14 potential pathogens causing severe infection in ICU patient [44]. Five "normal" bacteria are the causative agents in healthy individuals and include *S. pneumoniae,* MSSA*, H. influenzae, M. catarrhalis,* and *E. coli*. There are nine "abnormal" bacteria causing pneumonia. They include eight AGNB, such as *Klebsiella, Proteus, Morganella, Enterobacter, Citrobacter, Serratia, Acinetobacter, and Pseudomonas* species, and MRSA. Abnormal flora is uncommon in healthy people, whilst disease promotes oropharyngeal and gastrointestinal carriage of these microorganisms.

Control of oropharyngeal overgrowth of potential pathogens using antimicrobials and antifungal agents applied into the oropharynx is termed oropharyngeal decontamination, and has been shown to prevent lower respiratory tract infection and bloodstream infection [44, 84, 87]. Chlorhexidine is an antiseptic agent active against Gram-positive and Gram-negative, "normal" and "abnormal" bacteria [147]. The efficacy of oral chlorhexidine in preventing lower respiratory tract infection has been assessed in several RCTs and meta-analyses [82, 148-157]. A recent Belgian meta-analysis, including 12 RCTs, showed a significant 28% reduction in the relative risk for VAP [156]. However, none of those meta-analyses had bloodstream infection as morbidity endpoint, or the causative micro-organism as bacteriological endpoint.

The aim of this systematic review is to explore the effectiveness of oral chlorhexidine in the prevention of lower respiratory tract infection and bloodstream infection, to assess which microorganism causing LRTI and BSI is impacted by chlorhexidine, and to evaluate the effect on mortality in critically ill patients.

5.2 Materials and methods

5.2.1 Search strategy

The systematic review and meta-analysis has been performed in accordance with the PRISMA guidelines [109]. We searched PubMed, Embase and the Cochrane Register of Controlled Trials for RCTs with no language restriction. Search terms were chlorhexidine, oral care, oral hygiene, oral health, oral rinse, oral decontamination, mouthwashes, bloodstream infection, bacteraemia, lower airway (respiratory tract) infection, nosocomial pneumonia, ventilator-associated pneumonia, hospital-acquired pneumonia, dental plaque, with the search limits of "clinical trial" and "humans". Additionally, we searched abstracts from scientific meetings, crosschecked the references of papers and published meta-analyses, and searched unpublished studies in the register of clinical trials. Four investigators independently cooperated in performing the search and in screening titles and abstracts for relevant clinical trials published until June 2014. RCTs were analyzed based on the full text using a standardized data extraction form.

5.2.2 Selection criteria

We established inclusion and exclusion criteria before reviewing abstracts and articles. Only RCTs in critically ill patients receiving oral care with chlorhexidine were included. The control intervention could include placebo, standard therapy, or another product for oral care. We excluded RCTs in which topical oropharyngeal antibiotics or probiotics were used. We also excluded RCTs in which both arms received chlorhexidine and RCTs in cancer patients, neutropenic, stem cell and bone

marrow transplant patients. RCTs with usable information by outcome were finally included in the meta-analysis.

5.2.3 Data extraction

Three investigators independently retrieved the published findings from each clinical trial and compared the sets of data. Any disagreement was resolved by discussion. The following data were sought for each study: author, publication year, population included, description of the intervention/control arm, randomization, allocation concealment, blinding, and handling of dropouts/withdrawals, definition of lower respiratory tract infection, number of patients included, number of patients with lower respiratory tract infection, number of patients with the microorganism causing lower respiratory tract infection, number of patients with BSI, number of patients with the microorganism causing BSI (individual microorganism, Gram-positive, Gram-negative, "normal" flora, "abnormal" flora) and total mortality. We used the term 'lower respiratory tract infection' throughout the review; this comprised nosocomial pneumonia, hospital-acquired pneumonia, and VAP.

Microorganims causing infection have been classified with two methods: the Gram staining technique which distinguishes Gram-positive from Gram-negative microorganisms, and the method using the distinction between "normal" and "abnormal" flora. "Normal" flora included *S. pneumoniae, H. influenzae,* methicillin-sensitive *S. aureus, M. catarrhalis,* and *E. coli* [158]. Nine microorganisms belonged to the "abnormal" flora: eight AGNB including *Klebsiella, Proteus, Morganella, Enterobacter, Citrobacter, Serratia, Acinetobacter, and Pseudomonas* species, and MRSA [158]. Coagulase-negative staphylococci, enterococci and yeasts were not included in lower respiratory tract infection as they generally do not cause this

infection in immune-competent host, unless the authors stated that there was a histological evidence of lung infection due to these microorganisms [75, 159].

5.2.4 Quality assessment

The quality of the included RCTs was assessed by two investigators using the Jadad score [110]. The studies are considered of low quality if the score was ≤ 2 and high quality if the score was ≥ 3.

5.2.5 Endpoints and statistical analysis

The primary endpoint was the number of patients developing lower respiratory tract infection and bloodstream infection, and mortality. The secondary endpoint was the microorganism involved. We planned *a priori* the following subgroup analyses of the endpoints of lower respiratory tract infection, bloodstream infection and mortality: a) randomization/allocation procedures concealment (adequate or inadequate) (smaller treatment effect in concealed vs. unconcealed allocation); b) blinding of patients and caregivers to allocated treatment (blinded or unblinded) (smaller treatment effect in blinded compared with unblinded studies); c) quality of the studies (low or high quality) (smaller treatment effect in high quality studies); d) concentration of chlorhexidine (0.12%-0.2% or 1%-2%) (smaller treatment effect in lower concentrations); e) type of population (surgical, medical or mixed) (smaller treatment effect in medical/mixed population); f) age (adults, children) (smaller treatment effect in children).

Results were presented as odds ratio (OR) with 95% confidence interval (CI) using the random effects model. The random effects model provides a more conservative estimate of the 95% CI, taking heterogeneity into account. The Cochran *Q* statistic

for heterogeneity was used both for the outcome measures and through subgroup analyses; we considered heterogeneity to be significant if the *p* value was <0.10. I^2 was also evaluated with the formula 100% x (*Q*-df)/*Q*, where *Q* is Cochran's *Q* statistics and *df* is the degree of freedom (number of studies- 1). Negative values of I^2 are put equal to 0%; zero percent indicates no observed heterogeneity, whilst an I^2 of < 30% indicates mild heterogeneity, 30-50% moderate, and > 50% severe; we predefined significant heterogeneity as an I^2 measure greater than 50% [113]. A funnel plot was constructed to estimate potential publication bias. Computations were performed using the EasyMA software [114].

5.3 Results

5.3.1 Search findings and description of studies

The initial search resulted in 60 potentially relevant clinical trials (Figure 5.1). Of these, 28 were excluded: 18 clinical trials were not randomised, two RCTs were double publications, six RCTs received chlorhexidine in both study arms, and in two RCTs chlorhexidine was used in the control arm. Of the remaining 32 potentially appropriate RCTs, 10 were excluded: three because lower respiratory tract infection was not the endpoint and 7 because details on the number of patients with lower respiratory tract infection were absent, insufficient or unclearly reported. A final sample of 22 RCTs, totalling 4,277 patients (2,119 chlorhexidine, 2,158 controls), was the basis for the systematic review and meta-analysis [160-181].

Tables 5.1, 5.2 and 5.3 describe the main characteristics and data on outcomes extracted from the 22 RCTs. Studies were published during the time period from 1996 to 2012. Two studies were available only in abstract form [165, 174]. The

randomization procedure, including allocation concealment, was adequate in 8 studies [160, 164, 165, 170, 176-178, 181]. Eleven studies were double-blinded [160, 164-168, 170, 171, 173, 177-179, 181], one was single-blinded [160], nine were not blinded [161, 166-168, 171, 173-175, 179], and in one study the blinding method was not reported [163]. Nineteen RCTs included adults [160-176, 178, 180], and three paediatric patients [177, 179, 181]. Twelve studies were performed in mixed/trauma ICU population, four in cardiac surgery patients [160, 162, 165, 177], two in surgical patients [173, 180] and four in medical patients [161, 169, 178, 181]. Fifty-one percent of patients of this review (i.e. 2,168) were included in the six surgical studies. Half of the studies [160, 162, 165, 167, 170-172, 174, 175, 177, 179] used 0.12% chlorhexidine, seven studies employed 0.2% chlorhexidine [161, 163, 164, 169, 173, 176, 178], one study used 1% chlorhexidine [181], two studies used 2% chlorhexidine [166, 168], and in one study the chlorhexidine concentration was not clearly stated [180]. Chlorhexidine was applied one [175, 180], two [160, 162, 163, 167, 169, 170, 1727 178, 179], three [161, 164, 171, 173, 181] or four [165, 166, 168, 178] times a day. Different protocols of administration, or a combination of them, were described in the RCTs (e.g. mouth rinse, mouth cleansing, toothbrushing, gingival brushing, use of a gloved finger or swab, etc), as well as different chlorhexidine formulations (i.e. saline solution, alcoholic preparation, gel). Nosocomial lower respiratory tract infection was the endpoint of all included studies.

Studies were performed on mechanically ventilated subjects. In three studies only a portion of patients were ventilated, i.e. 39% and 34% [169], 65% and 72% [171], 57% and 77% [173], in chlorhexidine and control group, respectively. However, in two studies [169, 171], nearly all patients with lower respiratory tract infections

belonged to the mechanically ventilated group. Data on the type of microorganism causing lower respiratory tract infection were present in nine [160, 161, 164-166, 168, 169, 173, 181] and seven [161, 164-166, 169, 173, 181] RCTs which included data on the Gram stain and on “normal” and “abnormal” bacteria classifications, respectively. Mortality data were available from 16 studies [160-166, 168-171, 173, 177-179, 181].

All, but one [178], studies reported a definition of lower respiratory tract infection. Five studies [165, 170, 174, 177, 179] used the clinical pulmonary infection score, five studies [162, 165, 171, 177, 181] simply referred to the Centers for Disease Control and Prevention definition [182], and one [172] to the American Thoracic Society/Infectious Disease Society of America statement [183]. One study [167] did not provide a clear description of lower respiratory tract infection. The remaining nine studies [160, 161, 164, 166, 168, 171, 173, 176, 180] clearly defined lower respiratory tract infection.

The methodological quality assessment for all trials showed a median of 3.5 (quartile range 0-5). Accordingly, 14 RCTs were high-quality studies (160, 164-166, 169-171, 173, 175-179, 181).

The inspection of the funnel plot for lower respiratory tract infection suggested no evidence of publication bias. The funnel plot for bloodstream infection was not created due to the low number of studies.

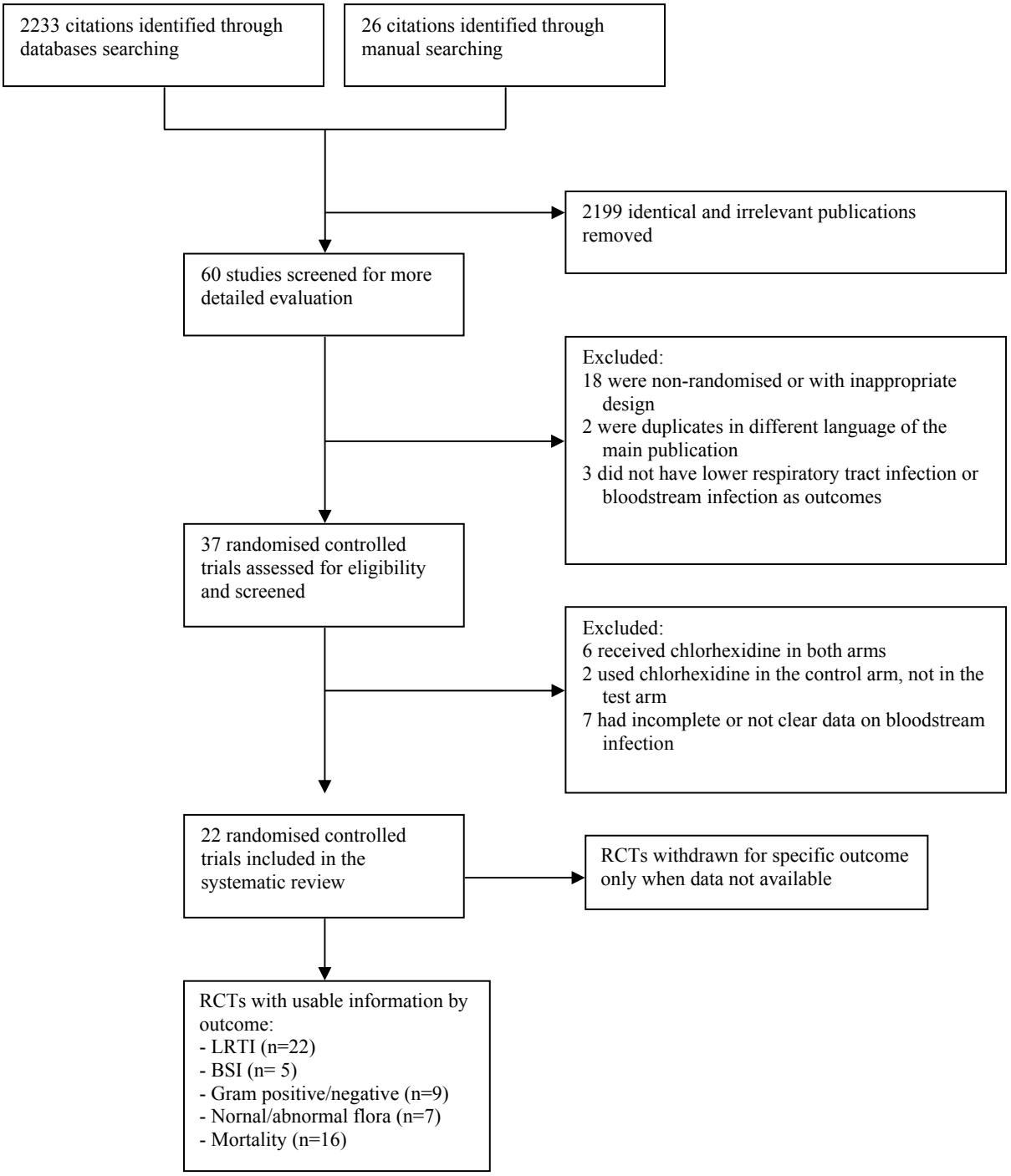

Figure 5.1 The flow diagram of the search strategy

Table 5.1 Summary of RCTs on oropharyngeal chlorhexidine for the prevention of infection

First Author	Year	Test	Control	Population	Allocation concealment	Randomization	Blinding	Jadad score
DeRiso [160]	1996	0.12% chx gluconate oral rinse preoperatively + two times a day until ICU discharge	Placebo, same colour, taste and smell	Cardiac surgery ICU	Central randomization (pharmacy)	Computer generated random numbers	Double blind	4
Fourrier [161]	2000	0.2% chx gel three times a day until ICU discharge	Standard oral care (mouth rinsing with bicarbonate isotonic serum)	Medical ICU	NR	Computer generated balanced randomization table	Single blind	2
Houston [162]	2002	15 ml of 0.12% chx gluconate oral rinse preoperatively + two times a day for 10 days or until extubation, tracheostomy, death, or diagnosis of lower respiratory tract infection	Solution of a phenolic mixture	Cardiac surgery ICU	NR	Patients' medical record numbers	No	0
Macnaughton [163]	2004	0.2% chx mouthwash two times a day	Placebo of identical appearance and smell	Medical/ surgical-ICU	NR	NR	NR	0
Fourrier [164]	2005	0.2% chx gel three times a day for max 28 days	Placebo, same colour, taste, odour and smell	Medical/ surgical ICU	Central randomization (pharmacy)	Block randomization	Double-blind	5
Segers [165]	2006	0.12% chx gluconate oral rinse and nasal gel four times a day until extubation	Placebo, same colour, taste and smell	Cardiac surgery ICU	Central randomization (pharmacy)	Computer generated table	Double-blind	5
Koeman [166]	2006	0.5 gr of 2% chx ointment four times a day 0.5 gr of 2% chx with 2% colistin ointment four times a day	Placebo, same taste, smell, and consistency	Mixed and surgical ICUs	NR	Computer generated schedule	Double-blind	5
Bopp [167]	2006	0.12% chx gluconate oral brushing two times a day	Standard care 6 times a day	Medical/ surgical ICU	NR	Coin flipped (heads, chx; tails, control)	No	0

Tantipong [168]	2008	15 ml of 2% chx solution four times a day	Oral care with normal saline	Medical and surgical ICUs and medical ward	NR	Randomization according to sex and hospital location of the patient	No	0
Panchabhai [169]	2009	10 ml of 0.2% chx gluconate solution oral cleansing two times a day	0.01% potassium permanganate solution two time a day	Medical neurology ICU	NR	Concealed simple randomization	No	3
Scannapieco [170]	2009	0.12% chx oral rinse two times a day 0.12% chx oral rinse once a day + placebo once a day	Placebo same colour, taste and smell two times a day	Trauma ICU	Central randomization (pharmacy)	Web-based enrolment system generating a set of SID that identified individual treatment assignments	Double-blind	5
Bellissimo-Rodrigues [171]	2009	0.12% chx solution three times a day	Placebo same colour, consistency, taste and smell	Medical/ surgical ICU	Central randomization (pharmacy)	A code number was selected from a box corresponding to a bottle containing placebo or test	Double-blind	4
Rujipong [172]	2009	0.12% chx toothbrushing and mouth wash two times a day	Routine oral care two times a day	Medical/ surgical ICU	NR	Lottery method	No	0
Cabov [173]	2010	0.2% chx gel three time/day	Placebo gel same taste, colour, and odour	Surgical ICU	NR	Computer generated balanced randomization	Double blind	4
Zouka [174]	2010	0.12% chx solution in saline (3:1)	Hexetidine 0.1% solution	Medical/ surgical ICU	NR	NR	No	0
Grap [175]	2011	5 mL of 0.12% chx solution once a day	Standard oral care without chx	Trauma ICU	NR	Block randomization scheme	No	3

Berry [176]	2011	0.2% chx solution oral rinse two times a day + sterile water oral rinse two hourly Sodium bicarbonate mouth wash rinsed two hourly	Sterile water oral rinse two hourly	Medical/ surgical ICU	Allocation of identical study packs remained blinded until the study pack was opened by the attending nurse	Computer generated table	No	3
Jacomo [177]	2011	0.12% chx gluconate alcoholic solution preoperatively and two times a day postoperatively	Placebo solution same texture, colour, flavour preoperatively and postoperatively	Paediatric cardiac surgery	Central randomization (pharmacy)	Computer generated table	Double-blind	5
Ozcaka [178]	2012	0.2% chx solution four times a day	Saline four times a day	Respiratory ICU	The head nurse assigned the treatment once received the SID	Set of SID that identified individual treatment assignments	Double-blind	5
Kusahara [179]	2012	0.12% chx gel two times a day	Placebo gel same colour, and odour	Medical/ surgical PICU	NR	Computer generated balanced randomization table	Double blind	5
Zaiton [180]	2012	15 ml chx diluted in 50 ml of water once a day	Oral care with normal saline 0.9% once a day	Surgical ICU	NR	NR	No	0
Sebastian [181]	2012	0.5 gr of 1% chx gel three times a day	Placebo gel same appearance, consistency, taste and smell	Medical PICU	Personnel not involved in the study and the allocation sequence remained concealed throughout the study	Computer generated random sequence	Double blind	5

Chx, chlorhexidine; ICU, Intensive Care Unit; PICU, Paediatric Intensive Care Unit; NR, not reported; SID, subject identification number.

Table 5.2 Summary of RCTs on oral chlorhexidine and the impact on lower respiratory tract infection, mortality and the type of microorganism causing lower respiratory tract infection

First Author	N° patients enrolled		N° patients with infection		Mortality		N° patients with infection due to Gram positive pathogens		N° patients with infection due to Gram negative pathogens		N° patients with infection due to normal flora		N° patients with infection due to abnormal flora	
	Chx	C	Chx	C	Chx	C	Chx	C	Chx	C	Chx	C	Chx	C
DeRiso [160]	173	180	3	9	2	10	1	2	3	8				
Fourrier [161]	30	30	5	15	3	7	0	3	5	11	0	3	5	11
Houston [162]	270	291	4	9	6	3								
Macnaughton [163]	91	88	34	27	36	33								
Fourrier [164]	114	114	13	12	31	24	1	2	11	8	2	3	10	7
Segers [165]	485	469	45	74	8	6	2	10	41	52	24	49	19	13
Koeman [166]	127	130	13	23	49	39	2	7	9	15	4	11	7	12
Bopp [167]	2	3	0	1										
Tantipong [168]	102	105	5	12	36	37	0	0	5	12				
Panchabhai [169]	224	247	16	19	78	70	2	3	11	13	3	3	10	13
Scannapieco [170]	50	49	7	12	8	8								
Bellissimo-Rodrigues [171]	98	96	19	20	35	33								
Rujipong [172]	12	12	0	2										
Cabov [173]	30	30	1	6	1	3	0	0	1	6	0	0	1	6
Zouka [174]	14	13	2	2										
Grap [175]	21	18	7	10										
Berry [176]	33	43	4	1										
Jacomo [177]	87	73	16	11	5	5								
Ozcaka [178]	29	32	12	22	17	19								
Kusahara [179]	46	50	15	16	8	12								
Zaiton [180]	40	40	13	24										
Sebastian [181]	41	45	12	14	16	21	1	0	10	14	2	1	9	13
Total	**2119**	**2158**	**246**	**341**	**339**	**330**	**8**	**28**	**96**	**139**	**35**	**70**	**61**	**75**

Chx, chlorhexidine, C, control.

Table 5.3 Summary of randomised controlled trials of oral chlorhexidine and the impact on bloodstream infection, and the type of microorganism causing bloodstream infection

First Author	N° patients enrolled		N° patients with BSI		N° patients with BSI due to Gram positive pathogens		N° patients with BSI due to Gram negative pathogens		N° patients with BSI due to normal pathogens		N° patients with BSI due to abnormal pathogens	
	Chx	C	Chx	C	Chx	C	Chx	C	Chx	C	Chx	C
DeRiso [160]	173	180	1	4								
Fourrier [161]	30	30	4	4	2	2	2	2	2	3	2	1
Fourrier [164]	114	114	7	3	3	4	0	3	3	4	0	3
Segers [165]	485	469	9	17								
Cabov [173]	30	30	1	2	0	2	1	0	0	2	1	0
Total number of patients	**832**	**823**	**22**	**30**	**5**	**8**	**3**	**5**	**5**	**9**	**3**	**4**

N°, number; Chx, chlorhexidine, C, control; BSI, bloodstream infection

5.3.2 Lower respiratory tract infection and VAP

Twenty-two trials included information on lower respiratory tract infection [160-181]. A total of 4,277 patients (2,119 chlorhexidine, 2,158 controls) were available for the analysis. There were 246 patients (11.61%) with lower respiratory tract infection in the chlorhexidine group and 341 (18.8%) in controls. Chlorhexidine significantly reduced the number of patients who developed lower respiratory tract infection (OR 0.66, 95% CI 0.51-0.85, p<0.001) (Figure 5.2). The test for heterogeneity yielded a non significant result (χ^2=21.66, p=0.42, I^2=3%). Twenty-one studies were used for the analysis of VAP [160-168, 170-181]. There were 240 patients with VAP in 2089 patients of the test group (11.5%), and 328 over 2128 patients in the control group (15.4%) (OR 0.68, 95% CI 0.53-0.87, p<0.001). Heterogeneity was not demonstrated (χ^2=20.37, p=0.44, I^2=1.8%).

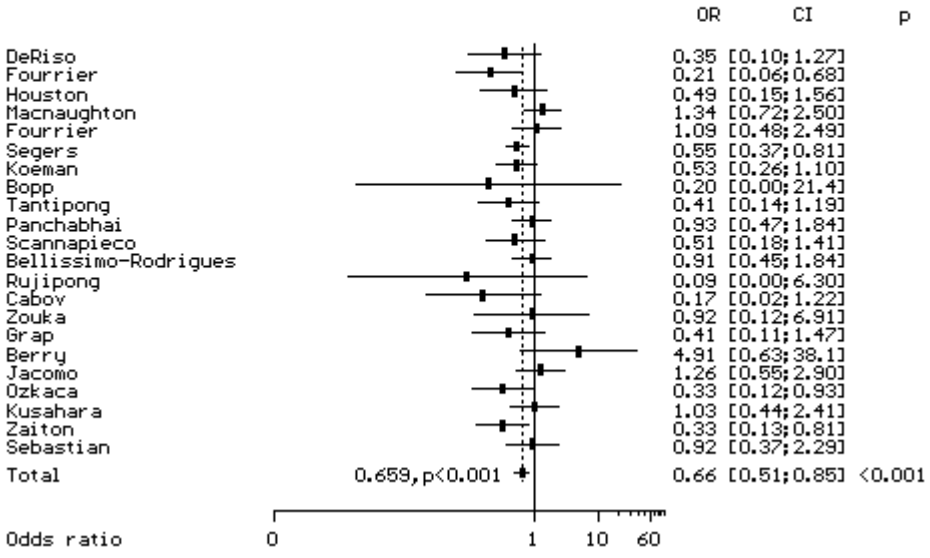

Figure 5.2 Impact of oral chlorhexidine on lower respiratory tract infection
OR, odds ratio; CI, 95% confidence interval. OR < 1 favours treatment; OR > 1 favours controls.

5.3.3 Bloodstream infection

Five studies [160, 161, 164, 165, 173] including 1,655 patients (832 chlorhexidine, 823 controls) were the basis for the meta-analysis of BSI. There were 22 patients (2.6%) with BSI in the chlorhexidine group, and 30 (3.6%) in the control group giving a non significant reduction of BSI (OR 0.74, 95% CI 0.37-1.50, p=0.40; I^2 =0%) (Figure 5.3).

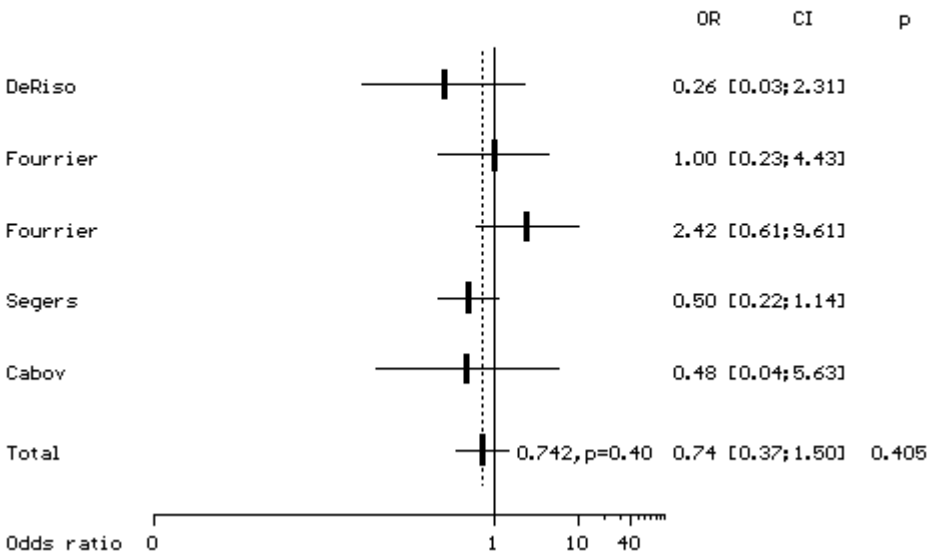

Figure 5.3 Forrest plot of the effect of oral chlorhexidine on bloodstream infection.
OR, odds ratio; CI, 95% confidence interval. OR < 1 favours treatment; OR > 1 favours controls.

5.3.4 Mortality

The overall mortality analysis was based on 16 trials [160-166, 168-171, 173, 177-179, 181] yielding a total of 4,026 patients (1,997 chlorhexidine, 2.029 control). There were 339 deaths in the chlorhexidine group (16.97%) and 330 among controls (16.26%). The analysis demonstrated a slight, not significant, increase in the ORs for

mortality in the chlorhexidine group (OR 1.11, 95% CI 0.92-1.33, p=0.28) (Figure 5.4). The test for heterogeneity was not significant (χ^2=24.20, p=0.51, I^2=0%).

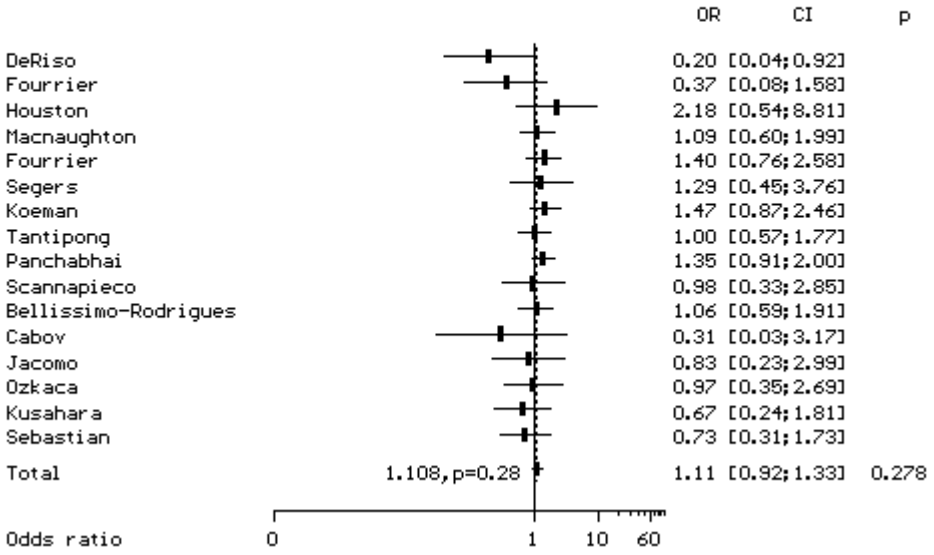

Figure 5.4 Impact of oral chlorhexidine on mortality
OR, odds ratio; CI, 95% confidence interval. OR < 1 favours treatment; OR > 1 favours controls.

5.3.5 Microorganisms causing lower respiratory tract infection

There were nine and 27 patients with lower respiratory tract infection due to Gram-positive bacteria in test and control group, respectively, indicating a protective effect of oral chlorhexidine (OR 0.41, 95% CI 0.19-0.85, p = 0.017) (Figure 5.5). The test for heterogeneity for the overall comparisons was not significant (χ^2=3.98, p=0.86, I^2=0%).

Gram-negative bacteria were the causative agents of lower respiratory tract infection in 96 patients of the chlorhexidine group and in 139 patients of the control group demonstrating a significant 32% reduction of Gram-negative lower respiratory tract infection with chlorhexidine use (OR 0.68, 95% CI 0.51-0.90, p=0.0073) (Figure 5.6). Heterogeneity was not significant (χ^2=8.0, p=0.43, I^2=0%).

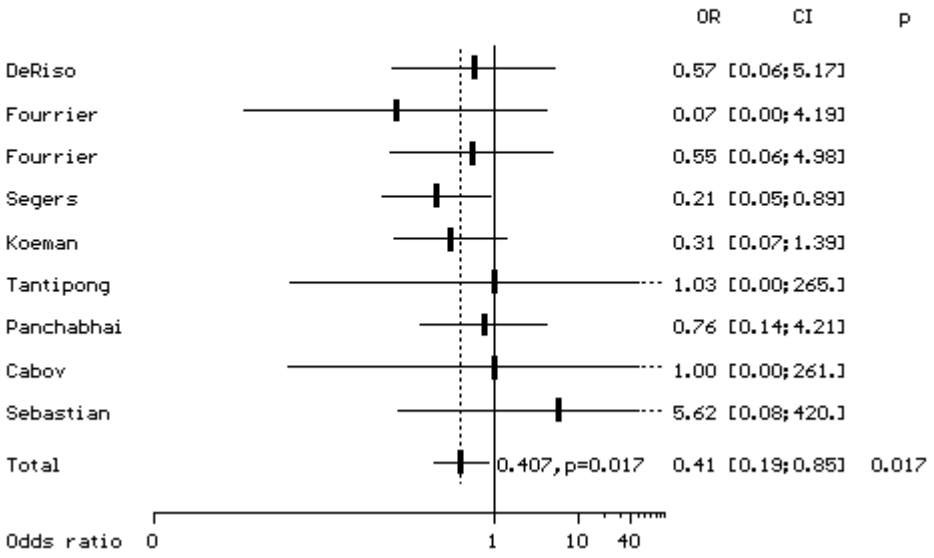

Figure 5.5 Impact of oral chlorhexidine on lower respiratory tract infection due to Gram-positive bacteria.
OR, odds ratio; CI, 95% confidence interval. OR < 1 favours treatment; OR > 1 favours controls.

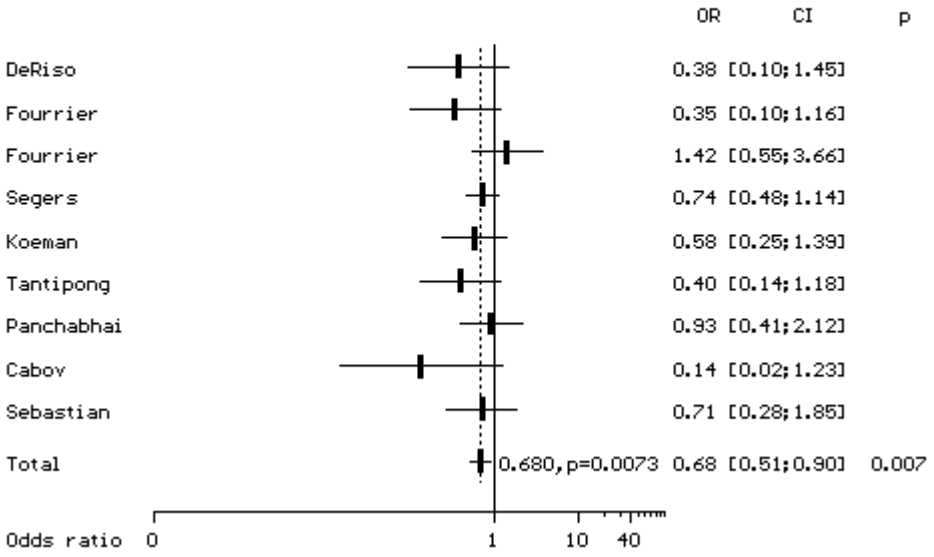

Figure 5.6 Impact of oral chlorhexidine on lower respiratory tract infection due to Gram-negative bacteria**.**
OR, odds ratio; CI, 95% confidence interval. OR < 1 favours treatment; OR > 1 favours controls.

Seven RCTs [161, 164-166, 169, 173, 181] enrolling 2,116 patients (1,051 chlorhexidine, 1,065 control) reported information on “normal” and “abnormal” bacteria. Thirty-five and 70 patients suffered from lower respiratory tract infection

due to "normal" flora in test and control group, respectively. Chlorhexidine significantly reduced lower respiratory tract infection due to these microorganisms (OR 0.50, 95% CI 0.33-0.75, p<0.001) (Figure 5.7). Heterogeneity was absent (χ^2=4.07, p=0.67, I^2=0%).

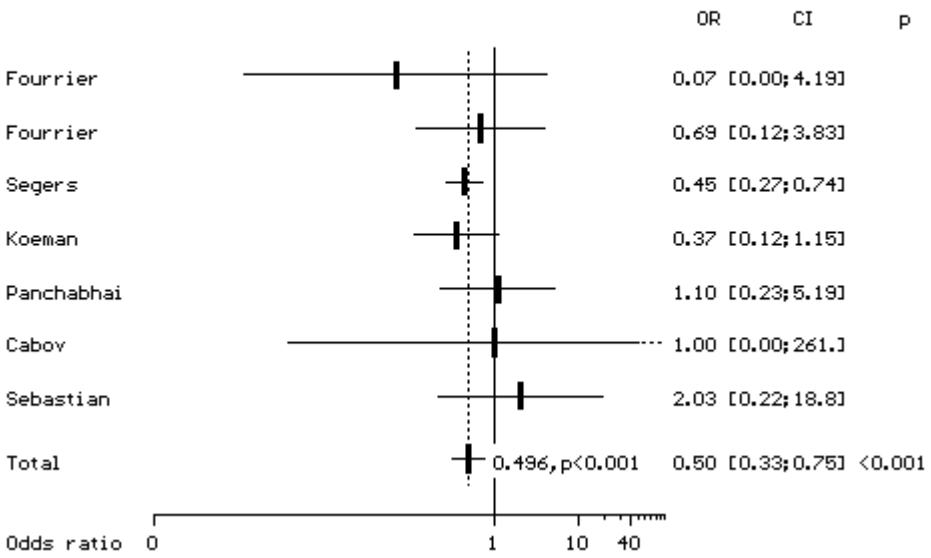

Figure 5.7 Impact of oral chlorhexidine on LRTI due to normal flora.
OR, odds ratio; CI, 95% confidence interval. OR < 1 favours treatment; OR > 1 favours controls.

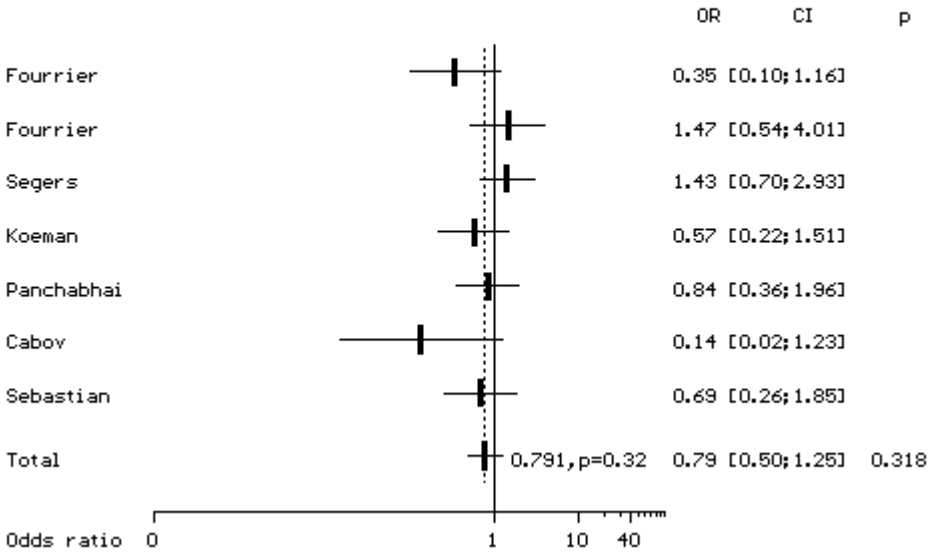

Figure 5.8 Impact of oral chlorhexidine on LRTI due to abnormal flora.
OR, odds ratio; CI, 95% confidence interval. OR < 1 favours treatment; OR > 1 favours controls.

"Abnormal" flora caused lower respiratory tract infection in 61 patients of chlorhexidine group and 75 patients of the control group. Chlorhexidine reduced, albeit not significantly, the odds of lower respiratory tract infection by these microorganims (OR 0.79, 95% CI 0.50-1.25, p=0.32) (Figure 5.8). Heterogeneity was not demonstrated (χ^2=6.36, p=0.38, I^2=5.6%).

5.3.6 Microorganisms causing bloodstream infection

Results from 3 trials including 348 patients (174 chlorhexidine, 174 controls) were available for the analysis of BSI classified according to both the Gram stain and normal and abnormal flora [161, 164, 173]. There were five (2.9%) and eight (4.6%) patients with BSI due to Gram-positive bacteria in test and control group, respectively, indicating a lack of effect of oral chlorhexidine (OR 0.72, 95% CI 0.23-2.22, p=0.57; I^2 = 0%). Gram-negative bacteria were the causative agents of BSI in 3 (1.7%) patients of the chlorhexidine group and in 5 (2.9%) patients of the control group demonstrating a non significant reduction of BSI (OR 0.83, 95% CI 0.16-4.41, p = 0.83; I^2 = 0.9%).

Five (2.9%) and 9 (5.2%) patients suffered from BSI due to "normal" flora in test and control group, respectively. Chlorhexidine did not significantly reduce BSI due to these microorganisms (OR 0.63, 95% CI 0.21-1.88, p = 0.49; I^2=0%). "Abnormal" flora caused BSI in 3 (1.7%) patients of chlorhexidine group and 4 (2.3%) patients of the control group (OR 1.12, 95% CI 0.14-8.66, p = 0.91; I^2=3%). No heterogeneity was demonstrated.

5.3.7 Type of microorganism causing LRTI and BSI

A separate analysis was performed for each individual microorganism (Tables 5.4 and 5.5). Table 5.4 shows the impact of chlorhexidine on the individual microorganism causing lower respiratory tract infection. Although most microorganisms were impacted by chlorhexidine, this effect was not significant for all comparisons. Additionally, the efficacy of oral chlorhexidine on MRSA lower respiratory tract infection was not significant in the only RCTs reporting these data. There were not lower respiratory tract infections due to MRSA in the chlorhexidine group compared with one patient with nosocomial lower respiratory tract infection due to MRSA in the control group (OR 0.20, 95% CI 0.01-14.64).

Table 5.4 Meta-analysis of the impact of chlorhexidine on the individual microorganism causing lower respiratory tract infection

Microorganisms	**N° patients randomised**		**N° patients with LRTI**		**OR (95% CI)**	***p***
	Chx	C	Chx	C		
MSSA	1021	1035	7	18	0.48 (0.20-1.16)	0.10
MRSA	114	114	0	1	0.20 (0.01-14.65)	NE
Streptococcus pneumoniae	612	599	1	6	0.24 (0.39-1.43)	0.12
Haemophilus influenzae	612	599	20	28	0.69 (0.38-1.23)	0.21
Moraxella spp.	485	469	4	9	0.42 (0.13-1.39)	NE
Escherichia coli	864	875	3	9	0.63 (0.15-2.74)	0.54
Pseudomonas spp.	1051	1065	19	30	0.80 (0.44-1.48)	0.48
Acinetobacter spp.	536	566	12	12	1.08 (0.47-2.48)	0.85
Citrobacter spp.	30	30	1	0	5.17 (0.07-390.74)	NE
Serratia spp.	515	499	4	2	1.67 (0.34-8.15)	0.53
Proteus spp.	30	30	0	1	0.19 (0.01-14.61)	NE
Enterobacter spp.	629	613	5	7	0.81 (0.25-2.60)	0.73
Klebsiella spp	780	791	13	12	1.05 (0.34-3.22)	0.93
S. maltophilia	30	30	0	2	0.10 (0.01-6.68)	NE
Enterobacteriaceae	127	130	7	8	0.89 (0.31-2.53)	NE

N°, number; Chx, chlorhexidine; C, control; MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; spp, species; LRTI, lower respiratory tract infection; OR, odds ratio; CI, confidence interval; NE, not evaluable.

Table 5.5 shows the impact of chlorhexidine on microorganisms causing bloodstream infection. Data on microorganisms were available only from three RCTs [161, 164, 173]. Although the majority of microorganisms were reduced by chlorhexidine (8

episodes in chlorhexidine group, 13 episodes in control group), this effect was not significant for all comparisons. Five episodes in both test and control group were caused by coagulase-negative staphylococci, anaerobes and enterococci. No MRSA bloodstream infections were found.

Table 5.5 Meta-analysis of the impact of chlorhexidine on individual microorganisms causing bloodstream infection

Microorganisms	**N° patients randomised**		**N° patients with BSI**		**OR (95% CI)**	***P***
	Chx	C	Chx	C		
MSSA	174	174	0	4	0.14 (0.01-2.03)	0.14
Pseudomonas aeruginosa	114	114	0	1	0.33 (0.01-8.19)	0.50
Acinetobacter baumannii	30	30	1	0	3.10 (0.12-79.23)	0.50
Serratia marcescens	30	30	1	0	3.10 (0.12-79.23)	0.50
Proteus mirabilis	114	114	0	1	0.33 (0.01-8.19)	0.50
Enterobacter aerogenes	30	30	0	1	0.32 (0.01-8.24)	0.49
Klebsiella pneumoniae	114	114	1	1	1 (0.06-16.19)	1
Coagulase-negative staphylococci	144	144	3	4	0.74 (0.16-3.40)	0.70
Anaerobes	30	30	1	1	1 (0.06-16.76)	1
Enterococcus faecalis	114	114	1	0	3.03 (0.12-75.08)	0.50

N°, number; Chx, chlorhexidine; C, control; MSSA, methicillin-sensitive *Staphylococcus aureus*; BSI, bloodstream infection; OR, odds ratio; CI, confidence interval.

5.3.8 Subgroup analysis

Table 5.6 shows the result of the subgroup analysis of lower respiratory tract infection. The impact of chlorhexidine on the reduction of lower respiratory tract infection was significant in studies with inadequate randomization procedures (OR 0.62, 95% CI 0.44-0.87, p=0.005), and both in blinded (OR 0.51, 95% CI 0.51-0.89, p = 0.005) and unblinded studies (OR 0.60, 95% CI 0.37-0.99, p=0.047), although in unblinded studies the significance was borderline. Chlorhexidine reduced lower respiratory tract infection both in low and in high quality studies, although the confidence interval in low quality studies was wider than in high quality studies. Chlorhexidine concentrations of 0.12%-0.2% and 1%-2% were associated with a significant lower respiratory tract infection reduction, although the latter showed a wide confidence interval with a borderline significance.

Table 5.6 Subgroup analysis of the endpoint of lower respiratory tract infection

Subgroups	N° RCTs	N° Patients		N° events		OR (95% CI)	*p*
		Chx	C	Chx	C		
Randomization/allocation							
- adequate	8	1012	1005	112	155	0.71 (0.46-1.07)	=0.1
- not adequate	14	1107	1153	134	186	0.62 (0.44-0.87)	=0.005
Blinding							
- double-blinded	11	1280	1268	156	219	0.68 (0.51-0.89)	=0.005
- not blinded	11	839	890	90	122	0.60 (0.37-0.99)	=0.047
Quality							
- low quality	8	561	582	63	92	0.49 (0.26-0.92)	=0.026
- high quality	14	1558	1576	183	249	0.70 (0.54-0.92)	=0.011
Chlorhexidine concentration							
- 0.12% - 0.2%	18	1809	1838	203	268	0.70 (0.52-0.94)	=0.018
- 1% - 2%	3	270	280	30	49	0.59 (0.35-0.97)	=0.04
Population type							
- surgical	6	1085	1083	82	133	0.52 (0.33-0.82)	=0.005
- medical	4	324	354	45	70	0.53 (0.26-1.09)	=0.083
- mixed	12	710	721	119	138	0.82 (0.60-1.12)	=0.22
- adult population	19	1945	1990	203	300	0.59 (0.45-0.79)	<0.001
- paediatric population	3	174	168	43	41	1.07 (0.65-1.77)	=0.79

RCTs, randomised controlled trials; Chx, chlorhexidine; C, control; OR, odds ratio; CI, confidence interval. The I^2 test for heterogeneity was not significant in all comparisons.

Six studies including 2,168 patients, i.e. half the population of this review, were performed in surgical patients; 4 of them were undertaken in heart surgery. The reduction in lower respiratory tract infection was significant (OR 0.52, 95% CI 0.33-0.82, *p*=0.005).

In four RCTs carried on medical population and in 12 studies in mixed ICU populations the lower respiratory tract infection reduction was not significant (OR 0.53, 95% CI 0.26-1.09, *p*=0.083, and 0.82, 95% CI 0.60-1.12, *p*=0.22, respectively). Rates of lower respiratory tract infection in the controls were 12.2%, 19.8%, and 19.1% in surgical, medical and mixed population, respectively.

Finally, chlorhexidine did not impact lower respiratory tract infection in three studies including a small subset of 342 paediatric patients.

The reduction of BSI was significant (OR 0.47, 95% CI 0.22-0.97, *p*=0.04) in three studies including 1,367 patients, i.e. more than 80% of the study population, and

performed in surgical patients; 2 of them including 1,307 patients, were undertaken in cardiac surgery (Table 5.7). Both in medical and in mixed population the impact of chlorhexidine on BSI was not significant (OR 1, 95% CI 0.23-4.43, and OR 2.4, 95% CI 0.61-9.61, respectively).

Table 5.7 Subgroup analysis of the endpoint of bloodstream infection

Subgroups	N° RCTs	N° patients		N° events		OR (95% CI)	*p*
		Chx	C	Chx	C		
Randomization/allocation							
- adequate	3	772	763	14	24	0.74 (0.23-2.45)	0.63
- inadequate	2	60	60	5	6	0.82 (0.23-2.94)	0.76
Blinding							
- Blinded	4	802	793	18	26	0.70 (0.28-1.79)	0.46
- Not blinded	1	30	30	4	4	1 (0.23-4.43)	1
Quality							
- High quality	4	802	793	18	26	0.70 (0.28-1.79)	0.46
- Low quality	1	30	30	4	4	1 (0.23-4.43)	1
Chlorhexidine concentration							
- Chx 0.12%	2	685	649	10	21	0.46 (0.22-0.99)	0.049
- Chx 0.2%	3	174	174	12	9	1.35 (0.53-3.45)	0.53
Population type							
- Surgical population	3	686	679	11	23	0.47 (0.22-0.97)	0.04
- Medical population	1	30	30	4	4	1 (0.23-4.43)	1
- Mixed population	1	114	114	7	3	2.4 (0.61-9.61)	0.21

RCTs, randomised controlled trials; Chx, chlorhexidine; C, control; OR, odds ratio; CI, confidence interval. The I^2 test for heterogeneity was not significant in all comparisons

The results of the subgroup analysis for the endpoint of mortality confirmed the previous pooled data. In particular, the analysis of subgroups of RCTs including adequate randomization, surgical and medical patients, and paediatric population demonstrated a not significant reduction in mortality, whilst in the other subgroups there was an increase, albeit not significant, in mortality. Interestingly, the baseline mortality rate in surgical patients, which represent 52% of the entire study population, was very low (2.6%) compared with medical (33%) or mixed population (29.4%) (Table 5.8).

Table 5.8 Subgroup analysis of the endpoint of mortality

Subgroups	N° RCTs	N° Patients		N° events		OR (95% CI)	*p*
		Chx	C	Chx	C		
Randomization/allocation							
- adequate	7	979	962	87	15935	0.97 (0.67-1.41)	=0.88
- not adequate	9	1018	1067	252	237	1.16 (0.93-0.44)	=0.18
Blinding							
- double-blinded	11	1280	1268	180	180	1.05 (0.82-1.36)	=0.68
- not blinded	5	717	761	159	150	1.17 (0.89-1.55)	=0.26
Quality							
- low quality	4	493	514	81	80	1.03 (0.69-1.52)	=0.90
- high quality	12	1504	1515	258	250	1.13 (0.92-1.40)	=0.24
Chlorhexidine concentration							
- 0.12% - 0.2%	13	1727	1749	238	233	1.10 (0.88-1.37)	=0.41
- 1% - 2%	3	270	280	101	97	1.13 (0.78-1.62)	=0.52
Population type							
- surgical	5	1045	1043	22	27	0.80 (0.35-1.81)	=0.59
- medical	4	324	354	114	117	0.99 (0.62-1.58)	=0.98
- mixed	7	628	632	203	186	1.15 (0.90-1.46)	=0.27
- adult population	13	1823	1861	310	292	1.16 (0.96-1.41)	=0.13
- paediatric population	3	174	168	29	38	0.73 (0.41-1.30)	=0.28

RCTs, randomised controlled trials; Chx, chlorhexidine; C, control; OR, odds ratio; CI, confidence interval. The I^2 test for heterogeneity was not significant for all comparisons

5.4 Discussion

Three main findings emerge from this systematic review and meta-analysis of 22 RCTs and including 4,277 patients:

1. Chlorhexidine significantly reduces lower respiratory tract infection, but not bloodstream infection; the subgroup analysis demonstrates that there is a significant reduction of lower respiratory tract infection and bloodstream infection only in the subgroup of surgical, mainly cardiac, patients.
2. Chlorhexidine shows a protective effect against lower respiratory tract infections caused by potential pathogens when these bacteria are classified using the Gram staining technique; remarkably, when the causative bacteria are classified into “normal” and “abnormal” bacteria, chlorhexidine reveals a significant reduction in lower respiratory tract infection due to “normal” flora only. The analysis for

each individual microorganism causing infections does not demonstrate any impact on MSSA and MRSA lower respiratory tract infection and bloodstream infection.

3. Mortality is not impacted by oral chlorhexidine.

Our observation that chlorhexidine is effective in reducing lower respiratory tract infections is in line with previous systematic reviews [149-157]. However, this result should be interpreted with caution. Half the population included in the analysis of lower respiratory tract infection, i.e. 2,168 of 4,277 patients, comes from only 6 RCTs performed on surgical, mainly cardiac (2,038 patients), individuals. The analysis revealed that chlorhexidine significantly reduced lower respiratory tract infection in this subgroup of patients, whereas in medical and in mixed ICU population it did not. These observations are in line with those of previous meta-analyses [148, 155, 156]. There are several reasons explaining the greater treatment effect in surgical population. Firstly, the severity of the underlying disease and the duration of mechanical ventilation are less in patients undergoing elective surgery than in medical or mixed population. Most surgical patients received 4 to 8 hours [162], 12 hours [165] or 24 hours [177] of mechanical ventilation after surgery, whereas non-surgical patients are ventilated for more than one week. Secondly, surgical patients are intubated in the operating theatre under optimal and controlled conditions rather than in emergency circumstances. Moreover, all cardiac surgery patients usually receive an adequate preoperative oral care. Finally, the sample size of medical and mixed population could be too small to detect an effect of chlorhexidine on lower respiratory tract infection. However, subgroup analyses are observational by nature and are not based on randomised comparisons. The results of

this subgroup analysis should be taken with caution and should be best viewed as hypothesis generating.

Recently, Helman et al. found that oral chlorhexidine reduced lower respiratory tract infection due to Gram-positive but not Gram-negative bacteria [184]. Conversely, an intriguing finding of this review is that oral chlorhexidine was beneficial in preventing lower respiratory tract infections due to both Gram-positive and Gram-negative potential pathogens, whereas, if the causative pathogens were classified into "normal" and "abnormal" flora, chlorhexidine covered only "normal" pathogens. The Gram staining technique is intrinsically unable to differentiate "normal" from "abnormal" flora: for example, Gram-negative bacteria comprise both "normal" (i.e. *E. coli*, *H. influenzae*, and *M. catarrhalis*) and "abnormal" pathogens (i.e. *Klebsiella, Proteus, Morganella, Enterobacter, Citrobacter, Serratia, Acinetobacter, and Pseudomonas* species). The "normal" Gram-positive and -negative bacteria usually cause primary endogenous lower respiratory tract infection in previously healthy individuals, such as trauma or surgical patients; in these patients lower respiratory tract infection develops "early" during the first week of ICU treatment [44]. On the contrary, the "abnormal" Gram-positive and negative microorganims are, in general, the causative agents of secondary endogenous lower respiratory tract infection which develops "late", after one week of ICU treatment [44]. The failure of oral chlorhexidine to protect against oropharyngeal carriage of "abnormal" bacteria may be due to the chlorhexidine inactivation by saliva resulting in non-bactericidal levels for these bacteria in the oropharynx [185]. Additionally, our finding that oral chlorhexidine is beneficial in surgical patients fits well with the result that chlorhexidine impacts only "normal" flora, and confirms the hypothesis of a previous systematic review [156] that oral chlorhexidine could have an effect on "early onset"

lower respiratory tract infection only. Additionally, this review investigated the impact of each individual microorganism causing LRTI and BSI. There were insufficient data from this review on MRSA infection, while there was no impact of chlorhexidine on MSSA LRTI and BSI. Additionally, this review was not design to detect the type of microorganims causing infection in the subsets of ICU patients, i.e. surgical or medical population.

Three reasons may explain the lack of the efficacy of oral chlorhexidine on BSI. First, owing to the pathogenesis of lower respiratory tract infection in the critically ill ICU patient, the application of antiseptics or antimicrobials into the oropharynx is expected to control only lower respiratory tract infection rather than BSI. Previous experiences with the use of oropharyngeal topical antibiotics in ICU patients confirm this hypothesis [186, 187]. A recent Dutch RCT demonstrated that ICU-acquired bacteraemia occurred in 4.6% of patients receiving the combination of oropharyngeal and gut antimicrobials (selective digestive decontamination, SDD) compared with 5.9% of patients receiving only oropharyngeal antimicrobials (selective oropharyngeal decontamination, SOD) (OR 0.77 95%, CI 0.65-0.91) [141]. AGNB bacteraemia has also been shown to be higher in patients receiving SOD than in those receiving SDD [187]. Second, the finding of our systematic review reinforces the critical role of gut decontamination in the control of BSI, despite the availability of reports showing that the oropharynx may be the source of *S. aureus* [146, 188] and AGNB bacteraemia [189]. Chlorhexidine applied into the oropharynx does not affect the gut flora and, therefore, overgrowth of potentially pathogenic microorganisms is the unavoidable consequence. Gut decontamination is important as it eradicates gut overgrowth, a phenomenon known to cause translocation [190], inflammation [191] and absorption of endotoxin [192]. Third, as oral chlorhexidine has been shown to

reduce lower respiratory tract infection, and lower respiratory tract infection may cause BSI [193-195], we would assume that BSI is also reduced. However, the link between lower respiratory tract infection and BSI is not robust. The sensitivity of blood cultures to diagnose lower respiratory tract infection is less than 25%, and, when positive, the microorganism may originate from an extra-pulmonary source, such as the urinary tract and vascular devices [75, 196, 197]. This information is supported by our analysis which found that 62% and 35% of microorganims causing BSI in test and control group, respectively, do not generally cause lower respiratory tract infection (e.g. coagulase-negative staphylococci, enterococci and anaerobes).

Similarly to the results of the subgroup analysis of lower respiratory tract infection, the subgroup analysis of BSI showed a significant reduction of bloodstream infection in surgical patients (OR 0.47; 95% CI 0.22-0.97; p=0.04). The majority of them, i.e. 1,300 patients (95%), underwent cardiac surgery.

Mortality was unaffected by oral chlorhexidine in previous meta-analyses, the main explanations being both the small sample size [148, 149] and the presence of heterogeneity [150, 155, 156]. Two meta-analyses showed an increase in mortality [82, 83]. Our mortality analysis included a large sample size of 4,026 patients, and did not show any significant heterogeneity using the random effects model, but was still unable to demonstrate a survival benefit and showed a non significant increase in mortality. There are some explanations for this finding. Firstly, none of the trials were powered to detect mortality differences, and, despite combining the RCTs, a final sample size with a baseline mortality rate of 16% could yet be inadequate to detect a survival benefit [198]. This hypothesis may be consistent with the very low baseline 2.6% mortality rate in surgical patients which are 52% (2,088 of 4,026) of subjects included in the analysis of mortality. The administration of parenteral

antibiotic prophylaxis may explain the low mortality rate in surgical, mainly cardiac, patients [199]. In the remaining population, the sample sizes of 678 medical and 1260 mixed patients with a mortality rate of 33% and 29%, respectively, may be not large enough to detect a survival benefit. Secondly, the infection-mortality relation may be difficult to demonstrate with the chlorhexidine manoeuvre which only impacts the oropharyngeal "normal" flora without affecting gut carriage of "abnormal" microorganisms, and thus, without reducing other severe infections, e.g. bloodstream infections. In the literature, an example of this issue is the survival benefit of SDD compared with SOD [44]. SDD reduced mortality by 29% (OR 0.71, 95% CI 0.61-0.82), reaching a 42% reduction (OR 0.58, 95% CI 0.45-0.77) when the gut was successfully decontaminated [85]. This may be due to the prevention of infections of the lower airways [86] and the bloodstream [87, 200] following the use of enteral antimicrobials which controls gut overgrowth [44]. On the contrary, SOD, in controlling only oropharyngeal carriage, failed to demonstrate any significant impact on survival in a meta-analysis [201], and, in a recent and large meta-analysis SOD was inferior to SDD in reducing mortality [88].

The strengths of this analysis are the comprehensive search for relevant RCTs, the assessment of methodological quality, the use of pre-specified subgroup analyses, and the use of the random effects model which takes heterogeneity into account. However, we acknowledge some important limitations. Firstly, we did not use an intention-to-treat analysis as some studies did not report whether the number of patients analysed reflected the total amount of patients randomised, and how withdrawals and dropouts were treated. Secondly, clinical heterogeneity was present because of: a) different types of patients included; b) different chlorhexidine concentrations, application forms, dosages, and duration of treatment; c) different

definitions of lower respiratory tract infection; d) the use of parenteral antibiotic prophylaxis. Moreover, there were only 14 high quality and 11 double-blinded RCTs. These variables might have impacted the results. However, we decided *a priori* to use the more conservative random effects model, which fits the distribution of the effects sizes, and takes into account the relevant source(s) of error [202]. Additionally, we addressed some of the aforementioned limitations by performing a pre-specified subgroup analysis of those variables, and we provided the clinical reason why a particular group of participants or studies needed to be looked at separately. Finally, although our search strategy was comprehensive, we could have missed some relevant trials.

5.5 Conclusion

This review demonstrates that oral care with chlorhexidine reduces overall lower respiratory tract infection, and lower respiratory tract infection due to Gram-positive and Gram-negative "normal" flora. Bloodstream infection is not impacted by chlorhexidine. Mortality is not affected. There are insufficient data to assess the efficacy of oral chlorhexidine on lower respiratory tract infection and bloodstream infection due to MRSA. Half the population of this review is surgical, and, therefore, the results can not be generalizable to the entire ICU population. This should warrant further large RCTs to test the effectiveness of oral chlorhexidine in preventing lower respiratory tract infection and in reducing mortality in medical and mixed critically ill patients. These RCTs should be adequately powered to detect the effect of chlorhexidine on the control of MRSA infection.

6 EFFECTIVENESS OF ENTERAL VANCOMYCIN TO CONTROL MRSA CARRIAGE AND INFECTION IN ICU PATIENTS. A SYSTEMATIC REVIEW AND META-ANALYSIS

6.1 Introduction

Control of MRSA carriage and infection is an important issue in the ICU. In an international study on the prevalence and outcomes of infection in ICUs the most common Gram-positive microorganism was *S. aureus* (20.5% of all isolates); MRSA accounted for 10% of all isolates [28]. The traditional approach to control MRSA spread in high-risk areas such as ICU includes several measures which have often failed because of the impracticalities in implementing them [21, 203-206]. They are: a) screening of MRSA carrier state in the nose, oropharynx or rectum in selected patients population such as cardiosurgical patients, orthopaedic patients, dialysis patients and intensive care unit patients in case of MRSA endemicity; b) high levels of hygiene and isolation to prevent transmission; c) topical mupirocin in the nose and throat to eradicate oropharyngeal and nasal carrier state; d) oropharyngeal chlorhexidine to clear the carrier state; e) chlorhexidine bathing to reduce skin carriage; f) parenteral vancomycin in case of infection.

Parenteral vancomycin is the first choice therapy of MRSA infection. However, vancomycin may be harmful in ICU patients particularly in those with impairment of the renal function and in subjects with septic shock. Moreover, penetration of vancomycin in some tissues, such as the lung, is limited. Additionally, there are concerns about the emergence of microorganisms resistant to vancomycin, such as VRE and VISA [205].

There is a large body of evidence from the literature that oropharyngeal and intestinal carriage and overgrowth of AGNB and yeasts can be controlled using SDD [44]. SDD includes a combination of polymyxin E, tobramycin and amphotericin B administered in the oropharynx and gut together with a short course of parenteral

antibiotic (e.g. cefotaxime). SDD has been shown to reduce AGNB and fungal carriage, lower respiratory tract infections, bloodstream infections, multiple organ failure and mortality, without the emergence of resistance [84, 89]. SOD, is a modified SDD regimen including only the oropharyngeal component. SOD demonstrated a reduction of lower respiratory tract infection and mortality, although bacteraemia was not controlled [44].

Using the same philosophy, the administration of vancomycin into the intestinal tract has been reported to eradicate *Clostridium difficile* from the gut [207]. Additionally, enteral vancomycin has been added to the classical SDD antimicrobials to control MRSA overgrowth or MRSA endemicity in the ICU [208, 209].

A systematic review and meta-analysis has been undertaken to assess the efficacy of enteral vancomycin to control MRSA carriage and infection in ICU patients. The secondary endpoint was to evaluate the emergence of resistance to vancomycin, in particular VRE and VISA.

6.2 Materials and methods

6.2.1 Search strategy

A systematic review and meta-analysis was performed using the PRISMA guidelines [109]. PubMed and the Cochrane Register of Controlled Trials were screened for randomised and non-randomised studies published until June 2016 with no language restriction. Search terms were selective decontamination of the digestive tract, selective bowel decontamination, selective gut decontamination, methicillin-resistant *Staphylococcus aureus*, vancomycin, topical vancomycin, vancomycin oropharynx, gut vancomycin, intestinal vancomycin, and oral vancomycin. The search limits were

"humans". A hand search of references from papers and published meta-analyses was done. Studies were analyzed using a standardized data extraction form. The search, the screening of the titles and abstracts, and the analysis of studies were made independently by two investigators.

6.2.2 Selection criteria

Inclusion and exclusion criteria were established before starting the review process. All studies in critically ill patients receiving enteral vancomycin (topical application to the oropharynx, intestine or both) in the test group and no vancomycin or placebo in the control group were included. Studies using vancomycin together with other antimicrobials to decontaminate the oropharynx and the gut were also included. Studies or interventions in neutropenic and bone marrow transplant patients were not included.

6.2.3 Data extraction

Two investigators independently retrieved and compared the sets of data from each trial. Any disagreement was resolved by discussion. The following data were sought: author, publication year, population included, description of the intervention in the test arm and in the control arm, randomization and allocation concealment, blinding, handling of dropouts and withdrawals, number of patients included, number of patients with infection, number of patients with *Staphylococcus aureus* infection and carriage, number of patients with MRSA infection and carriage, mortality, and antimicrobial resistance (e.g. presence of VRE and VISA).

6.2.4 Quality assessment

Two investigators assessed the quality of RCTs using the Cochrane Collaboration risk of bias tool [111]. The Newcastle-Ottawa quality assessment scale (NOS) was used to appraise the quality of non-randomised studies [112]. The Newcastle-Ottawa scale assigns a maximum of 4 points for selection of patients, two points for comparability of test and control groups, and 3 point for outcome assessment. A priori, we decided that randomised studies with a high or unclear risk of bias in less than two domains or observational studies with an NOS score greater than 7 would be considered to be high quality.

6.2.5 Endpoints and statistical analysis

The primary endpoints were the number of patients with infection, the number of patients with *Staphylococcus aureu*s carriage and infection, the number of patients with MRSA carriage and infection, and mortality; the secondary endpoint was the occurrence of resistant microorganisms (i.e. VRE and VISA). For the primary outcomes we decided *a priori* to separately analyse data from randomised and non-randomised studies, and from studies using vancomycin combined with other enteral antimicrobials. We did not assess statistical differences between these strata.

Results were presented as odds ratio (OR) with 95% confidence interval (CI) using the random effects model and *p* value. The Cochran *Q* statistic for heterogeneity has been used; heterogeneity is considered to be significant if the *p* was <0.10. Additionally, I^2 has been evaluated with the formula 100% x (*Q*-df)/*Q*, where *Q* is Cochran's *Q* statistics and *df* is the degree of freedom (number of studies- 1). Negative values of I^2 are equal to 0%; an I^2 of 0% indicates no observed heterogeneity, whilst < 30% indicates mild heterogeneity, 30-50% moderate, and >

50% severe (significant heterogeneity I^2 greater than 50%) [113]. The funnel plot to estimate potential publication bias has been explored. Computations were performed using the EasyMA software [114].

6.3 Results

6.3.1 Search findings and description of studies

After the exclusion of duplicates and irrelevant publications we considered 15 studies for more detailed evaluation. The subsequent screening yielded a final sample of 12 studies, 9 RCTs, including 1473 patients (711 vancomycin group, 762 controls) and 3 non-randomised studies including 1240 patients (654 vancomycin group, 586 controls). This set of studies was the basis for the systematic review and meta-analysis [210-221]. Figure 6.1 depicts the search strategy and the reason for exclusion of studies.

Tables 6.1, 6.2 and 6.3 describe the main characteristics and data extracted from RCTs and non-RCTs included in the systematic review.

All 9 RCTs included in the systematic review were European: three from France [211, 212, 214], two from Germany [213, 217], one from The Netherlands [210], one from Switzerland [215], one from Spain [216], and one from Italy [218]. Studies were published from 1990 to 2004. One study was published as abstract of a scientific meeting [216]. One study was performed in neonatal ICU [214], three studies were performed in surgical/trauma patients [213, 215, 217], one in neurosurgical ICU [212], four in mixed medical-surgical ICU [210, 211, 216, 218]. The oropharynx was decontaminated with vancomycin in 5 RCTs [210, 212, 214, 215, 218], the gut was decontaminated in 2 RCTs [211, 217], and in 2 RCTs

vancomycin was administered both in the oropharynx and in the gut [213, 216]. In general, vancomycin was administered four times a day, apart from one study in which it was given six times a day [215]. Different concentrations of vancomycin were used: one study used 2% [210], three studies used 4% [212, 217, 218], and the remaining studies employed different dosages of vancomycin solution in the oropharynx or in the gut.

In six RCTs vancomycin was combined with other enteral antimicrobials, such as polymyxin E or B, different aminoglycosides (gentamicin, tobramycin) and polyenes [210-213, 215, 217]. In two RCTs vancomycin was used alone [214, 216], and in one RCT both test and control group received the classical SDD protocol of polymyxin E, tobramycin and amphotericin B, but only the test group received vancomycin [218]. In only two RCTs [216, 218] the primary endpoint was the control of MRSA carriage and/or infection. In the remaining RCTs [214], which used a combination of different antimicrobials, vancomycin was included to control *S. aureus* carriage and/or infections, but no details were given about the inclusion of MRSA under *S. aureus* isolates.

Tables 6.2 and 6.3 (second part) show the main characteristics and data extracted from three non-randomised studies [219-221]. Two studies were from Spain [219, 220], and one study was Italian [221]. Studies were published between 2002 and 2007. One study was in burn ICU patients [219], and two studies were in medical-surgical ICUs [220, 221]. In two studies vancomycin was administered in both oropharynx and gut [219, 220], and one study in the intestinal tract only [221]. In two studies [219, 220] a 4% vancomycin paste was administered in the oropharynx together with vancomycin solution in the gut; one study employed only a

vancomycin solution in the gut [221]. In one study the test group received vancomycin together with SDD [219].

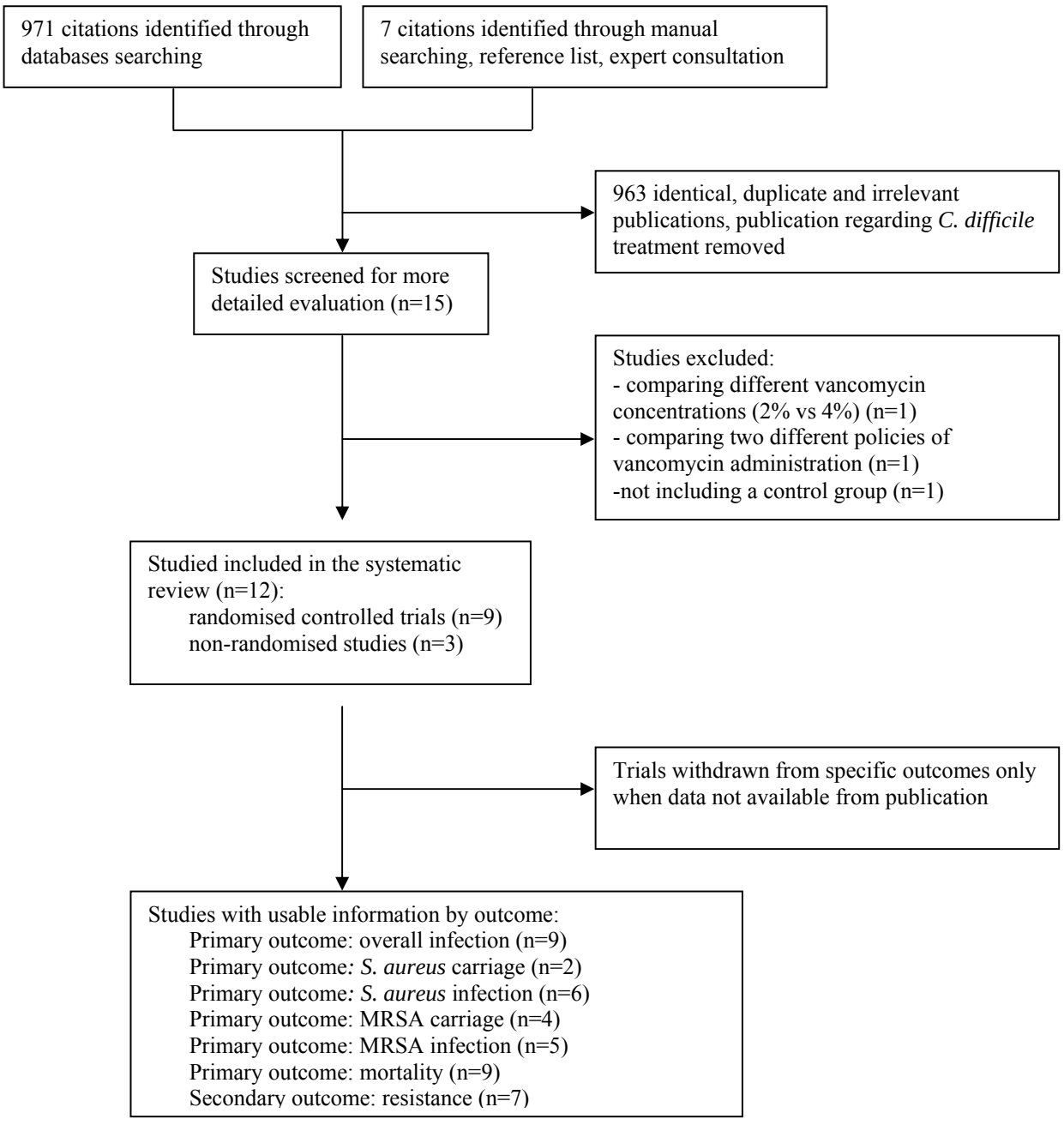

Figure 6.1 Diagram flow of study selection

Table 6.1 Characteristics of randomised controlled trials of enteral vancomycin

First Author	Year	Patients		Intervention		Population	Randomization	Allocation	Blinding
		Test	C	Test	C				
Bergmans [210]	2001	87	139	2% gentamicin + Pb + 2% van four times a day oropharynx. No parenteral antibiotics	Placebo	Medical, surgical, trauma and neurological patients	Yes, but not specified	Not specified	Double blind
Gaussorgues [211]	1991	59	59	Gentamicin 20 mg, colistin 36 mg, van 50 mg, amphotericin B 500 mg gut four times a day	Not reported	ICU ventilated patients	Yes, but not specified	Not specified	No
Korinek [212]	1993	63	60	PTA + 4% van oropharynx + PTA gut four times a day. No parenteral antibiotic	Placebo	Neurosurgery	Yes, but not specified	The pharmacist delivered the drugs	Double blind
Krueger [213]	2002	265	262	Gentamicin 80 mg, Pb 50 mg, van 125 mg four times a day nostrils, oropharynx and stomach	Sodium chloride 0.9%	Surgical population	Yes. A computer generated randomization scheme	The pharmacist assigned to treatment or placebo	Double blind
Marchand [214]	1990	20	20	Pharyngeal instillation of 4 drops of a 5% van solution 4 times a day	No local treatment	Neonate intensive care unit	Yes, but not specified	Not specified	No
Pugin [215]	1991	25	27	150 mg Pb+ 1 gr Neo+ 1 gr van solution 6 times a day oropharynx	Placebo	Surgical, trauma population	Yes, but not specified	Not specified	Double blind
Sanchez [216]	1997	48	50	4% oropharyngeal van paste + 250 mg van in the intestine four times a day	Placebo	Mechanically ventilated patients in general ICU	Yes, but not specified	Not specified	Double blind
Shardey [217]	1997	102	103	Pb 100 mg, tobramycin 80 mg, van 125 mg, AmB 500 mg four time a day gut	Placebo	Surgical population (total gastrectomy)	Yes, but not specified	Not specified	Double blind
Silvestri [218]	2004	42	42	0.5 gr of 4% van gel + PTA four times a day oropharynx	Only PTA	Mixed	Yes; computer generated random number table	Yes, statistician	No

C, control; ICU, intensive care unit; van, vancomycin; Pb, polymyxin B; Neo, neomycin; PTA, polymyxin E 100 mg + tobramycin 80 mg +amphotericin B 100 mg, AmB, amphotericin B.

Table 6.2 Characteristics of non-randomised studies of enteral vancomycin

First Author	Year	Patients		Intervention		Population and setting	Design	Endpoint(s)
		Test	C	Test	C			
Cerdà [219]	2007	375	402	4% van into the nose, 4% van in the oropharynx and 500 mg van in the gut, four times a day. All patients received PTA	Standard infection control policy	All patients admitted to the burn ICU	Prospective, sequential, cohort, 9-year period	To evaluate the effectiveness and safety of protocols to control endemicity of MRSA
de la Cal [220]	2004	258	140	Only patients with a MRSA carrier state received 4% van in the oropharynx and 500 mg van in the gut, four times a day	Standard infection control policy	Patients requiring mechanical ventilation. Medical-surgical ICU	Prospective, cohort, sequential, four-year period	To evaluate the effectiveness and safety of protocols to control endemicity of MRSA
Silvestri [221]	2002	21	44	500 mg van in the gut four times a day. All patients received PTA plus standard infection control policy	Standard infection control policy + PTA	Patients requiring mechanical ventilation. Medical-surgical ICU	Prospective, cohort, sequential, 8-month period during outbreak	Enteral van was evaluated as a measure to control an outbreak of MRSA infection

C, control group; van, vancomycin; MRSA, methicillin resistant *Staphylococcus aureus*; ICU, intensive care unit; PICU, paediatric intensive care unit; PTA, polymyxin E 100 mg + tobramycin 80 mg +amphotericin B 100 mg.

Table 6.3 Results of randomised (first part of the table) and non-randomised studies (second part of the table) of enteral vancomycin

First Author	N° patients enrolled		N° patients with infection		Mortality		N° patients with *S. aureus* carriage		N° patients with *S. aureus* infection		N° patients with MRSA carriage		N° patients with MRSA infection	
	Test	C	Test	C	Test	C	Test	C	Test	C	Test	C	Test	C
Bergmans [210]	87	139	31	66	25	53	0	9	3	11				
Gaussorgues [211]	59	59	5	15	29	59			1	2				
Korinek [212]	63	60	29	49	3	7								
Krueger [213]	265	262	91	149	52	75			15	63				
Marchand [214]	20	20	1	5			2	16	0	4				
Pugin [215]	25	27	4	21	10	11								
Sanchez [216]	48	50	0	3							0	9	0	3
Shardey [217]	102	103	21	36	5	11			9	40				
Silvestri [218]	42	42	16	24	12	15			9	21	8	20	8	12
Total	**711**	**762**	**198**	**368**	**136**	**231**	**2**	**16**	**37**	**141**	**8**	**29**	**8**	**15**
Cerdà [219]	375	402									14	102	10	76
de la Cal [220]	258	140											37	44
Silvestri [221]	21	44			7	17					13	39	2	22
Total	**654**	**586**			**7**	**17**					**27**	**141**	**49**	**142**

MRSA, methicillin-resistant *Staphylococcus aureus*; N°, number

In the Italian study both test and control group received SDD but only the test group received vancomycin [221]. The endpoint of all three studies was the efficacy of enteral vancomycin to control endemicity or outbreak due to MRSA. Therefore, the only available data were MRSA carriage and infection and only one study [221] reported the mortality rate.

6.3.2. Quality assessment

Nine studies were randomised, but in only two RCTs the randomization method was detailed [213, 218]. The allocation was adequate in three RCTs [212, 213, 218]; in the remaining the procedure was not specified. Six out of nine RCTs were double blinded [210, 212, 213, 215-217], one study was single blinded [218], and the remaining two studies were not blinded. The quality of RCTs assessed with the Cochrane collaboration risk of bias tool showed that the majority of information for the primary outcomes was from studies at high or unclear risk of bias. The quality of the three non-randomised studies was evaluated using NOS; all studies achieved 7 points, indicating a low quality.

6.3.3 Infection and carriage

Data on overall infection was available from 9 RCTs [210-218] including 1473 patients (711 test, 762 controls). There were 198 (27.8%) patients with infection in test group and 368 (48.3%) in controls, indicating a significant reduction of the odds of infection (OR 0.35, 95% CI 0.24-0.50, p=0.00199). Heterogeneity was mild (χ^2 9.5541, p=0.30, I^2=16%) (Figure 6.2).

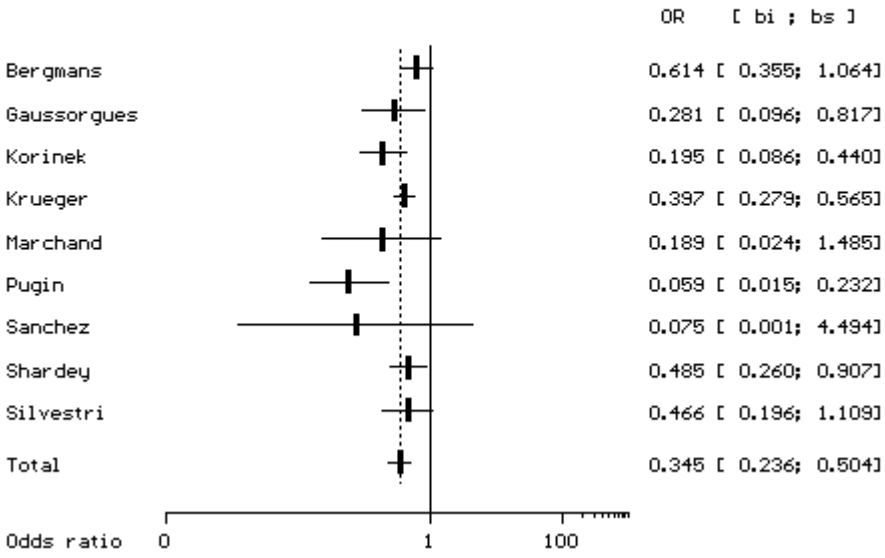

Figure 6.2 Forrest plot of the effect of enteral vancomycin on infection.
An odds ratio < 1 favours test; an odds ratio > 1 favours controls.

S. aureus carriage was described in two RCTs [210, 214] including 107 patients in test group and 159 in control group. Carriage developed in 2 (1.9%) and 25 (15.7%) patients in test and control group, respectively (OR 0.03, 95% CI 0.01-0.17, $p<0.001$). Heterogeneity was absent (χ^2 0.0102, $p=0.92$, $I^2=0\%$).

S. aureus infection was described in 6 studies [210, 211, 213, 214, 217, 218], including 1280 patients (575 test, 626 control), and affected 27 patients (2 test, 25 control), showing a significant reduction of infection (OR 0.21, 95% CI 0.15-0.32, $p<0.001$). There was not heterogeneity (χ^2 3.5143, $p=0.62$, $I^2=0\%$).

Four studies [216, 218, 219, 221] comprising a total of 1024 patients (486 test, 538 controls) provided data on MRSA carriage. There were 35 (7.2%) and 170 (31.6%) MRSA carriers in test and control group, respectively, demonstrating a significant impact of vancomycin (OR 0.15, 95% CI 0.09-0.25, $p<0.001$) with no heterogeneity (χ^2 2.9271, $p=0.40$, $I^2=0\%$ (Figure 6.3).

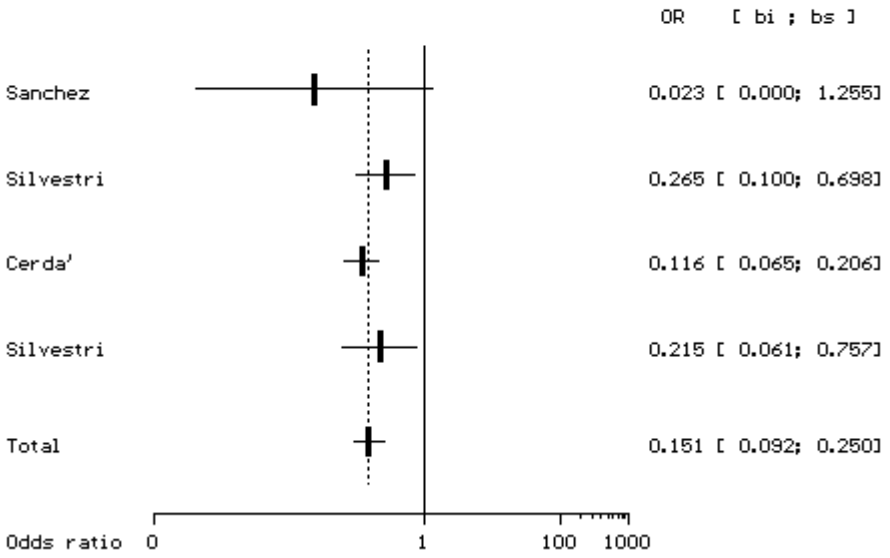

Figure 6.3 Forrest plot of the effect of enteral vancomycin on MRSA carriage.
An odds ratio < 1 favours test; an odds ratio > 1 favours controls.

In 5 studies [216, 218-221] reporting data on MRSA infection and including 1422 patients (744 test, 678 controls) there were 57 (7.66%) patients with MRSA infection in the vancomycin group and 157 (23.16%) in the control group, indicating a significant impact of vancomycin on MRSA infection (OR 024, 95% CI 0.12-0.50, $p< 0.001$). No heterogeneity was demonstrated (χ^2 3.5025, p=0.48, I^2=0%) (Figure 6.4)

6.3.4 Mortality

Mortality data were available from 9 studies in 2177 patients [210-213, 215, 217-219, 221], 1039 test and 1138 controls. Mortality was significantly reduced (184 test, 321 control) with an OR of 0.59 (95% CI 0.43-0.79, p<0.001). A mild heterogeneity was demonstrated (χ^2 10.3372, p=0.24, I^2=22.6%) (Figure 6.5).

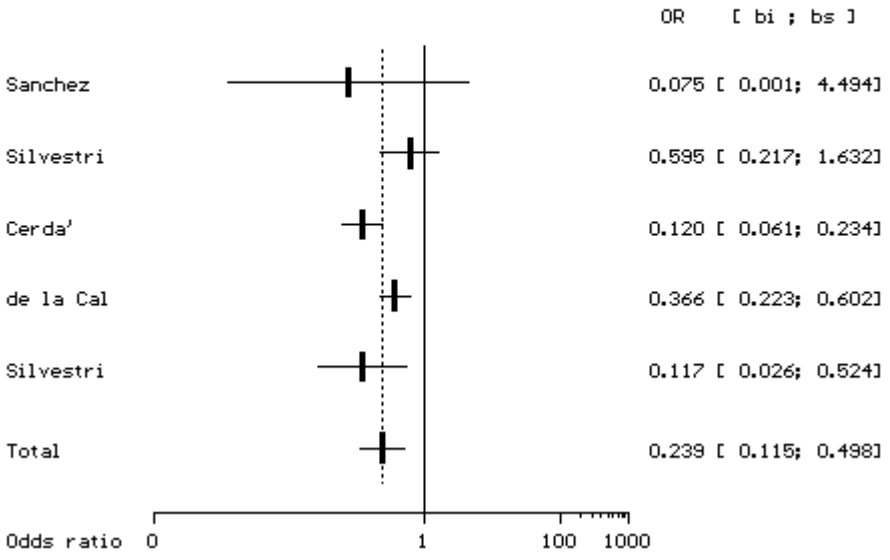

Figure 6.4 Forrest plot of the effect of enteral vancomycin on MRSA infection.
An odds ratio < 1 favours test; an odds ratio > 1 favours controls.

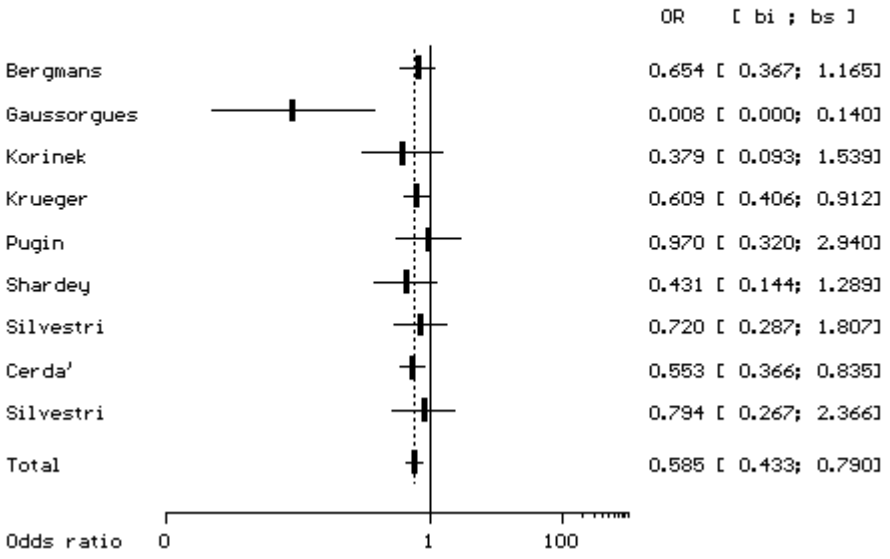

Figure 6.5 Forrest plot of the effect of enteral vancomycin on mortality.
An odds ratio < 1 favours test; an odds ratio > 1 favours controls

6.3.5 Resistance

Results on resistance were available from 7 studies (Table 6.4). In five studies VRE was not isolated [210, 212, 214, 218, 221]. In one study VRE carriage was reduced in vancomycin group compared with placebo [216]. In one study there were 4 VRE carriers only in the control group [219]. Finally, in another study VRE was imported and caused an outbreak of VRE infection in 13 patients which was brought under control [220]. Four studies gave details on VISA which has never been isolated [214, 218, 219, 221].

Table 6.4 Summary of resistance data from randomised and non-randomised studies of enteral vancomycin

First Author	Comments on resistance
Bergmans [210]	No VRE were isolated No cases of acquired resistance to the antibiotics used in the oropharyngeal paste
Gaussorgues [211]	No reported data
Korinek [212]	No VRE were isolated No evidence of increased incidence if resistant strain to the antibiotic used
Krueger [213]	No data
Marchand [214]	No VRE, VISA and VRSA
Pugin [215]	No reported data
Sanchez [216]	VRE was acquired in the oropharynx in 5 test and 9 placebo patients. The administration of vancomycin does not increase the incidence of VRE carriage
Shardey [217]	No data
Silvestri [218]	No VRE, VISA and VRSA
Cerdà [219]	There were 4 cases of VRE in control group. VISA was not detected.
de la Cal [220]	A VRE outbreak affecting 13 patients after two patients imported VRE into the unit. The outbreak resolved.
Silvestri [221]	A total of 895 microbiological samples were processed: neither VISA nor VRE were isolated.

6.3.6 Subgroup analysis

We did the analysis of the primary outcomes using data obtained from RCTs and non-randomised studies (Table 6.5). In RCTs overall infection, *S. aureus* carriage and *S. aureus* infection were significantly reduced; MRSA carriage and infection were reduced but not significantly (OR 0.17, 95% CI 0.03-1.06, p=0.057), and OR

0.53, 95% CI 0.20-1.41, p=0.20, respectively). Mortality was significantly reduced (OR 0.56, 95% CI 0.36-0.87, p=0.0011). Non-randomised trials did not report any data on overall infection, *S. aureus* carriage and *S. aureus* infection. Both MRSA carriage and infection were significantly reduced (OR 0.13, 95% CI 0.08-0.21, $p<$ 0.001, χ^2 0.7048; OR 0.19, 95% CI 0.07-0.46, p<0.001, respectively). Mortality was significantly reduced (OR 0.58, 95% CI 0.39-0.85, p=0.005).

Table 6.5 Subgroup analysis of randomised and non-randomised studies

Outcomes	N° RCTs	N° patients		N° events		OR (95% CI)	*P*	I^2
		Test	C	Test	C			
Randomised trials								
Overall infection	9	711	762	198	368	0.35 (0.24-0.50)	<0.001	16%
S. aureus carriage	2	107	159	2	25	0.03 (0.01-0.17)	<0.001	0%
S. aureus infection	6	575	625	37	141	0.21 (0.15-0.32)	<0.001	0%
MRSA carriage	2	90	92	8	29	0.17 (0.03-1.06)	= 0.057	0%
MRSA infection	2	90	92	8	15	0.53 (0.20-1.41)	= 0.20	0%
Mortality	7	643	692	136	231	0.56 (0.36-0.87)	= 0.011	36%
Non-randomised studies								
MRSA carriage	2	396	446	27	141	0.13 (0.08-0.21)	<0.001	0%
MRSA infection	3	654	586	49	142	0.19 (0.07-0.46)	<0.001	0%
Mortality	2	396	446	48	90	0.58 (0.39-0.85)	= 0.005	0%

C, control group; MRSA, methicillin-resistant *S. aureus.* Non-randomised studies did not provide data for overall infection, *S. aureus* carriage and infection

Table 6.6 shows the analysis of studies using vancomycin combined with the SDD regimen in the test group and studies using only vancomycin in the test group. Five studies were included in the group receiving vancomycin [214, 216, 218, 220, 221]. Two of them were included in this group although they used SDD in both test and controls, as the effect of SDD is equally distributed in both study groups [218, 221]. In these five studies there was a significant reduction of all outcome measures, apart from mortality. In studies in which the test group received vancomycin combined with antimicrobials of the SDD regimen all outcome measures were significantly reduced [210-213, 215, 217, 219]. A moderate heterogeneity was found for overall infection and mortality.

Table 6.6 Subgroup analysis of RCTs using enteral vancomycin combined with other enteral antimicrobials

Outcomes	N° RCTs	N° patients		N° events		OR (95% CI)	*P*	I^2
		Test	C	Test	C			
Vancomycin combined with other topical antimicrobials								
Overall infection	6	601	650	181	336	0.33 (0.21-0.53)	<0.001	33%
S. aureus carriage	1	87	139	0	9	0.08 (0.01-1.37)	= 0.08	-
S. aureus infection	4	513	563	28	116	0.20 (0.13-0.31)	<0.001	0%
MRSA carriage	1	375	402	14	102	0.11 (0.06-0.20)	<0.001	-
MRSA infection	1	375	402	10	76	0.15 (0.08-0.30)	<0.001	-
Mortality	7	976	1052	165	289	0.58 (0.38-0.80)	= 0.0017	38%
Vancomycin								
Overall infection	3	110	112	17	32	0.38 (0.17-0.84)	<0.016	0%
S. aureus carriage	1	20	20	2	16	0.03 (0.01-0.17)	<0.001	-
S. aureus infection	2	62	61	9	25	0.25 (0.10-0.64)	= 0.0035	0%
MRSA carriage	3	111	136	21	68	0.22 (0.11-0.48)	<0.001	0%
MRSA infection	4	369	276	47	81	0.35 (0.20-0.61)	<0.001	6%
Mortality	2	63	86	19	51	0.75 (0.37-1.52)	= 0.42	0%

C, control group; MRSA, methicillin-resistant *S. aureus;* I^2 was not considered when the outcome included only one study.

6.3.7 Publication bias

We did not inspect the funnel plot for publication bias for the primary outcomes due to the low number of studies [222].

6.4 Discussion

Four main findings emerge form this systematic review and meta-analysis of 9 randomised and 3 non-randomised trials in patients receiving enteral vancomycin:

1. Overall infections, *S. aureus* carriage and infection are reduced;
2. MRSA carriage and infection are reduced;
3. Mortality is reduced;
4. There is no emergence of VRE and VISA.

This is the first systematic review and meta-analysis evaluating the impact of enteral vancomycin on *S. aureus* and MRSA carriage and infection. Carriage or carrier state

is defined as the patient's state where *S. aureus* or MRSA are isolated from at least two consecutive surveillance samples (i.e. throat and/or rectal swabs taken on admission and two times a week, thereafter), in any concentration, over a period of at least one week. Transient presence or acquisition was defined if the microorganism is isolated only once. Overgrowth is defined as $\geq 10^5$ CFU/ml of oropharyngeal secretion or gram of faeces.

6.4.1 Overall infection

This systematic review demonstrated a significant reduction in overall infection. This result needs a careful appraisal. Although this endpoint was available from all 9 RCTs included in the systematic review, 6 of them [210-213, 215, 217] used vancomycin combined with the classical SDD regimen, including different antimicrobials such as polymyxins, aminoglycosides and polyenes. Therefore, the effect of vancomycin on overall infection could not be distinguished from that of the classical SDD protocol. However, we performed a subgroup analysis of studies in which the test group received vancomycin combined with other enteral antibiotic and of studies assessing the effect of only vancomycin. The results were not substantially different from those obtained from all pooled studies, although a moderate heterogeneity has been found in studies using vancomycin as a part of the SDD regimen (Table 6.6).

6.4.2 MRSA carriage and infection

The aim of the administration of enter vancomycin is the control of oropharyngeal and/or intestinal MRSA carriage and overgrowth. This meta-analysis demonstrated that MRSA carriage was reduced by 85% (OR 0.15, 95% CI 0.09-0.25) in patients

receiving topical vancomycin compared with those who did not. MRSA gut overgrowth is an independent risk factor for secondary endogenous MRSA infection. MRSA in overgrowth concentration in the oropharynx may migrate into the lower respiratory tract causing colonisation and subsequent infection of the lower respiratory tract (i.e. secondary endogenous infection). MRSA overgrowth is also a risk factor for transmission via hands of carers, promoting secondary endogenous carriage, colonisation and infection, and maintaining MRSA endemicity in the ICU. This meta-analysis demonstrated that vancomycin reduced MRSA infection by 76% (OR 024, 95% CI 0.12-0.50). Several studies included in this systematic review used vancomycin together with the SDD regimen. However, no changes are expected by the administration of antimicrobials directed against aerobic Gram-negative bacilli and fungi as they are not active against MRSA. The decrease in MRSA carriage and infection is only explained by the addition of vancomycin. The subgroup analysis of studies in which vancomycin was part of the SDD regimen confirmed this hypothesis (Table 6.6).

These findings are confirmed by the results of 3 non-randomised studies not included in this systematic review due to their study design, which, however, deserve some comments (Table 6.7). An Italian study [209] was undertaken in mechanically ventilated patients in an ICU where MRSA was endemic (i.e. one new case per month with a diagnostic sample positive for MRSA throughout a six-month period). The study was based on the hypothesis that the prevention of MRSA carriage using 4% vancomycin gel applied in the oropharynx was more effective in reducing carriage and lower respiratory tract infection than the treatment of established MRSA carriage. In period one, from July 2002 to December 2003, 98 patients received oropharyngeal vancomycin when they were MRSA carriers, i.e.

two consecutive surveillance samples positive for MRSA. In period two, from January 2004 to June 2005, 93 patients received vancomycin immediately on admission to the ICU. In period one there were 40 MRSA carriers while in period two there were no MRSA carriers ($p<0.001$). Patients with MRSA lower respiratory tract infection were less in period two (10 patients) compared with period one (30 patients), $p<0001$ due to the reduction of secondary endogenous MRSA infections ($p=0.01$). A total of 2321 microbiological samples were processed. Neither VRE nor VISA were isolated. MICs of vancomycin were always ≤ 1 μ/ml for MRSA and ≤ 4 μ/ml for enterococci. Viviani et al. [208] compared MRSA carriage and infections in two groups of patients receiving different concentrations of oropharyngeal vancomycin gel. One group received 4 ml of 2% gel and the other received 4 ml 4% gel divided into four doses. The vancomycin protocol was started as soon as surveillance cultures of the oropharynx were positive for MRSA. The 4% vancomycin protocol significantly reduced MRSA carriage and infection compared with the 2% vancomycin protocol. Neither VRE nor VISA were detected. A 4-year observational study [223] from the paediatric ICU of the Alder Hey Children Hospital, Liverpool, UK, assessed the effect of throat and gut surveillance combined with enteral vancomycin on gut overgrowth, transmission of MRSA, infection and mortality. Children identified as MRSA carriers received enteral vancomycin (0.5 gr of 2% oropharyngeal paste four times a day in the oropharynx, and 40 mg/kg/day oral vancomycin suspension in the gut). Enteral vancomycin reduced MRSA overgrowth, preventing secondary endogenous MRSA infections. Neither VRE nor VISA was isolated from any surveillance and diagnostic sample.

Table 6.7 Non-randomised studies of enteral vancomycin not included in the analysis due to study design

First Author	Year	Patients		Intervention		Population and setting	Design	Endpoint(s)
		Test	C	Test	C			
Silvestri [209]	2010	98	93	Only patients with a MRSA carrier state received 1 gr of 4% vancomycin gel in the oropharynx four times a day until carriage was abolished. All patients received SDD as infection control policy	All patients enrolled received 1 gr of 4% vancomycin gel in the oropharynx four times a day immediately on admission, irrespective of their MRSA carrier state. All patients received SDD as infection control policy	Patients requiring mechanical ventilation for more than 72 hours. Medical-surgical ICU	Prospective, cohort, sequential, 3-year study	To evaluate the effectiveness of two different policies of topical vancomycin on oropharyngeal carriage and lower respiratory tract infection due to MRSA
Thornburn [223]	2006	1241		Patients who were carriers, colonised or infected by MRSA received 0.5 gr of 2% vancomycin paste or gel, or 5 mg vancomycin lozenge four times a day, plus 40 mg/kg/day of oral vancomycin suspension. Patients who were carrier, colonised or infected by Gram-negative bacilli received also enteral polymyxin/tobramycin	There was no control group as the study was observational	Paediatric patients requiring mechanical ventilation for more than four days. Medical-surgical PICU	Prospective, observational, 4-year study	To assess the efficacy of enteral vancomycin on MRSA gut overgrowth, transmission, infections and mortality
Viviani [208]	2005	123	142	MRSA carriers received 1 ml of 2% vancomycin gel in the oropharynx four times a day. All patients received SDD as infection control policy	MRSA carriers received 1 ml of 4% vancomycin gel in the oropharynx four times a day. All patients received SDD as infection control policy	Patients requiring mechanical ventilation for more than 72 hours. Medical-surgical ICU	Prospective, cohort, sequential, 2-year study	To assess the efficacy of oropharyngeal decontamination with 2% vancomycin gel compared with 4% vancomycin on carriage and infection due to MRSA

MRSA, methicillin-resistant *Staphylococcus aureus*; C, control group; ICU, intensive care unit; SDD, selective decontamination of the digestive tract

6.4.3 Mortality

This systematic review showed a significant mortality reduction in 2177 patients (0.59, 95% CI 0.43-0.79). However, these results may be influenced by the concomitant use of SDD in 7 studies including 2028 patients. Selective digestive decontamination has been shown to reduce mortality by 27% (OR 0.73, 95% CI 0.64-0.84) to 29% (OR 0.71, 95% CI 0.61-0.82) [83, 85]. A subgroup analysis confirmed that in studies using vancomycin as part of the SDD protocol there was a mortality reduction (OR 0.58, 95% CI 0.38-0.80), albeit with a moderate level of heterogeneity. In contrast, in studies assessing the efficacy of only vancomycin the mortality reduction was not significant (OR 0.75, 95% CI 0.37-1.52). However, the sample size was too small (two studies, 149 patients) to detect any effect on mortality.

6.4.4 Resistance

This systematic review showed that the emergence of VISA and VRE was not a clinical problem, supporting the safety of enteral vancomycin. VRE was not isolated in 7 studies and in one study VRE was reduced in patients receiving enteral vancomycin. About twenty years ago the American recommendations on the spread of vancomycin resistance discouraged the enteral decontamination with vancomycin [224, 225]. These recommendations were based on the hypothesis that oral and intestinal administration of vancomycin may select for VISA and VRE. The updated strategies on the prevention of transmission and infection of MRSA in acute care setting do not include the use of vancomycin among decolonisation strategies [226]. However, enteral vancomycin is largely used to control *Clostridium difficile* colitis [227]; VRE has never been isolated in a large *C. difficile* outbreak despite the

administration of oral vancomycin [227], and has rarely been found in a recent trial of vancomycin versus fidaxomicin for the treatment of *C. difficile* infections [228]. On the contrary, two studies in which volunteers received low doses (i.e. 0.5 gr/day) of oral vancomycin suggested a selection of enterococci with reduced sensitivity to vancomycin [229, 230]. Another study demonstrated that the administration of parenteral vancomycin is a risk factor for the acquisition of VRE gut carriage [231]. It is important to appreciate that after 2 gr/day of parenteral vancomycin faecal vancomycin levels are between 6 and 11 μg per gram of faeces; in contrast, after 2 gr/day of oral vancomycin the intestinal concentration may vary between 3,000 and 24,000 μg per gram of faeces [98, 99]. High levels of faecal vancomycin may explain the absence of VRE when using enteral vancomycin, while non-lethal, low concentrations of vancomycin in the gut may promote the selection of VRE. This observation is in line with some studies showing that parenteral broad spectrum antibiotics that disregard the gut ecology, rather than high doses of enteral vancomycin, promote VRE [232-234].

This concept can be also applied for the selection of VISA. Repeated vancomycin exposure and suboptimal vancomycin concentrations are risk factor for VISA and heteroresistant-VISA [235]. Vancomycin creates a selection pressure that favours overgrowth of vancomycin-resistant clones leading to heteroresistant-VISA and VISA. Additionally, it should be recognized that enteral vancomycin may have an impact on the remaining gut microbiota. This issue will be considered in chapter 8.

6.4.5 Limitations

Some limitations of this systematic review and meta-analysis should be acknowledged. First, due to study design, both randomised and non-randomised

studies were included. This may reduce the robustness of findings. However, a subgroup analysis was performed on randomised and non-randomised studies. Second, although a significant statistical heterogeneity has not been demonstrated in this review, a possible clinical heterogeneity might be present due to different patients' population (e.g. paediatric, burns, medical, surgical, neurosurgical), different definitions of infection, different sites of vancomycin decontamination (oropharynx, intestine, or both), different vancomycin concentrations (2%, and 4%), and the use in both test and control groups of other antibiotics, such as SDD. Third, the test group of 9 out of 12 studies of this review received vancomycin together with polymyxins, aminoglycosides, and polyenes. Although this may have influenced the results of overall infection rate, polymyxins and aminoglycosides did not affect the results of MRSA carriage and infection, as they do not cover this microorganism. Nevertheless, a subgroup analysis on this issue was planned *a priori*. Fourth, the CLSI (Clinical and Laboratory Standards Institute) revised the criteria for susceptibility to vancomycin in 2006 [236]. The minimum inhibitory concentration (MIC) breakpoint for MRSA susceptible to vancomycin was lowered from 4 mg/L to ≤ 2 mg/L, and VISA from 8-16 mg/L to 4-8 mg/L. These values are maintained by EUCAST (European Committee on Antimicrobial Susceptibility Testing) [237]. The MIC breakpoint for enterococci sensitive to vancomycin has been held to 4 mg/L [237]. Therefore, the findings of the absence of VISA may be interpreted with caution, as all studies were carried out before the introduction of the new MIC breakpoints. Interestingly, in two Italian studies [218, 221] MICs of vancomycin were always ≤ 1 mg/L for MRSA and ≤ 4 mg/L for enterococci (personal communication). Finally, although the majority of studies included in this review are randomised controlled trials, and 6 of them are blinded, the quality

assessment revealed the presence of an unclear or high risk of bias for most outcome measures and a low quality of the non-randomised studies. Therefore, a possible bias may affect the interpretation of the results.

6.5 Conclusion

This systematic review and meta-analysis of randomised and non-randomised trials in critically ill patients showed that in studies using enteral vancomycin overall infection rates, *S. aureus* and MRSA carriage and infection, and mortality are significantly reduced. Enteral vancomycin is safe in terms of emergence of VISA and VRE. The results of this review should be interpreted with caution due to some limitations. Future large, high quality, randomised trials are warranted to confirm these findings.

7 ENTERAL VANCOMYCIN TO CONTROL MRSA INFECTION IN THE ICU.

A 16-YEAR OBSERVATIONAL STUDY IN MECHANICALLY VENTILATED PATIENTS

7.1 Introduction

During the last decades methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the most frequent pathogen causing infection and increasing morbidity, mortality and healthcare costs in the hospital setting: in the ICU the burden related to MRSA is even higher [28]. In the study of the prevalence and outcomes of infection in the ICU, MRSA was one of the most frequent microorganisms causing infection [28]. An Italian survey performed in 125 ICUs showed that MRSA was the main causative pathogen of ICU-acquired infections, including ventilator-associated pneumonia [238].

To contrast MRSA problem in the ICU, education programs for the healthcare personnel, hand hygiene, disinfection of the environment, isolation or cohorting of infected patients or carriers, use of protective equipment, have been recommended and implemented worldwide. However, these measures have often failed to control MRSA carriage, infection, and endemicity. A systematic review of the measures for the eradication of MRSA carriage, including both randomised and non-randomised studies, in non-ICU patients showed that mupirocin was an effective method for eradicating MRSA [107], although a recent meta-analysis of only randomised controlled trials in ICU patients was unable to demonstrate any significant benefit [see chapter 4].

Another approach to control MRSA carriage and infection is the administration of enteral vancomycin, both oropharyngeal and/or intestinal. The concept behind this measure is the control of oropharyngeal and intestinal overgrowth of MRSA, and, subsequently, the reduction of secondary endogenous infection due to MRSA transmission. Some studies in ICU patients demonstrated the efficacy and safety of

enteral vancomycin in controlling MRSA overgrowth, transmission, infection, and subsequent outbreak or endemicity [219-221]. Two randomised trials of enteral vancomycin showed that the administration of 4% vancomycin in the oropharynx controlled MRSA carriage and infection [216, 218].

Based on the results of these studies we undertook a retrospective analysis of prospectively collected data of the incidence of MRSA infection in a medical/surgical ICU in which oropharyngeal vancomycin was used to prevent MRSA carriage and infection.

7.2 Patients and Methods

7.2.1 Setting

The study was conducted on an eight-bed, medical-surgical ICU at the hospital in Gorizia, Italy. The ICU has an average annual admission rate of 310 patients. The ethical committee waived the need for patient's consent as the study is retrospective and observational.

7.2.2 Study design

From January 2000 to December 2015, in order to undertake the infection control program of the Friuli-Venezia-Giulia Regional Health Service, the policy of the Hospital Infection Control Committee was the acquisition of data on infection in ICU patients who required mechanical ventilation for ≥ 3 days. These data were obtained prospectively by two physicians who were responsible for the infection control project in the ICU. The observation period ended with the extubation, death or discharge of the patient. All data were captured on a record form which was sent to

the chief of the Hospital Infection Control Committee at the end of each year. All data needed for this study were extracted retrospectively from those record forms. Information used in the study existed in an electronic database in which patients' names and other details regarding the subjects' privacy were not available to the researcher.

Therefore, the design is longitudinal cohort retrospective study of prospectively collected data. Information was extracted in accordance with the retrospective chart review methodology [115, 239].

7.2.3 Patient population and data extraction

The study included data on MRSA infection from January 2000 through December 2015 amongst a cohort of ICU patients who were mechanically ventilated for ≥ 3 days.

The following data were extracted from the Hospital Infection Control Committee record forms. General characteristic of the study population, such as age, gender, simplified acute physiology score (SAPS) II, ICU mortality, days of ICU stay, number of patients with infection, number of infection episodes, and the day of infection onset, were recorded. Additionally, details on the type of infection, the classification of infection, and the microorganism causing infection were registered. Finally, patients with MRSA infection, episodes of MRSA infection, classification of MRSA infection and type of MRSA infection were retrieved.

7.2.4 Endpoints

The primary endpoint of the study was the incidence of infection due to MRSA. The secondary endpoints were the type of MRSA infection and its classification according to the carrier state method, and the emergence of VRE and VISA define.

7.2.5 Antibiotic policy

Infection prophylaxis using SDD was routinely employed in the study population as it is included in the antibiotic policy of the unit for all mechanically ventilated patients. The decontaminating protocol has been approved by the hospital committee for drug administration since 1998. The SDD protocol consisted of a combination of enteral and parenteral antimicrobials, hygiene and surveillance cultures as follows [44]:

1. a parenteral antibiotic was given for the first 4 days after admission (e.g. cefotaxime 80 mg/kg/day, or ceftazidime if the patient was a suspected pseudomonal carrier);
2. 0.5 g of a 2% gel of polymyxin E/tobramycin/amphotericin B was applied to the oropharyngeal mucosa four times a day, and 10 ml of suspension containing 100 mg of polymyxin E, 80 mg of tobramycin and 500 mg of amphotericin B (or 1.000.000 unit of nystatin) was administered in the digestive tract through the nasogastric tube four times a day;
3. surveillance samples of throat and rectum were taken on ICU admission and two times a week (e.g. Monday and Thursday) to detect the level of carriage;
4. high level of hygiene.

The main targets of the SDD protocol are aerobic Gram-negative bacilli and yeasts. By design the SDD manoeuvre is not active against MRSA. Due to the MRSA

endemicity in the ICU (i.e. at least one new case of MRSA infection per month) all ventilated patients also received oropharyngeal vancomycin as follows: from January 2002 to December 2015 all ventilated patients received 1 ml of a 4% vancomycin gel applied in the oropharynx 4 times a day. It is important to emphasize that from July 2002 to December 2003 and from January 2004 to June 2005 a prospective observational study of the efficacy of enteral vancomycin has been performed in the ICU. During the first period all patients received the enteral vancomycin protocol only when they were MRSA carriers, while in the second period all patients received the vancomycin protocol immediately on ICU admission, regardless of their carrier state. After the completion of the study, all ventilated patients continued to receive the same vancomycin protocol.

Systemic antibiotics were given in the case of infection based on clinical ground and laboratory results. In general, a five-day course of antibiotics was given, followed by clinical re-evaluation.

Surveillance samples of throat and rectum were taken on admission and twice weekly, thereafter. Diagnostic samples, i.e. tracheal aspirate, urine and blood, were obtained on clinical indication only.

Enteral feeding through a nasogastric or a naso-jejunum tube or a percutaneous gastrostomy was administered with progression to full nutritional support within 96 hours. Monitoring of gastric residual volume was performed every six hours. Stress gastric ulcer prophylaxis with proton pump inhibitors was routinely employed. Sucralfate was not used. Subglottic suctioning was not used. Ventilator circuits in the same patient were changed when necessary, heat moisture exchange filters every 1 week or when necessary. Patients requiring long-term ventilation for more than 10 days received a percutaneous or a surgical tracheostomy.

On 12 December 2008 the ICU moved to the new St. John of God Hospital in Gorizia.

7.2.6 Definitions

Lower respiratory tract infections included pneumonia and tracheobronchitis. Pneumonia was diagnosed on the presence of a new and/or progressive pulmonary infiltrate on chest radiograph persistent for > 48 h in addition to two clinical criteria and one confirmation criterion. Clinical criteria were the following: (1) core temperature ≥38.3°C or <36°C, (2) leucocytosis >12 x 10^9/L or leucopenia <4 x 10^9/L, (3) purulent tracheobronchial secretions. Confirmation criteria included (1) endotracheal aspirate with semiquantitative cultures at a concentration of ≥10^5, (2) positive blood culture in the absence of an extrapulmonary focus, (3) a response to antimicrobial therapy in the absence of alternative diagnosis. Tracheobronchitis was defined by the presence of all the above features without the radiographic findings [240]. Urinary tract infection (UTI) was defined as freshly voided catheter urine containing >10^5 CFU/ml and ≥5 leukocytes per high power light microscopic field. Bloodstream infection (BSI) was the presence of clinical signs of generalized inflammation combined with at least one positive blood culture for all potential pathogens apart from coagulase-negative staphylococci for which two consecutive blood cultures were required. Other infections were diagnosed according CDC criteria [241].

Infections were classified according to the criterion of the carrier state [44]. Primary endogenous infections are caused by both normal and abnormal PPMs carried by the patient in throat and/or gut on admission to the ICU. Secondary endogenous infections are caused by PPMs not carried in throat and/or gut at the time of

admission to the ICU, but acquired during ICU-treatment prior to the infection. Surveillance and diagnostic samples yield the same microorganism in infections of endogenous origin. Exogenous infections are caused by PPMs introduced into the patients from the environment, either animate or inanimate; bacteria are transferred directly into an internal organ, without previous carriage. In exogenous infections surveillance samples are negative for PPMs that readily appear in diagnostic samples. According to the criterion of carriage, only secondary endogenous and exogenous infections were labelled ICU-acquired infections, whilst primary endogenous infections were considered to be imported infections.

7.2.7 Sampling and microbiology

Diagnostic and surveillance samples were processed in a semiquantitative and qualitative way. Standard methods for identification, typing, and sensitivity patterns were used for all microorganisms. In particular, until 2006, to differentiate *S. aureus* from other species of staphylococci, production of deoxyribonuclease (by a DNA agar-plate method), a slide-agglutination test to detect clumping factor and protein A (Prolex™ Staph Latex Kit, Prolab Diagnostics, Neston, Wirral, UK), and a slide agglutination kit for the rapid detection of penicillin binding protein 2a for MRSA (MRSA Screen, Biogenetics, Padova, Italy) were used. If the results were inconclusive, staphylococcal species were identified by a biochemical method (ID 32 Staph, BioMerieux, Marcy l'Etoile, France). Sensitivity patterns were determined by the ATB and Vitek system (GPS-517 for staphylococci, GPS-516 for enterococci, GNS-502 for Gram-negative rods, BioMerieux, Marcy l'Etoile, France). In the following years a different method has been implemented using a Gram-positive card with the Vitek2® system to automatically identify Gram-positive microorganisms

(AST-P592). The breakpoint of vancomycin for VISA was 8 µg/mL and for VRE was 8 µg/mL until 2006. Afterwards the breakpoint of vancomycin for VISA and VRE were 2µg/mL and 4µg/mL, respectively [237, 242].

Standard methods for identification, typing and extended sensitivity patterns were used to confirm or refute the relatedness of microorganisms.

7.2.8 Statistical analysis

Data were presented as median with interquartile range, or as percentage, unless indicated otherwise. Exploratory data analyses were performed using the chi-square test with Yates correction for continuity, except when a small sample size required the Fischer's exact test. If necessary, comparisons between continuous variables amongst two groups were done with Student's t test for variables with normal distribution, and Mann-Whitney U test for non-normally distributed variables. The level of significance was set at 0.05. We decided *a priori* to compare the following sets of data: patients with MRSA infection and episodes of MRSA infection in the time periods from January 2000 - December 2001 (no oropharyngeal vancomycin) vs. January 2002 to December 2003 (use of oropharyngeal vancomycin only in MRSA carriers), and vs. January 2002 to December 2015 (use of oropharyngeal vancomycin in all ventilated patients). Odds ratio with 95% confidence interval (CI) was estimated. Computations were performed using MedCalc® Statistical software [243] and WinStat® for Excel.

7.3 Results

7.3.1 General characteristics

During the 16-year study period a total of 4,681 patients were admitted to the ICU. Among them, 1,113 patients (697 male, 62.6%) were ventilated for ≥ 72 hours, and were the basis of this retrospective cohort study. Patients had an ICU mortality of 33.4%, a median SAPS II of 43, and a median ICU stay of 15.75 days. General characteristics of the study population are reported in Table 7.1.

Table 7.1 General characteristics of the study population

Year	N° patients	Age (IQR)	Male (%)	SAPS II (IQR)	ICU stay (IQR)	Mortality (%)
2000	88	74.5 (67, 78)	56 (63)	44.5 (36, 45)	13 (9, 27)	31 (34.8)
2001	69	73 (65, 79)	43 (62.3)	44 (35, 53.7)	18 (10, 32.25)	36 (52.2)
2002	69	70 (57, 78)	48 (69.7)	43 (31.5, 59)	22 (11, 38.5)	26 (37.7)
2003	76	68.5 (61-68)	45 (59.2)	43 (33, 53.75)	14.5 (9.25, 29.5)	24 (31.6)
2004	74	74 (62, 79)	41 (55.4%)	39 (32, 47.5)	15.5 (9.75, 31,25)	26 (35)
2005	83	73 (64-79)	55 (66.5)	42 (35, 49)	15 (8, 23)	25 (30.1)
2006	38	73.5 (56.7, 78)	26 (68.4)	45 (38, 53)	14 (7-37.25)	15 (39.5)
2007	51	71 (64-79)	29 (56.9)	44 (37, 54)	20 (12, 37)	16 (35.3)
2008	74	73 (65, 78)	42 (65.6)	41 (36, 51.5)	16 (10, 25.5)	21 (32.8)
2009	62	76 (21, 81)	39 (66.1)	44 (34, 53.5)	17 (7.25, 34.5)	16 (25.8)
2010	74	76.5 (66.7, 82)	40 (54)	46 (39, 59)	11 (7, 23.5)	30 (41)
2011	74	74 (63, 79.7)	50 (67.5)	48.5 (40, 54.7)	18 (10, 25)	22 (29.7)
2012	72	74.5 /64, 81)	51 (70.8)	42.5 (37, 50)	14.5 (9, 25)	24 (33.3)
2013	68	75 (68, 80)	39 (57)	41 (32, 52.2)	16.5 (7.7, 25)	21 (31)
2014	68	76 (69, 81)	49 (72)	37.5 (33, 49)	15 (9, 24.75)	22 (32.3)
2015	83	75 (65.5, 81)	44 (53)	39 (34, 48)	17 (10, 31,5)	17 (20.5)
Total	**1113**	**73.75 (71.5, 75)**	**697 (62.6)**	**43 (41, 44.37)**	**15.75 (14.5, 17.75)**	**372 (33.4)**

SAPS, Simplified Acute Physiology Score; ICU, intensive care unit; IQR, interquartile range.

7.3.2 Overall infection and microorganisms

Table 7.2 describes the distribution of the number of patients with infection, the infection episodes and the day of infection onset. There were 701 patients with infection (63%) suffering from 983 infection episodes. In the majority of years, the median day of infection onset was the first day of ICU stay.

Table 7.2 Patients with infection and infection episodes over the study period

Year	Patients	Infection episodes	TB	PN	UTI	BSI	Other	PE	SE	Exo
2000	51	68	12	37	7	7	5	44	14	10
2001	49	68	11	40	2	3	12	55	10	3
2002	46	63	4	40	10	6	3	44	8	11
2003	41	56	1	30	9	6	10	42	0	14
2004	41	46	3	31	3	1	9	38	5	3
2005	52	62	6	39	4	4	9	55	3	4
2006	16	23	1	14	2	3	3	21	2	0
2007	41	54	15	21	3	2	13	48	1	5
2008	40	54	9	24	4	7	10	48	5	1
2009	42	59	8	26	7	12	6	47	8	4
2010	44	55	9	23	8	2	13	49	3	3
2011	42	59	8	18	5	13	15	47	7	5
2012	43	60	12	25	5	5	13	55	4	1
2013	49	78	14	34	9	12	9	68	6	4
2014	44	68	7	31	4	12	13	61	2	4
2015	60	110	16	38	14	24	18	90	10	10
Total	**701**	**983**	**136**	**471**	**96**	**119**	**161**	**812**	**88**	**83**

TB, tracheobronchitis; PN, pneumonia; UTI, urinary tract infection; BSI, bloodstream infection; PE, primary endogenous infection; SE, secondary endogenous infection; Exo, exogenous infection.

There were 136 episodes of tracheobronchitis, 471 pneumonias, 96 UTIs, 119 BSIs, and 161 episodes of other infections, such as peritonitis, meningitis or wound infection.

Microorganisms causing infection are reported in Table 7.3. In 389 episodes (39.6%) the causative microorganism was not isolated. Gram-positive microorganisms accounted for 251 infection episodes (25.5%), Gram-negative microorganisms caused 303 infection episodes (38.8%), yeasts were involved in 32 episodes (3.3%), and the remaining 8 episodes were divided among viruses, *Rickettsiae*, and *Chlamydiae*. *Pseudomonas aeruginosa* was the most frequently encountered microorganism (12.1%), followed by MRSA (10%) and *Escherichia coli* (5%).

Table 7.3 Microorganisms causing 983 episodes of infection

Microorganisms	Episodes	%
NA	389	39.6
Gram positive microorganisms	**251**	**25.5**
MRSA	98	10
Enterococcus spp	48	4.9
MSSA	46	4.7
CNS	27	2.7
Streptococcus pneumoniae	19	1.9
Streptococcus pyogenes	3	0.3
Clostridium spp	3	0.3
Listeria monocytogenes	2	0.2
Bacillus spp	2	0.2
Propionibacterium spp	1	0.1
Actinomyces spp	1	0.1
Corynebacterium spp	1	0.1
Gram-negative microorganisms	**303**	**30.8**
Pseudomonas aeruginosa	119	12.1
Escherichia coli	49	5
Klebsiella spp	22	2.2
Serratia spp.	19	1.9
Proteus spp	18	1.8
Enterobacter spp	17	1.7
Stenotrophomonas maltophilia	14	1.4
Haemophilus influenzae	10	1.0
Citrobacter spp	7	0.7
Acinetobacter spp	6	0.6
Bacteroides spp	6	0.6
Morganella spp	4	0.4
Burkolderia cepacia	2	0.2
Providencia spp	2	0.2
Salmonella spp	2	0.2
Legionella pneumoniae	2	0.2
Moraxella catarrhalis	2	0.2
Bordetella spp	1	0.1
Prevotella spp	1	0.1
Fungi	**32**	**3.3**
Candida spp	30	3.1
Aspergillus spp	2	0.2
Other	**8**	**0.8**
H_1N_1	3	0.3
Cytomegalovirus	2	0.2
Chlamydia pneumoniae	2	0.2
Coxiella burnetii	1	0.1

NA, not available; MRSA, methicillin-resistant *Staphylococcus aureus*; spp, species; MSSA, methicillin-sensitive *Staphylococcus aureus*; CNS, coagulase-negative staphylococci

7.3.3 MRSA infection

During the study period 145 (14%) infection episodes were due to *S. aureus*. MRSA was responsible for 98 episodes of infection (68.3% of *S. aureus* infections, 10% of all episodes) in 91 patients. Table 7.4 shows the infection episodes due to MRSA and the classification of infections. There were 27 (27.6%) episodes of tracheobronchitis,

44 (44.9%) pneumonias, 10 (10.2%) UTIs, 13 (13.3%) BSIs, and 4 (4%) episodes of other infections. Forty-eight (49%) episodes were primary endogenous, 29 (29.6%) were secondary endogenous, and 21 (21.4%) were exogenous.

Table 7.4 Patients with MRSA infection, infection episodes and classification of infections over the study period

Year	Patients	Infection episodes	TB	PN	UTI	BSI	Other	PE	SE	Exo
2000	27	29	8	14	1	4	2	13	13	3
2001	22	24	8	10	0	3	2	14	9	1
2002	9	9	3	4	1	1	0	4	2	3
2003	7	8	0	4	1	3	0	1	0	7
2004	4	4	0	3	1	0	0	3	1	0
2005	4	4	0	0	4	0	0	3	1	0
2006	0	0	0	0	0	0	0	0	0	0
2007	0	0	0	0	0	0	0	0	0	0
2008	3	3	2	1	0	0	0	3	0	0
2009	4	4	2	1	1	1	0	0	1	3
2010	1	1	0	1	0	0	0	1	0	0
2011	1	1	0	1	0	0	0	0	1	0
2012	1	1	1	0	0	0	0	1	0	0
2013	3	4	2	2	0	0	0	3	1	1
2014	2	2	0	1	1	0	0	0	0	2
2015	3	4	1	2	0	1	0	3	0	1
Total	**91**	**98**	**27**	**44**	**10**	**13**	**4**	**48**	**29**	**21**

TB, tracheobronchitis; PN, pneumonia; UTI, urinary tract infection; BSI, bloodstream infection; PE, primary endogenous infection; SE, secondary endogenous infection; Exo, exogenous infection.

MRSA infections were in decline over the study period (Table 7.4, Figure 7.1). In particular, there was a reduction of MRSA secondary endogenous infection during the use of oropharyngeal vancomycin from 2002 until 2015. Table 7.5 shows the comparison between the period in which oropharyngeal vancomycin was not used (2000-2001) and periods in which oropharyngeal vancomycin was included. A significant reduction in MRSA infected patients and secondary endogenous MRSA infection was found in periods using vancomycin. Exogenous MRSA infections increased in patients treated with vancomycin, and primary endogenous infections were reduced but not significantly.

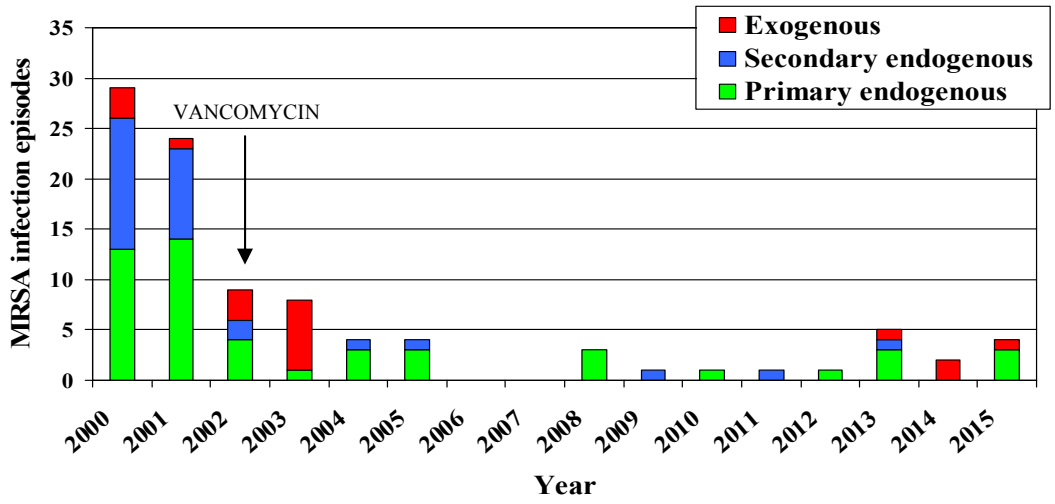

Figure 7.1 Incidence of MRSA endogenous and exogenous infections during the study period

Table 7.5 Comparison of data sets in patients treated with and without oropharyngeal vancomycin

	No enteral vancomycin (2000-2001) N (%)	Enteral vancomycin 2002-2003*			Enteral vancomycin 2002-2015*		
		N (%)	OR (95% CI)	*p*	N (%)	OR (95% CI)	*p*
Infected patients	100	87			601		
Patients with MRSA infection	49 (49)	16 (18.4)	0.23 (0.12-0.46)	< 0.001	42 (7%)	0.08 (0.05-0.13)	<0.001
MRSA infection episodes	53	17			45		
PE	27 (51)	5 (29.4)	0.4 (0.12-1.3)	= 0.127	21 (46.7%)	0.84 (0.38-1.87)	= 0.67
SE	22 (41.5)	2 (11.8)	0.19 (0.04-0.91)	= 0.037	7 (15.5%)	0.26 (0.1-0.69)	= 0.007
Exo	4 (7.5)	10 (58.8)	17.5 (4.3-71.26)	< 0.001	17 (37.8%)	7.44 (2.28-24.3)	< 0.001

Each group was compared with the group without enteral vancomycin; N, number; OR, odds ratio; CI, confidence interval; MRSA, methicillin-resistant *Staphylococcus aureus*; PE, primary endogenous infection; SE, secondary endogenous infection; Exo, exogenous infection.

No VISA was isolated during the study period. MRSA MICs for vancomycin were always <1 mg/L. Four infection episodes of urinary tract infection were due VRE; all four episodes were primary endogenous (i.e. imported in the ICU).

7.4 Discussion

Four important issues emerged from this long-term, observational, retrospective study in 1,113 patients requiring mechanical ventilation for ≥72 hours:

1. MRSA accounted for 98 (68.3%) of 145 *S. aureus* infectious episodes and most MRSA infections were lower respiratory tract infections;
2. the majority of MRSA infections were primary endogenous, although an exogenous infectious problem was found in the ICU;
3. there was a reduction of MRSA secondary endogenous infection after the implementation of oropharyngeal vancomycin;
4. The manoeuvre was safe in terms of emergence of VISA and VRE.

This study showed the emblematic picture of MRSA epidemiology in an Italian ICU. MRSA infection accounted for 10% of all infectious episodes in ICU, and for 68% of *S. aureus* infection. MRSA was the second most common microorganism causing infection after *Pseudomonas aeruginosa*, and was mainly isolated from the lower respiratory tract (72.5%), including 27 episodes of tracheobronchitis (27.6%) and 44 episodes of pneumonia (44.9%).

A survey in Italian ICUs showed that 22.7% of infectious episodes were due to *S. aureus*, and MRSA caused 53% of them [238]. Our findings are also in line with those of the Extended Prevalence of Infection in Intensive Care (EPIC) II study conducted in 75 countries in which MRSA was isolated in 8.7% of ICU infections in

Western Europe and 10.4% of ICU infections in Eastern Europe [28] Overall, 63.5% of ICU infections were located in the respiratory tract, with a significantly higher proportion of respiratory infections observed in Eastern Europe than Western Europe (71.6% versus 63.3%, *p* 0.05). Koulenti et al. [244] reported that MRSA was isolated in 16% of patients with nosocomial pneumonia. The majority of infections of the lower respiratory tract were usually due to MRSA, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter* species [245]. In the survey of European ICUs, the most common causes of ventilator-associated pneumonia were Enterobacteriaceae (43.0%), followed by *S. aureus* (32.6%), of which 18% of episodes were due to MSSA and 14.6% to MRSA [244]. Approximately 20% of VAP episodes were due to *P. aeruginosa* [244, 246] However, significant variability has been shown in the distribution of pathogens causing VAP across Europe. In a multicenter study of VAP, the prevalence of MRSA was shown to be significantly lower in patients from a Spanish hospital than from a French hospital [247].

In this study, about half of MRSA infections were primary endogenous, i.e. they were caused by MRSA imported in the ICU by the patients' own flora or the patient was admitted to the ICU with an MRSA infection. This is in line with previous studies showing that the majority of ICU infections are not due to bacteria acquired in the ICU, but to microorganisms imported in the ICU by the patient's admission flora [41, 42]. These infections can not be classified as ICU-acquired. Only secondary endogenous and exogenous MRSA infections can be considered infections acquired during the treatment in the ICU. An important finding of this study is that 21 MRSA infection episodes (21.4%) were exogenous. Interestingly, the majority of them (17 of 21 episodes) developed after the implementation of vancomycin. In

particular, MRSA exogenous infections were more common in 2002 (3 episodes), 2003 (7 episodes), 2009 (3 episodes), and 2014 (2 episodes). These results indicate a serious breach of hygiene measures, and are not related to the vancomycin use. Exogenous infections are caused by PPMs introduced into the patient's internal organs, such as the lower airways or the urinary tract, from the environment, either animate or inanimate, bypassing the carrier state.

This study showed that, after the introduction of topical oropharyngeal vancomycin, MRSA secondary endogenous infections were significantly reduced, and outbreak and endemicity of MRSA, typically present in 2000 and 2001, were under control. The administration of oropharyngeal topical vancomycin gel is expected to reduce MRSA oropharyngeal carriage in overgrowth concentrations, and, subsequently, to control secondary endogenous MRSA infection of the lower respiratory tract. From an epidemiological point of view the import of MRSA into the unit is an important factor contributing to its endemicity. However, a second important factor, maintaining the endemicity, is based on the prevention and control of MRSA acquisition due to transmission from patients with oropharyngeal and gut overgrowth of MRSA. The concept that overgrowth may promote the spread of MRSA via hands of carers, and may be an independent risk factor for secondary endogenous colonisation and infection is in line with recent observations showing that the implementation of enteral antimicrobials controls outbreaks and endemicity of MRSA following eradication and reduction of overgrowth [103, 208, 209, 216, 218-221, 223]. Two prospective randomised studies of enteral vancomycin have been published. An Italian study in mechanically ventilated patients showed that the administration of a 4% vancomycin gel in the oropharynx reduced lower respiratory tract infections due to MRSA acquired on the ICU and MRSA carriage (OR 0.26, CI

0.08-0.88, $p<0.001$; OR 0.25, CI 0.09-0.69, $p<0.01$, respectively) [218] A double-blind randomised placebo controlled Spanish study using enteral vancomycin applied in oropharynx and gut in mechanically ventilated patients prevented acquisition, carriage and infection due to MRSA [216]. Three prospective, before-after, studies were undertaken to evaluate the efficacy of enteral vancomycin for the prevention of MRSA infections. An Italian research team demonstrated that the administration of only intestinal vancomycin eradicated MRSA gut carriage and was effective in the control of an MRSA outbreak (OR 0.37, 95% CI 0.24-0.58) [221]. In a 9-year prospective study on a burn ICU in Getafe, Spain, oropharyngeal and intestinal vancomycin was added to the classical SDD protocol to control MRSA transmission [219]. There was a significant reduction of MRSA acquisition and infection in patients receiving vancomycin. Similarly, the same Spanish group in a prospective 49-month study found the same results in an ICU population of medical/surgical patients [220]. Finally, three observational studies were performed in ICU patients treated with enteral vancomycin. An Italian study [209] was based on the hypothesis that the prevention of MRSA carriage using 4% vancomycin gel applied in the oropharynx was more effective in reducing carriage and lower respiratory tract infection than the treatment of established MRSA carriage. In period one 98 patients received oropharyngeal vancomycin when they were MRSA carriers, i.e. two consecutive surveillance samples positive for MRSA. In period two 93 patients received vancomycin immediately on admission to the ICU. Patients with MRSA carriage were less in period two ($p<0.001$), as well as patients with MRSA lower respiratory tract infection, due to the reduction of secondary endogenous MRSA infections ($p = 0.01$). Another Italian research team [208] compared MRSA carriage and infection in two groups of mechanically ventilated patients receiving different

concentrations of oropharyngeal vancomycin gel. One group received a 2% gel and the other received a 4% gel. The 4% vancomycin protocol significantly reduced MRSA carriage, compared to the 2% vancomycin protocol. MRSA secondary endogenous infections were reduced by 4% vancomycin compared to 2% vancomycin. A 4-year observational study [223] from the paediatric ICU of the Alder Hey Children Hospital, Liverpool, UK, assessed the effect of throat and gut surveillance combined with enteral vancomycin on gut overgrowth, transmission of MRSA, infection and mortality. Children identified as MRSA carriers received enteral vancomycin (0.5 gr of 2% oropharyngeal paste four times a day in the oropharynx, and 40 mg/Kg/day oral vancomycin suspension in the gut). Enteral vancomycin reduced MRSA overgrowth, preventing secondary endogenous MRSA infections.

This study demonstrates that the use of vancomycin is safe in terms of emergence of VISA and VRE. These results are in line with previous findings showing no emergence of VISA and VRE during the use of enteral vancomycin [208, 209, 214, 219, 221, 223]. Other European studies, in which enteral vancomycin was combined with SDD, did not report any increased infection rate with VISA or VRE [210, 212, 218]. All these studies were conducted in ICUs without a history of VISA or VRE. A 5-year prospective study conducted in a Spanish ICU, in which enteral vancomycin was used, did not show any increase in the incidence of resistant microorganisms including MRSA [248]. The reason why enteral vancomycin does not select for VISA or VRE may be related to the high intestinal vancomycin levels between 3,000 and 24,000 μg/gram of faeces obtainable after 2 grams/day of enteral vancomycin. In contrast, after 2 grams/day of parenteral vancomycin faecal vancomycin levels are

between 6 and 11 μg/gram of faeces [98]. These non-lethal, low concentrations of vancomycin in the gut may promote the selection of VRE and VISA.

Although the strength of the study is the collection of data from a large number of patients during a 16-year period, this study has several limitations. Firstly, the design of the study is retrospective and most sources of error due to confounding factors and biases are common in retrospective studies. A weakness of retrospective studies is that they generate a great deal of missed data. A better design would be a cluster-randomised trial or a stepped-wedge trial. However, although this study is retrospective, it includes prospectively collected data, and, in designing the study, we followed the recommendations for chart review methodology, such as the *a priori* description of clear and well defined research outcomes, the development of a standardized data abstraction forms, the description of explicit inclusion and exclusion criteria. Secondly, the retrospective comparison of two consecutive periods may provide a low level of evidence in order to establish a causal relationship between the outcome and the intervention assessed. Thirdly, changes in patient population and patients' demographics, healthcare procedures, compliance with barrier precautions, different dynamics of endemic/epidemic strain transmission, and epidemiological changes in frequencies of MRSA may occur in a 16-year period. However, this study showed a small and not significant reduction in primary endogenous MRSA infections. Although data about MRSA carriage on admission and during ICU treatment were not available due to the study design, we could infer that the immediate use of oropharyngeal vancomycin may have brought under control MRSA carriage on admission, and, thus may have reduced primary endogenous MRSA infection. Fourthly, after 2006 the MIC breakpoint for MRSA susceptible to vancomycin was lowered from 4 mg/L to ≤ 2 mg/L, and VISA from 8-

16 mg/L to 4-8 mg/L [236]. Therefore, the findings of the absence of VISA should be interpreted with caution. However, MRSA MICs for vancomycin were always <1 mg/L.

7.5 Conclusion

This observational, retrospective study showed that MRSA infections were 10% of all ICU infections and that half of MRSA infections were primary endogenous due to MRSA present in the oropharyngeal and gut flora of the patients on ICU admission. The use of vancomycin significantly reduced MRSA secondary endogenous infections, avoiding MRSA endemicity. No emergence of VISA or VRE was demonstrated.

8 CONCLUDING REMARKS AND IMPLICATIONS FOR CLINICAL PRACTICE AND RESEARCH

8.1 Mupirocin and chlorhexidine failed to control MRSA infection and to reduce mortality

8.1.1 Mupirocin

The efficacy of oropharyngeal/nasal mupirocin on ICU overall infection and infection due to MRSA has been evaluated in a systematic review and meta-analysis of only randomised trials in critically ill patients (chapter 4). Although the analysis demonstrated a reduction of lower respiratory tract infection in patients receiving mupirocin, there was no effect of mupirocin on MRSA lower respiratory tract infection (OR 0.28, 95% CI 0.03-2.16, p=0.22). Mortality was not impacted.

8.1.2 Chlorhexidine

The efficacy of oropharyngeal chlorhexidine to control ICU infection, MRSA infection, and mortality, has been assessed in a systematic review of randomised controlled trials (chapter 5). Although the systematic review and meta-analysis demonstrated that oral care with chlorhexidine reduced overall lower respiratory tract infection, and lower respiratory tract infection due to Gram-positive and Gram-negative "normal" flora, such as methicillin-sensitive *S. aureus, S. pneumoniae, H. influenzae, and E. coli*, there were insufficient data to assess the efficacy of oral chlorhexidine on lower respiratory tract infection due to MRSA. Bloodstream infections were not reduced by oral chlorhexidine, and the reduction in BSI due to Gram-positive and Gram-negative microorganisms, and "normal" flora was not significant, as was the increase in bloodstream infection due to "abnormal flora". Bloodstream infections due to MRSA were not found in this review. Only in the subset of surgical, mainly cardiac, patients, chlorhexidine was effective in reducing

bloodstream infection. Mortality was not affected. Additionally, half the population of this review was surgical, and, therefore, the results can not be applied to the entire ICU population. This should warrant further large RCTs to test the effectiveness of oral chlorhexidine in preventing lower airway infection, in reducing mortality in medical and mixed critically ill patient, and in controlling MRSA infection.

8.1.3 Why did they fail?

8.1.3.1 Chlorhexidine concentration

Chlorhexidine is an antiseptic effective against Gram-positive bacteria, Gram-negative bacteria and fungi [147]. It has both bacteriostatic and bactericidal mechanisms of action, depending on its concentration. A recent systematic review assessed the efficacy of different concentrations of chlorhexidine mouthwash (i.e. 0.12%, 0.2% and 0.3%) on the microbial load and the effect on dental plaque [249]. The authors concluded that the effectiveness of this agent is dose dependent. The meta-analysis of chapter 5 explored this issue, and did not find any difference between 0.12%-0.2% and 1%-2% chlorhexidine concentrations (OR 0.70, 95% CI 0.52-0.94, and OR 0.59, 95% CI 0.35-0.97, respectively). However, these results should be taken with caution as clinical heterogeneity was present (e.g. most studies were surgical with a low period of ventilation, the number of daily applications varied from one to four, and the duration of treatment was different).

8.1.3.2 RCTs are underpowered

Price et al. [83], in a meta-analysis of the effect of chlorhexidine on mortality, found that it increased mortality (OR 1.25, 95% CI 1.05-1.50). Although the authors

acknowledged this issue, they were unable to explain this discrepancy of reduced pneumonia and higher mortality in patients treated with chlorhexidine. Similarly, the meta-analysis of chapter 5 showed a non significant increased mortality (OR 1.11; 95% CI 0.92-1.33). RCTs included in the systematic review were not powered to detect mortality differences, and, despite combining the RCTs, the final sample size with a baseline mortality rate of 16% could be not enough to detect a survival benefit. This hypothesis is consistent with the very low baseline 2.6% mortality rate in surgical patients which are 52% of subjects included in the mortality analysis. In the remaining sample sizes of 678 medical and 1260 medical/surgical/trauma patients with a mortality rate of 33% and 29%, respectively, may be too small to detect a survival benefit.

Similarly, the lack of any effect of mupirocin on mortality is due to the small sample size of the systematic review.

8.1.3.3 Chlorhexidine may reduce immunity

Several in-vitro studies have shown that chlorhexidine affects leucocytes and macrophages, and, thus, reduces immunity [250-254]. Although it is yet unclear how the reduced immunity may result in increased mortality in the critically ill, this hypothesis should be taken into account.

8.1.3.4 Biofilm-producing MRSA

A biofilm is a strong and complex microbiological structure with different distribution of cells, cell aggregates, bacterial toxins, polysaccharides and water. This structure protects microorganisms against host defence mechanisms and entry of antimicrobial agents and can be present on surfaces. Mupirocin, at standard

concentration, does not show sufficient bactericidal effect on MRSA in biofilm [255]. Similarly, chlorhexidine is less effective against biofilms of *A. baumannii*, *E. coli*, *P. aeruginosa*, and MRSA [256].

8.1.3.5 Mupirocin and chlorhexidine do not impact gut carriage

An important issue of the effectiveness of mupirocin and chlorhexidine is that they only impact lower respiratory tract infections following control of throat overgrowth of PPMs. Additionally, chlorhexidine mainly controls lower respiratory tract infection due to oropharyngeal "normal" flora, not MRSA; bloodstream infection was not affected. Similarly, the effect of mupirocin on MRSA infection was not demonstrated. It is highly unlikely that oropharyngeal chlorhexidine or mupirocin may control gut overgrowth of abnormal flora, and the subsequent bloodstream infection following bacterial translocation.

8.1.3.6 Damage of the lung and allergic reactions

Chlorhexidine can be aspirated into the lower airways developing an acute respiratory distress syndrome [257]. In addition, some patients may suffer from allergic reactions, including anaphylaxis [258].

8.2 Enteral vancomycin: a possible approach to control MRSA

8.2.1 Enteral vancomycin controls MRSA carriage and infection.

Control of MRSA infection usually relies on five pillars in order to reduce transmission and subsequent infection:

1. hand hygiene;

2. use of gloves, gowns and aprons (personnel protective equipment);
3. isolation;
4. care of patient equipment;
5. care of the environment

However, RCTs on gloves and gowns indicated that personal protective equipment had no effect on ICU infection and mortality [259, 260]. Handwashing alone can not control primary endogenous infections which are caused by MRSA already present in the patient's oropharynx and gut on admission to the ICU [70].

Therefore, the new approach of enteral vancomycin in ICU patients challenges the traditional recommendations, including isolation, barrier precautions, and the use of chlorhexidine and mupirocin [103].

The prophylactic use of antimicrobials to control oropharyngeal and/or digestive tract carriage of PPMs in ICU patients, i.e. SDD, has been introduced four decades ago, and has been a subject of debate. Advocates of the use of SDD have shown consistent benefits [44, 84, 186], but they have frequently encountered a strong opposition from detractors who initially refused the clear effect of SDD on mortality reduction, and, afterwards, emphasised the overwhelming risk of antimicrobial resistance [261, 262]. The addition of vancomycin to the SDD antimicrobials might raise even more concerns as vancomycin is a first line parenteral agent against MRSA infection.

Several studies have evaluated the efficacy of enteral vancomycin in decreasing MRSA carriage and infection rates. The core of this thesis is the systematic review and meta-analysis of randomised and non-randomised studies in ICU patients receiving enteral vancomycin (chapter 6). The endpoint was to assess the efficacy of enteral vancomycin to control MRSA carriage and infection in intensive care unit

patients. The review showed that in patients treated with enteral vancomycin overall infection rates, *S. aureus* carriage and infection, and MRSA carriage and infection were significantly reduced.

Additionally, another central section of this thesis is the retrospective study on the incidence of MRSA infection in a medical/surgical ICU using oropharyngeal vancomycin to prevent MRSA carriage and infection (chapter 7). The study is unique in its design as it embraces a period of 16 years, from 2000 to 2015. The study showed that, in patients receiving enteral vancomycin, MRSA secondary endogenous infections were significantly reduced compared with patients who did not receive vancomycin.

Enteral vancomycin aims to eradicate or reduce oropharyngeal and/or intestinal carriage of MRSA with two main endpoints: 1) a clinical endpoint of the control of endogenous MRSA infection in the individual patient; 2) an epidemiological endpoint of the control of MRSA dissemination to protect other patients in the ICU from acquiring MRSA following transmission via hands of carers. MRSA overgrowth is a risk factor for transmission via hands of carers, promoting secondary endogenous carriage, colonisation and infection, and maintaining MRSA endemicity in the ICU. Overgrowth of MRSA in the oropharynx may cause migration of the same microorganism into the lower respiratory tract causing colonisation and subsequent infection of the lower respiratory tract (i.e. secondary endogenous infection). Similarly, MRSA overgrowth in the gut may cause translocation and subsequent invasion of the bloodstream causing bloodstream infection.

8.2.2 Does enteral vancomycin impact mortality?

The systematic review (chapter 6) demonstrated a significant 41% mortality reduction in 2,177 patients. However, in the majority of studies (i.e. 7 out of 9 studies) enteral vancomycin was part of the SDD regimen, covering also AGNB and yeasts, and the reduction in mortality was still significant. In studies assessing the effect of only vancomycin the mortality reduction was not significant. Data on mortality are difficult to interpret due to the small sample size and the presence of confounding factors such as the concomitant use of SDD antimicrobials.

8.2.3 Safety of enteral vancomycin

8.2.3.1 Emergence of VRE and VISA

The secondary endpoint of the systematic review (chapter 6) was to evaluate the safety of the manoeuvre, i.e. the emergence of resistance to vancomycin, in particular the emergence of VRE and VISA. The systematic review showed that VRE and VISA were not a clinical problem. Additionally, the observational study (chapter 7) supports the safety of enteral vancomycin as the emergence of VISA and VRE was not demonstrated.

Enteral vancomycin has been successfully used in the treatment of MRSA post-operative enteritis [263], and for treating *Clostridium difficile*-associated infection [264]. In this context it has been reported that oral vancomycin was safe and well-tolerated.

Antimicrobial resistance often develops when antibiotics do not exert a full bactericidal activity, due to low concentrations. While non-lethal, low concentrations of vancomycin in the gut may promote the selection of resistant

clones, high levels of faecal vancomycin concentrations during enteral administration may explain the absence of VRE and VISA. However, early stages of vancomycin resistance are often not documented with the standard identification methods which do not identify h-VISA. Tests to identify h-VISA were not performed in the retrospective study of chapter 7. Similarly, studies included in the systematic review of chapter 6 did not mention the use of specific identification of heteroresistance. However, the retrospective study observed that vancomycin MIC for MRSA was always lower than 1 mg/L. Likewise, several studies included in the systematic review clearly reported that MIC for vancomycin was always lower than 1 mg/L. Owing to these finding it is unlikely that heteroresistant clones would have been identified if more sensitive test had been performed.

8.2.3.2 Impact on gut microbiota

The majority of antimicrobials may alter the microbiota composition. Data from critically ill patients receiving enteral vancomycin are limited to VRE and VISA. In the last years, research has focused on the effect of SDD on microbiota. Few studies have shown that SDD may affect the microbiota of the critically ill patient. However, the clinical impact has not yet been clarified.

Benus et al. [91] showed that in faecal samples of patients enrolled in a multicentre trial, AGNB were significantly reduced by SDD compared to SOD and standard care. *F. prausnitzii* was reduced by SDD regimen compared to SOD and standard care. Enterococci increased in SDD group compared to SOD and standard care. The decrease of butyrate production due to the decrease of *F. prausnitzii* group of bacteria may have some consequences. Butyrate is a primary source of energy for colonocytes and promotes the growth of colonocytes preventing mucosal atrophy

[265]. However, also enteral feeding, a common manoeuvre in critically ill patients, has been reported to reduce faecal butyrate concentrations in healthy volunteers [266]. Buelow et al. [92] evaluated the impact of SDD on the gut microbiota in one ICU patient during and after ICU stay, and searched for two common aminoglycoside resistance genes in twelve ICU patients who received SDD. Hospitalization and administration of SDD has large, but highly individualized, effects on the gut resistome. Selection for transferable antibiotic resistance genes in anaerobic commensal bacteria could impact the risk of transfer of antibiotic resistance genes to opportunistic pathogens. The same Dutch researchers [93] compared the gut microbiota of 10 ICU patients receiving SDD and 10 healthy subjects. The microbiota of SDD patients was characterized by lower microbial diversity, lower levels of *E. coli* and anaerobic Gram-positive butyrate-producing bacteria, and increased abundance of *Bacteroidetes* and enterococci. Four genes providing resistance to aminoglycosides, macrolides, disinfectants and tetracyclines were more present in ICU patients than in healthy subjects, but genes providing resistance to chloramphenicol and tetracycline were more present in healthy subjects. The risk associated with SDD on selection of antibiotic resistance was very limited. The limitation of this study is that the microbiota and microbiome of SDD ICU patients was compared with the microbiota and microbiome of healthy subjects. Other factors, including underlying critical illness, parenteral or enteral feeding and antibiotic therapy may affect the composition of the microbiota during ICU stay.

These studies have been undertaken in settings with low level of antibiotic resistance and can not be generalizable to other countries or hospitals where resistant bacteria are more prevalent. However, a recent, 4-year, cohort study in an ICU setting with high level of resistance found that SDD was effective in reducing infections due to

multi-drug resistant bacteria [267]. Colistin and tobramycin-resistant colonisation was not significantly increased.

An important consideration with the use of both SDD and SOD is the increase of enterococci in the gut in overgrowth concentrations, as they are intrinsically resistant to the SDD antimicrobials, and the reduction of Gram-negative bacteria. Antibiotic-resistant gene transfer has been shown in vivo between enterococci and other bacterial species [268]. In a study of 9 patients receiving SDD, although vancomycin resistance was not detected among *E. faecalis* and *E. faecium* isolates, multiple *Enterococcus* species carrying some antibiotic resistance and virulence genes were demonstrated [269]. However, it is unclear whether the clonal replacement observed in enterococci was due to nosocomial strains or populations that were previously present in lower abundances. Future studies are needed to test this hypothesis.

Knowledge of the impact of enteral vancomycin on the intestinal microbiota is scarce and is mainly associated with its use in *C. difficile* infection. Importantly, the majority of patients with *C. difficile* infection received antibiotics before vancomycin administration, limiting a true understanding of the vancomycin effects on intestinal microbiota. In a study by Isaac et al [100], nine patients, who were antibiotic free in the previous three months, received a two-week course of oral vancomycin. The control group did not receive the drug. Vancomycin treatment diminished the richness and diversity of human microbiota, reduced the level of *Bacteroidetes* phylum, whilst *Proteobacteria* and *Fusobacteria* phyla increased. Additionally, among *Proteobacteria*, abnormal AGNB increased. However, patients included in this study were not critically ill patients, and, therefore, these results can not be generalizable to the ICU population. In a recent report [270] the use of enteral vancomycin combined with SDD antimicrobials was associated with two cases of

vancomycin-dependent enterococci (VDE) carriage. VDE are enterococci which demonstrate specific requirements for glycopeptides antimicrobial agents. Although these microorganisms can cause nosocomial infection, the clinical significance of these isolates is not entirely clear.

8.3 Implications for clinical practice

Carriage and overgrowth of PPMs, including MRSA, in the oropharynx and gut are risk factors for transmission via hands of carers, promoting secondary endogenous MRSA carriage, colonisation of sterile organs and infection, and maintaining MRSA endemicity in the ICU [103].

The use of oral care with mupirocin or chlorhexidine to control lower respiratory tract infection and MRSA infection in ICU patients is not supported by the results of this thesis. Two systematic reviews (chapters 4 and 5) did not demonstrate any effect on MRSA infection, while the effect on overall lower respiratory tract infection was not robust.

The new concept of enteral vancomycin and surveillance cultures of throat and rectum in critically ill patients has been applied to only MRSA carriers by Spanish [220], Italian [221] and English [223] researchers. MRSA ICU endemicity has been limited using oropharyngeal and intestinal vancomycin both in adults [220] and in children [223], and MRSA outbreak has been controlled using intestinal vancomycin [221].

Subsequently, due to MRSA endemicity in their ICUs, the Spanish and Italian research groups employed this manoeuvre prophylactically in all ICU patients to prevent acquisition and carriage of MRSA [208, 209, 219]. These experiences were based on the hypothesis that the prevention of MRSA carriage using enteral

vancomycin was more effective in reducing MRSA carriage and lower respiratory tract infection than the treatment of established MRSA carriage. They rendered the unit virtually free of MRSA.

Interestingly, the study of chapter 7 confirmed these results and showed that in an ICU with MRSA endemicity the use of oropharyngeal vancomycin prophylactically in all ventilated patients was able to reduce overall MRSA infection, and MRSA secondary endogenous infection.

Additionally, patients with severe underlying diseases may be MRSA carriers prior to ICU admission [271], and contribute to maintain the MRSA endemicity in the ICU (i.e. primary endogenous carriage). The use of enteral vancomycin in all ICU patients is an attempt to reduce transmission of MRSA from MRSA index cases to the other patients of the ICU, i.e. secondary endogenous carriage.

Therefore, in case of an intensive care unit with MRSA endemicity or outbreak, the use of oropharyngeal and/or intestinal administration of vancomycin in all ventilated patients (combined with hygiene measures) may be considered a strategy to control MRSA carriage and subsequent infection. Surveillance cultures of throat and rectum, to evaluate the efficacy of the manoeuvre and to detect resistance at early stages, are mandatory.

There are no data about infection and re-colonisation of the digestive tract of ICU patients after the cessation of enteral vancomycin or the discharge from the ICU. The SDD experience demonstrated that once SDD was stopped, incidence of hospital acquired infection was higher than in patients not treated with SDD [97]. Similarly, it could be hypothesised that after the discontinuation of enteral vancomycin, patients may be re-colonised by MRSA and/or enterococci and MRSA or enterococcal infections may increase. Therefore, in patients who received enteral vancomycin (as

well as SDD/SOD) and are discharged from the ICU, a careful monitoring of the carrier state is mandatory to detect the possible emergence of resistant bacteria or enterococcal carriage.

8.4 Implications for research

The results of this thesis suggest some interesting fields of research.

Additional RCTs are needed to assess the effectiveness of mupirocin and oral chlorhexidine to prevent lower respiratory tract infection and to reduce mortality in critically ill patients. These RCTs should be adequately powered to detect the effect of these manoeuvres on lower respiratory tract infection, including MRSA infection, and mortality.

This thesis demonstrated that enteral vancomycin may control MRSA carriage and infection. However, the low quality of studies included in the systematic review and the presence of confounding factors may affect the robustness of the results. Therefore, large, high quality, randomised trials are warranted to confirm these findings.

There are no studies in ICU about the re-colonisation of the patient's digestive tract and the incidence of infection after the discontinuation of enteral vancomycin or after ICU discharge. A randomised trial should focus on this issue.

There are limited data on the impact of enteral vancomycin on the intestinal microbiota in ICU patients. Although the results of this thesis reject the hypothesis of a possible emergence of VISA and VRE during enteral vancomycin, changes in gut microbiota and the emergence of resistant microorganisms should be acknowledged. The potential consequences of enteral antimicrobials on the whole gut microbiota and microbiome need careful appraisal in RCTs or cluster-randomised trials in which

ICU patients receiving enteral vancomycin, with or without SDD, are compared to controls not receiving those antimicrobials.

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10 APPENDIX

10.1 Publications arising from this thesis

1. Silvestri L, Weir J, Gregori D, Taylor N, Zandstra DF, van Saene JJM, van Saene HKF. Effectiveness of oral chlorhexidine on nosocomial pneumonia, causative microorganisms and mortality in critically ill patients: a systematic review and meta-analysis. Minerva Anestesiol 2014; 80:805-820
2. Silvestri L, de la Cal MA, Cerda E, Viviani M, van Saene HKF. Use of enteral vancomycin for methicillin-resistant Staphylococcus aureus (MRSA) in intensive care. BMJ 2014;348:g2594
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4. Silvestri L, Weir WI, Gregori D, Taylor N, Zandstra DF, van Saene JJM, van Saene HKF. Impact of oral chlorhexidine on bloodstream infection in critically ill patients: systematic review and meta-analysis of randomized controlled trial. J Cardiothorac Vasc Anesth. 2017;31:2236-2244

10.2 Publications arising from the research area, but not including data from the thesis

1. Petros AJ, Silvestri L, Taylor N, Abecasis F, Damjanovic V, de la Cal MA, Zandstra D, van Saene HK. Comment on: Selective decontamination of the oropharynx and the digestive tract, and antimicrobial resistance: a 4 year

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