Candida and the Gram-Positive Trio: Testing the Vibe in the Icu Patient Microbiome Using Structural Equation Modelling of Literature Derived Data.

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Research article

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Abstract

Background

Whether *Candida* interacts with Gram-positive bacteria to enhance their invasive potential from the microbiome of ICU patients remains unclear. Several effective interventions to prevent ICU acquired infection would be expected to variably impact Candida colonization.

Methods

Four candidate generalized structural equation models (GSEM), using *Staphylococcus aureus, coagulase negative Staphylococci (CNS)* and *Enterococci* colonization as latent variables, were confronted with blood culture and respiratory tract isolate data derived from > 400 groups from >250 infection prevention studies.

Results

Introducing interaction terms between *Candida* colonization and each of *Staphylococcus aureus* (coefficient +0.34; 95% confidence interval 0.19 to 0.48) and *Enterococcal* (+0.55; 0.23 to 0.86) colonization (all as latent variables) improved the fit for each model. The magnitude and significance level of the interaction terms were similar to the positive associations between exposure to topical antibiotic prophylaxis (TAP) on *Enterococcal* (+0.52; 0.06 to 1.0) versus the negative association with *Staphylococcus aureus* (-0.42; -0.66 to -0.17) colonization.

Conclusions

GSEM modelling of published ICU infection prevention data implies interactions between *Candida* and Gram-positive bacteria in the human microbiome. This interaction might also account for the paradoxically high incidences among studies of TAP in ICU patients.

Key Points

- GSEM modelling of published ICU infection prevention data from >250 studies enables a test of and provides support to the interaction between Candida and Gram-positive bacteria.
- The various ICU infection prevention interventions may each broadly impact the patient microbiome.

Introduction

While *Candida* rarely causes ventilator associated pneumonia (VAP), and candidemia is uncommon in the ICU, surprisingly, *Candida* colonization is associated with high disease severity among high risk ICU patients.[1,2] The basis for this association remains unclear and an interaction between *Candida* colonization and invasive infection with Gram-positive bacteria such as *Staphylococcus aureus* remains

possible.[3-8] Several preclinical studies have implicated a potential interaction between Candida colonization and invasive infection with Gram-positive bacteria such as *Staphylococcus aureus*.[4-8] Moreover, Gram-positive bacteria account for the majority of candidemia associated mixed blood stream infections among ICU patients.[9]

Evaluating the possible clinical relevance of microbial interactions is unlikely to be achieved within the constraints of a single center study. Moreover, quantifying microbial colonization and the impact of the various interventions on it within the microbiome is not simple. Structural equation modelling of literature derived data offers a novel approach.[10-12]

Several anti-septic, antibiotic, anti-fungal, or non-decontamination based interventions have been studied for the prevention of ICU acquired infections. These methods variably target bacterial and *Candida* colonization.[12,13] Topical antibiotic prophylaxis (TAP) based methods appear to be the most effective.[14] Yet surprisingly, the incidences of candidemia, VAP and bacteremia with *Staphylococcus aureus,* coagulase negative Staphylococci (CNS) and *Enterococci* are unusually high among studies of methods using TAP and moreso among the concurrent control groups of these studies.[15-18] These high incidences are unexplained.

The objective here is to develop candidate generalized structural equation models (GSEM) of colonization with Candida and Gram-positive bacteria and then to confront these models using group level infection data from published studies of ICU patient groups with various group level exposures.

Materials And Methods

Being an analysis of published work, ethics committee review of this study was not required.

Study selection and decant of groups

The literature search and study decant used here is as described previously[13 ,17, 18] and is detailed in Supplementary Fig S1. The key inclusion criterion, being patient groups requiring prolonged (> 24 hours) ICU stay within studies of ICU infection prevention interventions, was expanded to include studies with group level *Candida,Staphylococcus aureus,* CNS and *Enterococcal* infection data. Studies without ICU infection prevention interventions were sourced to provide observational studies and summary benchmark incidence data. Most of the studies had been cited in one of 23 systematic reviews [19-41] with additional studies being found by snowball sampling using the 'Related articles' function within Google Scholar.[42]

In these GSEM models, the *Candida* and Gram-positive bacterial infection data serve as the measurement components, the group level exposure parameters serve as the structural components and colonization with *Candida*, and individual Gram-positive bacteria*,* each represented as latent variables, link the structural and measurement components.

Measurement components

The incidences of VAP with *Staphylococcus aureus* as well as the incidences of bacteremia with each of *Staphylococcus aureus,* CNS and *Enterococci* were extracted. As *Candida* is generally not counted as a cause of VAP, the count of *Candida* as a respiratory tract (RT *Candida*) isolate among patients with suspected VAP was recorded along with candidemia counts. The use of Center for Disease control (CDC) criteria, being the requirement for at least two positive culture for diagnosis of CNS bacteremia, was recorded. Counts for all subspecies of *Candida*, CNS and *Enterococci* were included. These were each expressed as a proportion using the number of patients with prolonged (>24 hours) ICU stay as the denominator. *Candida*, *Staphylococcus aureus,* coagulase negative Staphylococci (CNS) and *Enterococcal* colonization are each latent variables.

Structural components

The following data were used to form the structural components of the models; year of study publication, origin from trauma ICU's, whether more than 90% of patients of the group received more than 24 hours of MV, and the mean (or median) length of ICU stay (ICU-LOS) for the group. In the extraction of MV percentages, if this was not stated for any group, the percentage receiving MV was assumed to be less than 90%. In the extraction of ICU-LOS data from the studies, surrogate measures including mean (or median) length of mechanical ventilation were taken if the length of ICU-LOS was not available in order to generate broad categories of ICU-LOS of less than 5 days, 5 to 10 days and more than 10 days.

Also, the presence of any of the following group wide risk factors for candidemia and invasive *Candida* infection were noted; liver transplantation or liver failure, use of parenteral nutrition, surgery for intestinal perforation, pancreatitis and being colonized with *Candida*, however that was defined. An anti-septic exposure included use of agents such as chlorhexidine, povidone-iodine and iseganan. All anti-septic exposures were included regardless of whether the application was to the oropharynx, by tooth-brushing or by body-wash.

Topical antibiotic prophylaxis (TAP) is defined here as the group wide application of topical antibiotic prophylaxis to the oropharynx or stomach without regard to the specific antibiotic constituents. Protocolized parenteral antibiotic prophylaxis (PPAP) is the group wide use of any parenteral antibiotic used on a prophylactic basis. Group wide exposure to anti-fungal prophylaxis was identified whether this was as a single agent or as part of a decontamination regimen as used within selective digestive decontamination, without regard to the specific anti-fungal agent.

Structural equation modelling

Four candidate GSEM models were developed with colonization with *Candida* and the individual Gram-positive bacteria as latent variables. The models were constructed with and without the inclusion of studies with ICU-LOS less than 5 days, and with and without an interaction term between the colonization latent variables.

Because the observations are clustered by study, in each model a study identifier was used in order to generate a robust variance covariance matrix of the parameters of each coefficient estimate. The GSEM model with the lowest Akaike's information criterion (AIC) score was selected as having parsimony and optimal fit from among the candidate models using the 'GSEM' command in Stata (Stata 16, College Station Texas, USA).[43]

Visual benchmarking

Scatter plots of the *Candida*, *Staphylococcus aureus,* CNS and *Enterococcal* infection data were generated to facilitate a visual survey of the entire data as derived from the literature. To facilitate this visual survey, a benchmark for each outcome of interest was generated from the groups of the observational studies as described previously.[17,18]

Results

Characteristics of the studies

Of the 275 studies identified by the search, 135 were sourced from 23 systematic reviews (Table 1). Others were found during previous searches or by snowball sampling (Fig S1).Most studies were published between 1990 and 2010 and most had a mean ICU-LOS exceeding ten days. A minority originated from either North American or trauma ICU's. Twelve studies had more than one type of intervention groups and eight studies had no control group. The majority of groups from studies of infection prevention interventions had less than 150 patients per group versus more than 150 patients in the observational studies.

Of the 275 studies, there were 23 groups from 12 studies with mean ICU-LOS less than 5 days including the largest of which (>120,000 patients), being a study of targeted versus universal decontamination versus standard care.[44]

There was a broad range of infection prevention exposures. The majority of studies of anti-fungal prophylaxis as a single agent (i.e. without topical antibiotics) occurred in patient groups selected on the basis of risk factors for invasive candida infection and exposure to anti-fungal prophylaxis as a single agent occurred in only nine groups. The majority of data for anti-fungal exposures occurred in the context of a combined TAP and anti-fungal exposure in the context of a Selective Digestive Decontamination (SDD) regimen for which the antifungal was topical amphotericin being used in 50 groups. The TAP exposures included either topical polymyxin or a topical aminoglycoside or both in every case except four intervention groups. PPAP, most commonly a cephalosporin, was used within ten control groups and 44 intervention groups of TAP studies.

Infection data

Across all intervention categories among groups with non-zero counts, the incidences for Candidemia (Fig 1a and Fig S2a) and RT *Candida* (Fig 1b and S2b), *Staphylococcus aureus* VAP (Fig 2a and 3a) and bacteremia (Fig 2b and 3b) and CNS (Fig 3 and fig S4a) and *Enterococcal* bacteremia (Fig 4 and fig S4b) in each case varied by > 100 fold and ranging approximately tenfold above and below the respective literature derived benchmark. In general, the mean incidence among each category of intervention group was between up to 60% lower than the mean in the corresponding control groups.

The mean control and intervention group incidences of VAP and bacteremia for each of *Staphylococcus aureus* (Fig 2), CNS (Fig 3) and *Enterococci* (Fig 4) were generally similar to the benchmark derived from observational groups with the exception that *Staphylococcus aureus* VAP incidences among the control groups of TAP studies were generally approximately five percentage points above the respective benchmark and the mean incidences of infection for each of CNS bacteremia (Fig 3) and *Enterococcal* bacteremia (Fig 4) among the control and intervention groups of TAP studies were generally approximately two percentage points above the respective benchmarks.

GSEM modelling

Four candidate GSEM models were evaluated for fit and parsimony (see Table 2; Fig 5a and Fig 5b; Fig S5 – S8). The optimal model included an interaction term between the latent terms representing *Candida* colonization with either each of the three Gram-positive bacteria colonization latent variables (Figure 5b). The size and statistical significance of this interaction term was similar in magnitude in each case. The inclusion (models 1 & 2; Fig S5 and S6) or not (models 3 & 4; Fig S7 and S8) of 23 groups that had mean ICU-LOS less than 5 days made no material difference to the findings (Table 2).

In the optimal model (model 2; Table 2; Fig 5b), the following exposures; TAP, trauma ICU, mean ICU-LOS > 10 days and the interaction term with *Candida* colonization, displayed the strongest associations with *S aureus* colonization. Exposure to TAP displayed positive associations with CNS colonization and Enterococcal colonization but a negative association with *S aureus* colonization (Table 2). The magnitude of these associations was similar in each case to that with the *Candida* colonization interaction term.

In all models, exposure to candidemia risk factors, anti-septic, and antifungal interventions displayed strong and consistent associations with *Candida* colonization. Other exposures were not consistently strong or significant in association with any other variables (Table 2).

Discussion

There is a range of preclinical study evidence that suggests that interactions between *Candida* with other bacteria in the patient microbiome has the potential to promote invasive bacterial infections. The basis for the interaction may be molecular [7] or mechanical.[8]

However, there are several obstacles to defining the clinical relevance of any interactions between *Candida* and bacteria in the patient microbiome. *Candida* colonization has several predictors [1, 2] some of which, such as prolonged antibiotic exposure, have broad effects on the microbiome. *Candida* and bacterial colonization are problematic to define. VAP is an imprecise and somewhat subjective endpoint. Various specific Gram-positive bacteremias are each uncommon, as is candidemia. Data arising from single center clinical studies, and especially so infection data, will exhibit dependency and may not be generalizable. Finally, for *S aureus* bacteremia, being a relatively rare end point with a benchmark incidence of 1.8%, even multi-center studies may be underpowered to show any interaction with *Candida* colonization.[45] Moreover, other Gram-positive bacteria such as Enterococci are even less common than *S aureus* bacteremia. Finally, CNS bacteremia is variably defined in the studies with or without using CDC defining criteria.

Presumably as a result of these obstacles, attempts to define the clinical relevance of any interaction between *Candida* with other bacteria are scant and relate mostly to interactions with Gram negative bacteria. Some have reported conflicting results.

There is some evidence that the risk of VAP in association with *Pseudomonas aeruginosa* is more common in patients colonized by *C. albicans*[46] and that antifungal treatments can reduce this likelihood.[47] One study found that *Candida* colonization of the respiratory tract is associated with *Acinetobacter* VAP but not *Pseudomonas* VAP.[48]

In contrast, other attempts to define the clinical relevance of any interaction between *Candida* with other bacteria through either retrospective studies of the association with anti-fungal use or through studies of either pre-emptive or intensified prophylactic anti-fungal treatment [49-51] have failed to resolve the question. Several have questioned the specificity of the association and whether any association is simply a reflection of confounding by illness severity.[1,52]

The approach here is to circumvent these obstacles by using as a natural experiment data from > 400 patient groups from > 250 studies of infection prevention interventions among ICU patients. The various groups of these studies have been exposed to infection prevention interventions which, in conjunction with other exposures, modify the patient microbiome. Of note, any one group here could experience multiple concurrent exposures such as concomitant CRF, TAP, PPAP, anti-fungal and ICU-LOS>10 days. This is reflected in the wide range in incidences of infections across the >250 groups here.

SEM is emerging as a method to model the relationships among multiple simultaneously observed variables in order to provide a quantitative test of any theoretical model proposed within the literature.[10, 11, 53] An ability to test the validity and inferred relationship of conceptual variables that cannot be directly quantified is achieved by using latent variables within the model. GSEM allows generalized linear response functions in addition to the linear response functions allowed by SEM.

Limitations.

There are six key limitations to this analysis, the first being that this analysis is a group level modelling of four latent variables; colonization with each of *Candida*, *Staphylococcus aureus,* CNS and *Enterococci*. These latent variables and the coefficients derived in the GSEM models are indicative and intended for internal reference only. They have no counterpart at the level of any one patient or study and cannot be directly measured.

The GSEM analysis takes a structural rather than statistical approach to the question of any interactions between Candida and Gram-positive bacteria. The structural approach means that a limited number of conceptually key group level factors were entered as simple binary variables into intentionally simplistic GSEM models. There was no ability nor purpose to adjust for the underlying patient level risk. The true relationships between exposures and outcomes will likely be center specific, complex, graded and with multiple expoure interactions. A statistical approach would use more conventional analytic methods such as meta-analysis which, being based on an assumption of exchangability between control and intervention groups that randomized assignement of exposures provides, allows more precise effect size estimates for specific individual interventions under study. However, a randomized assignement of individual exposure to Candida colonization is neither an ethical nor a practical intervention outside of a natural experiement resulting from group level exposures to various ICU infection prevention interventions.

The second limitation is that there was considerable heterogeneity in the interventions, populations, and study designs among the studies here as the inclusion criteria for the various studies have been intentionally broadly specified. This breadth is both a strength, in that the breadth of the group wide exposures is the basis for the natural experiment here, and a limitation, in that the associations for a group wide exposure may not equate to associations at the level of an individual patient exposure.

Thirdly, several assumptions have been made for studies that failed to report key exposure and outcome variables in the analysis. For example, missing data for ICU-LOS and percent receiving MV has been broadly imputed. The extracted data is drawn mostly from studies located in systematic reviews. The data is provided in sufficient detail in the ESM to enable replication of the analysis.

Fourth, there are a large number of studies not included here because the required infection count data was not reported. However, the differences between control and intervention group mean infection incidences noted here in the scatter plots (Fig 1 - 4, Fig S2-S4) are similar to the summary effect sizes for each of the three broad categories of TAP, anti-septic and non-decontamination methods, against both overall VAP and against overall bacteremia which in turn are similar to prior published effect estimates sizes seen in systematic reviews of these interventions from which most of the studies examined here were derived.[19-41]

Fifth, the various regimens of TAP, anti-septic and anti-fungal intervention that have been used within the various studies have been considered as similar within each category. This is a deliberate simplification as some, for example the anti-fungal regimens, targeted different body sites. Also, the duration of

application of the regimens varied among the studies. On the other hand, a strength of this analysis is that it attempts to unpack the separate associations between the infection incidences and exposure to the variable exposure to the various SDD components (TAP, PPAP, anti-fungal).

Finally, the studies have been predominately undertaken within ICU's in first world countries. It is uncertain how representative of the microbiome elsewhere in the world. There is some evidence that the bacteria that cause VAP vary in different parts of the world.[54-56]

A strength of this analysis is that the sensitivity of the model to the inclusion of groups with ICU-LOS < 5 days, both with and without MV, is tested. These patient groups are of interest given the targeting of these interventions to broader categories of ICU patient groups including those with an overall shorter length of stay and with or without MV.

Conclusion

GSEM modelling of colonization with *Candida* and three Gram-positive bacteria, each as latent variables provide support to the possibility that these interact within the patient microbiome to enhance the potential of the bacteria to cause invasive infections. The magnitude of these interactions on the potential of the bacteria to cause invasive infections may be as large as that achieved with some of the infection prevention interventions. An interaction leading to enhanced invasive potential of Gram-positive bacteria might also account for the paradoxically high incidences among the groups of TAP studies.

Declarations

Acknowledgements and declarations.

Ethics approval and consent to participate:

Being an analysis of published work, ethics committee review of this study was not required.

Consent for publication:

Not applicable

Availability of data and material:

The datasets analysed during the current study are provided in the online appendix

Competing interests:

The author declares that he has no competing interests.

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Authors' contributions:

As sole author, JH produced the design of the study, performed the statistical analysis and wrote the manuscript. JH read and approved the final manuscript and is the guarantor of the paper.

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References

1. Terraneo S, Ferrer M, Martin-Loeches I, Esperatti M, Di Pasquale M, Giunta V, et al. Impact of *Candida* isolation in the respiratory tract in patients with intensive care unit-acquired pneumonia. Clin Microbiol Infect. 2016;22(1):94-e1.
2. Huang D, Qi M, Hu Y, Yu M, Liang Z. The impact of *Candida* spp airway colonization on clinical outcomes in patients with ventilator-associated pneumonia: A systematic review and meta-analysis. Am J Infection Control. 2019 pii: S0196-6553(19)30956-3. doi: 10.1016/j.ajic.2019.11.002.
3. Pendleton KM, Huffnagle GB, Dickson RP. The significance of *Candida* in the human respiratory tract: our evolving understanding. Pathog Dis. 2017;75:ftx029.
4. Harriott MM, Noverr MC. Candida albicans and *Staphylococcus aureus* form polymicrobial biofilms: effects on antimicrobial resistance. *Antimicrob Agents Chemother* 2009;53: 3914–22.
5. Carlson E. Effect of strain of *Staphylococcus aureus* on synergism with *Candida albicans* resulting in mouse mortality and morbidity. *Infect Immun* 1983;42: 285–92.
6. Hajishengallis G, Lamont RJ. Dancing with the stars: how choreographed bacterial interactions dictate nososymbiocity and give rise to keystone pathogens, accessory pathogens, and pathobionts. Trends Microbiol. 2016;24(6):477-89.
7. Todd OA, Fidel PL, Harro JM, Hilliard JJ, Tkaczyk C, Sellman BR, et al. *Candida albicans* augments *Staphylococcus aureus* virulence by engaging the staphylococcal agr quorum sensing system. MBio. 2019;10(3):e00910-9.
8. Schlecht LM, Peters BM, Krom BP, Freiberg JA, Hänsch GM, Filler SG, et al. Systemic *Staphylococcus aureus* infection mediated by *Candida albicans* hyphal invasion of mucosal tissue. Microbiol. 2015;161(Pt 1):168.

9. Kim SH, Yoon YK, Kim MJ, Sohn JW. Risk factors for and clinical implications of mixed Candida/bacterial bloodstream infections. Clin Microbiol Infect. 2013;19(1):62-8.
10. Carver S, Beatty JA, Troyer RM, Harris RL, Stutzman-Rodriguez K, Barrs VR, et al. Closing the gap on causal processes of infection risk from cross-sectional data: structural equation models to understand infection and co-infection. Parasites & Vectors. 2015;8(1):658.
11. Hurley JC. Structural equation modelling the 'control of gut overgrowth' in the prevention of ICU acquired Gram-negative infection. Crit Care. 2020;24:189.
12. Rushton SP, Shirley MD, Sheridan EA, Lanyon CV, O'Donnell AG. The transmission of nosocomial pathogens in an intensive care unit: a space–time clustering and structural equation modelling approach. Epidemiol Infect. 2010;138(6):915-26.
13. Hurley JC. Candida–Acinetobacter–Pseudomonas Interaction Modelled within 286 ICU Infection Prevention Studies. J Fungi. 2020;6(4):252.
14. Silvestri L, Miguel A, van Saene HK. Selective decontamination of the digestive tract: the mechanism of action is control of gut overgrowth. Intensive Care Med. 2012;38(11):1738-50.
15. Hurley JC. Incidence of coagulase-negative staphylococcal bacteremia among ICU patients: decontamination studies as a natural experiment. Eur J Clin Microbiol Infect Dis. 2019:1-8.
16. Hurley JC. Studies of selective digestive decontamination as a natural experiment to evaluate topical antibiotic prophylaxis and cephalosporin use as population-level risk factors for enterococcal bacteraemia among ICU patients. J Antimicrob Chemother. 2019;74(10):3087-94.
17. Hurley JC. Impact of selective digestive decontamination on respiratory tract *Candida* among patients with suspected ventilator-associated pneumonia. A meta-analysis. Eur J Clin Microbiol Infect Dis. 2016;35(7):1121-35.
18. Hurley JC. ICU-acquired candidemia within selective digestive decontamination studies: a meta-analysis. Intensive Care Med. 2015;41(11):1877-85.
19. Liberati A, D'Amico R, Pifferi S, Torri V, Brazzi L, Parmelli E. Antibiotic prophylaxis to reduce respiratory tract infections and mortality in adults receiving intensive care (Review). Cochrane Database Syst Rev 2009 ;4:CD000022.
20. Pileggi C, Bianco A, Flotta D, Nobile CG, Pavia M. Prevention of ventilator-associated pneumonia, mortality and all intensive care unit acquired infections by topically applied antimicrobial or antiseptic agents: a meta-analysis of randomized controlled trials in intensive care units. Crit Care 2011;15: R155.
21. Silvestri L, Van Saene HK, Casarin A, Berlot G, Gullo A. Impact of selective decontamination of the digestive tract on carriage and infection due to Gram-negative and Gram-positive bacteria: a systematic review of randomised controlled trials. Anaesthesia Intensive Care 2008;36(3):324-38.
22. Hurley JC. Prophylaxis with enteral antibiotics in ventilated patients: Selective decontamination or selective cross-infection? Antimicrob Agents Chemother. 1995;39:941–947.
23. Silvestri L, Van Saene HK, Milanese M, Gregori D, Gullo A. Selective decontamination of the digestive tract reduces bacterial bloodstream infection and mortality in critically ill patients. Systematic review

of randomized, controlled trials. J Hosp Infect. 2007;65(3):187-203.

24. Silvestri L, Weir WI, Gregori D, Taylor N, Zandstra DF, van Saene JJ, van Saene HK.. Impact of Oral Chlorhexidine on Bloodstream Infection in Critically Ill Patients: Systematic Review and Meta-Analysis of Randomized Controlled Trials. J Cardiothoracic Vasc Anesthesia. 2017;31(6):2236-44.
25. Labeau SO, Van de Vyver K, Brusselaers N, Vogelaers D, Blot SI. Prevention of ventilator-associated pneumonia with oral antiseptics: a systematic review and meta-analysis. Lancet Infect Dis. 2011;11:845-854.
26. Klompas M, Speck K, Howell MD, Greene LR, Berenholtz SM. Reappraisal of routine oral care with chlorhexidine gluconate for patients receiving mechanical ventilation: systematic review and meta-analysis. JAMA Intern Med. 2014;174(5):751-61.
27. Alhazzani W, Smith O, Muscedere J, Medd J, Cook D. Toothbrushing for Critically Ill Mechanically Ventilated Patients: A Systematic Review and Meta-Analysis of Randomized Trials Evaluating Ventilator-Associated Pneumonia. Crit Care Med.2013;41:646-655
28. Messori A, Trippoli S, Vaiani M, Gorini M, Corrado A. Bleeding and pneumonia in intensive care patients given ranitidine and sucralfate for prevention of stress ulcer: meta-analysis of randomised controlled trials. BMJ. 2000;321:1103–1106.
29. Huang J, Cao Y, Liao C, Wu L, Gao F. Effect of histamine-2-receptor antagonists versus sucralfate on stress ulcer prophylaxis in mechanically ventilated patients: a meta-analysis of 10 randomized controlled trials. Crit Care. 2010;14:R194.
30. Alhazzani W, Almasoud A, Jaeschke R, Lo BW, Sindi A, Altayyar S, Fox-Robichaud A. Small bowel feeding and risk of pneumonia in adult critically ill patients: a systematic review and meta-analysis of randomized trials. Crit Care. 2013;17:R127.
31. Melsen WG, Rovers MM, Bonten MJM. Ventilator-associated pneumonia and mortality: A systematic review of observational studies. Crit Care Med.2009;37:2709–2718.
32. Safdar N, Dezfulian C, Collard HR, Saint S. Clinical and economic consequences of ventilator-associated pneumonia: a systematic review. Crit Care Med.2005;33:2184–93.
33. Han J, Liu Y. Effect of ventilator circuit changes on ventilator-associated pneumonia: a systematic review and meta-analysis. Respiratory Care. 2010;55: 467-474.
34. Subirana M, Solà I, Benito S. Closed tracheal suction systems versus open tracheal suction systems for mechanically ventilated adult patients. Cochrane Database Syst Rev. 2007;4: CD004581;
35. Siempos II, Vardakas KZ, Kopterides P, Falagas ME. Impact of passive humidification on clinical outcomes of mechanically ventilated patients: A meta-analysis of randomized controlled trials. Crit Care Med.2007;35: 2843-51;
36. Muscedere J, Rewa O, McKechnie K, Jiang X, Laporta D, Heyland DK. Subglottic secretion drainage for the prevention of ventilator-associated pneumonia: a systematic review and meta-analysis. Crit Care Med.2011;39:1985–1991.
37. Delaney A, Gray H, Laupland KB, Zuege DJ. Kinetic bed therapy to prevent nosocomial pneumonia in mechanically ventilated patients: a systematic review and meta-analysis. Crit Care. 2006;10:R70;

38. Sud S, Friedrich JO, Taccone P, Polli F, Adhikari NK, Latini R, Gattinoni L. Prone ventilation reduces mortality in patients with acute respiratory failure and severe hypoxemia: systematic review and meta-analysis. Inten Care Med. 2010;36(4); 585-599.
39. Siempos II, Vardakas KZ, Falagas ME. Closed tracheal suction systems for prevention of ventilator-associated pneumonia. Brit J Anaesthesia. 2008;100(3): 299-306.
40. Playford EG, Webster AC, Sorrell TC, Craig JC. Antifungal agents for preventing fungal infections in non-neutropenic critically ill and surgical patients: systematic review and meta-analysis of randomized clinical trials. Journal of Antimicrobial Chemotherapy. 2006;57(4):628-38.
41. van Till JO, van Ruler O, Lamme B, Weber RJ, Reitsma JB, Boermeester MA. Single-drug therapy or selective decontamination of the digestive tract as antifungal prophylaxis in critically ill patients: a systematic review. Critical Care. 2007;11(6):R126.
42. Goodman L. Snowball sampling. Ann Math Statistics. 1961;32:148–70.
43. Stata Corporation. Stata structural equation modelling reference manual, in Stata 16 documentation. College Station, TX, USA. 2109 https://www.stata.com/bookstore/structural-equation-modeling-reference-manual/. Accessed 06 January 2020.
44. Huang SS, Septimus E, Kleinman K, Moody J, Hickok J, Avery TR, et al. Targeted versus universal decolonization to prevent ICU infection. *N Engl J Med.* 2013;368(24):2255-65.
45. Hurley JC. How the Cluster randomized trial 'works'. Clin Infect Dis. 2020;70:341–6.
46. Azoulay E, Timsit JF, Tafflet M, de Lassence A, Darmon M, Zahar JR, et al. *Candida* colonization of the respiratory tract and subsequent pseudomonas ventilator-associated pneumonia. Chest 2006;129(1):110-7.
47. Nseir S, Jozefowicz E, Cavestri B, Sendid B, Di Pompeo C, Dewavrin F, et al. Impact of antifungal treatment on *Candida*–Pseudomonas interaction: a preliminary retrospective case–control study. Intensive Care Med. 2007;33(1):137-42.
48. Tan X, Zhu S, Yan D, Chen W, Chen R, Zou J, et al. *Candida* airway colonization: A potential risk factor for *Acinetobacter baumannii* ventilator-associated pneumonia. Med Mycol 2016;21;54(6):557-66.
49. Albert M, Williamson D, Muscedere J, Lauzier F, Rotstein C, Kanji S, et al. *Candida* in the respiratory tract secretions of critically ill patients and the impact of antifungal treatment: a randomized placebo controlled pilot trial (CANTREAT study). Intensive Care Med. 2014;40:1313–22.
50. Ong DS, Klouwenberg PM, Spitoni C, Bonten MJ, Cremer OL. Nebulised amphotericin B to eradicate *Candida* colonisation from the respiratory tract in critically ill patients receiving selective digestive decontamination: a cohort study. Crit Care. 2013;17(5):R233.
51. Lindau S, Nadermann M, Ackermann H, Bingold TM, Stephan C, Kempf VA, et al. Antifungal therapy in patients with pulmonary *Candida* colonization may have no beneficial effects. J Intensive Care. 2015;3(1):31.
52. Timsit JF, Schwebel C, Styfalova L, Cornet M, Poirier P, Forrestier C, et al. Impact of bronchial colonization with *Candida* on the risk of bacterial ventilator-associated pneumonia in the ICU: the FUNGIBACT prospective cohort study. Intensive Care Med. 2019;45(6):834-43.

53. Bojan M, Duarte MC, Ermak N, Lopez-Lopez V, Mogenet A, Froissart M. Structural equation modelling exploration of the key pathophysiological processes involved in cardiac surgery-related acute kidney injury in infants. Crit Care. 2016;20(1):171.
54. Hurley JC. World-wide variation in incidence of *Acinetobacter* associated ventilator associated pneumonia: a meta-regression. BMC Infect Dis 2016;16(1):577.
55. Hurley JC. Worldwide variation in *Pseudomonas* associated ventilator associated pneumonia. A meta-regression. J Crit Care. 2019;51:88-93.
56. Hurley JC. World-wide variation in incidence of *Staphylococcus aureus* associated ventilator-associated pneumonia: A meta-regression. Microorganisms. 2018;6(1):18.

Tables

Table 1. Characteristics of studies [a]

	Observational studies				
	(no intervention)	Non-decontamination	Anti-septic	TAP ± PPAP	Anti-fungal
Study characteristics					
Sources [b]	Table S1	Table S2	Table S3	Table S4	Table S5
Number of studies	141	45	17	65	9
Origin from systematic review [c]	52	37	9	44	6
Study publication year (range)	1987-2018	1987-2017	2000-2016	1984-2018	1994-2014
North American ICU's [d]	29	9	7	6	2
Trauma ICUs [e]	24	9	3	12	0
Group characteristics					
number of groups	157	90	36	133	20
LOS < 5 days	10	0	8	6	0
LOS > 10 days	96	57	19	97	16
MV for >48 hours for <90% [f]	38	0	16	33	12
PPAP use in control group [g]	0	0	0	9	0
CRF [h]	11	0	0	17	12
Use of CDC criteria [i]	0	8	5	19	0
Numbers of patients per control group; (median; IQR) [j]	279 117-632	75 61-143	130 31-347	63 40-128	49 23-51

Footnotes

5. Source data is presented in Tables S1-S5. see Electronic Supplementary Material for additional ESM tables, ESM figures, and ESM references
6. Note, several studies had more than one control and or intervention group. Hence the number of groups does not equal the number of studies
7. Studies that were sourced from 16 systematic reviews (references in web-only supplementary)
8. Study originating from an ICU in Canada of the United States of America
9. Trauma ICU arbitrarily defined as an ICU with more than 50% of admissions for trauma.
10. Groups for which less than 90% of patients were reported to receive > 48 hours of MV
11. Use of PPAP for control group patients. PPAP is protocolized parenteral antibiotic prophylaxis
12. CRF is a term representing risk factors for either Candidemia or invasive *Candida* or patient groups selected on the basis of *Candida* colonization
13. CDC is the Center for Disease control criteria for defining a CNS bacteremia as being at least two blood cultures positive for CNS
14. Data is median and inter-quartile range (IQR)

Table 2: Development of GSEM model [a, b, c]

	All groups			Excluding groups with ICU-LOS<5days		
	Model 1	Model 2		Model 3	Model 4	
	Fig 5a	Fig 5b		Fig s7	Fig s8	
Factor [c - g]			95%CI			95%CI
b_S aureus_n						
S aureus col	0.94***	0.94***	0.71 to 1.16	0.94***	0.95***	0.72 to 1.21
ppap	0.45	0.44	-0.22 to 1.1	0.45	0.45	-0.2 to 1.13
_cons	-4.58***	-4.48***	-4.9 to -4.0	-3.93***	-3.89***	-5.04 to -4.11
v_ S aureus _n						
S aureus col	1	1	(constrained)	1	1	(constrained)
mvp90	0.46*	0.43*	0.05 to 0.8	0.44*	0.42*	0.02 to 0.82
non_D	-0.26*	-0.28*	-0.55 to -0.01	-0.26	-0.28*	-0.55 to -0.02
_cons	-4.02***	-3.92***	-4.5 to -3.3	-3.34***	-3.29***	-4.3 to -2.24
S aureus colonization [h]						
year80	-0.21***	-0.24***	-0.37 to -0.11	-0.20**	-0.26***	-0.38 to -0.13
crf	0.32	-0.38	-1. to 0.26	0.35	-0.29	-0.92 to 0.34
trauma50	0.99***	0.93***	0.64 to 1.2	0.99***	0.92***	0.64 to 1.2
los10	0.43***	0.47***	0.26 to 0.68	0.43***	0.46***	0.26 to 0.67
los5	0.68**	0.18	-0.3 to 0.67	-	-	-
TAP	-0.58***	-0.42***	-0.66 to -0.17	-0.57***	-0.41***	-0.64 to -0.17
a_S	-0.61**	-0.17	0.66 to 0.27	-0.63*	-0.16	-0.74 to 0.42

Candida col	-	0.34***	0.19 to 0.48	-	0.33***	0.2 to 0.47

Table 2: Development of GSEM model (continued) [a, b, c]

	All groups			Excluding groups with ICU-LOS<5days		
	Model 1	Model 2		Model 3	Model 4	
	Fig 5a	Fig 5b		Fig s7	Fig s8	
Factor [d – o]			95%CI			95%CI
b_CNS_n						
CNS col	1	1	(constrained)	1	1	(constrained)
cdc	-0.14	-0.12	-0.6 to 0.77	-0.07	-0.11	-0.71 to 0.61
ppap	-0.11	0.12	-0.6 to 0.77	-0.33	-0.01	-0.71 to 0.61
_cons	-4.87***	-4.72***	-6.2 to -3.84	-4.0***	-3.73***	-5 to -3.2
CNS colonization [i]						
year80	-0.02	-0.04	-0.29 to 0.22	0.03	-0.09	-0.31 to 0.18
crf	-0.11	-1.43*	-2.8 to -0.07	-0.33	-1.65*	-2.92 to -0.33
trauma50	-0.17	-0.61	-1.3 to 0.08	-0.21	-0.68*	-1.4 to -0.4
los10	0.33	0.45	-0.04 to 0.94	0.38	0.48*	0.2 to 1.0
los5	1.10*	0.28	-0.51 to 1.06	-	-	-
TAP	0.68	0.83**	0.32 to 1.44	0.9*	0.9**	0.45 to 1.61
a_S	-0.42	0.01	-0.64 to 0.66	-0.33	0.23	-0.38 to 0.81
Candida col	-	0.54**	0.16 to 0.92	-	0.58**	0.21 to 0.96
b_Ent_n						
Enterococcal col	1	1	(constrained)	1	1	(constrained)
ppap	-0.12	0.41	-0.15 to 0.96	-0.2	0.33	-0.28 to 0.98
_cons	-6.49***	-6.09***	-6.8 to -5.3	-5.36***	-5.0***	-6.5 to -4.47

Enterococcal colonization [j]						
year80	0.18	0.10	-0.09 to 0.3	0.31*	0.15	-0.1 to 0.38
crf	0.16	-1.28	-2.67 to 0.08	0.32	-1.1	-2.5 to 0.28
trauma50	0.06	-0.50	-1.36 to 0.37	0.06	-0.54	-1.39 to 0.32
los10	-0.2	-0.14	-0.63 to 0.35	-0.18	-0.12	-0.63 to 0.38
los5	1.51***	0.64	-0.33 to 1.6	-	-	-
TAP	0.53	0.52*	0.06 to 1.0	0.62*	0.57*	0.02 to 1.12
a_S	-0.36	0.03	-0.25 to 0.32	-0.49	0.01	-0.49 to 0.54
Candida col	-	0.54***	0.23 to 0.86	-	0.55***	0.27 to 0.86

Table 2: Development of GSEM model (continued) [a, b, c]

	All groups			Excluding groups with ICU-LOS<5days		
	Model 1	Model 2		Model 3	Model 4	
	Fig 5a	Fig 5b		Fig s7	Fig s8	
Factor [d - o]			95%CI			95%CI
b_ *Candida* _n						
Candida col	0.50***	0.54***	0.33 to 0.67	0.50***	0.56***	0.36 to 0.77
_cons	-5.62***	-5.62***	-6.23 to -5.01	-4.9***	-5.0***	-6.2 to -4.8
v_ *Candida* _n						
Candida col	1	1	(constrained)	1	1	(constrained)
mvp90	0.02	0.24	-0.84 to 0.89	0.12	0.4	-0.33 to 1.11
non_D	-0.29	-0.41	-0.96 to 0.38	-0.32	-0.46	-1.09 to 0.16
_cons	-6.53***	-6.57***	-8.1 to -4.97	-5.23***	-5.57***	-7.8 to -5.0
Candida colonization [k]						
year80	-0.01	0.07	-0.33 to 0.31	0.09	0.15	-0.14 to 0.43
crf	2.31***	2.23***	1.33 to 3.28	2.06***	1.98***	1.1 to 2.85
trauma50	0.23	0.25	-0.69 to 1.14	0.24	0.27	-0.57 to 1.1
los10	-0.08	-0.12	-0.59 to 0.42	-0.07	-0.11	-0.56 to 0.35
los5	1.61**	1.35**	0.57 to 2.64	-	-	-
TAP	0.64	0.60	-0.2 to 1.48	0.53	0.51	-0.17 to 1.18
a_S	-1.44**	-1.36**	-2.44 to -0.44	-1.41*	-1.46*	-2.58 to -0.35
AF	-1.57***	-1.40***	-2.48 to -0.65	-1.52**	-1.30**	-2.07 to -0.52

Table 2: Development of GSEM model (continued) [a, b, c]

	All groups			Excluding groups with ICU-LOS<5days		
	Model 1	Model 2		Model 3	Model 4	
	Fig 5a	Fig 5b		Fig s7	Fig s8	
Factor [d – o]			95%CI			95%CI
Error terms						
var (e. S aureus col)	0.45***	0.30***	0.34 to 0.58	0.45***	0.30***	0.21 to 0.43
var (e. CNS col)	0.79***	0.43**	0.5 to 1.33	0.75*	0.29*	0.12 to 0.75
var (e. Ent col)	0.46*	0.13	0.17 to 1.24	0.58	0.21	0.04 to 0.86
var (e. *Candida* col)	1.48***	1.32***	0.98 to 2.24	1.49***	1.32***	0.87 to 1.9
Model fit [p]						
AIC	5076	5002	-	4541	4476	-
Groups(n)	434	434	-	410	410	-
Clusters (n)	268	268	-	255	255	-
Factors (n)	53	56	-	49	52	-

Footnotes

1. Legend: * p<0.05; ** p<0.01; *** p<0.001
2. Shown in this table are models derived with all studies including those with LOS<5 days (models 1 and 2) and models derived after excluding studies with LOS< 5 days (models 3 and 4).
3. v _sr_n is the count of *Staphylococcus aureus* VAP; and v_can_n is the count of *Candida* isolates from patients with VAP; b_sr_n is the count of *Staphylococcus aureus* bacteremia; and b_can_n is the count of Candidemia; b_cns_n is the count of coagulative negative *Staphylococcus* bacteremia and b_ent_n is the count of *Enterococcal*

4. PPAP is the group wide use of protocolized parenteral antibiotic prophylaxis; TAP is topical antibiotic prophylaxis; non-D is a non-decontamination intervention; year = year of study publication in units of ten (decade); Crf = Candidemia risk factor; Trauma ICU arbitrarily defined as an ICU for which >50% of admissions were for trauma; cdc is the use of CDC cirteria for CNS bacteremia counts.
5. MVP90 is use of mechanical ventilation by more than 90% of the group
6. LOS5 is a mean or median length of ICU stay for the group of <5 days
7. LOS10 is a mean or median length of ICU stay for the group of >10 days
8. *S aureus* colonization (*S aureus* col) is a latent variable
9. CNS colonization (CNS col) is a latent variable
10. Enterococcal colonization (Enterococcal col) is a latent variable
11. *Candida* colonization (*Candida* col) is a latent variable
12. Model fit; AIC is Akaike's information criteria. This indicates model fit taking into account the statistical goodness of fit and the number of parameters in the model. Lower values of AIC indicate a better model fit. Groups is the number of patient groups; clusters is the number of studies; N is the number of parameters in the model.

Figures

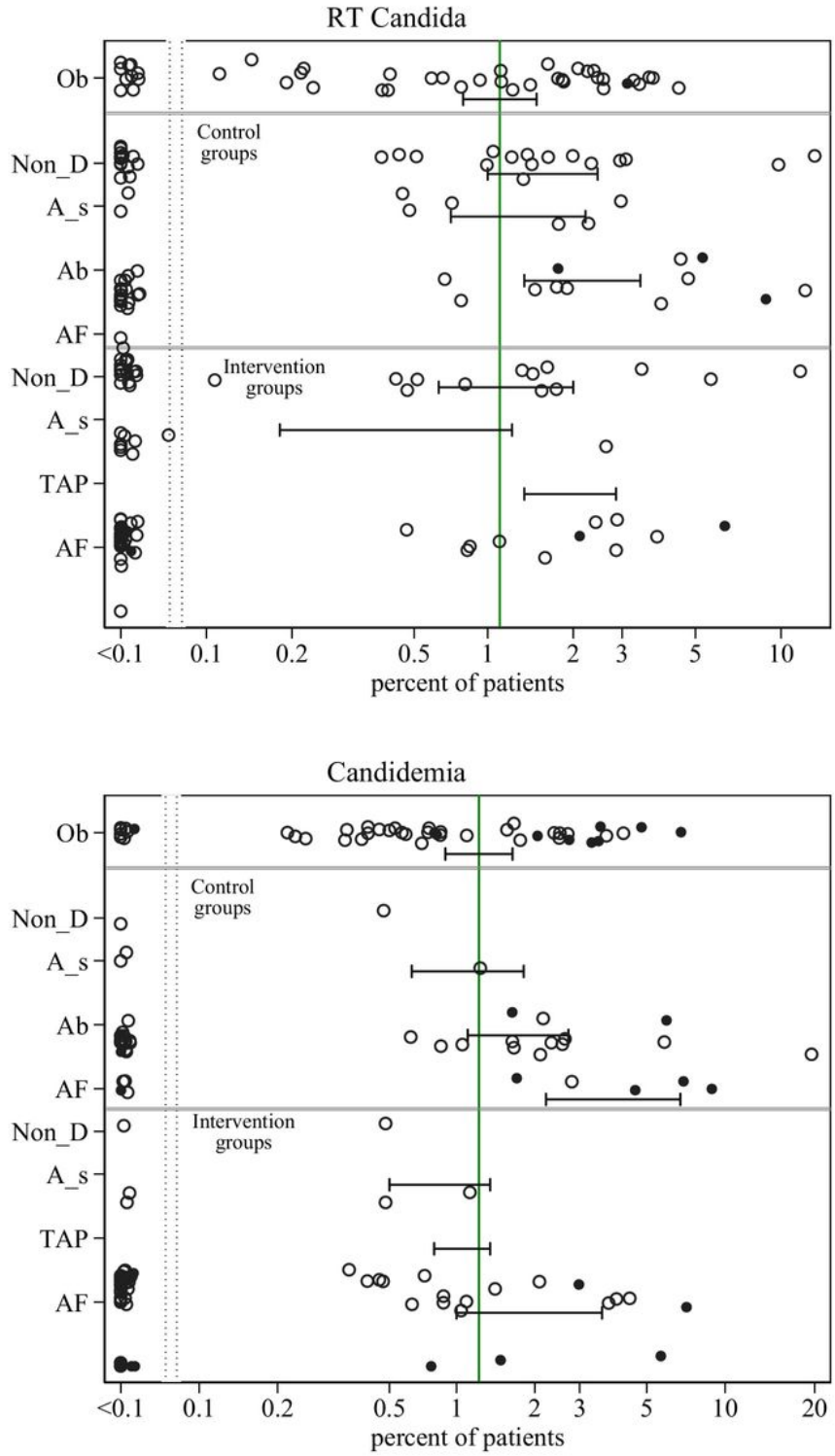

Figure 1

a and b. Scatter plots (logit scale) & 95% CI of RT Candida incidence (Fig 1a) and Candidemia (Fig 1b) in component (control and intervention) groups of various methods of infection prevention in the ICU. The benchmark incidence in each plot is the summary mean derived from the observation studies (central vertical line). The groups wide presence of candidemia risk factors (CRF) is identified by solid symbols versus not (open). Abbreviations; non-D is non-decontamination, A_s is anti-septic, TAP is topical

antibiotic prophylaxis and AF is anti-fungal. Equivalent scatter plots excluding studies with ICU-LOS<5 days are shown in the ESM (Fig S2a & S2b).

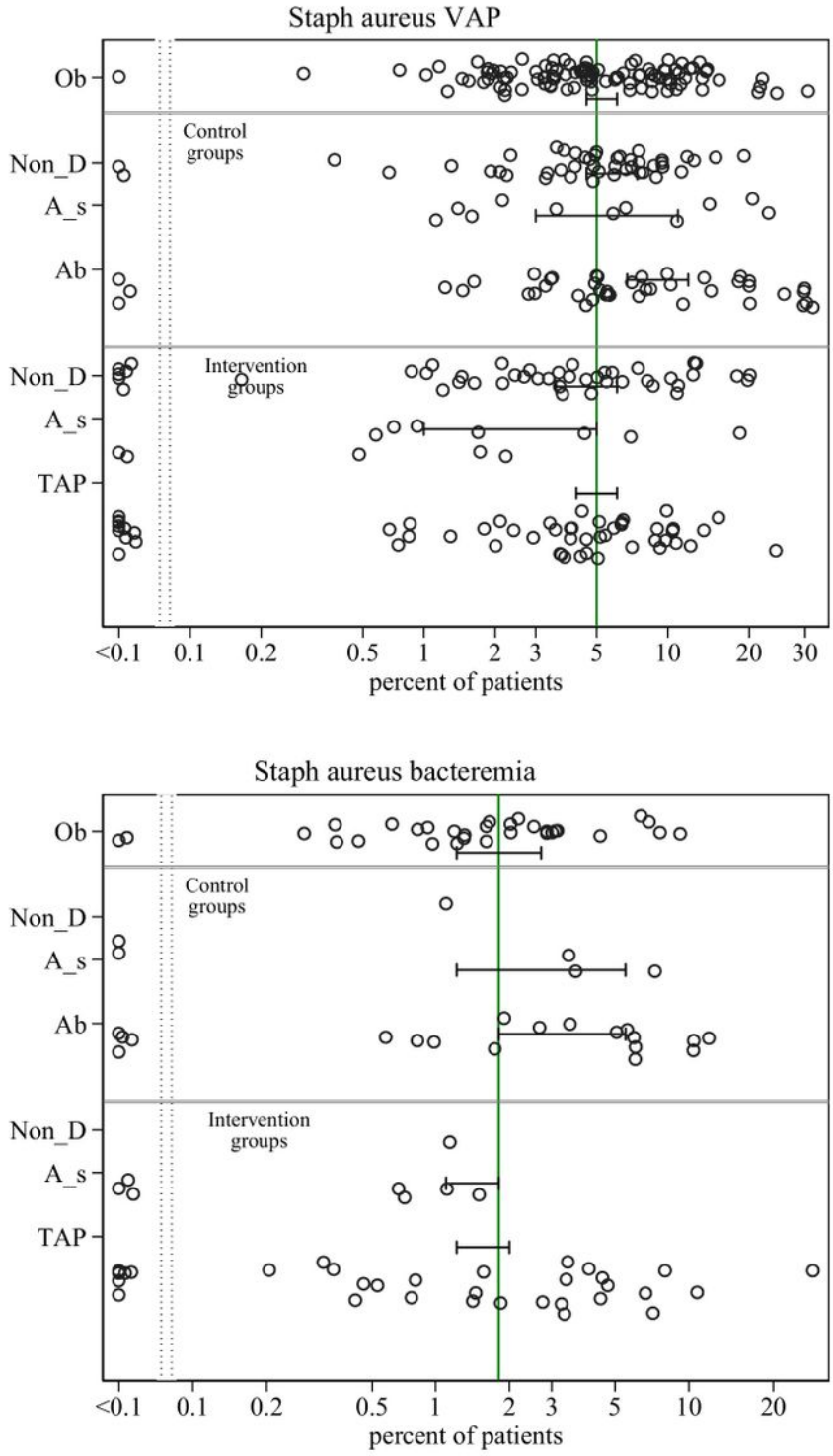

Figure 2

a and b. Scatter plots (logit scale) & 95% CI of Staph aureus VAP incidence (Fig 2a) and Staph aureus bacteremia (Fig 2b) in component (control and intervention) groups of various methods of infection prevention in the ICU. The benchmark incidence in each plot is the summary mean derived from the

observation studies (central vertical line). Abbreviations; non-D is non-decontamination, A_s is anti-septic, TAP is topical antibiotic prophylaxis and AF is anti-fungal. Equivalent scatter plots excluding studies with ICU-LOS<5 days are shown in the ESM (Fig S3a & S3b).

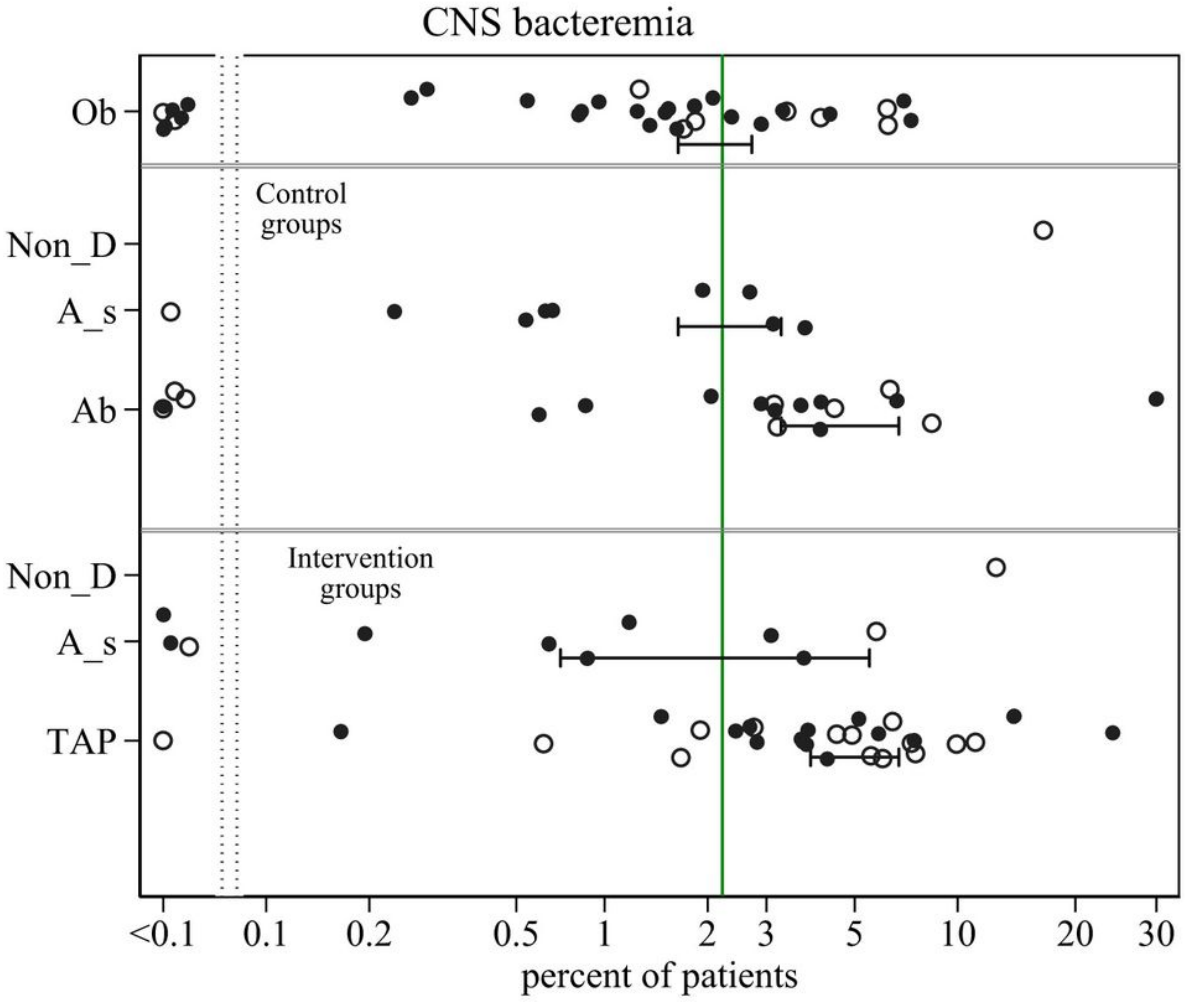

Figure 3

Scatter plots (logit scale) & 95% CI of CNS bacteremia in component (control and intervention) groups of various methods of infection prevention in the ICU. The benchmark incidence in each plot is the summary mean derived from the observation studies (central vertical line). Those studies using (solid) versus not using (open) CDC criteria (at least 2 positive blood cultures) for CNS bacteremia diagnosis are indicated. Abbreviations; non-D is non-decontamination, A_s is anti-septic, TAP is topical antibiotic prophylaxis and AF is anti-fungal. Equivalent scatter plots excluding studies with ICU-LOS<5 days are shown in the ESM (Fig S4a).

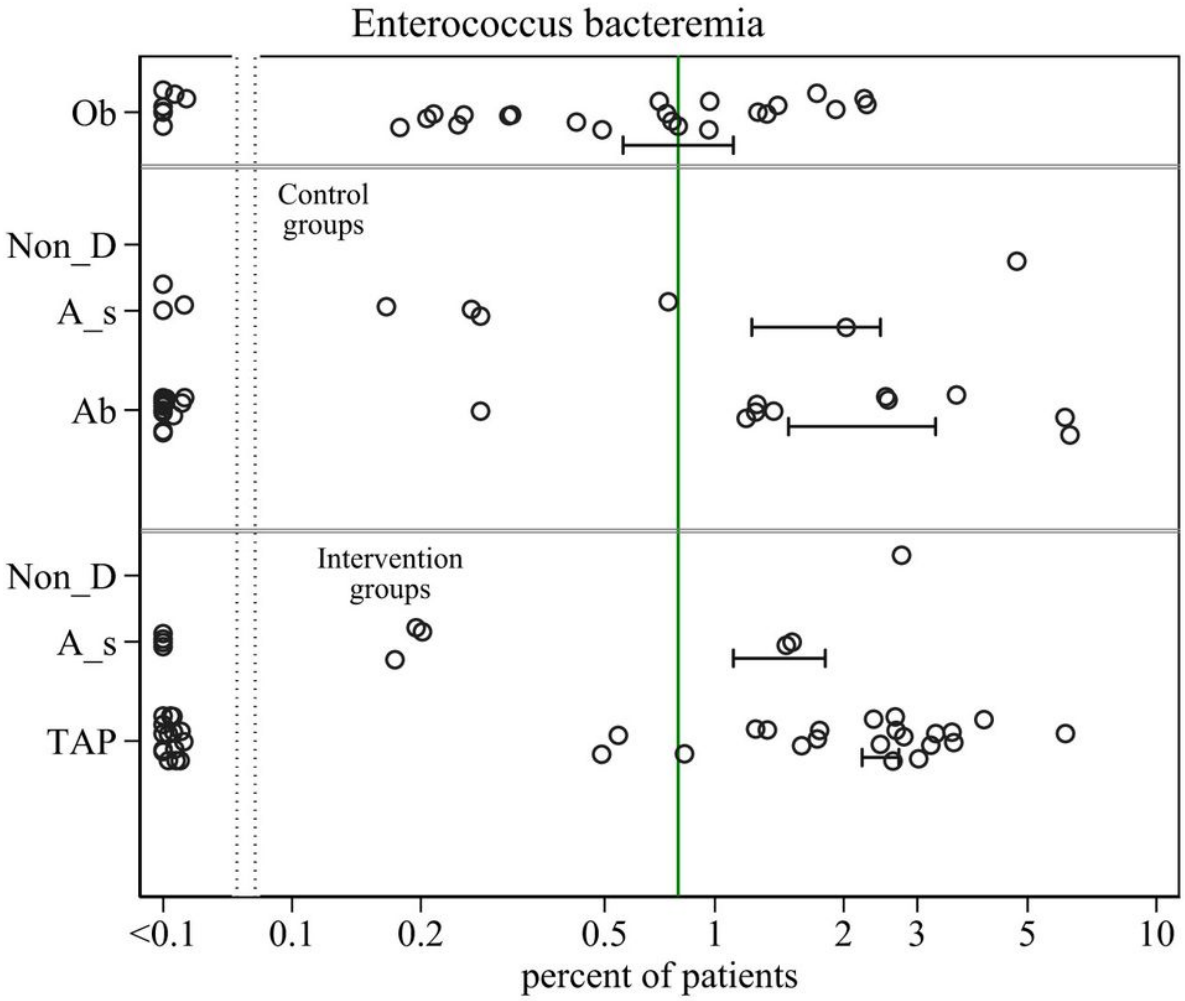

Figure 4

Scatter plots (logit scale) & 95% CI of Enterococcal bacteremia in component (control and intervention) groups of various methods of infection prevention in the ICU. The benchmark incidence in each plot is the summary mean derived from the observation studies (central vertical line). Abbreviations; non-D is non-decontamination, A_s is anti-septic, TAP is topical antibiotic prophylaxis and AF is anti-fungal. Equivalent scatter plots excluding studies with ICU-LOS<5 days are shown in the ESM (Fig S4b).

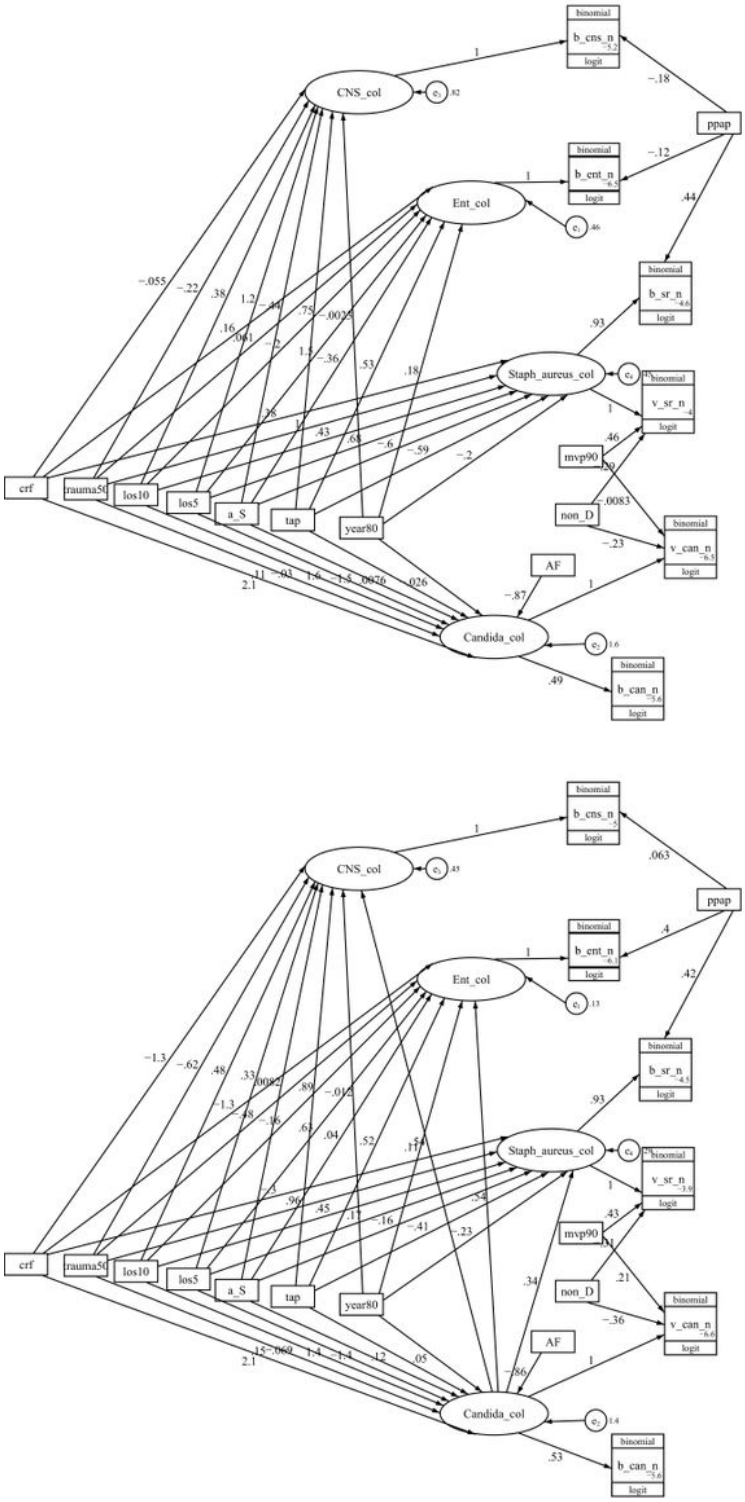

Figure 5

A GSEM without (Fig 5a left, model 1) and with (Fig 5b right, model 2) an interaction term between Candida colonization and colonization with each of three Gram-positive bacteria. The model with the interaction term (Fig 5b right; Model 2) is the optimal model. Candida_col, S aureus_col, CNS_col and Ent_col (ovals) are latent variables representing Candida, S aureus, CNS and Enterococcal colonization, respectively. The variables in rectangles are binary predictor variables representing the group level

exposure to the following; a trauma ICU setting (trauma50), mean or median length of ICU stay < 5 days (los5), mean or median length of ICU stay ≥ 10 days (los10), exposure to a topical anti-septic based prevention method (a_S), exposure to a TAP based prevention method (tap), year of study publication, exposure to a non-decontamination based prevention method (non-D), use of mechanical ventialtion more for than 90% of the group (mvp90), use of CDC criteria to define CNS bactereia (cdc) or exposure to PPAP (ppap). The circles contain error terms. The three-part boxes represent the count data for Candida, and S aureus, CNS and Enterococci as VAP (v_can_n, v_S aureus_n) and bacteremia (b_can_n, b_S aureus_n, b_cns_n, b_Ent_n) isolates. These counts are logit transformed with the total number of patients in each group as the denominator using the logit link function in the generalized model of the GSEM. The equivalent models not including studies with ICU-LOS<5 days are shown in the ESM (Fig S7 & S8).

Supplementary Files

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