

Bacterial Infections

Brig Ajay Sharma

Dept of Clinical Hematology &

Centre for Stem Cell Transplantation & Research,

Paras Hospital, Panchkula

Transplant PTS are at substantial risk for a variety of infections due to

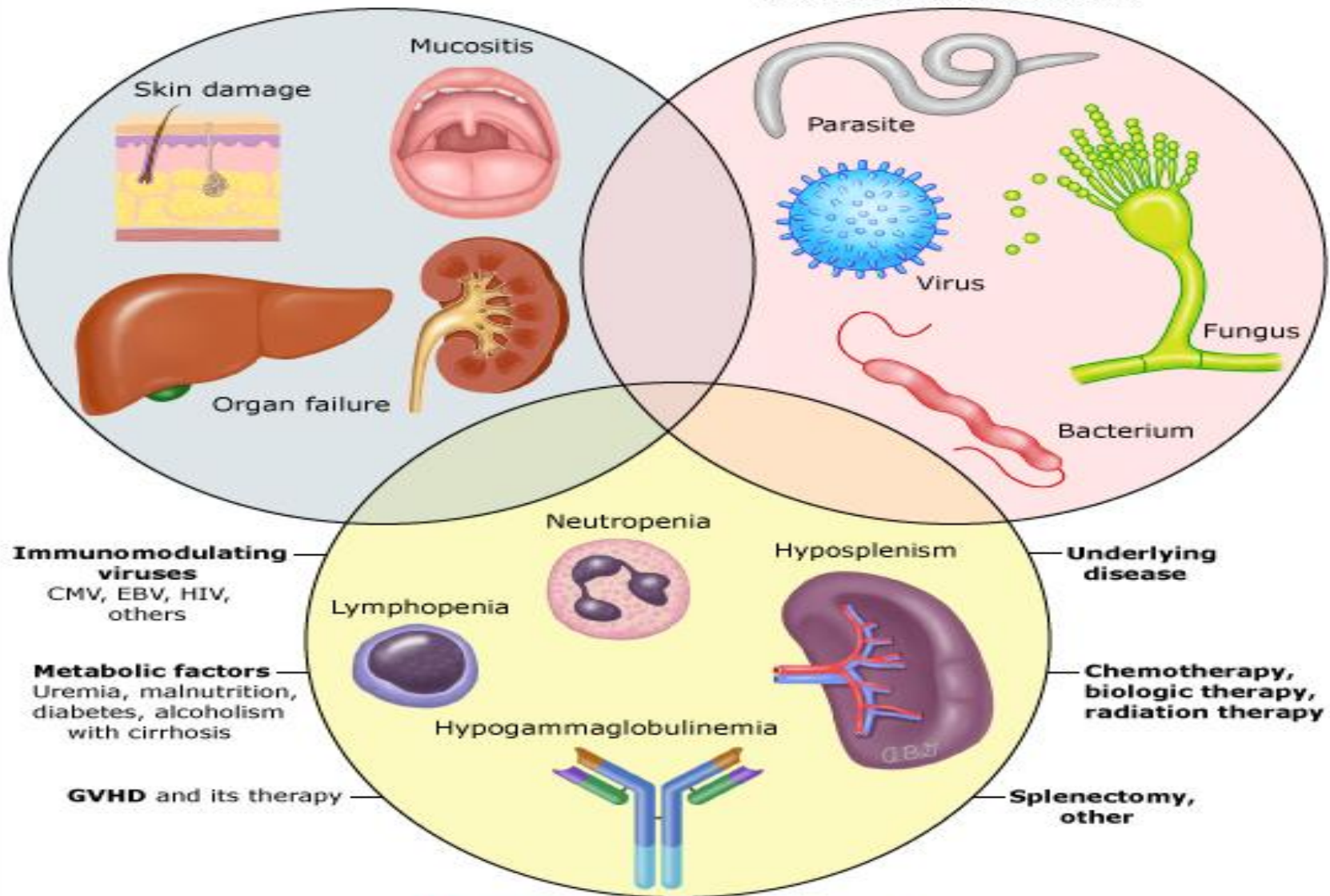
Bacterial Infections carry higher risks of morbidity & mortality than in Immunocompetent individuals

**THE RISK OF INFECTION RESULTS FROM
THE INTERACTION OF AT LEAST 3
FACTORS:**

Organ dysfunction

Pathogen/environment

Exposure intensity and virulence
Community-acquired
Hospital-acquired
Reactivation of latent infection



Net state of immunosuppression

Factors related to Transplant:

Immunosuppression:

- **Prior Transplant, within a year**
- **Immunosuppressive Regimens:**
 - ATG: Profound T-cell depletion
 - MTX: Mucosal injury, prolonged neutropenia
 - PTCY: More bacterial (& viral) infections,
- **H/O documented infection within 90 days**
- **Relapse Disease**
- **Neutropenic Phase**
- **CD4 cytopenia (<200/cmm)**
- **Steroid Therapy: >1mg/Kg/D**
- **Immunosuppressive Viruses**
 - CMV, HIV, HHV
- **Age > 45 Vs < 19**

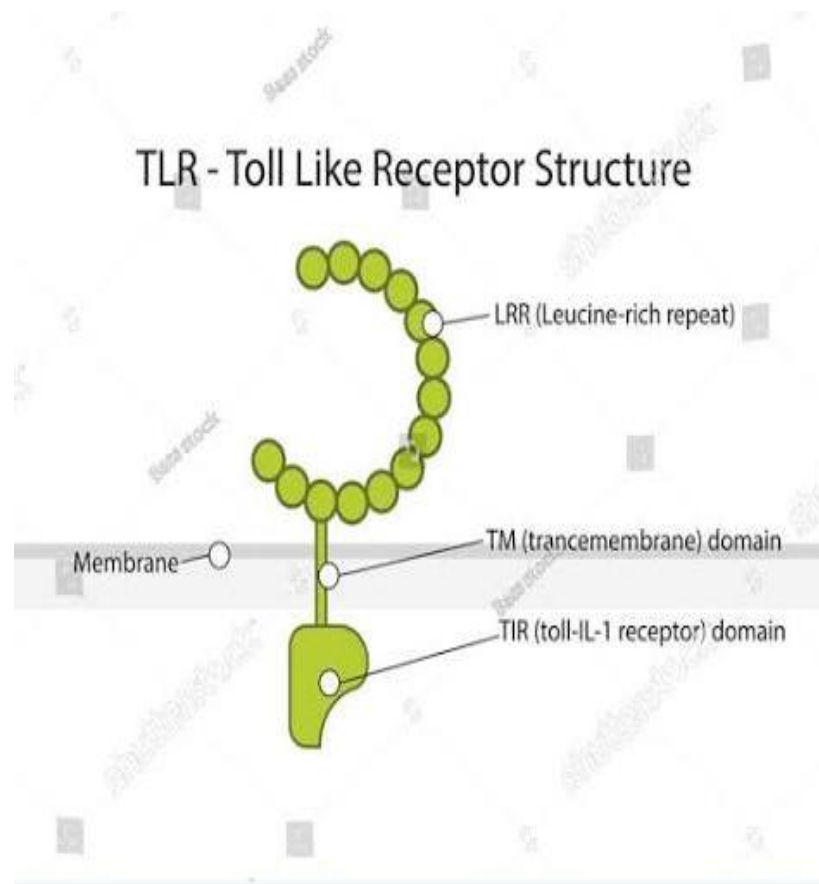
Graft Characteristics:

- **Graft Source:**
 - PBSC : Rapid Engraftment, but more cGVHD
 - UCB: Slow Engraftment & less GVHD
- **Type of Tx:**
 - MRD
 - MUD
 - Haplo
- **T- Cell Depletion:**
 - High risk for prolonged neutropenia & neutropenic infections, though,
 - Low risk of GVHD & related infections.
- **CD34 Cell Dose :**
 - <2 Vs
 - > 2.5

Immuno-genetics factors...

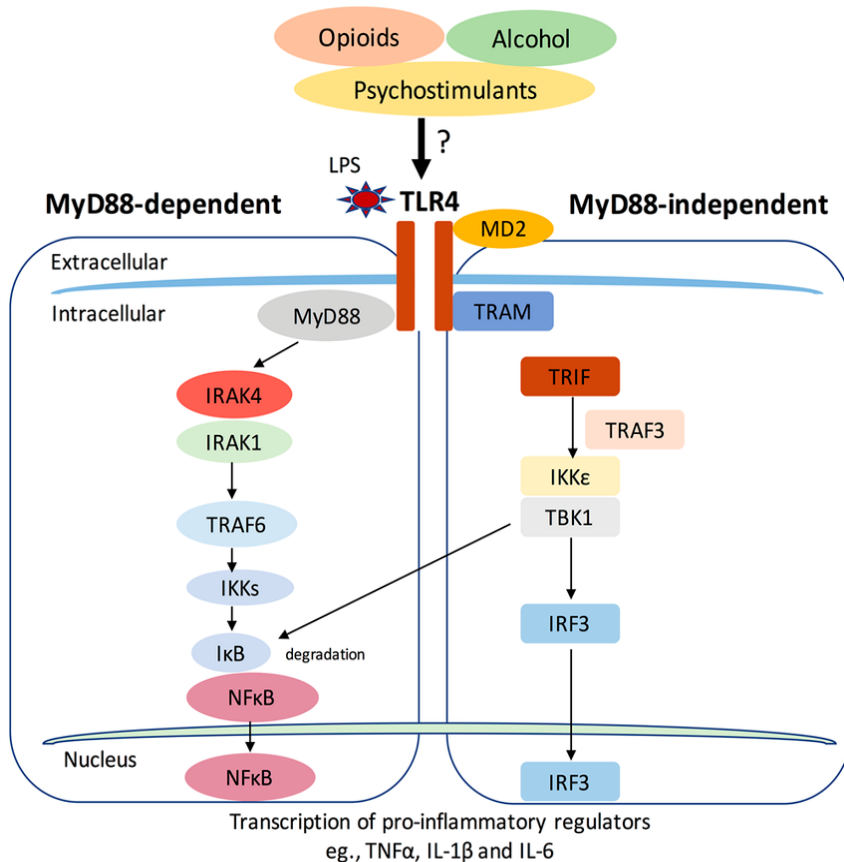
The gene polymorphism in recipients or donors can increase the risk of infections in recipient

- **Gene Polymorphisms in donors/ recipients**
 - TLR gene for Toll-like receptor 4

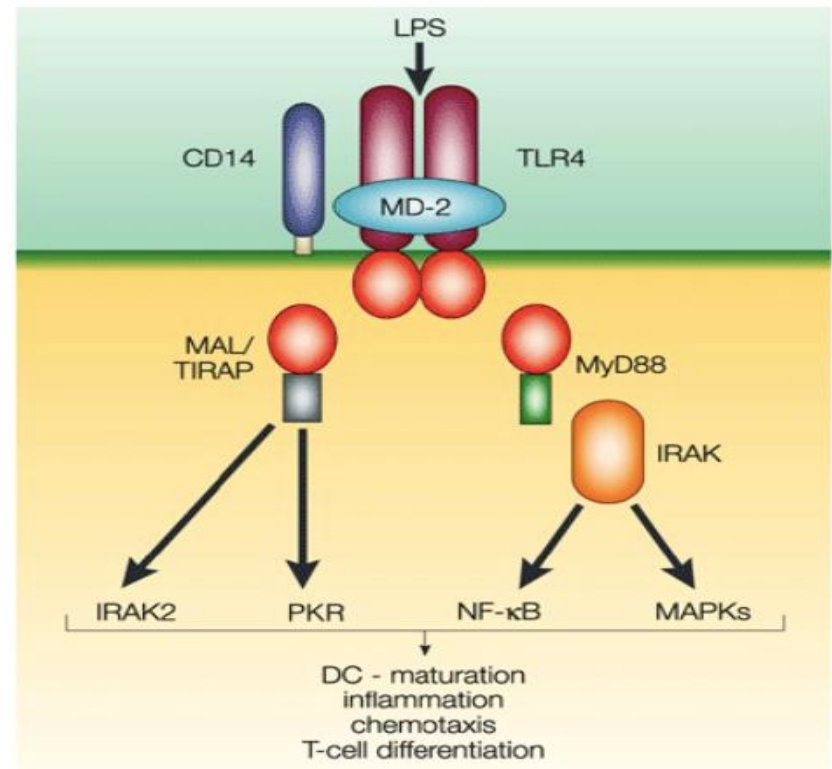


Toll-like Receptor 4(TLR-4) signaling pathway mutations can increase susceptibility to infections

Health

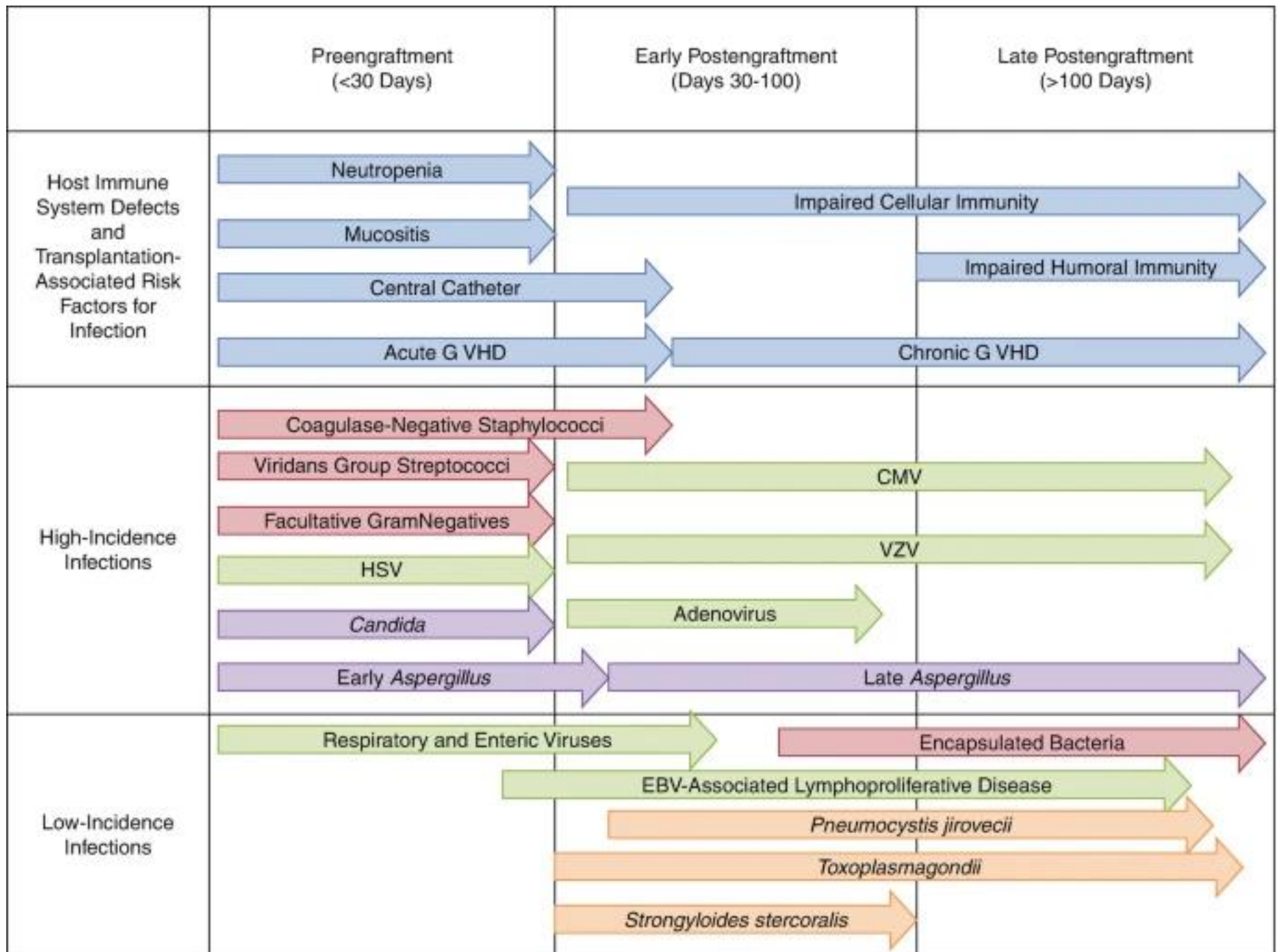


Bacterial infections

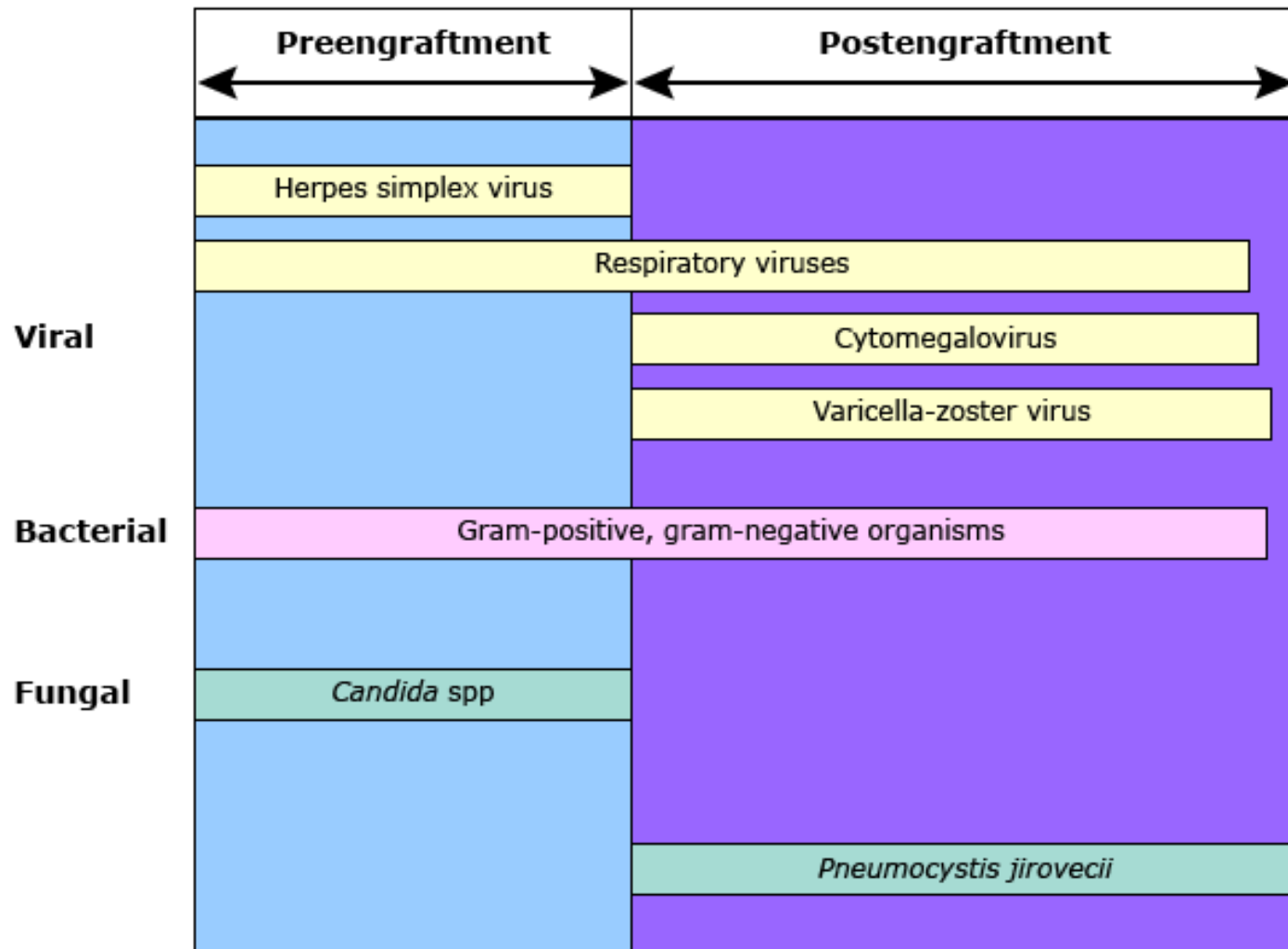


Bacterial infections are more common early during Post BMT period

TIMELINE OF BMT INFECTIONS



Typical timing of infections among autologous hematopoietic cell recipients receiving antimicrobial prophylaxis



Epidemiology of BSIs has been changing over the years..

- **Late 20th century: Gm+ve infections were common, due to**
 - Fluoroquinolone prophylaxis: Ciprofloxacin/ Levofloxacin
 - Intravascular devices/Central Lines

Early 21st century: Gm –ve infections increased in prevalence and dominated due to

- **Antibiotic overuse**

- Widespread use of antibiotics that were more effective against Gm+ve bacteria, thus
- Creating a selective pressure that favored the survival and proliferation of Gram-negative bacteria

- **Intrinsic Resistance:**

- Gm- ve bacteria have a unique cell envelope with an outer membrane that acts as a formidable permeability barrier.
- Presence of Efflux pumps that actively expel antibiotics, makes them intrinsically less susceptible to antibiotics.

- **Acquisition of Resistance Genes:**

- Gm-ve bacteria can easily transfer genetic material, including antibiotic resistance genes, between different species and genera via plasmids

More recently...

- **Significant rise in bacterial blood-stream Infections**
- **Mortality ranges from 5% up to 60% in cases of MDR infections.**
- **Gradually resistant infections have been rising:**
 - MDR-GNB
 - ESBL
- **3 newer MDROs have appeared more recently**
 - CR-E
 - CR-PA
 - CR-AB
- **All these can increase the morbidity & mortality**



Article

Bacterial Bloodstream Infections after Allogeneic Hematopoietic Stem Cell Transplantation: Etiology, Risk Factors and Outcome in a Single-Center Study

Jessica Gill ^{1,†}, Alessandro Busca ^{2,†} , Natascia Cinatti ³, Roberto Passera ⁴ , Chiara Maria Dellacasa ², Luisa Giaccone ² , Irene Dogliotti ², Sara Manetta ², Silvia Corcione ^{5,*} and Francesco Giuseppe De Rosa ^{5,‡}

¹ Division of Hematology, Department of Molecular Biotechnology and Health Sciences, University of Torino, A.O.U. Città della Salute e della Scienza di Torino, 10126 Turin, Italy; jessica.gill@edu.unito.it

² Department of Oncology and Hematology, SSD Stem Cell Transplant Center, A.O.U. Città della Salute e della Scienza di Torino, 10126 Turin, Italy

³ Division of Internal Medicine, Department of Medical Sciences, University of Torino, A.O.U. Città della Salute e della Scienza di Torino, 10126 Turin, Italy

⁴ Department of Medical Sciences, A.O.U. Città della Salute e della Scienza di Torino, University of Torino, 10126 Turin, Italy

⁵ Division of Infectious Diseases, Department of Medical Sciences, A.O.U. Città della Salute e della Scienza di Torino, University of Torino, 10126 Turin, Italy

* Correspondence: silvia.corcione@unito.it

† These authors contributed equally to this work.

‡ These authors contributed equally to this work.

Abstract: Background—Allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients are subject to major risks for bacterial bloodstream infections (BSIs), including emergent multidrug-resistant (MDR) organisms, which still represent the main cause of morbidity and mortality in transplanted patients. Methods: We performed an observational, retrospective, single-center study on patients undergoing allo-HSCT between 2004 and 2020 at the Stem Cell Transplant Unit in Turin to assess the incidence, etiology, and outcomes of BSIs and to explore any risk factors for bacteriaemia. Results: We observed a total of 178 bacterial BSIs in our cohort of 563 patients, resulting in a cumulative incidence of 19.4%, 23.8%, and 28.7% at 30, 100, and 365 days, respectively. Among isolated bacteria, 50.6% were Gram positive (GPB), 41.6% were Gram negative (GNB), and 7.9% were polymicrobial infections. Moreover, BSI occurrence significantly influenced 1-year overall survival. High and very high Disease Risk Index (DRI), an haploidentical donor, and antibacterial prophylaxis were found as results as



Citation: Gill, J.; Busca, A.; Cinatti, N.; Passera, R.; Dellacasa, C.M.; Giaccone, L.; Dogliotti, I.; Manetta, S.; Corcione, S.; De Rosa, F.G. Bacterial Bloodstream Infections after Allogeneic Hematopoietic Stem Cell Transplantation: Etiology, Risk

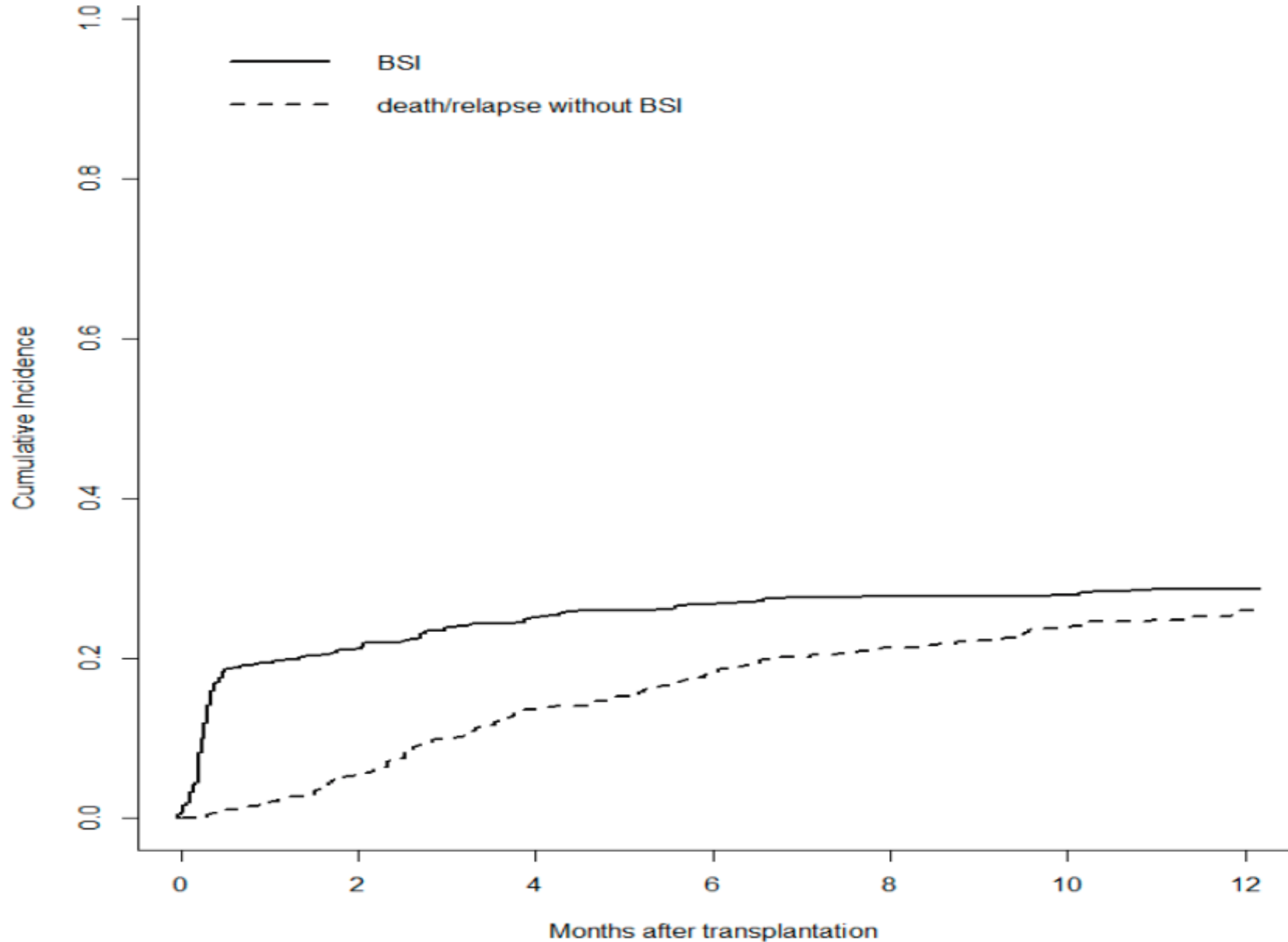
Our present study aimed to estimate the incidence rates and the current clinical features of BSIs during the first year post-HSCT and to explore the impact of BSIs on HSCT outcomes. This study has been enriched with the analysis of risk factors for BSI that might potentially guide a tailored approach for allotransplanted patients.

2. Materials and Methods

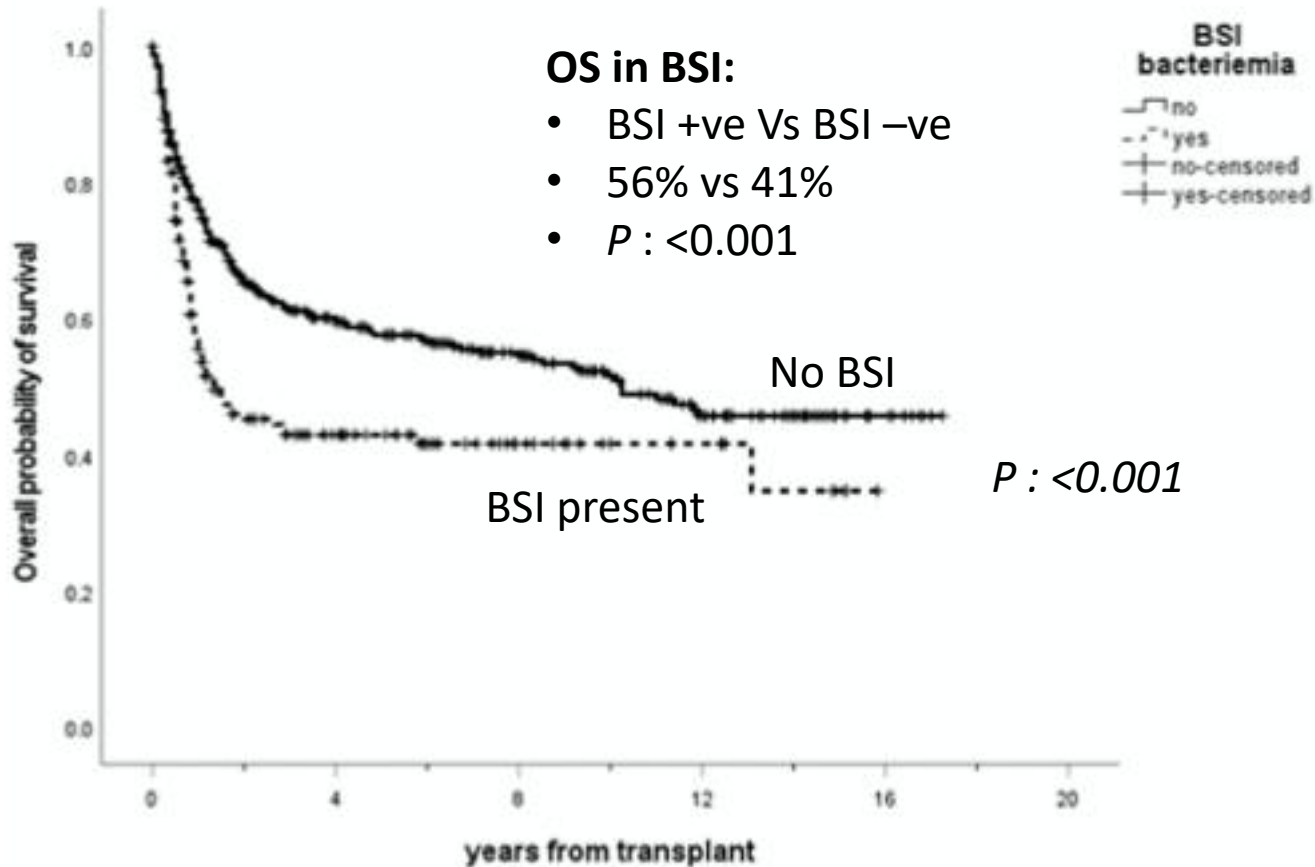
2.1. Study Design and Data Collection

This observational retrospective study analyzed all consecutive episodes of BSIs occurring in adult patients who had undergone an allo-HSCT for hematologic malignancies between January 2004 and December 2020 at the Stem Cell Transplant Center, AOU Città

The CI rate of Bacterial Blood Stream infections (BSIs) is 19.4%, 23.8% & 28.7% at 1, 3 and, months after transplant for the whole cohort.



OS was better in non-BSI situations



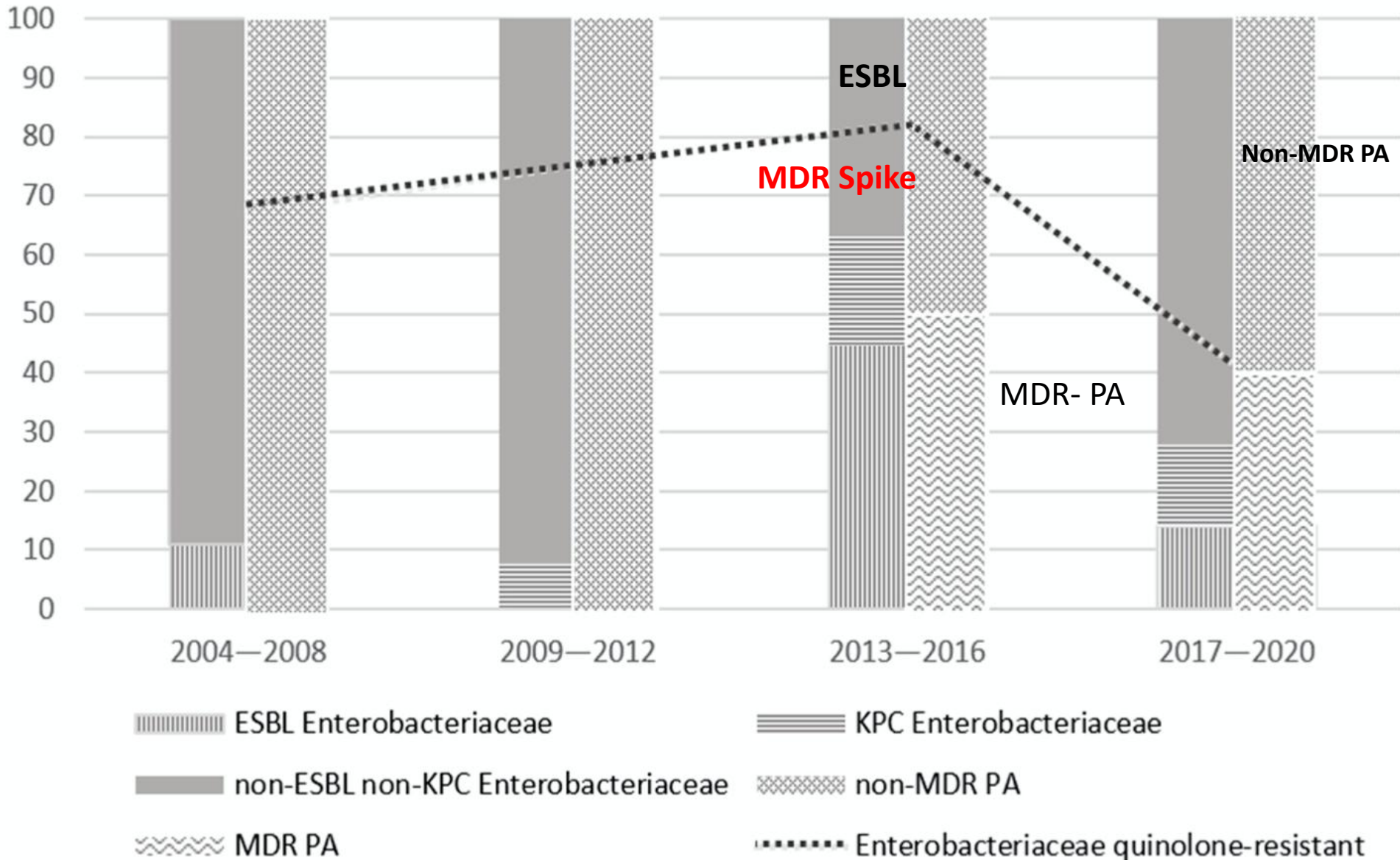
Etiology of BSIs

	Whole Cohort (n = 178)	Pre-Engraftment (day 0–30) (n = 109)	Post-Engraftment (day ≥ 31) (n = 69)
Gram-positive Bacteria	90	52	38
Coagulase-negative <i>staphylococci</i> ^a ★	66	34	32
<i>Staphylococcus aureus</i> (n. MRSA)	3 (2 MRSA)	1 (1 MRSA)	2 (1 MRSA)
<i>Streptococci mitis</i> ★	8	7	1
Other streptococci	4	2	2
<i>Enterococcus</i> (n. VRE)	8 (1 VRE)	7 (1 VRE)	1 0
<i>Corynebacterium</i> spp.	1	1	-

Etiology of BSI...

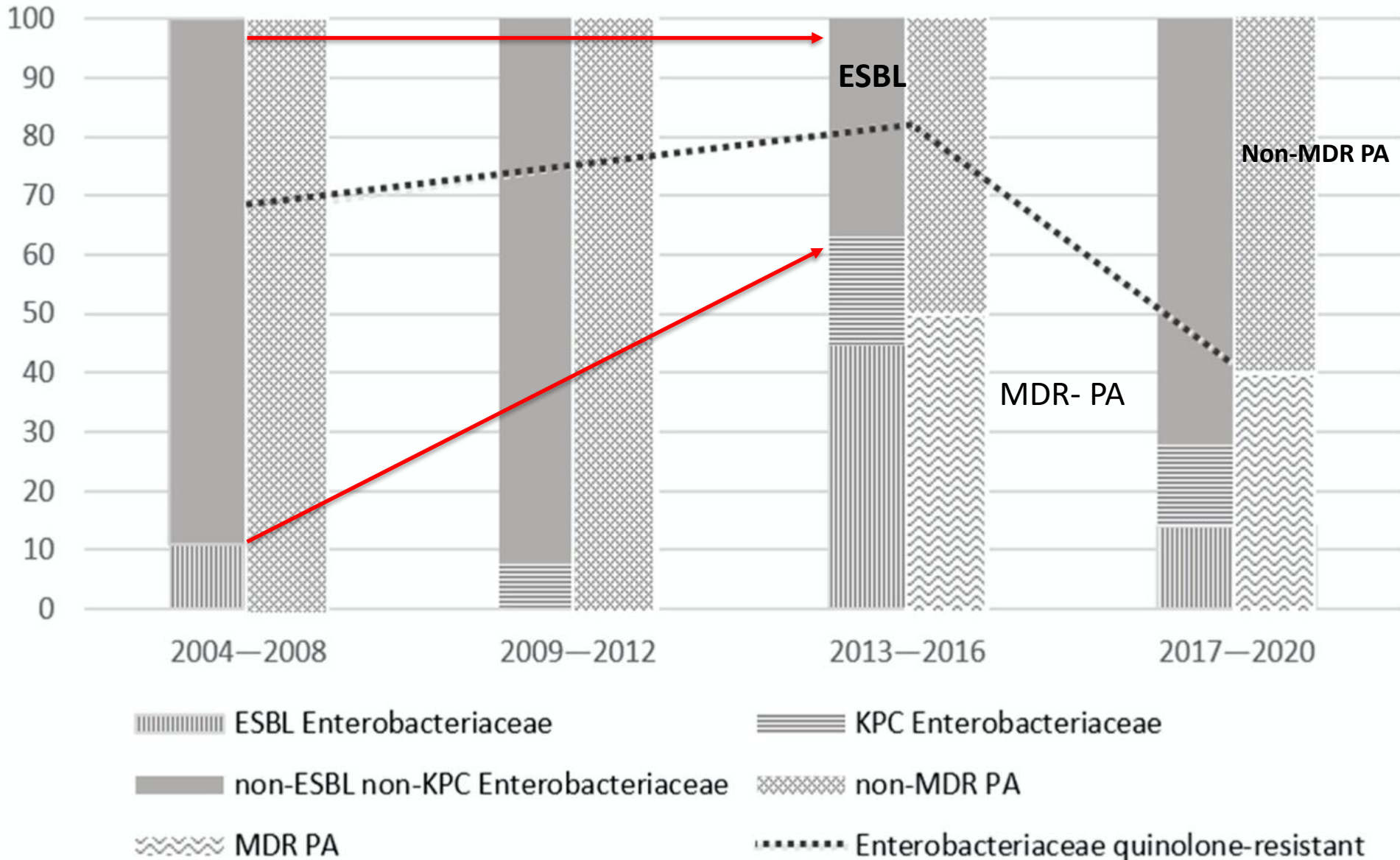
	Whole Cohort (n = 178)	Pre-Engraftment (day 0–30) (n = 109)	Post-Engraftment (day ≥ 31) (n = 69)
Gram-negative Bacteria	74	47	27
<i>Escherichia coli</i> (n. ESBL) ★	39 (7 ESBL)	30 (6 ESBL)	9 (1 ESBL)
<i>Klebsiella</i> (n. ESBL) ★	12 (2 ESBL)	7 (0 ESBL)	5 (2 ESBL)
(n. KPC)	(7 KPC)	(5 KPC)	(2 KPC)
<i>Enterobacter</i> (n. ESBL)	4 (1 ESBL)	3 (1 ESBL)	1
<i>Stenotrophomonas maltophilia</i>	3	-	3
<i>Citrobacter</i>	1	1	-
<i>Proteus mirabilis</i>	2	1	1
<i>Pseudomonas aeruginosa</i>	10	3	7
(n. MDR) ★	(3 MDR)	(1 MDR)	(2 MDR)
<i>Fusobacterium</i>	2	2	-
<i>Bacteroides</i>	1	-	1
Polymicrobial BSI ★	14	10	4
MDR-gram-negative bacteria ★	20	13	7

Susceptibility Patterns of GNB isolates



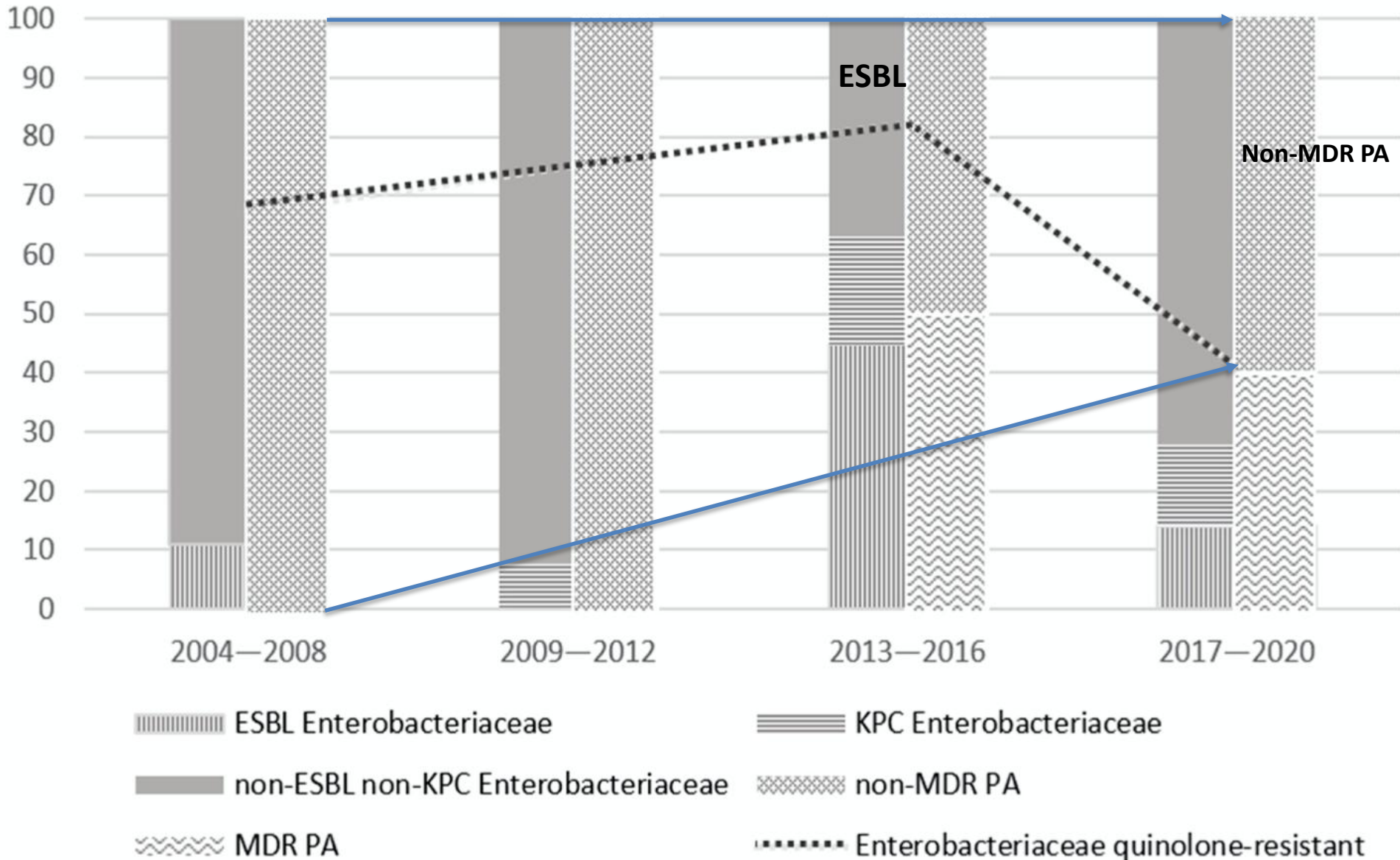
1. Decrease in ESBL infections, 2. Rise in MDR PA

Susceptibility Patterns of GNB isolates



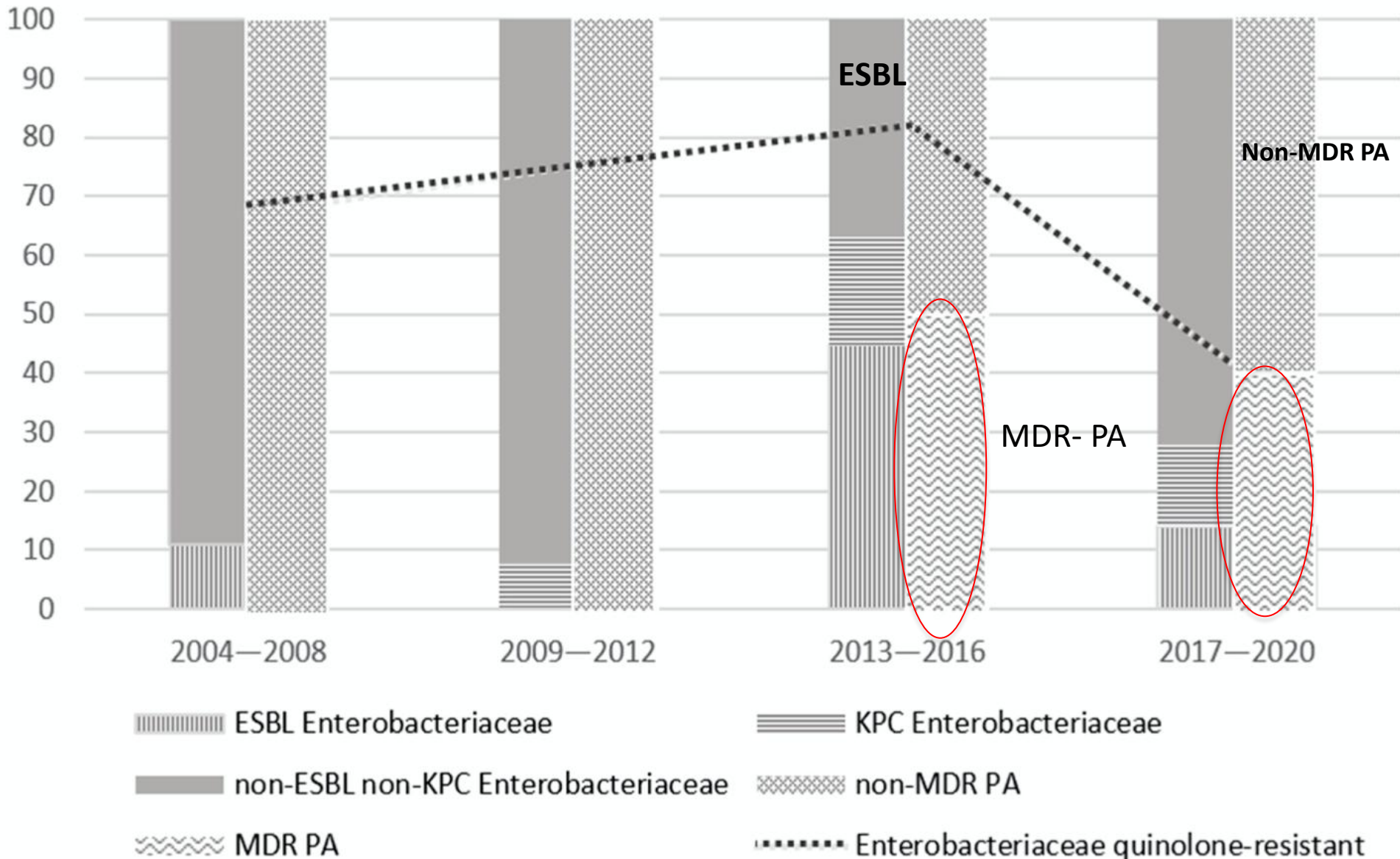
1. Decrease in ESBL infections, 2. Rise in MDR PA

Susceptibility Patterns of GNB isolates



1. Decrease in ESBL infections, 2. Rise in MDR PA

Susceptibility Patterns of GNB isolates

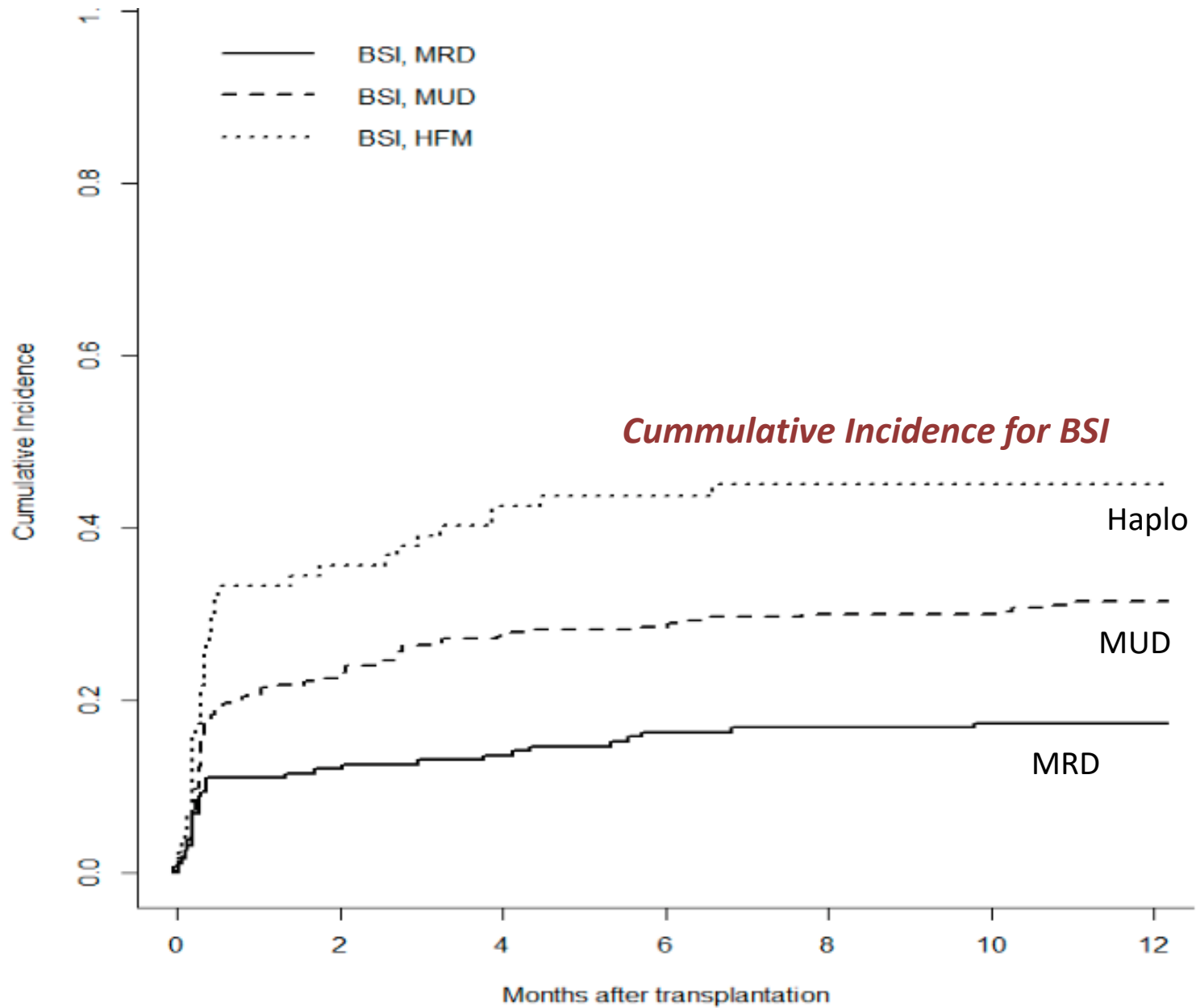


1. Decrease in ESBL infections, 2. Rise in MDR PA

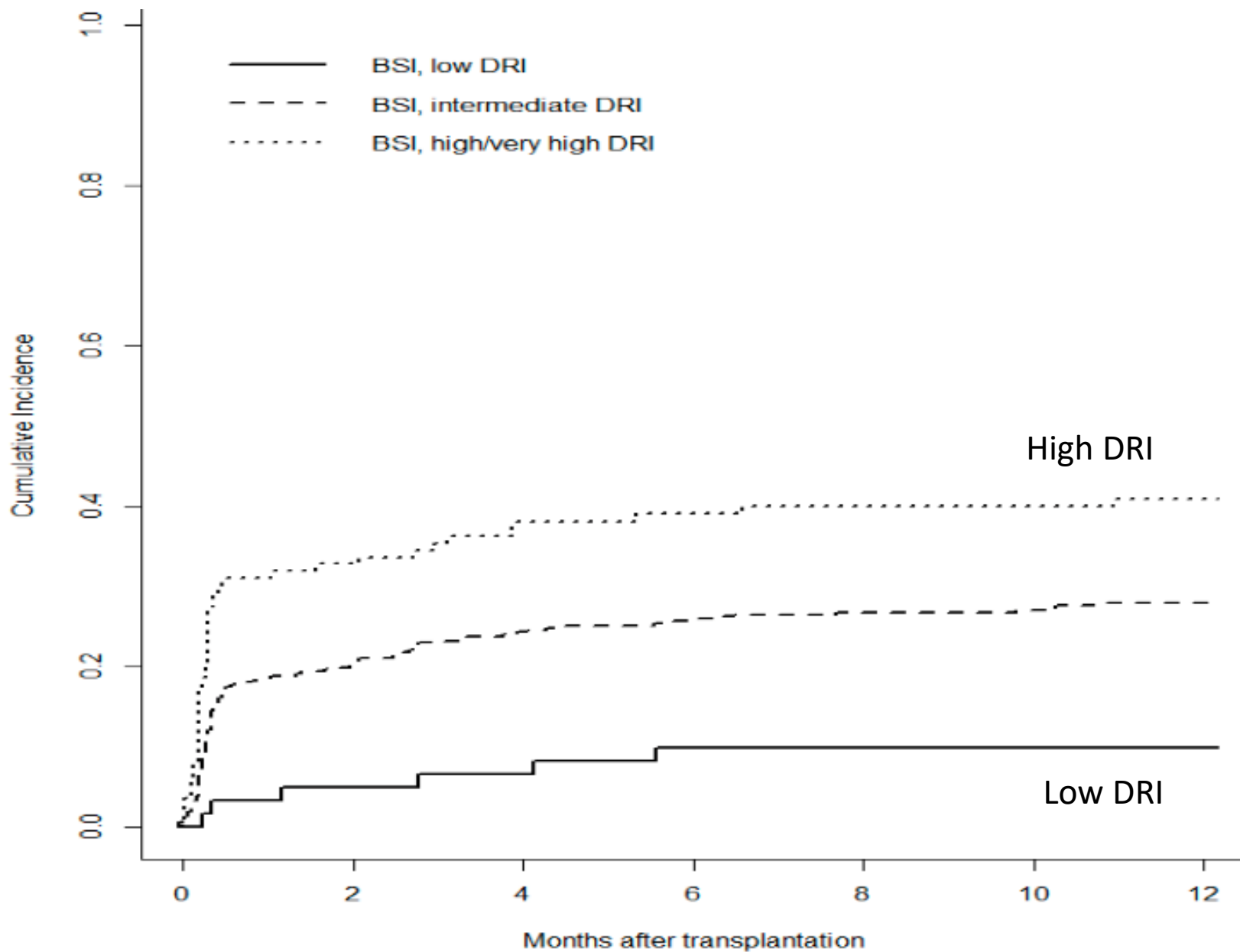
Blood Stream Infections (BSIs)

3 FACTORS ARE SIGNIFICANTLY ASSOCIATED

1. Graft Source: Type of Tx



2. Premorbid conditions (Risk Index DRI)



3. Antibiotic Prophylaxis



antibiotics



Article

Impact of Discontinuing Levofloxacin Prophylaxis on Bloodstream Infections in Neutropenic Hematopoietic Stem Cell Transplantation Patients

Thaís Guimarães ¹, Igor Carmo Borges ², Fernanda de Souza Spadão ¹, Livia Mariano ³, Marina de Mattos Nascimento ³, Hermes Higashino ², Flavia Rossi ⁴, Vanderson Rocha ³ and Silvia Figueiredo Costa ^{2,*}

¹ Department of Infection Control, Instituto Central, Hospital das Clínicas, University of São Paulo, São Paulo 05508-220, Brazil

² Infectious Diseases Department, Hospital das Clínicas, University of São Paulo, São Paulo 05508-220, Brazil

³ Hematology Department, Hospital das Clínicas, University of São Paulo, São Paulo 05508-220, Brazil

⁴ Microbiology Laboratory, Central Laboratory Division, Hospital das Clínicas, University of São Paulo, São Paulo 05508-220, Brazil

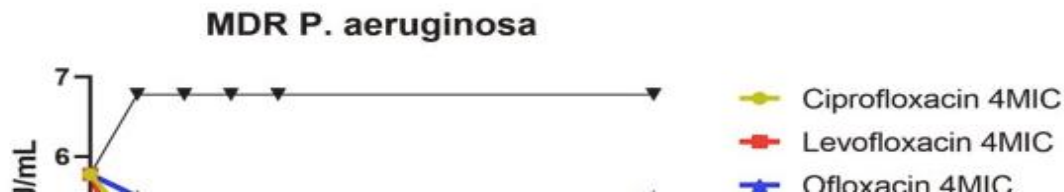
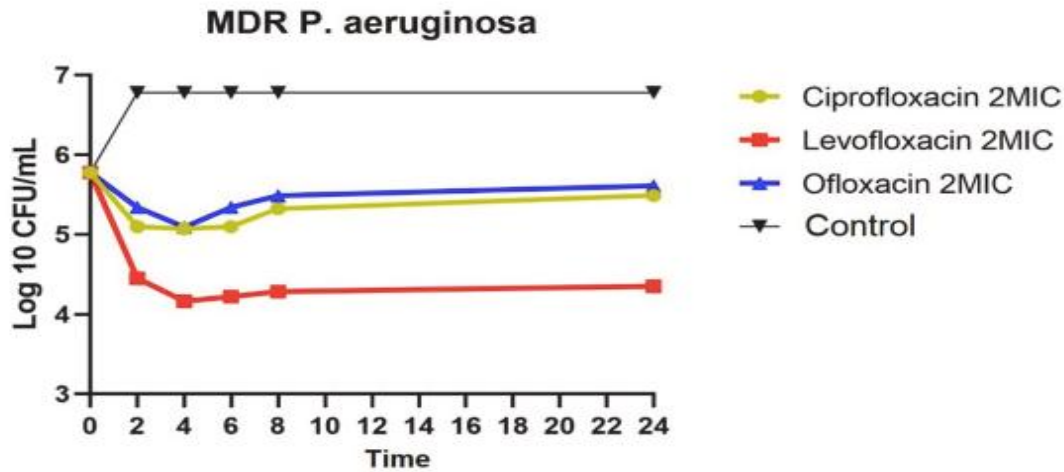
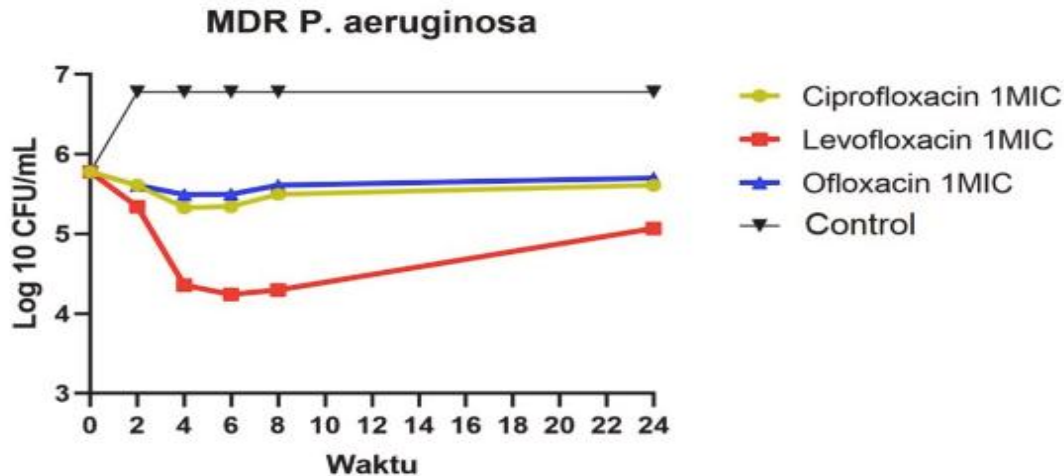
* Correspondence: silviacosta@usp.br; Tel.: +55-11-3061-7011

Abstract: Multidrug-resistant pathogens have emerged worldwide. We have driven the hypothesis that the non-use of fluoroquinolone prophylaxis during neutropenia could reduce antibiotic resistance in Gram-negative bacteria that cause bloodstream infections (BSIs) in hematopoietic stem cell transplantation (HSCT) patients and that this change in resistance pattern could lead to an impact on BSI mortality. This is a quasi-experimental study comparing BSI incidence, resistance patterns of bacteria that cause BSI, and BSI mortality when levofloxacin prophylaxis was routine for neutropenic HSCT patients (2016–2018) to when fluoroquinolone prophylaxis was discontinued in our center (2019). Bivariate comparisons and multivariate logistic regression models were used for analyses. A total of 310 HSCTs (66 (21%) allogeneic and 244 (79%) autologous) were performed during the study period. Sixty (19%) patients had BSIs, 30 in each evaluated period. The discontinuation of levofloxacin prophylaxis was associated with an increase in BSI incidence and a decrease in the resistance rates of causative BSI bacteria and in BSI 30-day mortality. The increase in the rate of resistant bacteria causing BSI and in BSI mortality might outweigh the benefits of a decrease in BSI



Citation: Guimarães, T.; Borges, I.C.; Spadão, F.d.S.; Mariano, L.; Nascimento, M.d.M.; Higashino, H.; Rossi, F.; Rocha, V.; Costa, S.F. Impact of Discontinuing Levofloxacin Prophylaxis on Bloodstream Infections in Neutropenic

Infection Rates are better in prophylaxis group



your rese...

But, Bacterial Infections continue to occur at an highrate among Tx recipients.

- **Leading to high morbidity & mortality**
- **CI of 19% & 28% at Day-30 as well as 1 Yr post Tx. (21%- 55%)**
- **There is progressive increase in GNB over GPB, with a preponderance of Enterobacteriaceae &**
- **This is when prophylaxis was being uniformly followed.**

Antibiotic prophylaxis in Transplant

***DOES IT REALLY HELP ? OR,
DOES IT HARM, ACTUALLY!***

721.CLINICAL ALLOGENEIC TRANSPLANTATION: CONDITIONING REGIMENS, ENGRAFTMENT, AND ACUTE TRANSPLANT TOXICITIES | NOVEMBER 5, 2020

Volume 136, Issue Supplement 1

November 5 2020

Omitting Fluoroquinolones Antibiotic Prophylaxis in Allogeneic Hematopoietic Stem Cell Transplantation Does Not Increase Gram-Negative Bacteremia Rate or Transplant-Related Mortality

Israel Henig, MD, Oryan Henig, Haggai Bar-Yoseph, Hanin Daoud, Dana Yehudai-Ofir, MD, Ilana Oren, Tsila Zuckerman, MD



Blood (2020) 136 (Supplement 1): 34-35.

<https://doi.org/10.1182/blood-2020-142357>

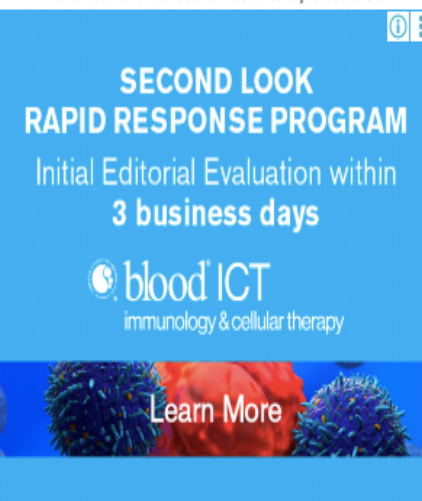


Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is widely utilized as a curative treatment for malignant and non-malignant hematological conditions. Fluoroquinolone prophylaxis (FQ-P) is demonstrated to reduce the rate of blood stream infections (BSI) caused by gram-negative bacteria (GNB) during allo-HSCT and increases overall survival (OS), making this approach the standard of care. The available data show that during the transplantation period, the intestinal microbiome diversity profoundly decreases, which is associated with a significant increase in transplant-related mortality (TRM), acute graft-versus-host disease (aGVHD) related mortality and decrease in OS. FQ-P is reported to be a dominant factor in the perturbation of the gut microbiota, leading some centers to omit or modify transplant antibiotic prophylaxis regimens. The aim of the present study has been to evaluate the effects of FQ-P omission on the prevalence of gram-negative bacteria blood stream infections (GNB-BSI), GNB susceptibility to antibiotic treatment, mortality of patients with sepsis and overall TRM.


[< Previous Article](#)

[Next Article >](#)

Advertisement intended for health care professionals



**SECOND LOOK
RAPID RESPONSE PROGRAM**
Initial Editorial Evaluation within
3 business days

 **blood ICT**
immunology & cellular therapy

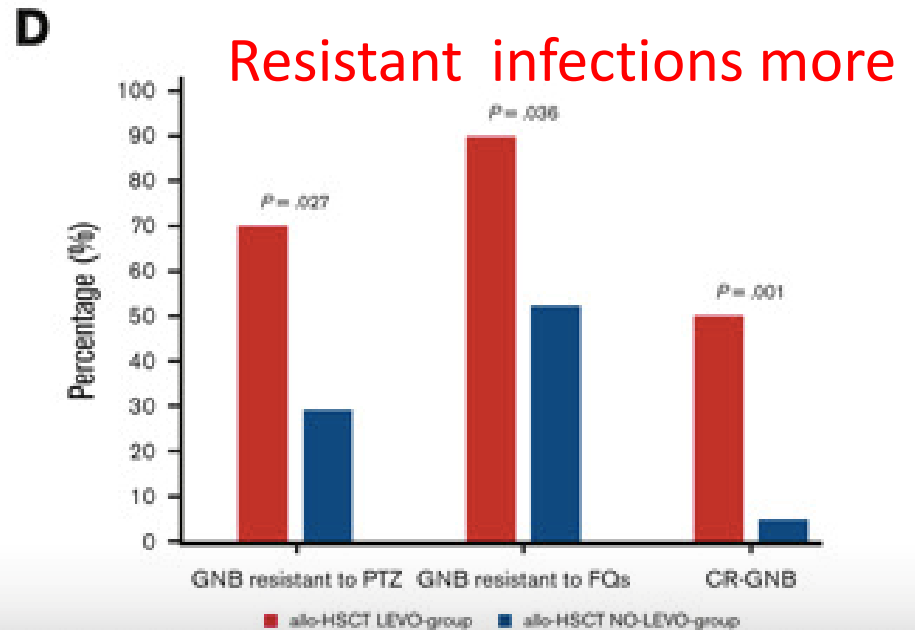
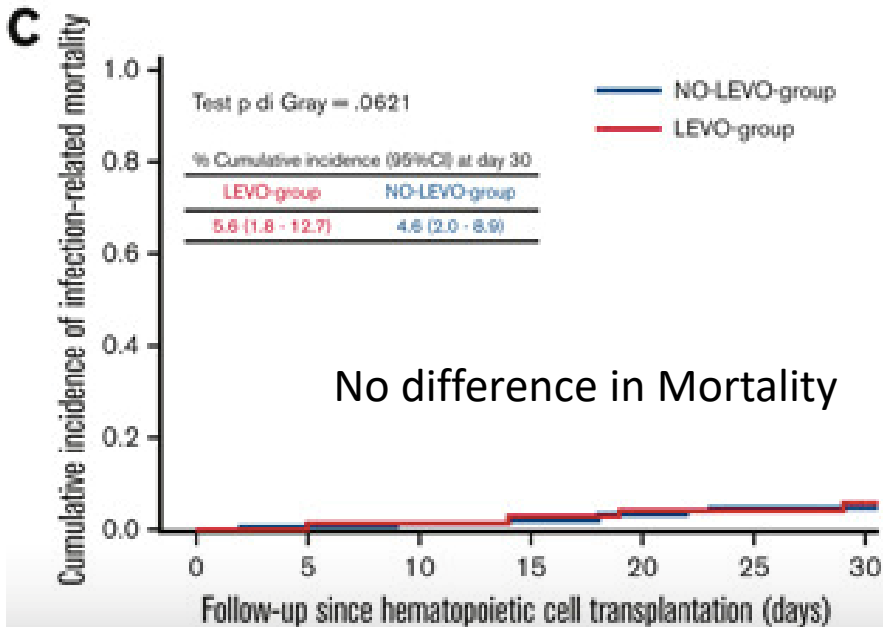
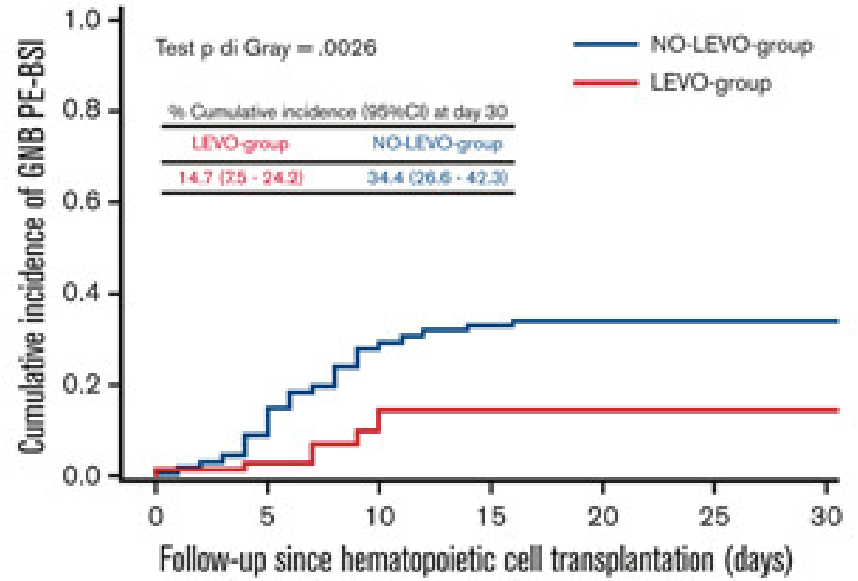
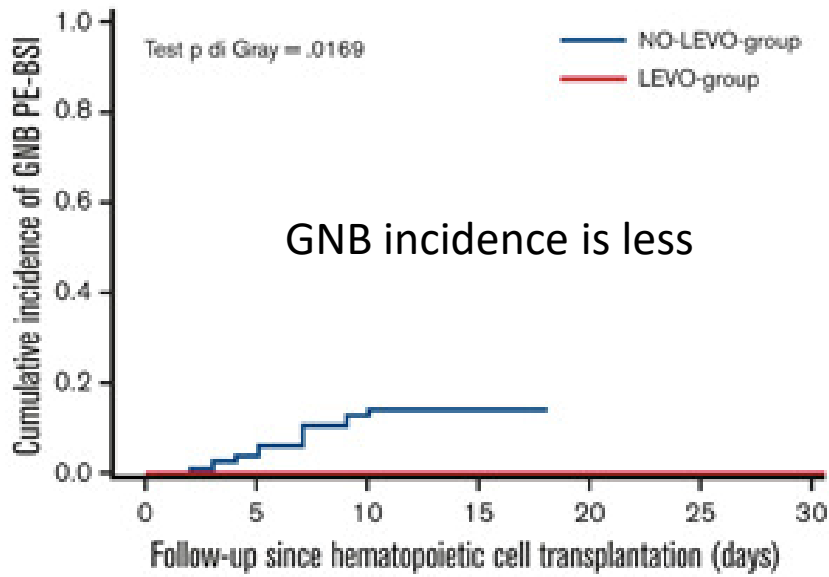
[Learn More](#)

[Sign in via your Institution](#)

Potential Articles of Interest

Impact of Omitting Prophylactic Antibiotics, to

Fluoroquinolone Prophylaxis





ELSEVIER

Transplantation and Cellular Therapy

journal homepage: www.astctjournal.org

ASTCT

American Society for
Transplantation and Cellular Therapy

Full Length Article

Allogeneic – Adult

Antibiotic Prophylaxis During Allogeneic Stem Cell transplantation—A Comprehensive Single Center Retrospective Analysis



Charlotte K.F. Neuerburg^{1,2,†}, Friederike Schmitz^{1,2,†}, Marie-Therese Schmitz³, Susanne Rehnelt^{1,2}, Martin Schumacher^{1,2}, Marjio Parčina⁴, Matthias Schmid³, Dominik Wolf^{1,5}, Peter Brossart^{1,2}, Tobias A.W. Holderried^{1,2,*}

¹ Department of Oncology, Hematology, Immuno-Oncology and Rheumatology, University Hospital Bonn, Bonn, Germany

² Center for Integrated Oncology (CIO) ABCD, Aachen Bonn Cologne Düsseldorf, Germany

³ Institute for Medical Biometry, Informatics and Epidemiology, University Hospital Bonn, Bonn, Germany

⁴ Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany

⁵ Department of Hematology and Oncology, Internal Medicine V, Comprehensive Cancer Center Innsbruck (CCCI), Tyrolean Cancer Research Institute (TKFI), Medical University Innsbruck, Innsbruck, Austria

Conclusion: Our study supports previous reports of noninferiority of allo-HSCT without use of antibiotic prophylaxis with close monitoring and rapid intervention, if infection is suspected. The trend towards improved outcomes without antibiotic prophylaxis, however, might not only be due to the absence of antibiotic prophylaxis but also due to additional progresses in the field over the recent years. While the present study is too small to draw definite conclusions, these results strongly warrant further multicenter studies addressing the potential benefit of omitting antibiotic prophylaxis during allo-HSCT.

© 2024 The American Society for Transplantation and Cellular Therapy. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

How does prophylaxis actually affect the outcome..!

“The antibiotic paradox” in allogeneic stem cell transplantation

Bone Marrow Transplantation (2025) 60:1541–1543; <https://doi.org/10.1038/s41409-025-02706-y>

TO THE EDITOR:

In allogeneic stem cell transplantation (ASCT), antibiotic treatment of neutropenic and organ infections is widely used and needed: Blood stream infections (BSI) account for the most severe manifestations and occur either during neutropenia or are associated with organ complications such as acute gastrointestinal (GI) Graft-versus-Host Disease (GvHD) or pneumonia. Patients' neutropenia and immunodeficiency demand immediate diagnosis and rapid empiric treatment until microbial culture results allow adapted treatment [1]. BSI affect between 13 and 30% of transplant recipients, markedly increasing mortality [2]. Due to the high BSI risk and the uncertain etiology of fever in the early transplant period, broad spectrum antibiotic treatment is started usually at the time of febrile neutropenia or fever of unknown origin (FUO) [3]. Current guidelines recommend de-escalation and discontinuation of antibiotics during neutropenia once infections clear [4]. Yet, a recent European survey, found only 36% of centers stop antibiotics before neutrophil recovery, resulting in long-term application.

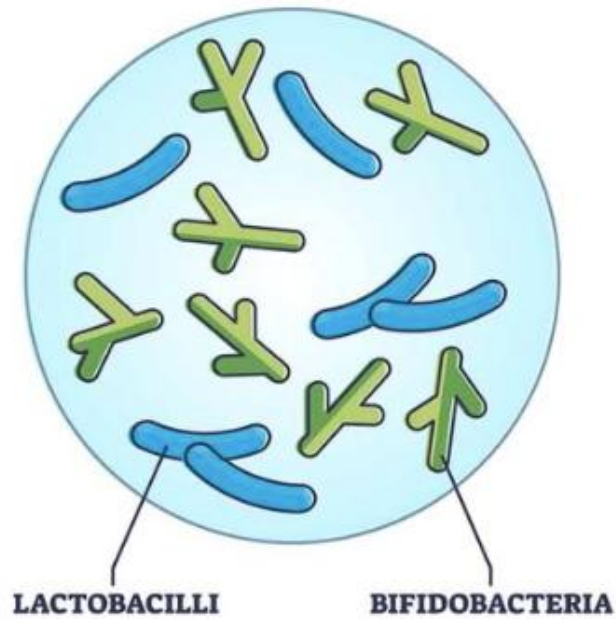
With the introduction of 16s rRNA sequencing refined microbiota analyses showed, that this early and prolonged use of antibiotics resulted in intestinal dysbiosis with loss of commensal, protective bacteria, abundance of facultative patho- gens such as Enterococci [5] as well as resistant pathogens such as Klebsiella, E. coli and Pseudomonas [6]. Dysbiosis induces increased translocation and inflammation and patients who developed dysbiosis consistently experience worse outcomes compared to patients with diverse microbiota at neutrophil engraftment indicating the double-edged nature of early antibiotic treatment. The poorer outcome is mainly explained by an elevated risk of severe acute GvHD. Mechanistically, losing metabolites such as short chain fatty acids (SCFA) and indoles increases epithelial damage [7, 8] and undermines immunoregulation of regulatory T cells and thus explain this

The Antibiotic Paradox :

(Explained by differential microbial targets)

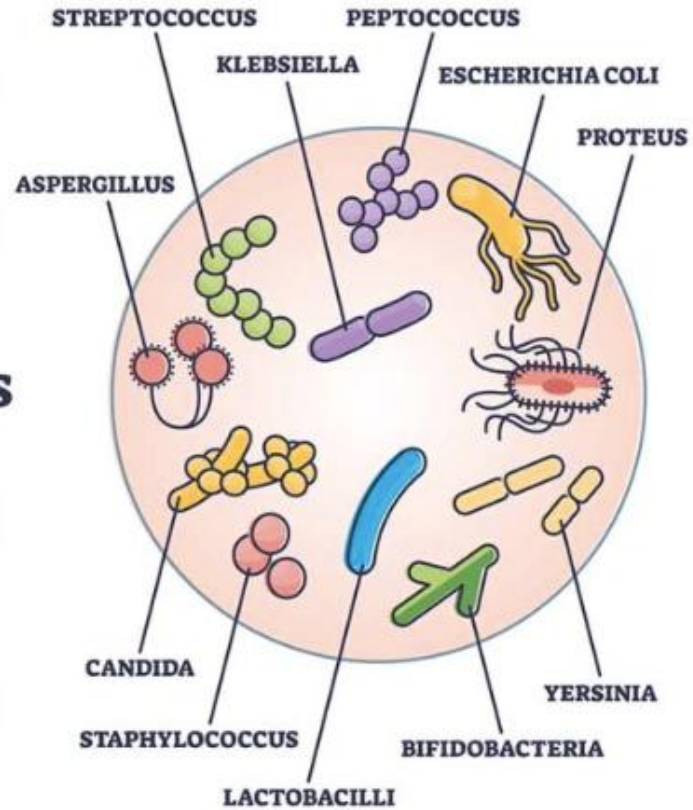
- **Early and prolonged antibiotic therapy :**
 - Affects luminal microbiota composition
 - Resulting in severe intestinal dysbiosis and
 - An increased risk for acute GI GvHD and
 - Infection associated mortality.
- **Antibiotics are not de-escalated mostly (upto 70%)**

Prophylactic antibiotics lead to Gut Dysbiosis

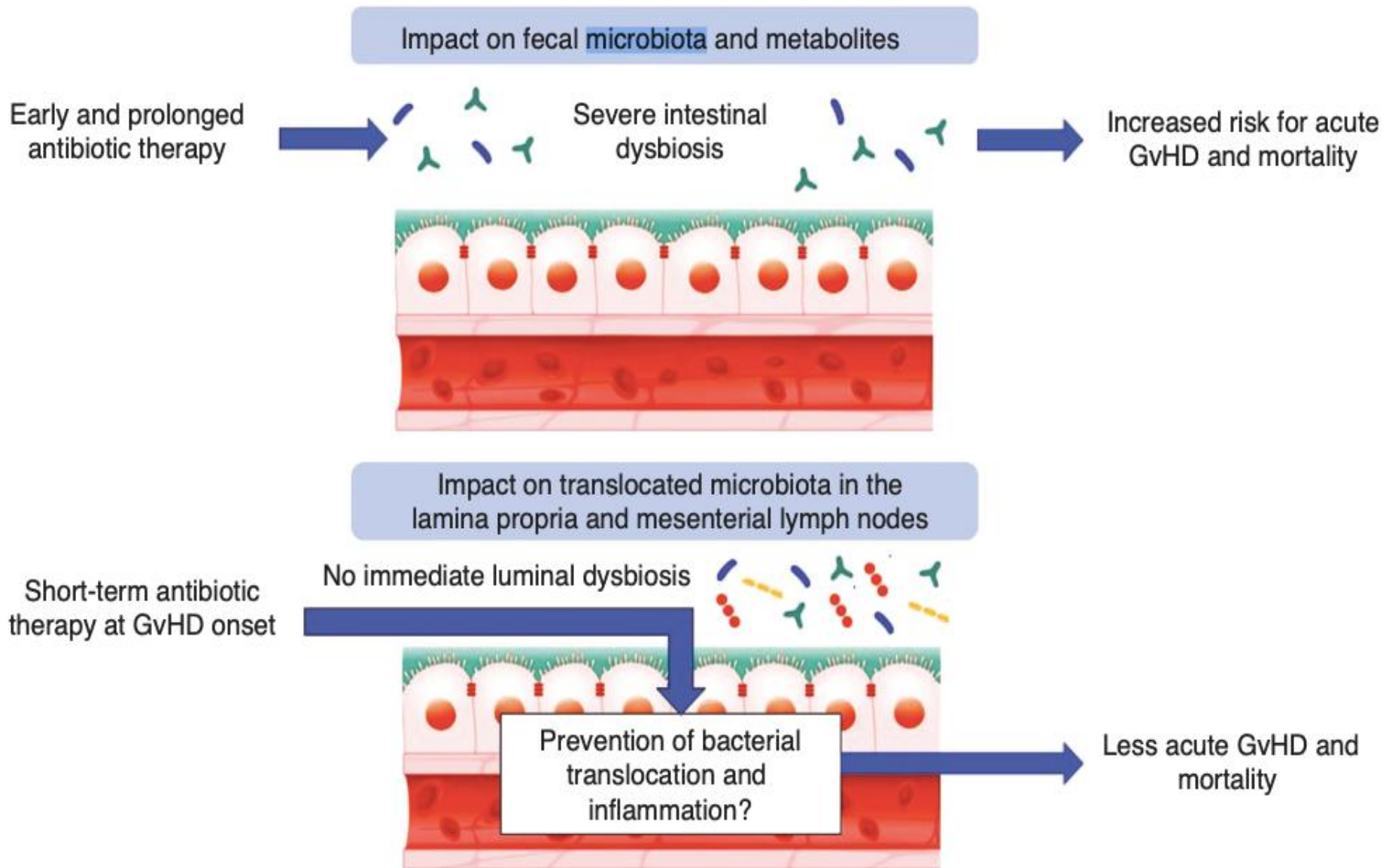


NORMAL

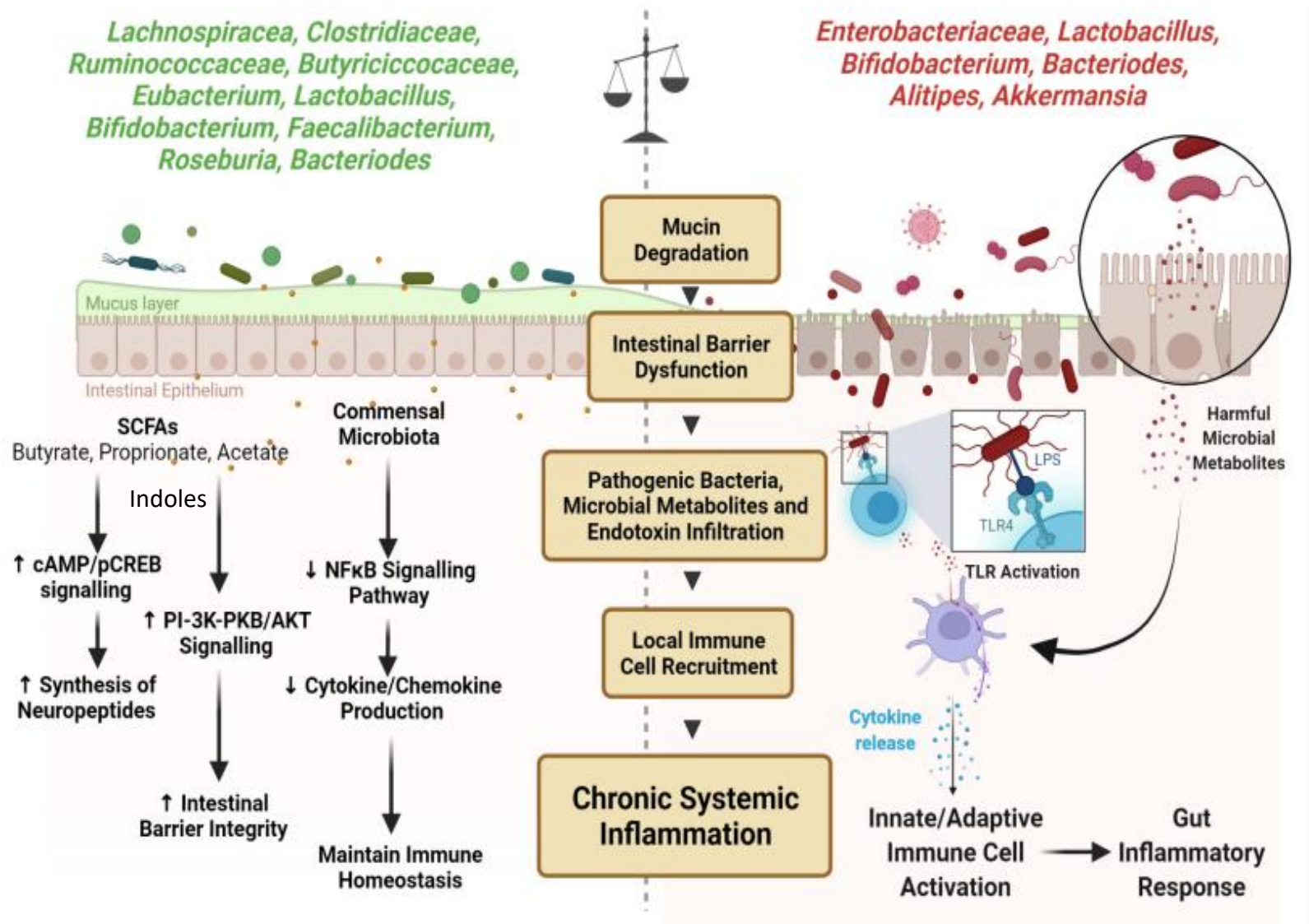
VS



DYSBIOSIS



Intestinal Dysbiosis



Epithelial Damage

Translocation




Inflammation



ORIGINAL ARTICLE



Microbiota as Predictor of Mortality in Allogeneic Hematopoietic-Cell Transplantation

Authors: Jonathan U. Peled, M.D., Ph.D. , Antonio L.C. Gomes, Ph.D. , Sean M. Devlin, Ph.D., Eric R. Littmann, B.A., Ying Taur, M.D., Anthony D. Sung, M.D., Daniela Weber, M.D.,  **+****44**, and Marcel R.M. van den Brink, M.D., Ph.D. [Author Info & Affiliations](#)

Published February 26, 2020 | N Engl J Med 2020;382:822-834 | DOI: 10.1056/NEJMoa1900623 | **VOL. 382 NO. 9**

Copyright © 2020



Abstract

BACKGROUND

Relationships between microbiota composition and clinical outcomes after allogeneic hematopoietic-cell transplantation have been described in single-center studies. Geographic variations in the composition of human microbial communities and differences in clinical practices across institutions raise the question of whether these associations are generalizable.



RELATED ARTICLES

CORRESPONDENCE | JUN 10, 2020

Microbiota and Allogeneic Hematopoietic-Cell Transplantation



PHYSICIAN JOBS

MAY 1, 2025

Hematology / Oncology Presque Isle, Maine
[Flexible Hematology/Oncology Opportunity in Maine](#)

Pediatrics, General Braintree, Massachusetts
[Pediatric Urgent Care Physician - Weekends](#)

Physical Medicine & Rehabilitation Searsport, Maine
[Interventional Pain Physician - Searsport, Maine](#)

High vs Low Diversity of Bacteria

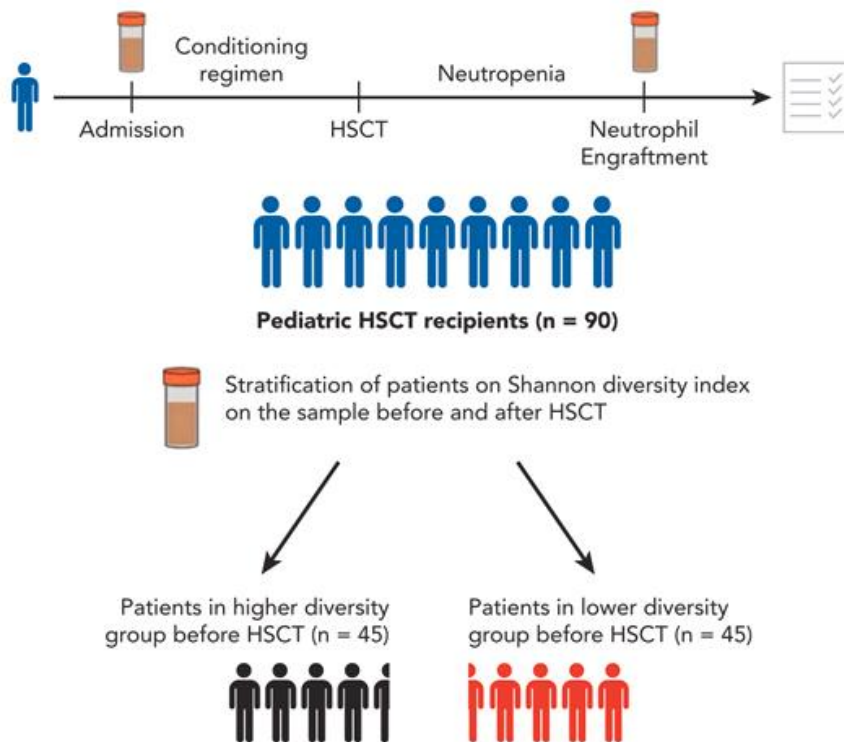
- **The study profiled >8000 fecal samples from 1300 patients &**
- **Used ribosomal RNA sequencing to stratify patients into HD (high-diversity) & LD (low- diversity) groups.**
 - HD group had low mortality as shown by the HR for death of 0.49(95% CI 0.27-0.92) as compared to
 - LD group with HR of 0.72(95% CI 0.55-0.92)
 - HD group also had a low incidence of GVHD

Composition of GUT microbiota has an impact on the health & immune response

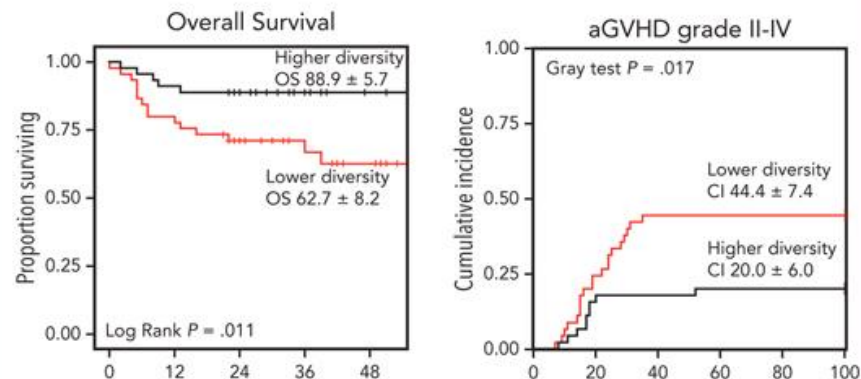
- **‘Higher Diversity (HD)’ of intestinal bacteria is associated with lower mortality & lower rates of deaths attributable to GVHD**
- **Increase in potentially pathogenic bacteria & loss of diversity in bacterial taxa is associated with higher incidence of GVHD**

Gut Microbiota Diversity Before Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) as a Predictor of Mortality in Children

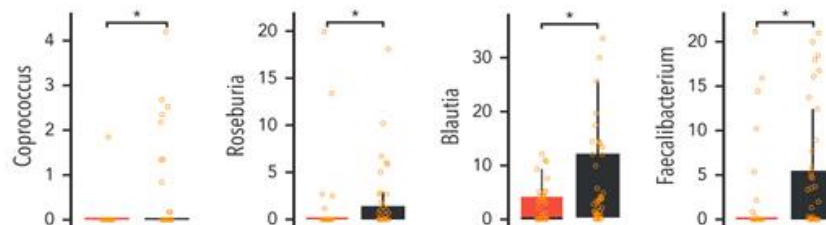
Patients and Methods



Main Outcomes



Gut microbiota composition at genus



SCFA producing commensals

Conclusion: Higher pre-transplant microbiota diversity correlates with better overall survival, a lower incidence of acute GVHD, and a higher abundance of short-chain fatty acid (SCFA)-producing taxa.

Antibiotic Prophylaxis

**IS NOT THE BEST WAY TO CONTROL INFECTIONS
DURING TRANSPLANT**

[Home](#) > [Annals of Hematology](#) > [Article](#)

Interventional antibiotic treatment replacing antibiotic prophylaxis during allogeneic hematopoietic stem cell transplantation is safe and leads to a reduction of antibiotic administration

Research | Published: 06 September 2024

Volume 103, pages 4687–4699, (2024) [Cite this article](#)



[Annals of Hematology](#)

[Aims and scope](#) →

[Submit manuscript](#) →

[Rosa Toenges](#) , [Fabian Lang](#), [Rakhshinda Ghaffar](#), [Sarah Lindner](#), [Vera Schlipfenbacher](#), [Julia Riemann](#), [Salem Ajib](#), [Khoulood Kouidri](#), [Anjali Cremer](#), [Bodo Weber](#), [Ngoc Thien Thu Nguyen](#), [Antje Knoch](#), [Janne Vehreschild](#), [Hubert Serve](#) & [Gesine Bug](#)


 678 Accesses  2 Citations  12 Altmetric  2 Mentions [Explore all metrics](#) →

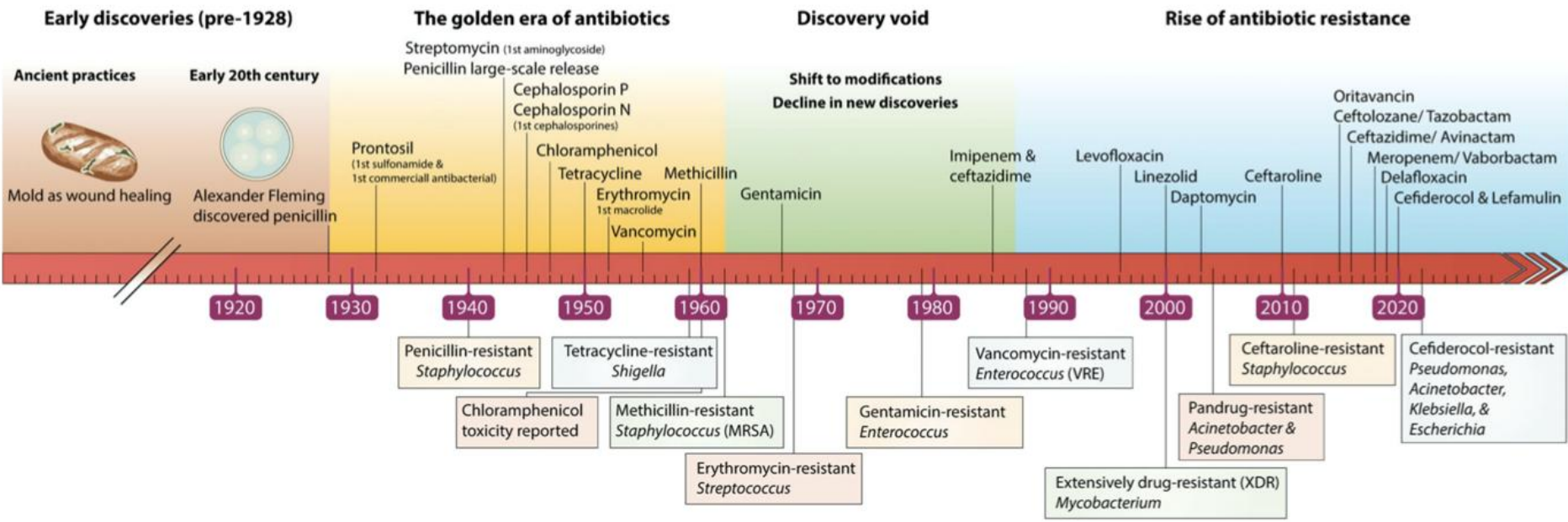
Abstract

Access this article

[Log in via an institution](#) →

Subscribe and save

 Springer+ from €37.37 /Month



Appropriateness of Empirical Antibiotic Therapy in Hospitalized Patients with Bacterial Infection: A Retrospective Cohort Study

Yuting Luo^{1,*}, Zhaowang Guo^{2,*}, Ying Li^{1,*}, Hui Ouyang¹, Shanfeng Huang¹, Yuanli Chen³, Kenan Li¹, Yuxin Ji¹, Hongqiong Zhu¹, Wentao Luo¹, Xu Liu^{1,4}, Xinghua Li¹, Jinyu Xia¹, Xi Liu¹

¹Department of Infectious Diseases, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, People's Republic of China; ²Clinical Laboratory, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, People's Republic of China; ³Department of Hospital Infection Control, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, People's Republic of China; ⁴Guangdong Provincial Key Laboratory of Biomedical Imaging and Guangdong Provincial Engineering Research Center of Molecular Imaging, Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xi Liu; Xinghua Li, Department of Infectious Diseases, The Fifth Affiliated Hospital, Sun Yat-sen University, 52 Meihua East Road, Xiangzhou District, Zhuhai, 519000, People's Republic of China, Tel/Fax: +86-756-252-8592, Email: liuxi26@mail.sysu.edu.cn; lixh79@mail.sysu.edu.cn

Objective: The incidence of inappropriate and excessive empirical antibiotic therapy is unclear. The aim of this study was to determine the prevalence of different empirical antibiotic therapy prescriptions, related factors, and outcomes in hospitalized patients with bacterial infection.

Methods: A retrospective cohort study was performed and patients with bacterial infection who were admitted between October 1, 2019, and September 30, 2020, were included. Multivariable analysis was performed by the logistic regression model.

Results: A total of 536 (42.6%) of the 1257 included patients received inappropriate empirical antibiotic therapy (IEAT), and 368 (29.3%) patients received appropriate but unnecessarily broad-spectrum empirical antibiotic therapy (AUEAT). MDRO (adjusted OR

Empirical Therapy can be appropriate or inappropriate:

- **IEAT: Inappropriate Empirical Antibiotic Therapy**
- **AEAT (Appropriate Empirical Antibiotic Therapy):**
 - Empirical therapy with a regimen active against the identified pathogen based on susceptibility testing.
- **AUEAT:**
 - **A**ppropriate but **U**nnecessarily broad-spectrum **E**mpirical **A**ntibiotic **T**herapy
- **IEAT & AUEAT:** Are harmful, as they would lead to:
 - Increase in Antibiotic resistance
 - Presence of C difficile infection
 - Antibiotic related toxicities
 - High morbidity & mortality

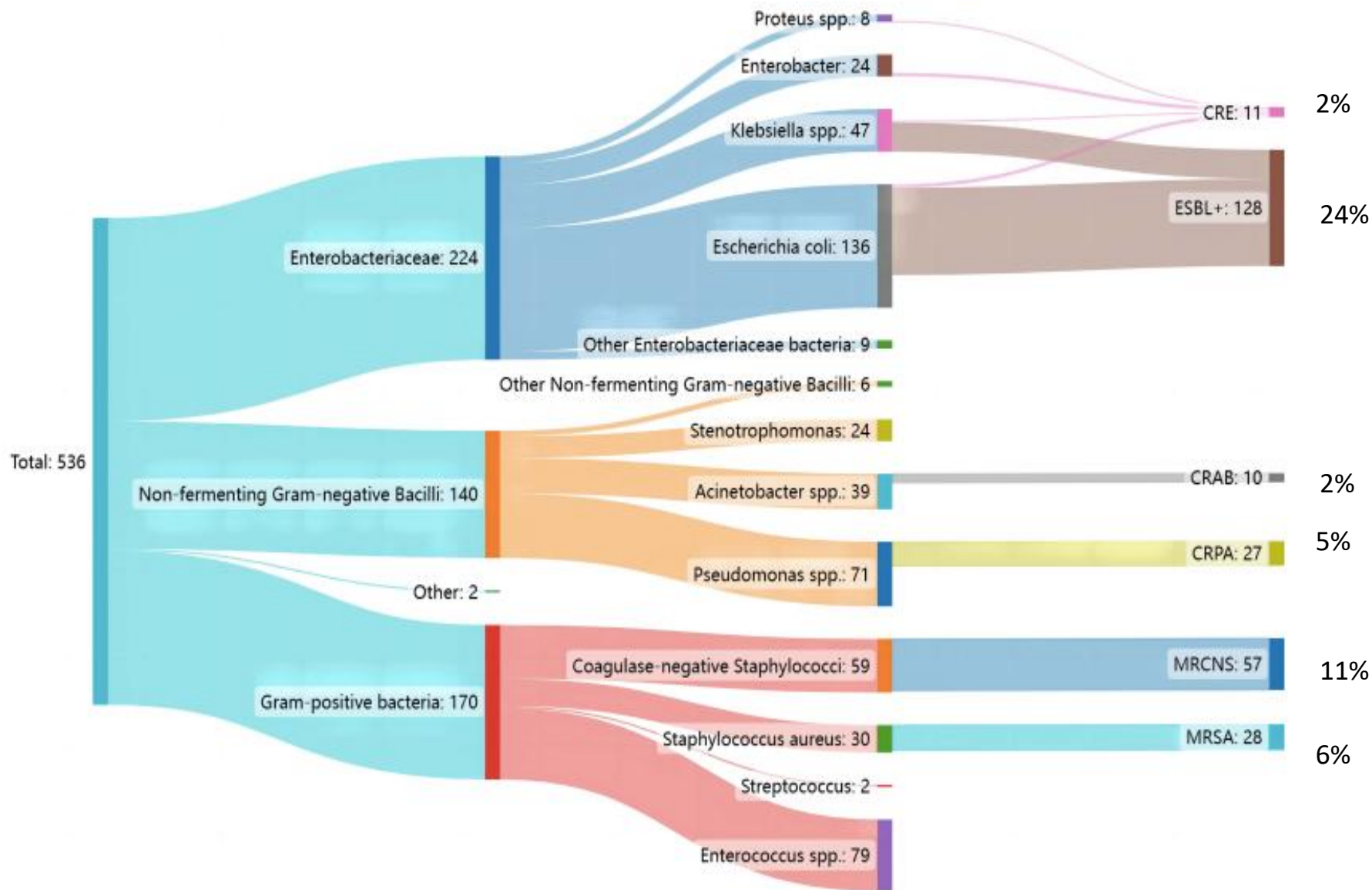


Figure 3 Pathogen distribution in patients received IEAT.

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; ESBL, extended-spectrum β -lactamase; CRE, carbapenem-resistant *Enterobacteriaceae*; CRPA, carbapenem-resistant *Pseudomonas aeruginosa*; CRAB, carbapenem-resistant *Acinetobacter baumannii*; MRCNS, methicillin-resistant coagulase negative *Staphylococcus*.

Many Risk Factors were identified for MDRO:

- **Age >65 yrs**
- **Co-morbidities**
- **Previous therapies**
- **Central lines**
- **H/O documented infections during previous chemo**
- **Prior MDRO colonization/ infection**

Choosing Appropriate Empirical Therapy during transplant

CONTINUES TO BE CHALLENGE

Antimicrobial resistance : emerging problems

- **Resistance to Imipenem has been found in:**
 - 28% of E Coli
 - 55% of K pneumoniae
 - 80% of Acenitobacter baumannii

ICMR AMR surveillance Network

Potential problems with 3rd Gen Cephalosporins:

- 1. Sub-optimal activity against Gm POS organisms**
- 2. Emergence of penicillin resistant viridans streptococci**
- 3. Bush gp-1 Beta Lactamase producing Enterobacteriaceae**
- 4. Bacteria producing ES Beta Lactamase**
- 5. Resistance among nonfermenters**

A few terms

- **Beta Lactamases:**

- Enzymes produced by bacteria to result in resistance to Beta Lactam antibiotics

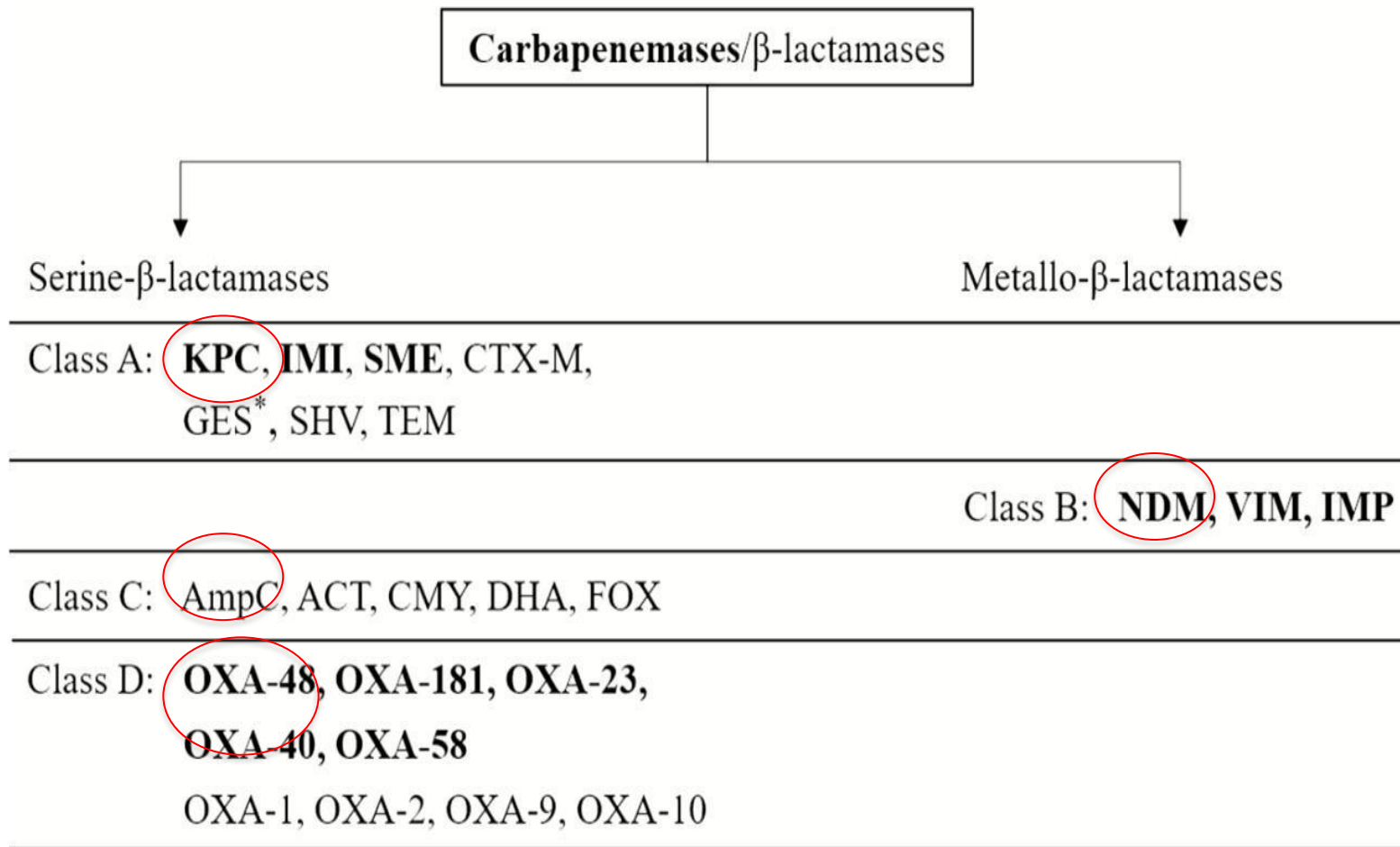
- **ESBLs:**

- B-Lactamases that hydrolyze the Extended spectrum cephalosporins like CFZ & Oxymino-monobactam
Aztreonam

- **Carbapenemases:**



- Diverse group of Beta- Lactamases that are active not only against cephalosporins, but also against Carbapenems

Classification of Carbapenemases or β -lactamases



* Some variants may possess carbapenemase activity

Ambler Classification	C	A†	D	B
Functional group	1	2	2 ^{yl}	3
Inhibitory Profile*		Clavulanic acid		EDTA
ESBLs	[Blue bar spanning C, A†, and D]			
Carbapenemases		[Purple bar spanning A†, D, and B]		
Classical examples	ESBL: AmpC	Penicillinase: TEM-1, SHV-1 ESBLs: CTX-M, TEM-3 Carbapenemase: KPC	ESBL: OXA-11 Carbapenemase: OXA-23, OXA-48	Carbapenemase: IMP, VIM, NDM

 Serine β-lactamase
 Metallo β-lactamase

MDR Gram negative organisms

- **ESBL-E**
- **CRE (Carbapenem Resistant Enterobacteriaceae)**
- **DTR- Pseudomonas Aeruginosa**
- **CRAB – Carbapenem Resistant Acinetobacter Baumannii**

Initiating Appropriate Empirical Therapy

ALGORITHMIC APPROACH

Strategies for EAT should consider:

- **Site of Infection: BSI or Lung or Catheter**
- **Epidemiological data of center**
- **In vitro sensitivity data of center**
- **Pharmacokinetic properties of antibiotic**

Antibiotics

have variable bioavailability in different organs

Penetration of antibiotics in Lungs	
Fluoroquinolones (High Intracellular Concentration)	+++
Carbapenems	+ / ++
Broad spectrum Cephalosporins	+ / ++
Ureidopenicillins	+ / ++
Aminoglycosides (Inactivated by low pH of bronchial secretions)	--

Initial Empirical therapy:

- **Monotherapy with antipseudomonal beta-lactam agent:**
 - Cefepime: High mortality ?? Not really!!
 - Meropenem
 - Piperacillin- tazobactam
- **Second agent :**
 - Aminoglycoside : High risk of GNB, Hypotension, Focal infection
 - Fluoroquinolone: Lung infection
 - Teicoplanin: central line infection
- **High Risk of resistance:**
 - Meropenem

Initial therapy....

- **Addition of Gm POS Cover at outset:**
 - Line sepsis likely
 - No definite evidence of benefit on routine use
- **Monotherapy Vs Combination at initial therapy**
- **Cefepime Vs Piperacillin-tazobactam**
- **Piperacillin- tazobactam Q8H or Q6H**
- **Infusion time:**
 - 3 hrly better than ½ hr
 - Continuous infusion

Persistent Fever

- **Median time to defervescence in HSCT:**
 - 5 days
- **Need to look for appropriate therapy:**
 - Culture results
 - Biofire/ PCR multiplex
 - Review for the causes & foci of infection
 - Addition of antifungals
- **Upgrade the antibiotics according to resistance pattern in the center**

Antibiotic Resistance:

Increasing frequency of antibiotic resistant organisms

- **Gram Positive Organisms:**
 - Coagulase negative Staph
 - MRSA
 - VRE
 - Penicillin & Ceftriaxone resistant Strepto pneumoniae
 - Intrinsic resistance to Vancomycin: Lactbacillus
- **Gram Negative Organisms:**
 - GNB: Pseudomonas aeruginosa, E coli
 - Acinetobactor

The treatment of infections caused by ESBL-E

- **Meropenem, imipenem-cilastatin, or ertapenem** are preferred for the treatment of infections caused by ESBL-E.
- For patients who are critically ill and/or experiencing hypoalbuminemia, **Imipenem-cilastatin** is the preferred carbapenems.

***Treatment of infections CRE if KPC
(Klebsiella pneumoniae carbapenemases)
production is present***

- **Ceftazidime-avibactam**
- **Meropenem-vaborbactam, and imipenem-cilastatin-relebactam** are preferred treatment options for KPC-producing infections.
- **Cefiderocol** is an alternative option.

The role of Tetracycline derivatives for the treatment of infections caused by CRE

- **Beta Lactams** are commonly used.
- **Tigecycline and Eravacycline** are alternative options when β -lactam agents are either not active or unable to be tolerated.

CRE infection*

Molecular identification

Ambler Class A CRE
(KPC, NMC, SME)

Ambler Class B CRE
(NDM, VIM, IMP)

Ambler Class D CRE
(OXA-48-like)

- Ceftazidime-avibactam
- Imipenem-relebactam
- Meropenem-vaborbactam
- Cefiderocol

- Ceftazidime-avibactam +
aztreonam
- Cefiderocol

- Ceftazidime-avibactam
- Cefiderocol

Other available antibiotics after in-vitro analysis: Tetracyclines, Colistin, Aminoglycosides, Fosfomycin, Fluoroquinolones, TMP-STX

Treatment of infections caused by MDR P. aeruginosa

- For critically ill patients **ceftolozane-tazobactam, ceftazidime-avibactam, imipenem-cilastatin-relebactam** is a reasonable treatment.

*Treatment of infections by DTR-*P. aeruginosa**

- Ceftolozane-tazobactam, **ceftazidime-avibactam**, and imipenem-cilastatin-relebactam are preferred options for the treatment of infections by DTR-*P. aeruginosa*.
- **Cefiderocol** is an alternative treatment.

the general approach for the treatment of infections caused by CRAB

- **Sulbactam- Dulobactam**
- In combination with **imipenem or meropenem**.
- The use of high-dose **ampicillin-sulbactam (total daily dose of 6-9 grams of the sulbactam component)** in combination with at least one other agent .
- **Polymyxin B** can be considered in combination with at least one other agent .
- **High dose minocycline or high-dose tigecycline .**

- **Strepto MRD:** Meropenem
- **MRSA:**
 - Vancomycin, Linezolid or Daptomycin (Exc Lungs)
- **VRE:**
 - Linezolid, Daptomycin
- **ESBL-producing GNB:**
 - Meropenem, Imipenem
- **CRE (Carbapenemase-producing Enterobacteriaceae)**
 - NDM: Polymyxins, Tigecycline,
 - Ceftazidime- avibactam + Aztreonam
 - Non-NDM: CFZ-avibactam
- **KPCs (K.pneumoniae carbapenemases):**
 - Ceftazidime-avibactam





Bacterial Identification

BEYOND

ROUTINE BLOOD CULTURES

Review

Impact of Multiplex PCR on Diagnosis of Bacterial and Fungal Infections and Choice of Appropriate Antimicrobial Therapy

Francesca Serapide ¹, Rita Pallone ¹, Angela Quirino ², Nadia Marascio ², Giorgio Settimo Barreca ²,
Chiara Davoli ¹, Rosaria Lionello ¹, Giovanni Matera ² and Alessandro Russo ^{1,*}

¹ Infectious and Tropical Disease Unit, Department of Medical and Surgical Sciences, “Magna Græcia” University of Catanzaro, 88100 Catanzaro, Italy; f.serapide@unicz.it (F.S.); pallonerita@gmail.com (R.P.); chiara.davoli93@gmail.com (C.D.); rosarialionello0@gmail.com (R.L.)

² Unit of Clinical Microbiology, Department of Health Sciences, “Magna Græcia” University of Catanzaro, 88100 Catanzaro, Italy; quirino@unicz.it (A.Q.); nmarascio@unicz.it (N.M.); gbarreca@unicz.it (G.S.B.); mmatera@unicz.it (G.M.)

* Correspondence: a.russo@unicz.it; Tel.: +39-09613647552 Apr 2025

Abstract: Multiplex Polymerase Chain Reaction (PCR) has significantly impacted the field of infectious disease diagnostics, offering rapid and precise identification of bacterial and fungal pathogens. Unlike traditional culture methods, which may take days to yield results, multiplex PCR provides diagnostic insights within hours, enabling faster, targeted antimicrobial therapy and reducing the delay in treating critical infections like sepsis. The technique’s high sensitivity and broad pathogen coverage make it ideal for both single and polymicrobial infections, improving outcomes across respiratory, bloodstream, and bacterial/fungal infections. However, multiplex PCR is not without challenges; initial high costs and the need for specialized training can limit its adoption, especially in low-resource settings. This review discusses the clinical advantages and limitations of multiplex PCR, highlighting its influence on diagnostic accuracy, antimicrobial stewardship, and the global fight against antimicrobial resistance (AMR). Furthermore, recent innovations in multiplex PCR, such as digital PCR and portable devices, are explored as potential tools for expanding access to rapid diagnostics worldwide.

Table 3. Advantages and limitations of multiplex PCR.

Advantages	Limitations
Rapid pathogen detection	Risk of false positives
High sensitivity	Potential bias to distinguish between infection/colonization/previous infection
Multiple pathogen detection	High cost of equipment and assays
Effective in polymicrobial cases	Requirement for specialized training
Reduced antibiotic misuse	Limited access in low-resource settings

Table 2. Key statistics on diagnostic delays and impacts on AMR.

Parameter	Traditional Culture	Multiplex PCR
Diagnostic Time	48–72 h	1 h
Average Time to Treatment	48+ h	<6 h
Impact on AMR (Reduction)	Moderate	High



Usefulness of BioFire FilmArray BCID2 for Blood Culture Processing in Clinical Practice

 Benjamin Berinson,^a Anna Both,^a Laura Berneking,^a Martin Christner,^a Marc Lütgehetmann,^a Martin Aepfelbacher,^a
 Holger Rohde^a

^aInstitute for Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

ABSTRACT Rapid pathogen characterization from positive blood cultures (BC) can improve management of patients with bloodstream infections (BSI). The FilmArray blood culture identification (BCID) assay is a molecular test approved for direct identification of BSI causing pathogens from positive BC. A recently updated version of the panel (BCID2) comprises improved species identification characteristics and allows for the detection of one expanded-spectrum β -lactamase (ESBL)- and several carbapenemase-encoding genes. Here, the clinical performance of the BCID2 assay for species identification in 180 positive BCs was evaluated. BCID2 results were concordant with the standard of care (SOC) in 159/180 (88.3%) BCs; 68/74 (91.9%) and 71/74 (96.0%) of all samples growing monobacterial, Gram-positive or Gram-negative pathogens, respectively, were identified, in agreement with SOC results. Nonconcordance was related to the detection of additional pathogens by the BCID2 assay ($n = 4$), discrepant species identification ($n = 4$), or failure of BCID2 to detect on-panel pathogens ($n = 1$). A number (12/31; 38.7%) of discordant results became evident in polymicrobial BC specimens. BCID2 identified the presence of bla_{TEM}-carrying species in 12 BC specimens but failed to predict third

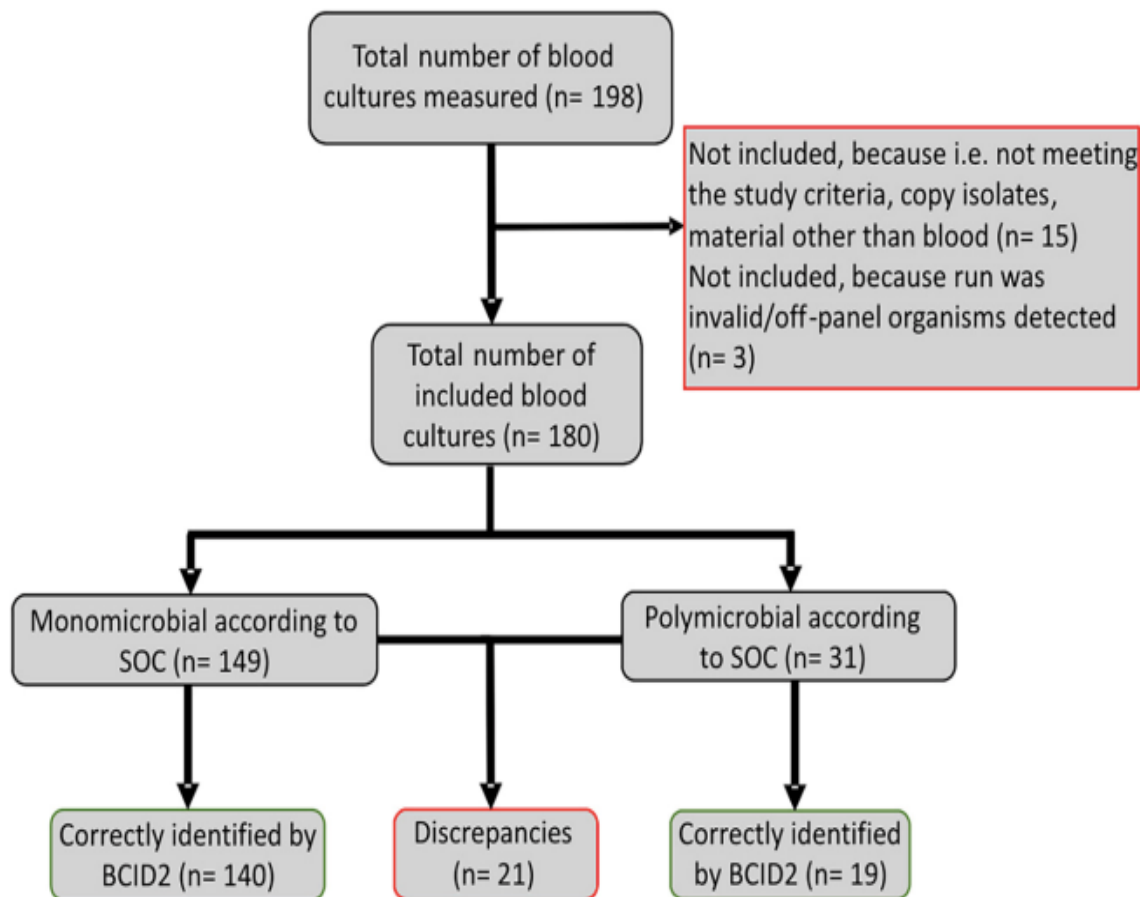


FIG 1 Flowchart showing the inclusion and results/interpretation of isolates. The discrepancies are presented in more detail in Table 1.

identified by the BCID2 panel can be found online (<https://www.biomerieux-diagnostics.com/biofire-bcid-panel>).

TABLE 1 Overview on discordant species identification by SOC analytics and the BCID2 assay system

Study no.	SOC identification	BCID2 identification
Monomicrobial Gram positive		
6	<i>E. faecalis</i>	<i>E. faecalis</i> , <i>Staphylococcus</i> spp.
47	<i>S. haemolyticus</i>	<i>S. epidermidis</i>
54	<i>E. faecalis</i>	<i>E. faecalis</i> , <i>S. epidermidis</i>
62	<i>S. haemolyticus</i>	<i>S. epidermidis</i>
97	<i>S. haemolyticus</i>	<i>S. epidermidis</i>
118	<i>S. haemolyticus</i>	<i>S. epidermidis</i>
Monomicrobial Gram negative		
17	<i>K. pneumoniae</i>	None
28	<i>E. coli</i>	<i>E. coli</i> , <i>S. epidermidis</i>
70	<i>E. coli</i>	<i>E. coli</i> , <i>S. epidermidis</i>
Polymicrobial culture		
5	<i>K. pneumoniae</i> , <i>S. capitis</i>	<i>K. pneumoniae</i> group
14	<i>P. aeruginosa</i> , <i>S. maltophilia</i>	<i>P. aeruginosa</i>
20	<i>E. faecium</i> , <i>S. haemolyticus</i>	<i>E. faecium</i> , <i>S. epidermidis</i>
51	<i>E. faecium</i> , <i>S. epidermidis</i>	<i>E. faecium</i>
58	<i>E. coli</i> , <i>A. veronii</i>	<i>E. coli</i> , <i>K. pneumoniae</i> group
73	<i>E. coli</i> , <i>S. epidermidis</i>	<i>E. coli</i> , <i>Staphylococcus</i> spp.
75	<i>S. haemolyticus</i> , <i>C. krusei</i>	<i>S. epidermidis</i> , <i>C. krusei</i>
82	<i>E. coli</i> , <i>S. anginosus</i> group	<i>E. coli</i> , <i>B. fragilis</i> , <i>Streptococcus</i> spp.
123	<i>C. perfringens</i> , <i>S. epidermidis</i>	None
127	<i>E. faecalis</i> , <i>E. faecium</i> , <i>Candida albicans</i>	<i>E. faecalis</i> , <i>E. faecium</i>
129	<i>K. oxytoca</i> , <i>E. faecium</i>	<i>K. oxytoca</i>
178	<i>P. agglomerans</i> , <i>S. haemolyticus</i>	<i>Enterobacterales</i> , <i>S. epidermidis</i>

TABLE 2 Distribution of resistance markers detected by BCID2

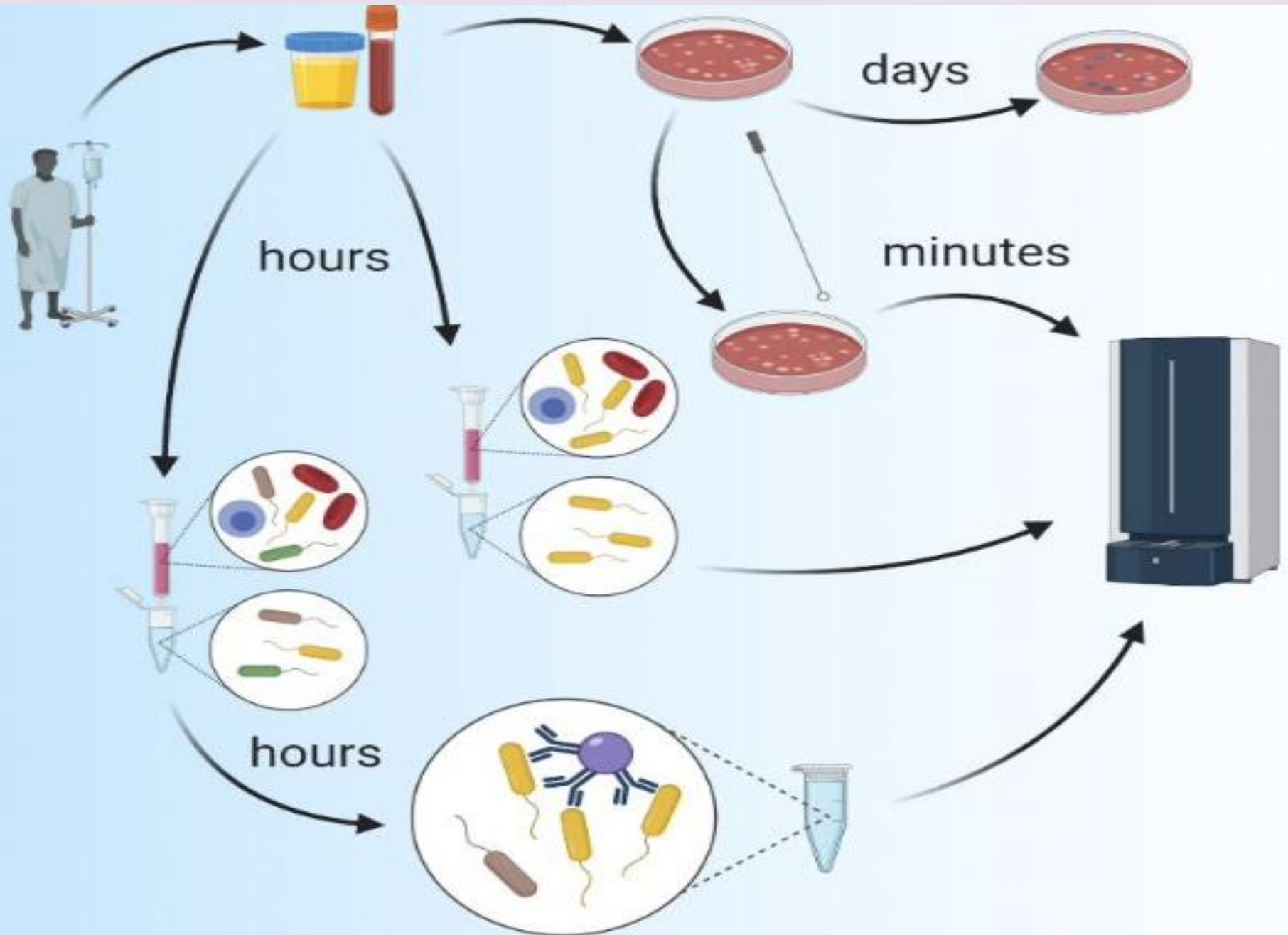
Isolate	Resistance marker detected by BCID2 (n)			None detected
	<i>bla</i> _{CTX-M}	<i>bla</i> _{OXA-48} -like	<i>bla</i> _{VIM}	
Phenotypic third-generation cephalosporin resistance				
<i>E. coli</i> (n = 12)	11	0	0	1 ^a
<i>K. pneumoniae</i> group (n = 3)	1	0	0	2 ^b
<i>K. oxytoca</i> (n = 1)	0	0	0	1 ^c
Carbapenem-resistant isolates				
<i>P. aeruginosa</i> (n = 1)	0	0	1	0
<i>K. pneumoniae</i> group (n = 1)	1	1	0	0

^aMolecular analysis revealed the presence of *bla*_{TEM}.

^bMolecular analysis revealed the presence of *bla*_{SHV} or a combination of *bla*_{SHV} and *bla*_{TEM}.

^cMolecular analysis did not reveal the presence of a *bla*_{TEM}, *bla*_{SHV} or *bla*_{CTX-M}.

MALDI : Matrix Assisted Laser Desorption - Time Of Flight MS



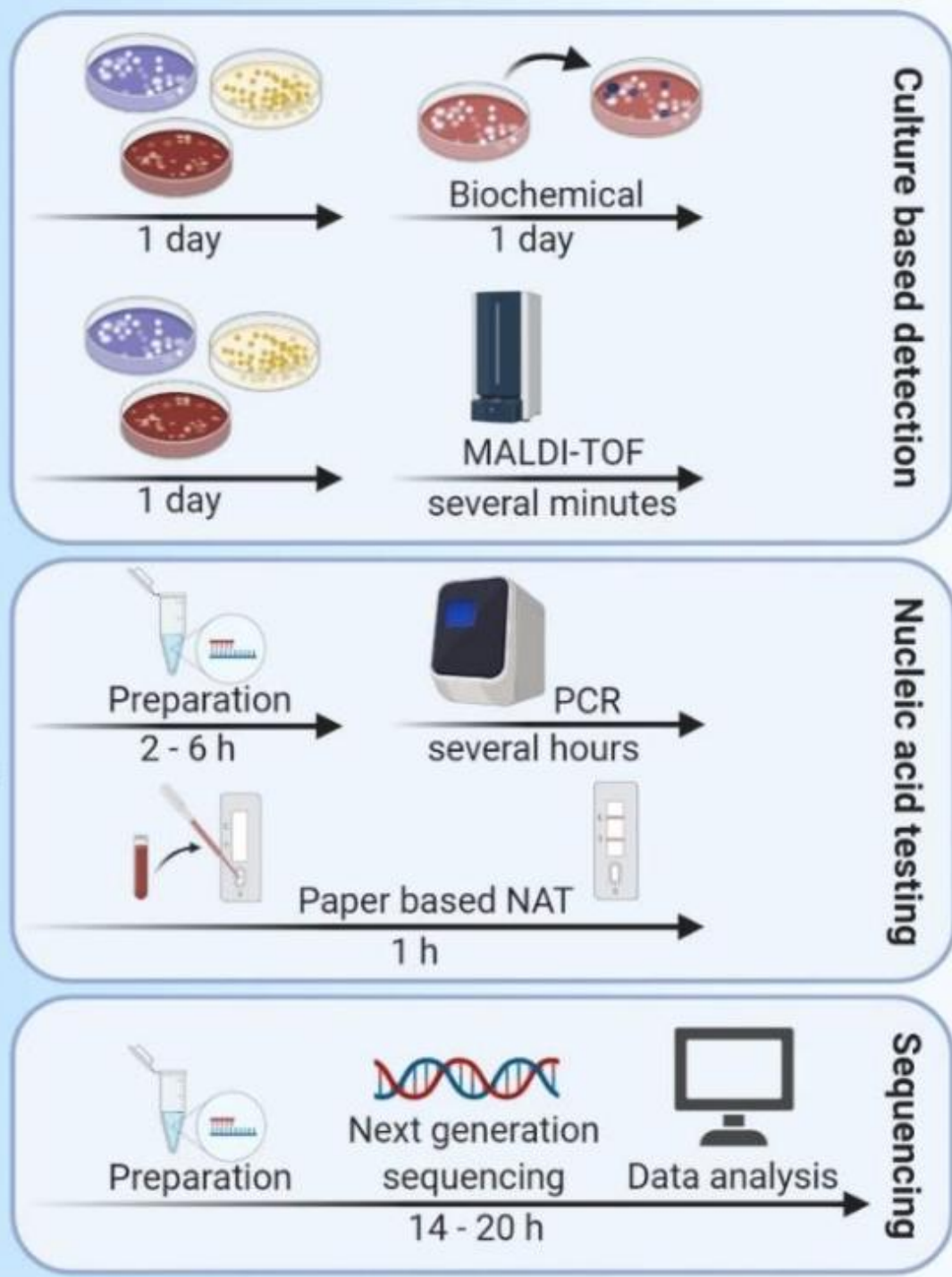


Table 1. Comparison of methods applicable for pathogen detection. (RD: Resistance Determination, AST: Antimicrobial susceptibility testing, POC: Point-of-Care).

Method	Pathogen Identification (ID)	Time	RD	AST	Advantages and Disadvantages	POC	Ref
Cell culture	Growth based; all culturable bacteria	24–72 h cultivation + 18–24 h for biochemical ID	-	✓	+ Cost-effective + Good specificity – Long turnaround times – Lacking sensitivity – Prone to errors in workflow – Difficulties with fastidious organisms – Unculturable organisms not detectable	-	[5,6,13,14,16,21,27, 133–135]
PCR-analysis and real-time PCR	Sequence dependent amplification of bacterial genes > pathogen-specific	One to several hours	✓	-	+ No cultivation + Good performance – Expensive – A priori knowledge on suspected pathogens necessary – Turnaround time – High-end instrumentation	-	[8,13,16,17,19,21,27, 136–142]
Next-generation sequencing	Simultaneous sequencing of billions of nucleic acid fragments contained in heterogenous samples > identification on subspecies or strain level based on SNPs	14–20 h	✓	-	+ Primer independent + Identification without a priori knowledge or suspicion + Faster adaption to new resistance mechanisms – Complex workflow with experimental pitfalls and biases – High overall error rate – Differentiation between colonization and infection critical	(✓)	[5,22–24,27–29,31– 33,136,143–146]
MALDI-TOF; Direct sample testing	Generated mass spectrum of molecular sample composition compared to spectral database containing spectra from pure colonies (pre-cultivation); Cell enrichment followed by specific isolation	2–50 h	(✓)	(✓)	+ Automatable + Low costs per test + Fast analysis – Pre-cultivation necessary – Several resistance mechanisms not detectable – Identification of subspecies limited – Polymicrobial analysis difficult + No pre-cultivation – A priori knowledge necessary	-	[15,38,39,41,43– 55,147–152]

Conclusions:

- **There is no ideal antibiotic**
- **Resistance is a big problem, we can't escape**
- **Only judicious use of Empirical therapy is the solution**
- **Optimal use of lab is the key to successful antibiotic therapy**

Client Name : C03 / Paras Hospitals, Panchkula

Sample Type : BAL Fluid

TEST NAME	RESULT	UNIT	BIOLOGICAL REF. INTERVAL
Bio FirePneumonia Plus Panel <i>(Multiplex PCR, Closed System)</i>			
<u>BACTERIA (SEMI QUANTITATIVE)</u>			
Acinetobacter calcoaceticus-baumannii complex	DETECTED (>=10⁷)		NOT DETECTED
Enterobacter cloacae complex	NOT DETECTED		NOT DETECTED
Escherichia coli	NOT DETECTED		NOT DETECTED
Haemophilus influenza	NOT DETECTED		NOT DETECTED
Klebsiella aerogenes	NOT DETECTED		NOT DETECTED
Klebsiella oxytoca	NOT DETECTED		NOT DETECTED
Klebsiella pneumoniae group	NOT DETECTED		NOT DETECTED
Moraxella catarrhalis	NOT DETECTED		NOT DETECTED
Proteus spp.	NOT DETECTED		NOT DETECTED
Pseudomonas aeruginosa	NOT DETECTED		NOT DETECTED
Serratia marcescens	NOT DETECTED		NOT DETECTED
Staphylococcus aureus	NOT DETECTED		NOT DETECTED
Streptococcus agalactiae	NOT DETECTED		NOT DETECTED
Streptococcus pneumoniae	NOT DETECTED		NOT DETECTED
Streptococcus pyogenes	NOT DETECTED		NOT DETECTED

- **CFZ-avibactam**
- **Sulbactam- Durolobactam**
- **High dose Mino/ Tigecyclin –Sulbactam (6-9 Gm)**
- **Polymyxin + Mino/Tigecyclin**

CARBAPENEMASES

IMP	NOT DETECTED	NOT DETECTED
KPC	NOT DETECTED	NOT DETECTED
OXA-48-like	N/A	NOT DETECTED
NDM (E Coli)	DETECTED	NOT DETECTED
VIM	DETECTED	NOT DETECTED

ESBL

CTX-M	NOT DETECTED	NOT DETECTED
-------	--------------	--------------

METHICILLIN RESISTANCE

mecA/C and MREJ (MRSA)	N/A	NOT DETECTED
------------------------	-----	--------------

Quiz Questions

1. High Diversity biota is associated with
 - A. High mortality
 - B. 2. Low mortality

2. Bacterial infections are most common during

A. Pre engraftment period

B. B. Post engraftment period

3. MALDI-TOF , though very sensitive takes longer time to culture the organism as compared to SOC cultures

- A. True
- B. False

4. The new antibiotic from an already known antibiotic class that has emerged for treating CR-E, CR-PA or CR-AB infections.

A. CFZ -Avibactam

B. Cefiderocol

NDTM E Coli

1. First line:

- Ceftazidime –avibactam + Aztreonam
- Cefiderocol

2. Second line:

- Colistin- Tigecycline

Thank
you

