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CARBAPENEMASE PRODUCING ENTEROBACTERIACAE RECTAL COLONIZATION IN DIALYSIS PATIENTS: A LARGE MONOCENTRIC RETROSPECTIVE ANALYSIS

Presentata da: Matteo Righini

Coordinatore Dottorato

Niccolò Daddi

Supervisore

Gaetano La Manna

Abstract:

Carbapenemase-producing Enterobacteriaceae (CPE) represent a growing global public health concern due to their increasing prevalence and resistance to carbapenems, a group of last-resort antibiotics. Dialysis patients, who often have compromised immune systems, are particularly vulnerable to infections that represent the second cause of death in dialysis' cohorts. Presenting a rectal colonization by CPE has a significative impact on patients in dialysis? Are there factors that can help us understand which patients are at a higher risk of developing CPE colonization? How can we treat a CPE colonized patient who develop fever? Our study aim to reviews the challenges posed by CPE in dialysis settings and explores current diagnostic, therapeutic, and infection control strategies on a large cohort of dialyzed patients.

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Introduction

1. OVERVIEW OF CARBAPENEMASE PRODUCTION

Carbapenemase production is a crucial mechanism that leads to resistance against carbapenem antibiotics, which are commonly used as a last resort in treating severe bacterial infections. Carbapenemases are enzymes produced by certain bacteria, particularly Enterobacteriaceae, that can hydrolyze and inactivate carbapenem antibiotics, rendering them ineffective. The emergence and spread of carbapenemase-producing Enterobacteriaceae (CPE) have become a major global health concern due to limited treatment options and the potential for widespread resistance [1].

Classification of Carbapenemases:
Carbapenemases are classified into three major classes based on their structural and functional characteristics: class A, class B, and class D [2].

1.1 Class A Carbapenemases:
Class A carbapenemases, also known as Klebsiella pneumoniae carbapenemases (KPCs), are most commonly encountered in Enterobacteriaceae. They are often plasmid-mediated and have a broad spectrum of activity against multiple beta-lactam antibiotics.

1.2 Class B Carbapenemases:
Class B carbapenemases, also referred to as metallo-beta-lactamases (MBLs), are zinc-dependent enzymes that hydrolyze carbapenems. The most clinically significant MBLs include the New Delhi metallo-beta-lactamase (NDM), Verona integron-encoded metallo-beta-lactamase (VIM), and imipenemase (IMP).

1.3 Class D Carbapenemases:
Class D carbapenemases, also known as oxacillinases (OXAs), are predominantly produced by Acinetobacter baumannii. They exhibit hydrolytic activity against carbapenems and some other beta-lactam antibiotics.

Mechanisms of Carbapenemase Production:
Carbapenemase production can occur through different mechanisms, including genetic acquisition and mutation within the bacterial genome. The most common mechanism involves the acquisition of mobile genetic elements, such as plasmids and transposons, that carry the genes encoding carbapenemases.

2.1 Plasmid-Mediated Carbapenemases:
Plasmids play a significant role in the dissemination of carbapenemase genes among different bacterial species. They can transfer between bacteria, including Enterobacteriaceae, Acinetobacter spp., and Pseudomonas aeruginosa, facilitating the rapid spread of carbapenem resistance.

2.2 Chromosomally Encoded Carbapenemases:
Some bacteria, particularly Acinetobacter baumannii, possess chromosomally encoded carbapenemases. These enzymes are usually associated with intrinsic resistance and contribute to the development of multidrug-resistant strains.

Clinical Implications:
Carbapenemase production poses a significant clinical challenge, as it severely limits treatment options for infections caused by CPE. The resistance conferred by carbapenemases extends not only

to carbapenems but also to other beta-lactam antibiotics, further complicating the choice of appropriate therapy. Production of carbapenemases is the most common Carbapenemase Resistant Enterobacteriacae (CRE) resistance mechanism, other mechanisms include outer membrane proteins deletion or alteration, excessive activation of efflux pumps, alteration of penicillin binding proteins and biofilm components, and these mechanisms could function individually or together. The horizontal transmission of drug resistance genes, mutations in the genome and related regulatory factors are important directions for the study of drug resistance mechanisms.

Treatment Options:
Due to the limited effectiveness of carbapenems against CPE, alternative treatment strategies must be considered. These may include the use of combination therapy, employing other antibiotic classes, such as polymyxins, aminoglycosides, or tigecycline, or the exploration of novel antimicrobial agents under development.

Infection Control Measures:
Infection prevention and control measures play a crucial role in containing the spread of CPE in healthcare settings. Strict adherence to standard precautions, active surveillance, early detection, and prompt implementation of appropriate infection control measures are essential.

2. EPIDEMIOLOGY AND RISK FACTORS IN DIALYSIS

Hemodialysis (HD) and peritoneal dialysis (PD) patients are a high-risk population for various healthcare-associated infections, including those caused by CPE. Understanding the epidemiology and risk factors associated with CPE infections in dialysis settings is crucial for effective prevention, surveillance, and management. By focusing on surveillance, adherence to infection control practices, antimicrobial stewardship, and education, the risk of CPE transmission in dialysis units can be mitigated, thereby improving patient outcomes

Epidemiology of CPE: from Macro to Micro

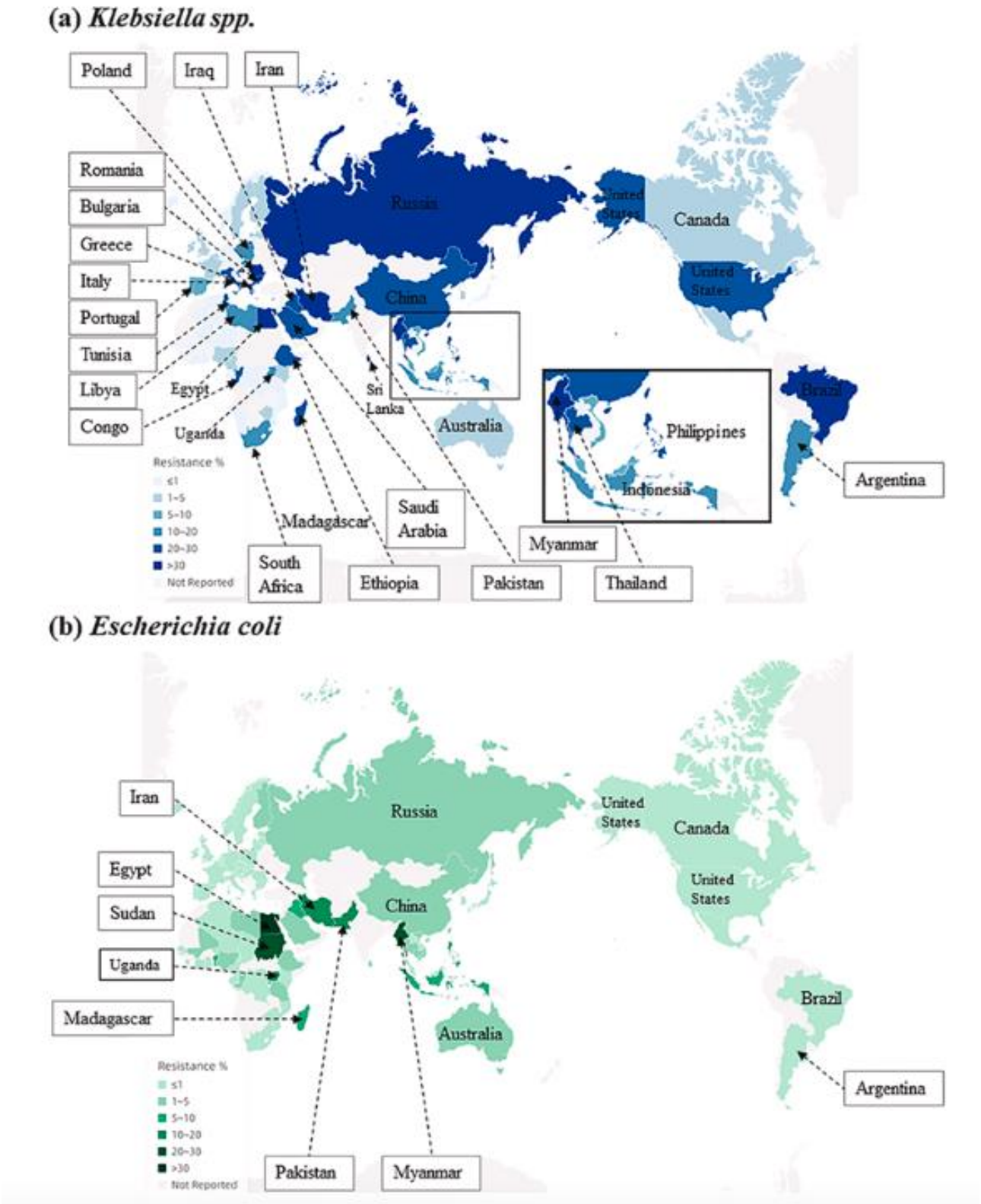


Figure 1. Global distribution of carbapenem-resistant (a) Klebsiella spp. and (b) E. coli. Data from (Han et al., 2017; CARA, 2018; Mitgang et al., 2018; Malchione et al., 2019; Vinogradova, 2019; ECDC, 2020; Fupin et al., 2021; Garza-Gonzalez et al., 2021; Kuzmenkov et al., 2021; WHO, 2021)

CRE exhibits varying prevalence rates globally (Figure 1). In Europe, CRE cases are predominantly found in the 19-64 age group, and there's a higher incidence among males. Similarly, in China, the gender distribution mirrors that of Europe, and the average age of affected individuals is around 62

years [3]. In a multicenter global study, a total of 8,787 Enterobacteriaceae samples were collected from 64 medical centers around the world. The overall resistance rate of CRE in 2019 was 4.5%. According to data from the WHO Global Antimicrobial Surveillance System (GLASS) in 2021 [4], the resistance rates for meropenem and imipenem in Carbapenem-Resistant Klebsiella pneumoniae (CRKP) were 12.34% and 10.63%, respectively. Additionally, meropenem exhibited a resistance rate of 0.9%, and imipenem had a resistance rate of 1.3% for Carbapenem-Resistant Escherichia coli (CREco) [5]. In the WHO GLASS data, Egypt stands out with notably high resistance rates for meropenem, imipenem, ertapenem, and doripenem. In other parts of Africa, Uganda reported a 10% resistance rate to ertapenem in urine E. coli isolates and a 10.34% resistance rate to imipenem in urine Klebsiella pneumoniae isolates. Madagascar detected even higher resistance rates, with 28.77% of Klebsiella pneumoniae and 6.2% of E. coli being resistant to imipenem. However, it's worth noting that a 2018 meta-analysis of CRE in Africa, conducted by Mitgang et al., revealed relatively low (<1%) to moderate (1–5%) resistance proportions throughout the continent. This suggests a high transmission capacity of CRE. [6]

The situation with CRE in Asia is also a cause for concern. The prevalence of CRE in China has been on the rise in recent years. Data from China's national bacterial surveillance in 2020 indicated a significant increase in drug resistance rates of Enterobacter to imipenem and meropenem since 2016. Notably, 24.2% of Klebsiella pneumoniae strains were resistant to meropenem [7].
In Southeast Asia, CRE dissemination appears to be less severe compared to China. Malchione et al. (2019) conducted a meta-analysis of CRE epidemiology in Southeast Asia [8]. They found that CREco and carbapenem-resistant Klebsiella species accounted for high rates among CRE strains. Carbapenem-resistant Klebsiella species showed high resistance rates (>5%) in Indonesia, the Philippines, Thailand, and Vietnam. Rates were moderate (1–5%) in Malaysia and Singapore, and low (<1%) in Cambodia and Brunei. For CREco, high rates were observed in Indonesia and Myanmar, while they were low in Cambodia, Malaysia, the Philippines, Singapore, Thailand, and Vietnam.

In the Americas, both Brazil and the United States have shown high rates of CRKP at 35.29% and 24.6%, respectively. [9]
Canada and Mexico, neighboring the United States, have experienced a noticeable decline in carbapenem-resistant Klebsiella species rates, with figures of 3.6% and 2.1%, respectively [10-12]. Argentina, which borders Brazil, has a relatively lower rate of carbapenem-resistant Klebsiella species at 12.26% [4].

In contrast, most European regions and Australia have lower resistance rates compared to the aforementioned regions. Data from the WHO GLASS showed that in the United Kingdom of Great Britain and Northern Ireland, resistance rates for K. pneumoniae and E. coli against ertapenem were 1.2% and 0.14%, respectively. Australia had corresponding resistance rates of 1.49% for K. pneumoniae and 3.75% for E. coli [4]. A report from the European Centre for Disease Prevention and Control [13] identified Greece, Romania, and Bulgaria, three neighboring countries, with high rates of carbapenem-resistant Klebsiella pneumoniae, suggesting that environmental factors might contribute to the spread of drug-resistant strains in this region.
Russia has a notable CRKP rate of 41.87%, and CREco has a rate of 3.47% [14, 15].
Overall, the global distribution of carbapenem-resistant Klebsiella species and E. coli varies by region, with some areas experiencing high resistance rates.

The Romagna situation
Romagna is a region located in the northern part of Italy. The region encompasses approximately 1.1 million people distributed across the provinces of Ravenna, Rimini, and Forlì-Cesena. Each province has its own Level III hospital and refers to a centralized laboratory dedicated to the

processing of microbiological tests. In 2022, a total of 276,100 tests were conducted, with roughly 80% originating from hospital departments (Figure 2). Every year, a report is prepared on infections and the microbiological situation within the organization. This report is discussed with healthcare professionals to assess areas of improvement and areas that require enhancement.

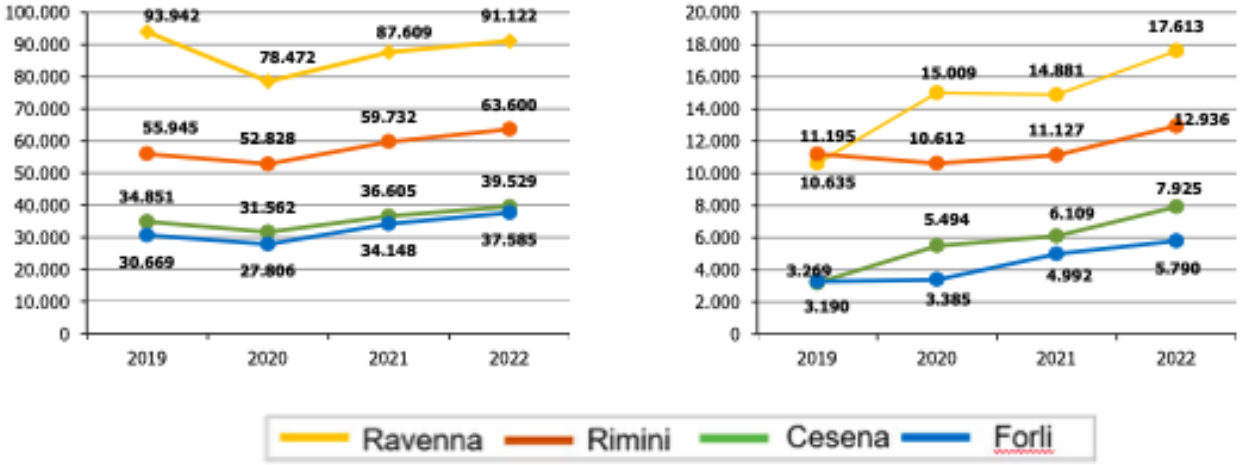


Figure 2. Number of bacteriaemias in AUSL-Romagna per year from 2019 to 2022. Different colour lines represent different provinces. Ravenna: 386.723 habitants, Rimini: 325.139 habitants, Cesena: 203.042 habitants. Forlì: 188.030 habitants.

Patogeni	Numero episodi di batteriemia			
	2019	2020	2021	2022
Escherichia coli	1507	1266	1348	1506
Staphylococcus aureus	609	653	698	716
Klebsiella pneumoniae	377	427	414	461
Enterococcus faecalis	293	313	374	356
Enterococcus faecium	116	123	150	166
Pseudomonas aeruginosa	204	259	234	238
Acinetobacter baumanii	70	52	62	53
Candida spp.	136	162	192	223

Patogeni	Tasso per 100.000 abitanti			
	2019	2020	2021	2022
Escherichia coli	134.4	113.0	120.7	135.0
Staphylococcus aureus	54.3	58.3	62.5	64.2
Klebsiella pneumoniae	33.6	38.1	37.1	41.3
Enterococcus faecalis	26.1	27.9	33.5	31.9
Enterococcus faecium	10.3	11.0	13.4	14.9
Pseudomonas aeruginosa	18.2	23.1	20.9	21.3
Acinetobacter baumanii	6.2	4.6	5.6	4.8
Candida spp.	12.1	14.5	17.2	20.0

Figure 3. Number of bacteriaemias and 100.000 habitants/rate in AUSL-Romagna.

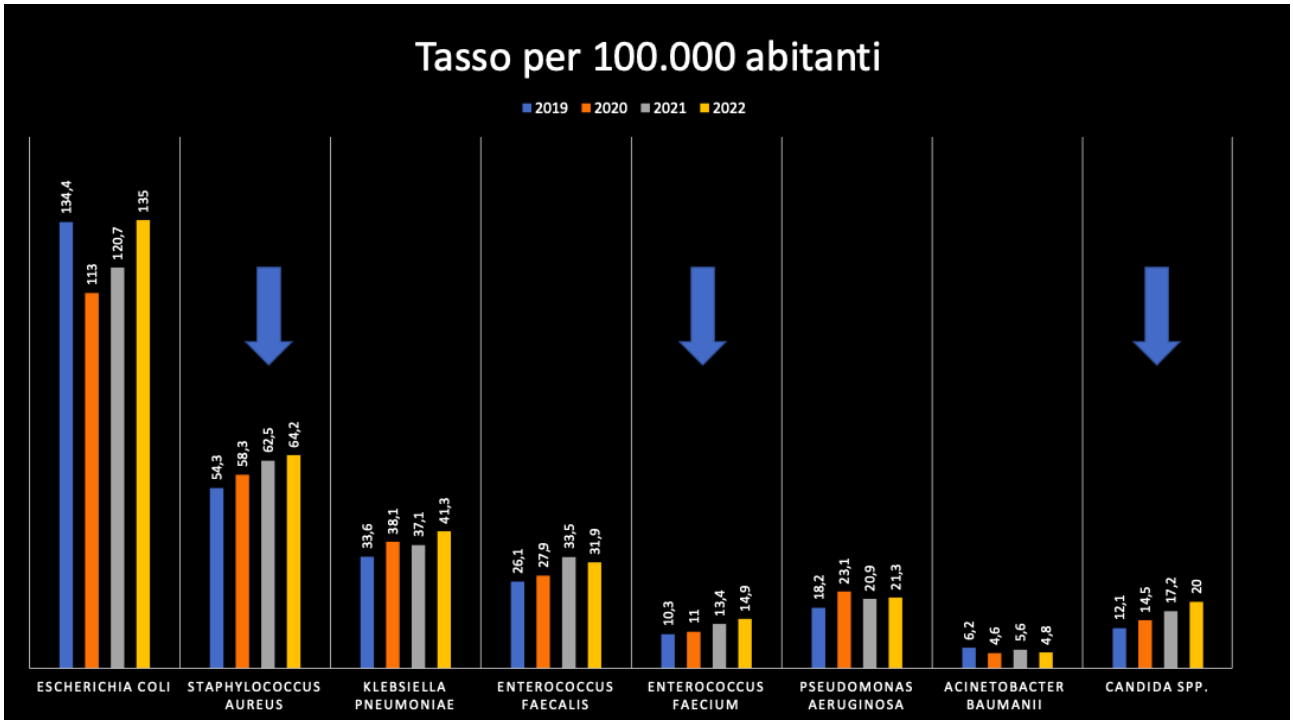


Figure 4. Number of bacteriaemias and 100.000 habitants/rate in AUSL-Romagna. Arrows show which infections improved in the last years.

Figures 3 and 4 depict infection rates for major bacteria and their trends in recent years. Considering the burden of CPE, Figure 5 provides a comparison of Klebsiella Pneumoniae bacteremias between the Emilia Romagna Region (RER) and AUSL-Romagna, Figure 6 of Pseudomonas Aeruginosa and Acinetobacter Baumanii. It's noteworthy that there are distinct trends in bacteremia rates between the two groups. RER experienced a decline in carbapenem resistance in 2021, while AUSL-R observed it in 2022. Generally, the resistance percentage tends to be lower in AUSL-R.

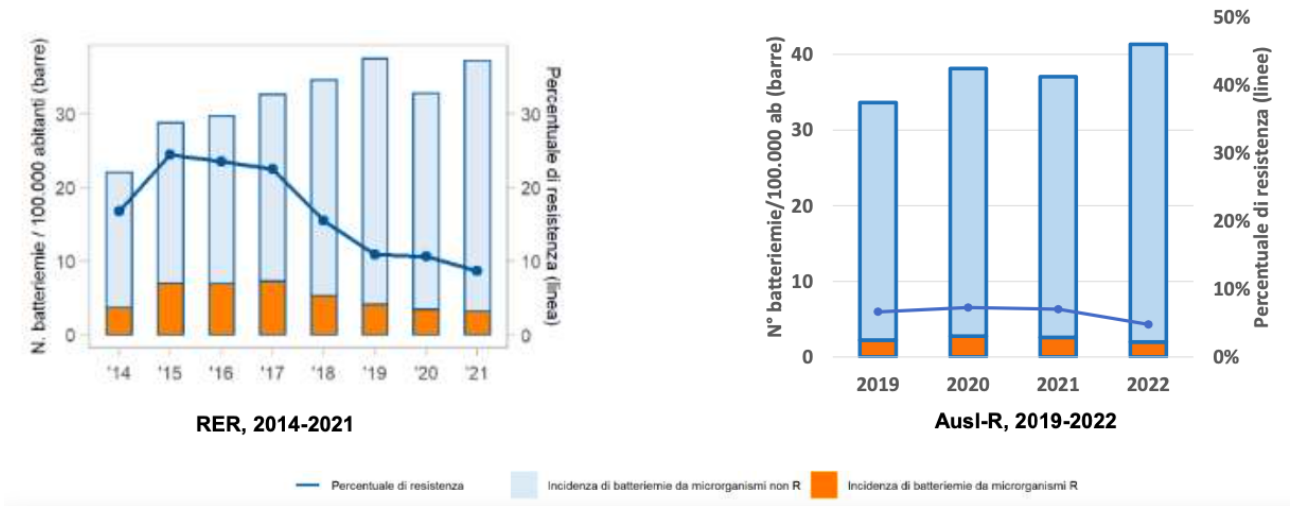
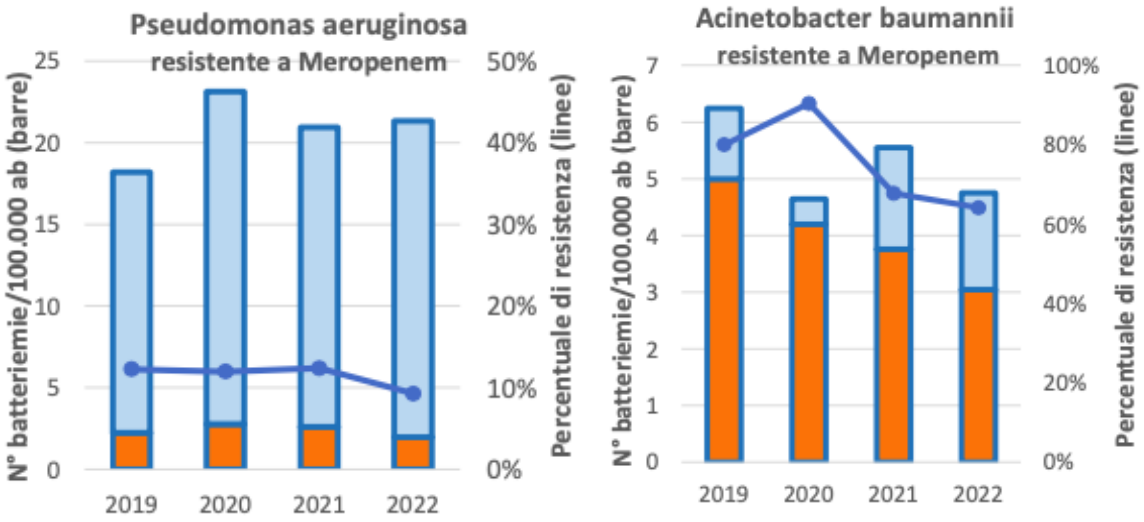


Figure 5. Comparison between the trend of infections by Klebsiella Pneumoniae in Emilia Romagna Region (RER) and AUSL Romagna in the last years and the percentage of resistance. We can appreciate that CPE experienced a decline in both cohorts and that the percentage of resistance is lower in AUSL-Romagna.



Figures 6. Rates of Meropenem-resistant bacteremias caused by Pseudomonas Aeruginosa (on the left) and Acinetobacter Baumanii (on the right) in AUSL-Romagna.

Epidemiology of CPE in dialysis:

- Prevalence:

Studies have shown an increasing trend in the prevalence of CPE among dialysis patients. The prevalence rates can vary depending on geographical location and the level of infection control practices implemented in dialysis units.

- Transmission Dynamics:

CPE can spread within dialysis units through various routes, including direct contact with contaminated surfaces, equipment, or healthcare personnel, as well as through person-to-person transmission among patients.

- Outbreaks and Clonal Spread:

Outbreaks of CPE infections in HD units have been reported, indicating the potential for clonal spread within these settings. Factors such as overcrowding, inadequate infection control practices, and the presence of colonized or infected patients can contribute to the occurrence and propagation of outbreaks. Even in PD units, outbreaks of CPE have been reported. These outbreaks often result from cross-transmission within the unit, emphasizing the importance of strict infection control measures

Risk Factors for CPE Infections in Hemodialysis Patients:

- Underlying Comorbidities:

HD patients often have multiple comorbidities, including end-stage renal disease (ESRD), diabetes mellitus, immunosuppression, and frequent healthcare exposures. These conditions increase their susceptibility to infections, including CPE.

- Frequent Healthcare Exposures:

HD patients typically require frequent hospitalizations, outpatient clinic visits, and invasive procedures, which increase their risk of exposure to healthcare-associated pathogens, including CPE.

- Prolonged Hospital Stays:

Longer hospital stays, either due to the underlying medical condition or for HD-related procedures, increase the likelihood of CPE acquisition, especially in settings with high CPE prevalence.

- Invasive Procedures and Catheter Use:

HD patients often require the placement of central venous catheters for vascular access, which can serve as a portal of entry for CPE. Additionally, invasive procedures, such as dialysis catheter exchanges or interventions, can increase the risk of CPE transmission.

- Antibiotic Exposure:

The prolonged and recurrent use of antibiotics in HD patients, including prior exposure to carbapenems and other broad-spectrum agents, can promote the selection and dissemination of CPE strains.

- Dialysis Unit Factors:

The specific characteristics of the HD unit, such as the patient-to-staff ratio, adherence to infection control practices, water treatment quality, and environmental contamination, can significantly influence the risk of CPE acquisition and transmission.

Risk factors for CPE infections in peritoneal dialysis patients:

- Frequent Healthcare Exposures:

PD patients have frequent healthcare exposures, including dialysis sessions, clinic visits, and hospitalizations. These exposures increase the risk of acquiring CPE, especially if infection control practices are not rigorously followed.

- Prolonged Hospital Stays:

Patients on PD may require prolonged hospital stays for various reasons, such as surgery or treatment of complications. Extended hospitalizations increase the risk of exposure to healthcare-associated pathogens, including CPE.

- Immunosuppression:

Some PD patients may be immunosuppressed due to underlying medical conditions or medications. Immunosuppression can increase susceptibility to infections, including those caused by CPE.

- Antibiotic Exposure:

PD patients are often exposed to antibiotics for the management of various infections and complications. Prolonged or inappropriate antibiotic use can promote the selection and dissemination of CPE strains.

- Invasive Procedures:

Invasive procedures, such as peritoneal catheter insertions or catheter exchanges, can create opportunities for CPE acquisition. Strict aseptic techniques and infection control practices are critical during these procedures.

- Dialysis Unit Factors:

The characteristics of the PD unit, including its adherence to infection control measures, water treatment quality, and environmental contamination, can influence the risk of CPE acquisition and transmission.

- Transfer of Patients:

Patients may transfer between different healthcare facilities, including HD and PD units. These transfers can introduce new patients into the PD unit, potentially carrying CPE with them.

Strategies for Prevention and Control:

- Surveillance and Screening:

Routine surveillance for CPE colonization among HD patients, including screening of both new admissions and existing patients, enables early detection, isolation, and implementation of appropriate infection control measures.

- Adherence to Infection Control Practices:

Strict adherence to infection control practices, including hand hygiene, proper disinfection of equipment and surfaces, and appropriate use of personal protective equipment, is critical to prevent the transmission of CPE within HD units.

- Antimicrobial Stewardship:

Promoting judicious use of antibiotics, optimizing the choice and duration of therapy, and discouraging unnecessary antibiotic use can help minimize the selection pressure for CPE and reduce the risk of infections.

- Patient and Staff Education:

Providing education and training to both HD patients and healthcare personnel about infection control practices, hand hygiene, and the importance of antimicrobial stewardship can enhance awareness and adherence to preventive measures.

3. IMPACT ON PATIENT OUTCOMES

CPE infections can have a significant impact on patient outcomes, particularly in HD patients who often have compromised immune systems. Understanding the potential consequences of CPE infections is crucial for effective management and prevention strategies.

Increased Morbidity and Mortality:

CPE infections are associated with higher rates of morbidity and mortality compared to infections caused by susceptible bacteria. HD patients are particularly susceptible to severe infections and are more likely to experience adverse outcomes, including sepsis, organ failure, and death, when infected with CPE.

Delayed and Inadequate Treatment:

CPE infections pose a therapeutic challenge due to limited treatment options. The resistance conferred by carbapenemases makes these infections more difficult to manage, often leading to delays in initiating appropriate therapy. Delayed and inadequate treatment can further worsen patient outcomes and increase the risk of complications.

Treatment Failure and Recurrent Infections:

Due to the limited effectiveness of carbapenem antibiotics against CPE, treatment failure is more common. Inadequate treatment can result in persistent or recurrent infections, requiring prolonged hospitalization, additional interventions, and increased healthcare costs. Recurrent infections can also contribute to the development of chronic carriage or colonization with CPE.

Impact on Dialysis Adequacy:

CPE infections in HD patients can affect the adequacy of dialysis treatment. Patients with active infections may experience hemodynamic instability, necessitating alterations in the dialysis prescription. In some cases, temporary interruption or modification of dialysis sessions may be required, leading to potential complications associated with inadequate clearance of waste products and fluid overload.

Transmission to Other Patients:

HD units are high-risk settings for the transmission of healthcare-associated infections, including CPE. Infected patients can serve as sources of transmission, leading to outbreaks and the spread of CPE within the dialysis unit. This not only impacts the infected patient but also poses a risk to other vulnerable individuals undergoing HD, further exacerbating the overall impact on patient outcomes.

Economic Burden:

CPE infections in HD patients impose a significant economic burden on healthcare systems. The prolonged hospitalizations, increased use of healthcare resources, and the need for specialized

infection control measures contribute to higher healthcare costs. Moreover, the indirect costs associated with lost productivity and potential long-term complications further contribute to the economic impact.

Prevention and Mitigation Strategies:
Given the adverse impact of CPE infections on patient outcomes, prevention and mitigation strategies are crucial. Implementing effective infection control measures, including surveillance, early detection, appropriate isolation precautions, and antimicrobial stewardship, can help reduce the risk of CPE transmission and improve patient outcomes.

4. IMPORTANCE OF SURVEILLANCE PROGRAMS

Surveillance programs are essential for effective detection, prevention, and management of CPE infections. They facilitate early detection, guide infection control measures, monitor trends, identify high-risk patients, aid outbreak management, evaluate prevention strategies, support public health reporting, contribute to research efforts, and guide antimicrobial stewardship initiatives. By implementing robust surveillance programs, healthcare facilities can mitigate the impact of CPE and protect vulnerable populations, including HD patients.

Early Detection and Timely Intervention:
Surveillance programs enable the early detection of CPE colonization or infection in patients, allowing for prompt initiation of appropriate infection control measures and therapeutic interventions. Timely identification of CPE cases facilitates early treatment, reducing the risk of transmission and improving patient outcomes.

Monitoring the Epidemiology and Trends:
Surveillance programs provide valuable data on the epidemiology and trends of CPE infections. By monitoring the prevalence, incidence, and distribution of CPE strains, surveillance programs help identify emerging resistance patterns, high-risk populations, and potential outbreaks. This information can guide infection control strategies and resource allocation.

Risk Assessment and Identification of High-Risk Patients:
Surveillance programs aid in identifying high-risk patients and populations who are more susceptible to CPE infections, such as HD patients. By assessing risk factors, such as prior hospitalizations, antibiotic exposure, and comorbidities, surveillance programs can target interventions and preventive measures to those who need them most.

Infection Control and Outbreak Management:
Surveillance programs provide essential data for designing and evaluating infection control measures. By identifying CPE carriers or infected individuals, surveillance programs help implement appropriate isolation precautions, cohorting strategies, and environmental cleaning protocols. Additionally, surveillance data contribute to the prompt detection and management of CPE outbreaks within healthcare facilities.

Evaluation of Prevention and Control Measures:
Surveillance programs allow for the evaluation of the effectiveness of prevention and control measures implemented to mitigate CPE transmission. By monitoring CPE rates over time and assessing the impact of interventions, surveillance data can inform the refinement of infection control policies and guide evidence-based practices.

Public Health Reporting and Communication:

Surveillance programs facilitate the reporting of CPE cases to public health authorities, which is essential for tracking the epidemiology of CPE at regional, national, and global levels. Rapid and accurate reporting enables public health agencies to monitor the overall burden, detect emerging resistance patterns, and coordinate appropriate response measures.

Research and Antimicrobial Stewardship:
Surveillance programs generate valuable data for research purposes and antimicrobial stewardship efforts. Longitudinal surveillance data can contribute to understanding the dynamics of CPE transmission, the impact of interventions, and the effectiveness of new treatment strategies. Furthermore, surveillance data can guide antimicrobial stewardship programs by identifying patterns of antibiotic resistance and informing optimal antibiotic prescribing practices.

5. DIAGNOSTIC APPROACHES AND CHALLENGES

Diagnostic approaches for CPE include phenotypic methods, such as carbapenem susceptibility testing and modified Hodge test, as well as molecular methods like Polymerase Chain Reaction (PCR) and Next Generation Sequencing (NGS). Each method has its advantages and challenges in terms of sensitivity, specificity, speed, and accessibility. Overcoming the challenges associated with CPE detection, such as heterogeneity of resistance mechanisms, variability in carbapenemase genes, coexistence of other resistance mechanisms, and the need for rapid and accessible diagnostic methods, is crucial for effective CPE management and infection control.

Phenotypic Methods:

- Carbapenem Susceptibility Testing:

Phenotypic methods, such as antimicrobial susceptibility testing (AST), are commonly used to identify CPE. Reduced susceptibility or resistance to carbapenems, often detected through disk diffusion or broth microdilution methods, can indicate the presence of CPE. However, phenotypic testing alone may not differentiate between carbapenemase production and other resistance mechanisms.

- Modified Hodge Test (MHT):

The MHT is a phenotypic test used to detect carbapenemase production. It involves inoculating a carbapenem-susceptible indicator organism around a test organism and observing the growth pattern. Positive results indicate carbapenemase production, but false-negative or inconclusive results can occur due to the presence of non-carbapenemase mechanisms or weak carbapenemase production.

- Other Phenotypic Tests:

Additional phenotypic tests, such as the Carba NP test, CarbaR Strip, or CarbaLux, utilize colorimetric or fluorogenic substrates to detect carbapenemase activity. These tests can provide rapid results, but they may have varying sensitivity and specificity, and they may not detect all carbapenemase types.

Molecular Methods:

- Polymerase Chain Reaction (PCR):

PCR-based methods, targeting specific carbapenemase genes, are highly sensitive and specific for the detection of CPE. Multiplex PCR assays can simultaneously identify multiple carbapenemase genes, aiding in rapid and accurate detection. However, these tests require specialized equipment, expertise, and may have limitations in detecting emerging or unknown carbapenemases.

- Next-Generation Sequencing (NGS):

NGS technologies enable comprehensive genomic analysis, allowing the identification of carbapenemase genes, resistance determinants, and genetic relatedness of CPE strains. NGS provides valuable insights into the molecular epidemiology of CPE and can aid in outbreak investigations. However, it requires sophisticated bioinformatics analysis and may not be readily available in all settings.

Challenges in CPE Detection:

- Heterogeneous Resistance Mechanisms:

CPE can possess diverse carbapenemase genes or other resistance mechanisms, making their detection challenging. Phenotypic methods may not reliably differentiate between carbapenemase production and other resistance mechanisms, leading to false-negative or inconclusive results.

- Variability in Carbapenemase Genes:

The genetic diversity of carbapenemase genes poses challenges for molecular detection methods. New or emerging carbapenemases may not be included in commercial PCR assays, leading to potential missed detections. Ongoing surveillance and monitoring of emerging resistance mechanisms are necessary to update diagnostic assays accordingly.

- Coexistence of Resistance Mechanisms:

CPE strains can harbor multiple resistance mechanisms, such as extended-spectrum beta-lactamases (ESBLs) or AmpC enzymes, in addition to carbapenemases. The presence of these mechanisms can complicate the interpretation of phenotypic tests and require a comprehensive approach to identify and characterize CPE accurately.

- Turnaround Time and Accessibility:

Rapid diagnosis of CPE is essential for timely initiation of appropriate infection control measures. However, some diagnostic methods, particularly molecular techniques or NGS, may have longer turnaround times or limited accessibility, especially in resource-limited settings.

- Interpretation and Reporting:

Interpreting and reporting CPE diagnostic results require expertise and a thorough understanding of the available methods. Standardization of interpretation criteria, quality control measures, and reporting guidelines is necessary to ensure accurate and consistent reporting of CPE detection results.

6. THERAPEUTIC CONSIDERATIONS

Therapeutic considerations for CPE infections are complex due to limited treatment options and the emergence of multidrug resistance.

Antimicrobial Options:

- Carbapenems:

Carbapenems, such as meropenem or imipenem, are commonly considered the first-line agents for treating Enterobacteriaceae infections. However, CPE strains often exhibit resistance to carbapenems due to the production of carbapenemases. Therefore, the empirical use of carbapenems may not be effective in CPE infections unless susceptibility is confirmed.

- Alternative Beta-Lactam Agents:

Some CPE strains may retain susceptibility to certain beta-lactam agents, such as ceftazidime/avibactam or ceftolozane/tazobactam. These agents possess beta-lactamase inhibitors that can overcome certain carbapenemases and provide activity against CPE. However,

susceptibility testing should guide the choice of therapy, and local susceptibility patterns should be considered.

- Polymyxins:

Polymyxins, including colistin and polymyxin B, are considered as treatment options for CPE infections. They exhibit activity against many CPE strains, but their use is limited due to potential nephrotoxicity and neurotoxicity. Dosing adjustments based on renal function are crucial, especially in HD patients.

- Aminoglycosides:

Aminoglycosides, such as gentamicin or amikacin, can be considered in combination therapy regimens for CPE infections. They have synergistic activity with carbapenems or polymyxins and can enhance treatment efficacy. However, their use may be limited by renal toxicity and the need for therapeutic drug monitoring.

- Tigecycline:

Tigecycline, a glycylcycline antibiotic, is an option for CPE infections when other agents are not suitable. It exhibits activity against some CPE strains; however, its use as monotherapy is generally discouraged due to suboptimal pharmacokinetics and limited clinical data.

Combination Therapy:

Combination therapy is often recommended for severe CPE infections, particularly when limited treatment options are available. It aims to enhance treatment efficacy, prevent resistance emergence, and broaden the spectrum of activity. Combinations of agents with different mechanisms of action, such as carbapenems with aminoglycosides or polymyxins, are commonly used. However, the choice of combination therapy should be guided by susceptibility testing, individual patient factors, and local resistance patterns.

- Newer Antimicrobials:

Research and development of novel antimicrobial agents, including beta-lactam/beta-lactamase inhibitor combinations and other agents targeting resistant Gram-negative pathogens, are ongoing. These newer agents hold promise for treating CPE infections and may expand treatment options in the future.

- Therapeutic Drug Monitoring (TDM):

Therapeutic drug monitoring can be considered for certain agents, such as polymyxins or aminoglycosides, to optimize dosing regimens and minimize toxicity. TDM helps achieve therapeutic drug levels while avoiding supratherapeutic concentrations and reducing the risk of treatment failure or adverse effects. Nowadays, Romagna’s laboratory are improving the assays available for several antibiotics.

- Adjunctive Therapies:

Adjunctive therapies, such as immune-based therapies (e.g., monoclonal antibodies or phage therapy), may be explored in specific cases, although their clinical utility in CPE infections is still under investigation.

- Individualized Approach and Consultation:

Given the complexity of managing CPE infections, an individualized approach is crucial. Consultation with infectious disease specialists or antimicrobial stewardship teams can provide guidance on appropriate treatment options, combination therapy, dosing adjustments, and monitoring strategies based on local resistance patterns and patient-specific factors.

7. INFECTION PREVENTION AND STRATEGIES

Effective infection prevention and control (IPC) strategies are crucial for mitigating the spread of CPE HD settings. Surveillance and screening, isolation precautions, hand hygiene, environmental cleaning, antibiotic stewardship, education, and collaboration are key components of a comprehensive IPC approach. By implementing these measures consistently and involving all stakeholders, healthcare facilities can minimize the risk of CPE transmission, protect vulnerable patients, and improve patient outcomes.

Surveillance and Screening:

- Active Surveillance:

Implementing active surveillance programs for CPE colonization among HD patients helps identify asymptomatic carriers and prevent transmission. Regular screening using appropriate sampling methods, such as rectal or perianal swabs, can detect colonization early and guide infection control measures.

- Admission Screening:

Performing CPE screening for all newly admitted HD patients helps identify those colonized upon admission. This enables prompt implementation of appropriate precautions and prevents further transmission within the unit.

Isolation Precautions:

- Contact Precautions:

Adhering to contact precautions for patients colonized or infected with CPE is essential. These precautions include wearing gloves and gowns during patient care and ensuring proper hand hygiene before and after patient contact.

- Cohorting:

Considering cohorting of CPE-positive patients can help prevent cross-transmission within the HD unit. Grouping CPE-positive patients together and segregating them from CPE-negative patients can minimize the risk of transmission.

- Dedicated Equipment:

Using dedicated equipment, such as blood pressure cuffs, dialysis machines, and other reusable medical devices, for CPE-positive patients reduces the risk of cross-contamination and transmission to other patients.

- Hand Hygiene:

Promoting and ensuring proper hand hygiene practices among healthcare personnel is essential for preventing CPE transmission. Healthcare providers should perform hand hygiene with soap and water or an alcohol-based hand sanitizer, particularly before and after patient contact.

- Environmental Cleaning:

Implementing rigorous environmental cleaning and disinfection protocols is necessary to reduce the environmental reservoir of CPE. Regular cleaning of patient care areas, equipment, and high-touch surfaces using appropriate disinfectants helps eliminate CPE and prevent transmission.

- Antibiotic Stewardship:

Implementing antibiotic stewardship programs is crucial to minimize the selection pressure for CPE and reduce the risk of infections. Promoting appropriate antibiotic use, optimizing therapy, and

discouraging unnecessary antibiotic prescriptions can help prevent the emergence and spread of CPE strains.

- Education and Training:

Providing education and training to both healthcare personnel and patients is vital for effective IPC. Healthcare providers should receive regular training on hand hygiene, proper use of personal protective equipment, and adherence to infection control protocols. Patients should be educated about the importance of IPC measures, including hand hygiene and adherence to isolation precautions.

- Collaboration and Communication:

Effective collaboration and communication between different healthcare teams, including infection control, laboratory, and HD units, are essential for a comprehensive IPC approach. Sharing surveillance data, outbreak information, and implementing coordinated strategies help prevent and control CPE transmission.

- Surveillance of Environmental Sources:

Regular surveillance of environmental sources, such as water and surfaces in the HD unit, is crucial. Monitoring water quality, implementing appropriate water treatment strategies, and ensuring proper disinfection of surfaces contribute to reducing CPE transmission.

8. MICROBIOME ALTERATIONS

Colonization of the intestine by nonhost niche microorganisms can lead to a permeable gut through various mechanisms, by either being directly responsible for the features of inflammation or favoring microbe-microbe and microbe- host interactions such as regulating the diversity and community composition of the cecal microbiome, manipulating bacterial metabolites, participating the innate and the adaptive immune responses, and penetrating host barriers, allowing colonizing microorganisms to invade the intestinal tissue and leading to subsequent translocated infection. The diversity and composition of the gut microbiome were found to be linked to markers of inflammation and gut permeability in both the jejunum and colon. An increase in alpha diversity microbiome was associated with elevated levels of IL-6 in both the jejunum and plasma measurements. The association of similar bacteria with differences in plasma IL-6 and jejunum occludin indicate that a relationship may exist between systemic inflammation and intestinal tight-junction integrity. The alterations in cytokine secretion, characterized by multiple inflammatory markers, suggest that inflammation in this preclinical model spreads from the bloodstream to various organ systems [16].

Particularly, elevated plasma IL-6 has been shown to enhance the tissue expression of the receptor for the complement activation product C5a in a model of septic shock. Moreover, intestinal epithelial cells are known to respond to C5a, which can trigger all classic signs of inflammation, by increasing the permeability of monolayers like the gut mucosae. An elevated production of C5a in intestinal tissues can, in turn, lead to impairments in gut-barrier function and integrity. This increases the risk of bacterial translocation through opsonization, microbial dysbiosis, and bacteremia, all of which could potentially result in fatal sepsis.

It's plausible that immediate changes in the gut microbiome following an injury induce dysfunction in the intestinal barrier, allowing bacterial translocation into the bloodstream. This triggers a robust systemic proinflammatory immune response before the onset of sepsis. Furthermore, preexisting individual variations in the gut microbiome at the time of injury may influence the physiological response. For instance, a microbiome with a high relative abundance of LPS-containing gram-negative bacteria might result in increased inflammation in response to trauma.

There is a scarcity of studies that systematically monitor alterations in the gut microbiome before the onset of sepsis. Nevertheless, these initial investigations suggest that individuals with low diversity in their gut microbiome and a substantial presence of pathogenic gram-negative bacteria and enterococci are at an increased risk of developing sepsis. Similar risk factors have been observed in other studies for the development of bacteremia in hematopoietic stem cell transplant recipients and for infections in patients within the ICU.
Just as it is evident that the aforementioned alterations in the intestinal microbiome predispose to the onset of sepsis, it is equally established that sepsis reduces the variability of the gut microbiome. However, it is not straightforward to predict, and there are no conclusive studies demonstrating the specific nature of alterations in the intestinal microbiota following a sepsis episode, leaving numerous questions unanswered. [16 - 24]

Disrupting the balance of intestinal microbial communities can lead to immune dysregulation and increased susceptibility to diseases. The microbiota plays a crucial role in maintaining the host's functional innate immunity. The host's immune system continually monitors the intestinal environment to assess metabolic status and colonization. In a steady-state condition, metabolites and components from the commensal microbiota are recognized by various Pattern Recognition Receptors (PRRs), including Toll-like receptors (TLRs) and NOD-like receptors (NLRs). This recognition regulates the function of the intestinal epithelial barrier, the lifespan of phagocytes, and triggers the secretion of antimicrobial peptides (AMPs) and immunoglobulin A (IgA). Furthermore, beneficial bacteria ferment dietary fibers, producing short-chain fatty acids (SCFA), which stimulate the production of anti-inflammatory cytokines and the generation of regulatory T cells (Tregs) [25] [26].

The microbiota also influences the priming of the inflammasome activation, leading to the transcription of inflammatory factors like tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), pro-IL-1beta, and pro-IL-18. The gut microbiota contributes to maintaining intestinal immune homeostasis by modulating different facets of the T-cell response. For instance, segmented filamentous bacteria are potent inducers of Th17 cells in the intestine, while polysaccharide A from the commensal Bacteroides fragilis promotes the generation of Tregs. Alternatively, pattern recognition by TLRs and NLRs can also induce the maintenance of tolerance.

Lastly, it is evident that the intestinal immune system can detect the metabolic state of the microbiota by recognizing microbial metabolites through their respective PRRs [27].

9. FUTURE DIRECTIONS

Enhanced Diagnostic Techniques:
Continued research and development of diagnostic techniques for CPE are essential. Improvements in phenotypic methods, such as modified Hodge tests and colorimetric/fluorogenic assays, can enhance sensitivity and specificity. Additionally, advancements in molecular techniques, including point-of-care testing and rapid molecular assays, can provide more rapid and accurate detection of CPE.

Novel Therapeutic Approaches:
The development of new antimicrobial agents targeting CPE is a critical area of research. Novel beta-lactam/beta-lactamase inhibitor combinations, siderophore-conjugated antibiotics, and other agents with activity against resistant Gram-negative pathogens hold promise. Expanding the armamentarium of effective antibiotics is vital in combating CPE infections and improving treatment outcomes.

Antimicrobial Stewardship:
Continued emphasis on antimicrobial stewardship programs is crucial in preventing the emergence and spread of CPE. Robust antimicrobial stewardship efforts can optimize antibiotic use, promote judicious prescribing, and prevent the selection of resistant strains. Regular monitoring of prescribing practices and feedback to healthcare providers can lead to improved antimicrobial use and reduced CPE incidence.

Vaccines and Immunotherapies:
Exploring the potential for vaccines and immunotherapies targeting CPE is an area of ongoing research. Vaccines targeting common carbapenemases or outer membrane proteins can stimulate the immune response and potentially prevent CPE colonization or infection. Immunotherapies, including monoclonal antibodies, bacteriophages, or immune modulators, may also hold promise in augmenting the host immune response against CPE.

Infection Control Measures:
Continued emphasis on robust infection control measures is essential in preventing and containing CPE transmission. This includes strict adherence to hand hygiene practices, enhanced environmental cleaning, and implementation of innovative technologies for surface disinfection. Ongoing research on effective strategies for environmental decontamination and engineering solutions to minimize transmission risk can further improve infection control.

Surveillance and Genomic Epidemiology:
Advancements in genomic epidemiology and surveillance can provide valuable insights into the molecular epidemiology, transmission dynamics, and resistance mechanisms of CPE. Whole-genome sequencing and phylogenetic analysis can help identify transmission clusters, track outbreaks, and guide infection control interventions. Integrating genomic data with clinical and epidemiological information can enhance understanding of CPE epidemiology and inform targeted prevention strategies.

International Collaboration:
International collaboration is crucial in addressing the global challenge of CPE. Sharing data, knowledge, and best practices across different healthcare settings and countries can foster a better understanding of CPE epidemiology, antimicrobial resistance mechanisms, and innovative approaches. Collaborative efforts can accelerate research, harmonize surveillance strategies, and inform global policies for CPE prevention and control.

10. CPE INFECTIONS IN SPECIFIC POPULATION

10a. Intensive care units

CPE infections in intensive care units present a significant epidemiological and clinical challenge. Preventing and managing these infections requires a comprehensive approach, including stringent infection control practices, judicious antibiotic use, and effective surveillance to detect and respond to outbreaks promptly. The high stakes involved in ICU care make it imperative to address CPE infections effectively [28-31].

Epidemiology of CPE Infections in Intensive Care Units (ICUs):

CPE infections have become a significant concern in ICUs globally. These units often house critically ill patients with multiple comorbidities, central lines, and urinary catheters, making them more vulnerable to healthcare-associated infections. The problems in these kind of setting is that

ICUs can experience both endemic and outbreak scenarios of CPE. Endemic cases are continuously present, while outbreaks can result from cross-transmission, often fueled by lapses in infection control measures.
As seen in the previous pages, the epidemiology of CPE infections varies by region. Some areas experience a higher prevalence due to differences in antimicrobial use, local resistance patterns, and the effectiveness of infection control measures. CPE infections in ICUs are burdened by a high mortality rates, especially when initial antibiotic therapy is delayed or ineffective.

Risk Factors for CPE Infections in ICUs:

Antibiotic Exposure: The extensive use of broad-spectrum antibiotics in ICUs can select for multidrug-resistant organisms, including CPE. Prolonged antibiotic courses and inappropriate use contribute to resistance.
Invasive Procedures: Patients in ICUs often undergo invasive procedures, such as mechanical ventilation, urinary catheterization, and central venous catheter placement. These procedures can serve as entry points for CPE.
Prolonged Hospitalization: Lengthy stays in ICUs, characterized by frequent healthcare interventions, can increase the risk of CPE acquisition and infections. The longer the exposure to the healthcare environment, the higher the risk.
Comorbidities: Patients in ICUs frequently have multiple comorbidities, such as diabetes, immunosuppression, or chronic kidney disease (CKD). These conditions can compromise their immune response and increase susceptibility to infections.
Transfer Between Facilities: Patients may be transferred between healthcare facilities, potentially carrying CPE with them. Communication and effective infection control practices between facilities are crucial.
Environmental Contamination: The high density of patients and shared equipment in ICUs can lead to environmental contamination with CPE. Inadequate environmental cleaning and disinfection can contribute to transmission.
Antibiotic Pressure: The presence of CPE in ICUs can exert antibiotic pressure on healthcare providers, leading to the use of last-resort antibiotics, which can further drive resistance.
Clinical Implications of CPE Infections in ICUs:
Limited Treatment Options: CPE infections often exhibit resistance to multiple antibiotics, limiting treatment options. Clinicians may need to rely on less effective or more toxic antibiotics.
Increased Mortality: CPE infections are associated with a higher risk of mortality, especially among critically ill patients in ICUs.
Extended Hospital Stays: Patients with CPE infections may experience longer hospital stays, including periods of isolation to prevent transmission.
Infection Control Challenges: Managing CPE infections in ICUs can be challenging due to high patient turnover, shared spaces, and the need for stringent infection control measures.

10b. Hematological malignancies

Hematological patients, particularly those with hematological malignancies or those undergoing stem cell transplantation, are at heightened risk for infections due to compromised immune systems resulting from their disease and treatment. CPE infections often occur in hospital settings, including specialized hematological units. These units can serve as reservoirs for multidrug-resistant pathogens, including CPE. Preventing and managing these infections requires a multifaceted approach, including strict infection control measures, careful antibiotic stewardship, and close monitoring to promptly detect and respond to outbreaks. The critical condition of hematological patients necessitates effective strategies to address CPE infections [32- 35].

This population present some specifics features that makes it much more susceptible of CPE infection:

Immunosuppression: Hematological patients are often profoundly immunosuppressed due to their underlying condition and the aggressive treatments they receive, which can include chemotherapy and stem cell transplantation. This makes them highly susceptible to infections, including CPE.

Prolonged Hospitalization: Many hematological patients require extended hospitalization, often involving multiple healthcare interventions. Prolonged exposure to the healthcare environment increases the risk of CPE acquisition.

Invasive Procedures: Hematological patients frequently undergo invasive procedures, including bone marrow biopsies, central line insertions, and stem cell transplants. These procedures can serve as entry points for CPE.

Broad-Spectrum Antibiotics: Patients with hematological conditions often receive broad-spectrum antibiotics, which can select for resistant organisms, including CPE.

Comorbidities: Hematological patients frequently have underlying comorbidities, such as neutropenia, which further compromise their immune defenses.

Antibiotic Pressure: The presence of CPE in hematological units can lead to antibiotic pressure, where healthcare providers turn to last-resort antibiotics, potentially driving resistance further.

Clinical Implications of CPE Infections in Hematological Patients:

Limited Treatment Options: CPE infections are often resistant to multiple antibiotics, limiting therapeutic options. Clinicians may need to consider alternative treatments, which may be less effective or more toxic.

High Mortality Rates: CPE infections in hematological patients are associated with increased mortality, especially among those with compromised immune systems.

Extended Hospital Stays: Patients with CPE infections may experience prolonged hospital stays, which can include periods of isolation to prevent transmission.

Infection Control Challenges: Managing CPE infections in hematological units can be challenging due to the immunosuppressed nature of the patient population, high patient turnover, and shared spaces.

10c. Transplant recipients

Transplanted patients, including those who have received solid organ, are profoundly immunosuppressed to prevent organ rejection. This immunosuppression makes them highly susceptible to various infections, including CPE. These units house patients with complex medical needs, providing opportunities for CPE transmission. The incidence of CPE infections can vary in transplant units, reflecting regional differences in CPE prevalence, resistance patterns, and the effectiveness of infection control measures [36-39].

Risk Factors for CPE Infections in Transplanted Patients:

Immunosuppression: Immunosuppressive regimens are a necessity for transplant patients to prevent graft rejection. These regimens weaken the immune system, increasing the risk of infections, including CPE.

Invasive Procedures: Transplanted patients often undergo invasive procedures, including organ transplantation surgery. These procedures create entry points for CPE and other healthcare-associated pathogens.

Prolonged Hospitalization: Many transplant patients require extended hospitalization both pre- and post-transplant. This prolonged exposure to healthcare environments can increase the risk of CPE acquisition.

Broad-Spectrum Antibiotics: Post-transplant, patients receive immunosuppressants and broad-spectrum antibiotics to prevent and treat infections. The use of antibiotics can select for resistant organisms, including CPE.

Comorbidities: Transplant patients may have underlying comorbidities related to their primary disease or organ failure, which can further compromise their immune defenses.

Antibiotic Pressure: The presence of CPE in transplant units can lead to antibiotic pressure, causing healthcare providers to resort to last-resort antibiotics, potentially driving resistance further.

Clinical Implications of CPE Infections in Transplanted Patients:

Limited Treatment Options: CPE infections are often resistant to multiple antibiotics, limiting therapeutic choices. Clinicians may need to explore alternative treatments, which may be less effective or more toxic.

High Mortality Rates: CPE infections in transplanted patients are associated with increased mortality, especially among those with compromised immune systems.

Prolonged Hospital Stays: Patients with CPE infections may experience extended hospital stays, sometimes in isolation to prevent transmission.

Infection Control Challenges: Managing CPE infections in transplant units can be complex due to the immunosuppressed nature of the patient population, the need for close monitoring, and stringent infection control measures.

In summary, CPE infections in transplanted patients pose a unique challenge due to the profound immunosuppression required to prevent graft rejection. Effective prevention and management of these infections necessitate rigorous infection control, judicious use of antibiotics, and vigilant monitoring to promptly detect and respond to outbreaks. The heightened vulnerability of transplanted patients underlines the importance of addressing CPE infections in transplant settings.

11. HEMODIALYSIS

Hemodialysis (HD): HD is a well-established and widely utilized technique in which blood is withdrawn from the patient's body through a vascular access site, passed through a dialyzer, and then returned to the patient. The dialyzer acts as an artificial kidney, utilizing a semipermeable membrane to remove waste products, excess fluids, and toxins from the blood. HD primarily relies on diffusion to accomplish solute clearance.

Hemodiafiltration (HDF): Hemodiafiltration is an advanced variation of HD that combines diffusion and convection to achieve enhanced solute removal. In HDF, a convective component is introduced by infusing a sterile substitution fluid into the extracorporeal circuit. This fluid, along with the diffusive component, helps in the removal of middle and larger molecular weight substances more efficiently. The combination of diffusive and convective clearance in HDF has been shown to provide better solute removal compared to conventional HD.

Advantages of Hemodiafiltration:

Improved Solute Clearance: By incorporating convective clearance, HDF offers better removal of middle and larger molecular weight toxins that are not efficiently cleared by diffusion alone. This can contribute to improved overall solute clearance and potentially better clinical outcomes.

Removal of Inflammatory Mediators: HDF has been shown to remove pro-inflammatory substances, cytokines, and other uremic toxins that are implicated in chronic inflammation and associated complications in ESRD patients.

Potential Cardiovascular Benefits: Some studies have suggested that HDF may have advantages in reducing cardiovascular events, such as improved blood pressure control, decreased left ventricular hypertrophy, and better control of fluid balance.

It is important to note that HDF requires additional infrastructure, such as a high-quality water system and reliable substitution fluid delivery. It also demands closer monitoring to ensure proper fluid and electrolyte balance, especially in patients with cardiovascular instability or low blood pressure.

12. PERITONEAL DIALYSIS

Peritoneal dialysis (PD) is a renal replacement therapy option for patients with ESRD. When considering the epidemiology and risk factors related to PD, several key points should be emphasized:

Globally, PD makes up 9% of all kidney replacement therapy (KRT) and 11% of all dialysis procedures. As per data from the 2018 International Society of Nephrology Global Kidney Health Atlas (ISN-GKHA), the median worldwide prevalence of PD stood at 38.1 per million population (pmp). However, this prevalence exhibited an extraordinary range, spanning over 5,000-fold, from 0.1 pmp in Egypt to 531 pmp in Hong Kong. A significant portion of PD patients, more than half, resided in just four countries: China, the United States, Mexico, and Thailand. Notably, PD was not accessible in 30 countries, with 20 of them located in Africa. The survey findings further highlighted that the utilization of PD was substantially lower in low-income countries (LICs) at 0.9 pmp (with a 95% confidence interval of 0.7–1.5) compared to high-income countries (HICs) where it was 53.0 pmp (with a 95% confidence interval of 40.6–89.8 pmp). [40-44]. Although PD is a well established treatment modality it is underused in Western countries, with a prevalence in Italy of 15%. Despite there being contraindications for each treatment, nowadays the choice of the kind of treatment depends on several features, mainly the specific experience of the Clinical Unit and the patient’s choice. [45,46].
The preservation of residual kidney function is a key factor in PD. This can impact patient outcomes and complications. Efforts to maintain residual kidney function, such as avoiding nephrotoxic drugs or insults that can determine Acute kidney injury are important.

Monitoring peritoneal membrane function is vital. High or low transporter status can affect the effectiveness of PD treatment. Assessing transporter status and making necessary adjustments can optimize therapy. The nutritional status is another key point in this kind of patients. Obesity is associated with complications such as catheter exit site infection and mechanical issues during PD. Conversely, malnutrition can increase the risk of peritonitis and affect patient outcomes. Proper nutritional management is crucial.

PD offers distinct advantages when compared to HD. These advantages encompass the convenience of home-based treatment, improved quality of life, technical simplicity, reduced reliance on highly trained staff, greater cost-effectiveness in most countries, enhanced accessibility to dialysis in resource-limited settings, and improved survival rates, particularly in the initial years of therapy. Key outcomes associated with PD encompass not only clinical outcomes, which are typically defined as medical outcomes based on assessments or diagnoses by healthcare providers. These clinical outcomes include aspects like PD-related infections, technique survival, mechanical complications, hospitalizations, and PD-related mortality. However, an equally crucial dimension lies in patient-reported outcomes. These outcomes are reported directly by patients and focus on how they function or feel, often related to their quality of life or symptoms. Patient-reported outcomes, though highly valuable, are somewhat underutilized in routine day-to-day healthcare compared to clinical outcomes. [47]

13. MORTALITY IN HEMODIALYSIS PATIENTS

Mortality in HD patients remains a significant concern within the field of nephrology. Compared to the general population, individuals undergoing HD have a higher mortality rate and face a number of challenges that contribute to this increased risk. Here are some key factors associated with mortality in HD patients:

Cardiovascular Disease: Cardiovascular disease is the leading cause of death in HD patients. These individuals have a higher prevalence of risk factors such as hypertension, diabetes, dyslipidemia, and left ventricular hypertrophy. Additionally, the presence of chronic inflammation, volume overload, vascular calcification, and anemia further contribute to cardiovascular complications.

Infection: Infections are a significant cause of morbidity and mortality in HD patients. Factors such as immunosuppression, vascular access-related infections, comorbid conditions, and frequent exposure to healthcare settings increase the susceptibility to infections. Bloodstream infections (BSIs), pneumonia, and soft tissue infections can lead to sepsis, resulting in severe complications and death (Figure 8).

Malnutrition and Inflammation: HD patients often experience malnutrition and chronic inflammation, commonly referred to as the malnutrition-inflammation complex syndrome. Inadequate nutrient intake, protein-energy wasting, chronic inflammation, and increased oxidative stress contribute to the overall increased mortality risk.

Cardiovascular Events: HD patients are at a higher risk of experiencing cardiovascular events such as myocardial infarction, congestive heart failure, arrhythmias, and sudden cardiac death. Factors such as electrolyte imbalances, fluid overload, vascular calcification, and uremic toxins contribute to cardiovascular events.

Dialysis Adequacy and Access Complications: Insufficient dialysis adequacy, indicated by low urea reduction ratios and inadequate removal of uremic toxins, is associated with increased mortality

risk. Complications related to vascular access, such as infections, thrombosis, and stenosis, can lead to vascular access failure, contributing to morbidity and mortality.

Other Factors: HD patients are also at an increased risk of developing metabolic bone disease, mineral imbalances, hyperparathyroidism, and anemia. These conditions, if not properly managed, can further impact patient outcomes and contribute to mortality.

	ESRD patients, 2019				ESRD patients, 2020			
	Dialysis		Transplant		Dialysis		Transplant	
Age	Female	Male	Female	Male	Female	Male	Female	Male
40-44	10.2	11.1	29.7	28.0	8.9	9.4	26.7	24.4
45-49	9.1	9.6	25.8	24.1	7.7	8.2	23.0	20.8
50-54	7.8	8.1	22.1	20.5	6.8	7.0	19.6	17.4
55-59	6.8	6.9	18.7	17.1	5.9	6.0	16.5	14.5
60-64	5.9	5.8	15.5	14.1	5.1	5.0	13.6	11.9
65-69	5.0	4.8	12.6	11.5	4.4	4.2	10.9	9.7
70-74	4.2	4.1	10.1	9.3	3.7	3.6	8.7	7.7
75-79	3.7	3.5	8.2[a]	7.5[a]	3.3	3.0	6.9[a]	6.0[a]
80-84	3.2	2.9			2.8	2.6		
85+	2.6	2.4			2.4	2.1		

Figure 7. Life expectancy of dialysis and transplant population allocated for age. Dialysis patients shown a reduced life expectancy compared to transplanted.

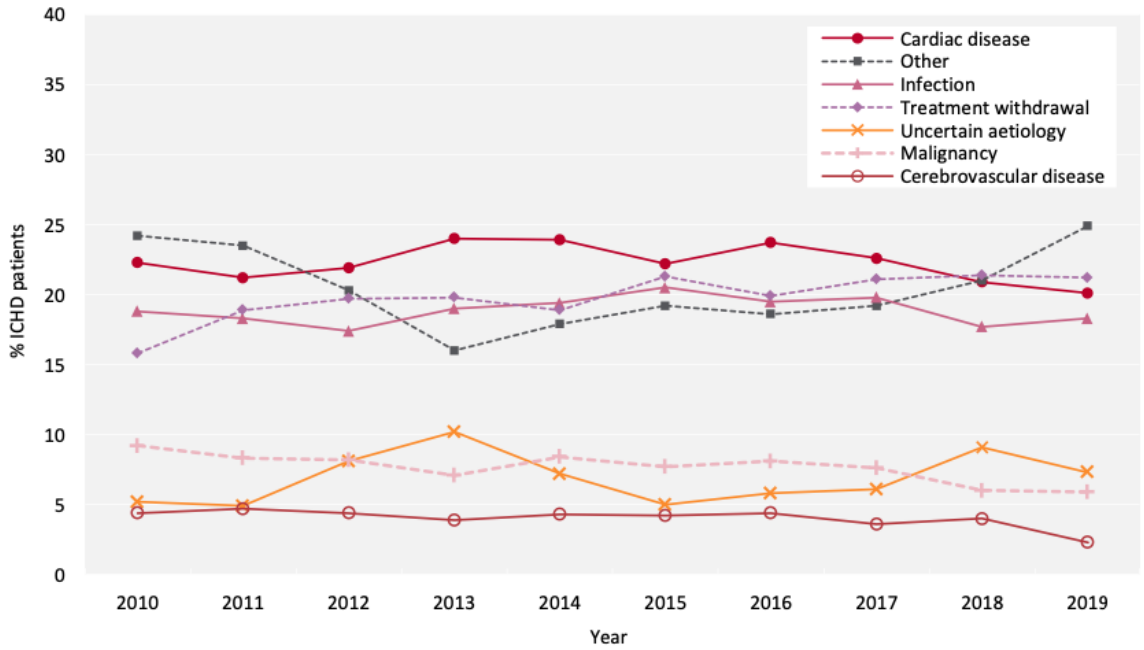


Figure 8. Causes of mortality in HD patients according to 23°Annual report of UKKA, 2019 [48]. Infections represent the 2°cause of mortality in HD patients (25%).

14. INFECTIONS IN HEMODIALYSIS

Infections are a significant concern in HD patients and can lead to increased morbidity and mortality. Several factors contribute to the increased risk of infections in this population, including

compromised immune function, repeated exposure to healthcare settings, and the presence of vascular access sites.
HD patients are susceptible to BSIs, which can be caused by pathogens introduced through access sites or from other sources, such as urinary tract infections or respiratory infections. Prompt identification, appropriate antimicrobial therapy, and catheter removal (if indicated) are critical in managing BSIs.
Vaccination plays a crucial role in preventing infections in HD patients. It is recommended to provide routine immunizations, including influenza, pneumococcal, hepatitis B, and other vaccines, as per guidelines and patient-specific factors.
Implementing strict infection control measures is crucial in preventing infections in HD patients. Key strategies include proper hand hygiene, regular disinfection of dialysis equipment and surfaces, appropriate catheter care and dressing changes, adherence to aseptic techniques during catheter insertion and manipulation, optimization of vascular access care, appropriate antimicrobial therapy and patient education. Multidisciplinary collaboration among nephrologists, nurses, infection control practitioners, and other healthcare professionals is essential in reducing the burden of infections and improving patient outcomes.

Pathogens involved in hemodialysis infections

HD patients are susceptible to various pathogens that can cause infections. The choice of pathogen can depend on factors such as the type of access, local epidemiology, patient-specific characteristics, and infection prevention practices. These are the most common pathogens involved in HD-related infections:

Staphylococcus aureus: Staphylococcus aureus is a gram-positive bacterium and a leading cause of infections in HD patients. It can cause access site infections, BSIs, and infections at other sites. Methicillin-resistant Staphylococcus aureus (MRSA) is a particular concern due to its resistance to multiple antibiotics.

Coagulase-Negative Staphylococci (CoNS): Coagulase-negative staphylococci, such as Staphylococcus epidermidis, are commonly associated with HD-related infections. They can colonize vascular access sites and cause infections, including catheter-related BSIs.

Escherichia coli: Escherichia coli is a gram-negative bacterium that can cause urinary tract infections, BSIs, and other infections in HD patients. Infections with multidrug-resistant strains of E. coli can pose significant challenges in treatment.

Klebsiella pneumoniae: Klebsiella pneumoniae is another gram-negative bacterium associated with HD-related infections. It can cause urinary tract infections, pneumonia, and BSIs. Some strains of K. pneumoniae have developed resistance to multiple antibiotics.

Pseudomonas aeruginosa: Pseudomonas aeruginosa is a gram-negative bacterium that can cause severe infections in HD patients. It is commonly associated with access site infections, respiratory tract infections, and BSIs. Pseudomonas infections can be difficult to treat due to their intrinsic resistance to many antibiotics.

Candida species: Fungal infections, particularly those caused by Candida species, are a concern in HD patients. Candida infections can occur at vascular access sites, causing access-related infections and BSIs. The use of central venous catheters and long-term broad-spectrum antibiotic use can increase the risk of Candida infections.

Other pathogens, including gram-negative bacteria such as Enterobacter spp., Acinetobacter spp., and Serratia marcescens, as well as other fungal species, such as Aspergillus spp., can also cause infections in HD patients. It is important to consider local epidemiology and antibiotic resistance patterns when managing infections in these patients.

Prevention measures, including proper hand hygiene, catheter care, aseptic techniques during catheter insertion, adherence to infection control protocols, and antimicrobial stewardship, are crucial in reducing the risk of infections and minimizing the spread of multidrug-resistant pathogens in the HD setting.

Infection rate in hemodialysis patients

Infection rates in HD patients can vary depending on various factors such as geographic location, patient population, healthcare practices, infection prevention measures, and the presence of comorbidities. Infections are a significant concern in this population due to their increased susceptibility and exposure to healthcare settings.

Vascular Access Infections: Infections related to vascular access, including central venous catheters (CVCs), arteriovenous fistulas (AVFs), and arteriovenous grafts (AVGs), are a common source of infections in HD patients. The infection rates can vary depending on the type of access used, with CVCs being associated with the highest infection rates. Studies have reported infection rates ranging from 0.5 to 6.5 infections per 1000 catheter-days for CVCs.

Catheter-Related Bloodstream Infections (CRBSIs): CRBSIs are a serious complication associated with CVCs. The rate of CRBSIs in HD patients can range from 1.5 to 6.0 episodes per 1000 catheter-days.

Exit Site Infections: Exit site infections, which occur at the point where the catheter exits the skin, can also contribute to infection rates in HD patients. The incidence of exit site infections varies, with rates reported between 0.2 and 1.0 infections per 1000 catheter-days.

Other Infections: In addition to vascular access-related infections, HD patients are also at risk of other types of infections, including urinary tract infections, respiratory tract infections, soft tissue infections, and BSIs from non-access-related sources. The rates of these infections can vary widely depending on factors such as local epidemiology, infection prevention practices, and patient-specific characteristics.

Urinary tract infections in hemodialysis patients

Urinary tract infections (UTIs) can occur in HD patients, although the incidence and risk factors may differ from the general population. Addressing UTIs in HD patients requires a comprehensive approach that includes infection prevention strategies, appropriate catheter care, vigilant monitoring, and prompt diagnosis and treatment of UTIs when they occur. Multidisciplinary collaboration among nephrologists, infectious disease specialists, urologists, and healthcare providers involved in the care of HD patients is crucial to optimize outcomes and reduce the burden of UTIs.

Risk Factors: HD patients have several unique risk factors for UTIs, including the presence of indwelling urinary catheters, frequent healthcare exposures, compromised immune function, underlying urological abnormalities, and residual urine in the bladder due to impaired kidney function. Female HD patients may be at a higher risk due to anatomical factors.

Catheter-Associated UTIs: HD patients with indwelling urinary catheters, commonly used in cases of urinary retention or urinary incontinence, have an increased risk of developing catheter-associated UTIs. These infections can occur due to bacterial colonization and biofilm formation on the catheter surface.

Pathogens: The most common pathogens causing UTIs in HD patients are similar to those in the general population and include gram-negative bacteria such as Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis. However, there may be a higher prevalence of multidrug-resistant organisms, including extended-spectrum beta-lactamase (ESBL)-producing bacteria and methicillin-resistant Staphylococcus aureus (MRSA), in the HD setting.

Diagnosis: UTIs in HD patients are typically diagnosed based on clinical symptoms (e.g., urinary frequency, urgency, dysuria) and confirmed by urine culture. Due to the presence of chronic inflammation and colonization of the urinary tract, it is important to interpret urine culture results in the context of clinical presentation to differentiate between asymptomatic bacteriuria and true UTIs requiring treatment.

Prevention and Management: Preventive measures are crucial to reduce the incidence of UTIs in HD patients. Strategies include appropriate catheter care, regular catheter maintenance and replacement, avoidance of unnecessary catheter use, optimizing fluid balance, and promoting proper hygiene practices. Prompt treatment with appropriate antibiotics based on urine culture and sensitivity results is essential for managing UTIs in this population.

Complications and Recurrence: Untreated or recurrent UTIs in HD patients can lead to complications such as pyelonephritis (kidney infection), sepsis, and urosepsis. Recurrent UTIs may necessitate further evaluation for underlying urological abnormalities and consideration of long-term preventive measures.

Respiratory tract infections in hemodialysis patients

Respiratory tract infections are a significant concern in HD patients, as they can lead to complications and increased morbidity and mortality.

Increased Susceptibility: HD patients often have a higher susceptibility to respiratory tract infections due to several factors. These include compromised immune function, frequent healthcare exposures, underlying comorbidities such as diabetes or cardiovascular disease, and the presence of chronic inflammation associated with ESRD.

Pneumonia: Pneumonia, an infection of the lungs, is a common respiratory tract infection in HD patients. It can be caused by a variety of pathogens, including bacteria (such as Streptococcus pneumoniae and Staphylococcus aureus), viruses (such as influenza and respiratory syncytial virus), and less commonly, fungi. Pneumonia can be community-acquired or healthcare-associated, depending on the setting where the infection is acquired.

Upper Respiratory Tract Infections: HD patients are also susceptible to upper respiratory tract infections, including sinusitis, pharyngitis, and upper respiratory viral infections (such as the common cold). These infections are often caused by viral pathogens, including rhinovirus, influenza viruses, and respiratory syncytial virus.

Dialysis-Related Factors: HD procedures can potentially contribute to respiratory tract infections. The close proximity of patients in dialysis units and the shared environment may increase the risk of transmission of respiratory pathogens. Additionally, exposure to dialysis equipment and surfaces, if not properly disinfected, can contribute to the spread of infections.

Influenza and Pneumococcal Vaccination: Vaccination plays a crucial role in preventing respiratory tract infections in HD patients. Annual influenza vaccination is recommended for all HD patients and their healthcare providers. Pneumococcal vaccination, including the pneumococcal conjugate vaccine (PCV13) and pneumococcal polysaccharide vaccine (PPSV23), is also recommended to protect against pneumococcal infections.

Prevention and Management: Preventive measures are vital in reducing the risk of respiratory tract infections in HD patients. These include promoting hand hygiene, implementing respiratory etiquette, ensuring appropriate ventilation in dialysis units, adherence to infection control protocols, and timely administration of vaccinations. Early recognition, prompt diagnosis, and appropriate treatment of respiratory tract infections are essential in managing these infections and preventing complications.

Covid infection in dialysis patients

COVID-19, caused by the novel coronavirus SARS-CoV-2, has had a significant impact on dialysis patients.

Increased Vulnerability: Dialysis patients are considered a high-risk population for severe illness from COVID-19 due to their compromised immune system, underlying comorbidities (such as cardiovascular disease and diabetes), and frequent healthcare exposures. They may also have limited physiological reserve, making them more susceptible to complications.

Higher Mortality Rates: HD patients who contract COVID-19 have been found to have higher mortality rates compared to the general population. Factors such as advanced age, presence of comorbidities, and the burden of CKD contribute to this increased risk.

Transmission: COVID-19 can be transmitted through respiratory droplets and close contact. HD units, where patients receive treatment in close proximity, can be potential sites for transmission. Strict infection control measures, including screening, testing, use of personal protective equipment (PPE), and environmental disinfection, are critical in reducing the risk of transmission.

Symptom Presentation: HD patients with COVID-19 may present with a range of symptoms, including fever, cough, shortness of breath, fatigue, and loss of taste or smell. However, some individuals, including older adults or those with immunosuppression, may have atypical or milder symptoms.

Management: The management of COVID-19 in HD patients involves a multidisciplinary approach, including nephrologists, infectious disease specialists, and other healthcare professionals. Treatment typically includes supportive care, monitoring of fluid and electrolyte balance, and management of respiratory symptoms. Close attention should be given to potential drug interactions with medications used in HD, such as anticoagulants and erythropoiesis-stimulating agents.

Vaccination: COVID-19 vaccination is strongly recommended for HD patients as part of global vaccination efforts. Vaccination can help reduce the risk of severe illness, hospitalization, and

mortality from COVID-19. HD patients should receive the COVID-19 vaccine as per local guidelines and recommendations.

Given the heightened vulnerability of HD patients to COVID-19, strict adherence to infection control measures, vaccination, and ongoing surveillance are crucial in minimizing the risk of transmission and optimizing patient outcomes. Regular communication and coordination among healthcare providers, dialysis units, and public health authorities are vital in managing COVID-19 infections in HD patients.

Bloodstream infections non related to vascular access in hemodialysis patients

BSIs can occur in HD patients from non-access-related sources, meaning infections that do not originate directly from the vascular access site.

Sources of Infection: Non-access-related BSIs in HD patients can originate from various sources, including urinary tract infections, respiratory tract infections, skin and soft tissue infections, intra-abdominal infections, and infections associated with other medical devices or surgical sites. These infections can lead to bacteria or other pathogens entering the bloodstream.
Pathogens: The pathogens causing non-access-related BSIs in HD patients can vary. Gram-positive bacteria, such as Staphylococcus aureus, Streptococcus species, and Enterococcus species, are commonly implicated. Gram-negative bacteria, including Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, can also cause these infections. Fungal infections, such as those caused by Candida species, may occur as well.

Risk Factors: HD patients have specific risk factors that contribute to non-access-related BSIs, including immunocompromised status, frequent healthcare exposures, presence of comorbidities (e.g., diabetes, cardiovascular disease), impaired immune function, and prolonged hospital stays. HD patients may have multiple risk factors simultaneously, increasing their susceptibility to BSIs.

Clinical Presentation: Non-access-related BSIs can present with symptoms such as fever, chills, malaise, and signs of sepsis. The specific symptoms and severity may vary depending on the source of infection and the causative pathogen.

Diagnosis and Treatment: Diagnosis of non-access-related BSIs involves blood cultures to identify the causative pathogen. Regular surveillance and monitoring for signs of infection are important for early detection and intervention. Prompt initiation of appropriate antibiotic therapy is crucial for effective treatment. The choice of antibiotics should be guided by culture and sensitivity results, along with consideration of local resistance patterns and the patient's individual characteristics.

15. IMMUNE SYSTEM DYSFUNCTION IN HEMODIALYSIS

Compromised immune-system in hemodialysis

HD patients often have a compromised immune system, which increases their vulnerability to infections. Several factors contribute to immunocompromised status in these patients:

Uremic Toxins: CKD and ESRD result in the accumulation of uremic toxins in the body. These toxins have immunosuppressive effects, impairing immune cell function and reducing the body's ability to mount an effective immune response against infections.

Nutritional Deficiencies: Malnutrition and inadequate nutrient intake are common in HD patients. Deficiencies in essential nutrients, such as vitamins, minerals, and trace elements, can weaken the immune system and compromise its ability to fight infections.

Dialysis-Related Factors: HD itself can impact the immune system. During the dialysis process, there is a loss of certain immune components, including immunoglobulins and complement proteins, which are important for defense against infections. HD can also lead to immune cell dysfunction and alterations in cytokine levels, further compromising immune function.

Vascular Access: The presence of vascular access sites, such as central venous catheters (CVCs), arteriovenous fistulas (AVFs), or arteriovenous grafts (AVGs), increases the risk of infection. Infections at these access sites can directly introduce pathogens into the bloodstream and impair immune function locally.

Comorbidities and Medications: HD patients often have comorbid conditions such as diabetes, cardiovascular disease, and autoimmune disorders. These conditions and their associated medications, including immunosuppressants and corticosteroids, can further weaken the immune system and increase infection susceptibility.

Frequent Healthcare Exposure: HD patients require regular visits to healthcare settings, exposing them to potential pathogens. Dialysis centers and hospitals can be reservoirs for healthcare-associated infections, increasing the risk of acquiring infections during dialysis sessions or hospital stays.

Immune cells dysfunction during hemodialysis

During HD, immune cell dysfunction can occur, leading to alterations in immune response and increased susceptibility to infections. Several factors contribute to immune cell dysfunction in HD patients:

Uremic Environment: CKD and ESRD result in the accumulation of uremic toxins in the body. These toxins, such as urea, guanidines, and cytokines, have immunosuppressive effects and can directly impair the function of immune cells. Uremic toxins can affect various aspects of immune cell function, including altered cytokine production, impaired phagocytosis, decreased lymphocyte proliferation, and reduced antigen-presenting capability.

Oxidative Stress: HD patients often experience increased oxidative stress due to the accumulation of reactive oxygen species (ROS) and reduced antioxidant defense mechanisms. This oxidative stress can cause damage to immune cells and impair their function, leading to decreased immune response and increased susceptibility to infections.

Inflammation: Inflammation is a common feature of CKD and ESRD. The chronic inflammatory state seen in these conditions can lead to immune cell dysfunction. Inflammatory mediators, such as interleukins and tumor necrosis factor-alpha (TNF-alpha), can alter immune cell signaling, impair cellular function, and disrupt the balance between pro-inflammatory and anti-inflammatory responses.
Dialysis-Related Factors: HD itself can contribute to immune cell dysfunction. During dialysis sessions, there is a loss of certain immune components, including immunoglobulins and complement proteins, which are essential for immune defense. Additionally, the interaction of blood with the artificial surfaces of dialysis equipment can trigger activation of immune cells and the release of pro-inflammatory cytokines, further contributing to immune dysregulation.

Medications: HD patients often require medications to manage comorbid conditions such as hypertension, diabetes, and cardiovascular disease. Some of these medications, such as immunosuppressants or corticosteroids, can directly impact immune cell function and suppress immune responses.

The immune cell dysfunction observed in HD patients can lead to increased susceptibility to infections, impaired wound healing, and altered immune surveillance against malignancies. It is crucial to manage this immune dysfunction through a comprehensive approach, including nutritional support, control of inflammation, optimization of dialysis techniques, vaccination, and appropriate medication management.

Oxidative stress during hemodialysis

HD can contribute to oxidative stress in patients with ESRD. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them with antioxidants. Here's briefly described how HD can contribute to oxidative stress:

Ischemia-Reperfusion Injury: During HD, the circulation of blood is temporarily interrupted when it is diverted from the patient's body to the dialysis machine. This interruption of blood flow followed by reperfusion during the return of blood can lead to ischemia-reperfusion injury. The restoration of blood flow causes an increased generation of ROS, contributing to oxidative stress.

Contact with Bioincompatible Surfaces: The interaction of blood with the surfaces of dialysis equipment and materials, such as dialyzer membranes and tubing, can trigger the activation of immune cells and the release of pro-inflammatory cytokines. This immune activation and inflammation can result in the production of ROS and oxidative stress.

Changes in Antioxidant Systems: HD can alter the antioxidant defense mechanisms in the body. It can lead to a decrease in antioxidant levels, such as reduced levels of glutathione, superoxide dismutase, and catalase, which are crucial in neutralizing ROS. This imbalance between ROS production and antioxidant defense contributes to oxidative stress.

Uremic Toxins: ESRD patients have an accumulation of uremic toxins due to impaired kidney function. These uremic toxins, such as advanced glycation end-products (AGEs), urea, and guanidines, can directly contribute to oxidative stress by promoting ROS production and impairing antioxidant systems.

The consequences of oxidative stress during HD can have various implications for patients. It can lead to damage to lipids, proteins, and DNA, and contribute to tissue injury, inflammation, and complications such as cardiovascular disease and accelerated atherosclerosis. Oxidative stress can also impact immune cell function and contribute to immune dysfunction, as mentioned in the previous response.

Efforts to mitigate oxidative stress during HD include strategies such as the use of biocompatible dialysis membranes, optimization of dialysis techniques, and antioxidant supplementation. Additionally, lifestyle modifications including a well-balanced diet rich in antioxidants and regular exercise can help reduce oxidative stress.

Close monitoring of oxidative stress markers and ongoing research into antioxidant therapies are essential to better understand and address the impact of oxidative stress during HD, with the goal of improving patient outcomes and reducing long-term complications.

Inflammation during hemodialysis

Inflammation is a common feature observed in patients undergoing HD. Multiple factors contribute to the development and persistence of inflammation during HD.

Uremic Toxins: In ESRD, the kidneys are unable to effectively remove waste products and toxins from the body. Accumulation of uremic toxins, such as advanced glycation end-products (AGEs), cytokines, and pro-inflammatory molecules, contributes to a chronic inflammatory state. These toxins stimulate immune cells and promote the release of inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-alpha).

Activation of Immune Cells: HD procedures involving blood contact with foreign surfaces and the dialyzer membrane can activate immune cells, such as monocytes and neutrophils. Activation of these cells triggers the release of pro-inflammatory mediators, contributing to inflammation.

Endotoxins: Endotoxins, which are components of the cell wall of certain bacteria, can be present in the blood of HD patients due to gut translocation. These endotoxins can initiate an inflammatory response, stimulating the production of pro-inflammatory cytokines.

Vascular Access-Related Infections: Infections related to vascular access sites, such as central venous catheters (CVCs), arteriovenous fistulas (AVFs), or arteriovenous grafts (AVGs), can lead to local and systemic inflammation. The presence of infections or biofilm on the access site triggers an immune response, contributing to inflammation.

Oxidative Stress: HD itself can induce oxidative stress, leading to inflammation. The process of ischemia-reperfusion during HD, contact of blood with artificial surfaces, and imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms contribute to oxidative stress, which can promote inflammation.

Chronic inflammation in HD patients has been linked to increased cardiovascular disease, malnutrition, muscle wasting, and accelerated atherosclerosis. Managing inflammation during HD is important for improving patient outcomes. Strategies to address inflammation include:

Biocompatible Dialysis Materials: The use of biocompatible dialysis membranes and materials can reduce immune activation and inflammation during HD.

Adequate Dialysis Adequacy: Achieving optimal dialysis adequacy, including adequate removal of uremic toxins and maintenance of fluid balance, can help minimize inflammation.
Nutritional Support: Ensuring appropriate nutritional support, including an adequate protein and calorie intake, can help address inflammation and reduce the risk of malnutrition and muscle wasting.

Control of Vascular Access Infections: Strict adherence to infection prevention protocols and prompt treatment of vascular access-related infections can help minimize inflammation associated with infections.

Medications: In some cases, anti-inflammatory medications or medications targeting specific inflammatory pathways may be prescribed to manage inflammation in HD patients. However, their use should be carefully evaluated and individualized based on the patient's specific needs and potential risks.

Dialysis related factors improved cells dysfunction

Dialysis-related factors can contribute to immune cell dysfunction in patients undergoing HD. However, certain strategies and interventions can help improve immune cell function and mitigate immune dysfunction. Here are some dialysis-related factors that can positively impact immune cell dysfunction:

Biocompatible Dialysis Membranes: The choice of dialysis membrane can play a role in immune cell activation and function. Biocompatible membranes have been developed to minimize immune activation and reduce the release of pro-inflammatory cytokines during HD. Using biocompatible dialysis membranes can help mitigate immune cell dysfunction and inflammation.

Optimal Dialysis Adequacy: Adequate dialysis adequacy, including appropriate removal of uremic toxins and maintenance of fluid balance, is essential for immune cell function. Proper dialysis dosage and achieving target clearance values can help optimize immune cell health and function.

High-Flux HD: High-flux HD involves the use of dialyzers with a more permeable membrane, allowing for enhanced clearance of larger molecular weight toxins. High-flux HD has been shown to improve immune cell function, reduce inflammatory markers, and potentially enhance immune response compared to conventional HD.

Avoidance of Repeated Access Trauma: Repeated access trauma, such as frequent cannulation or repeated access site infections, can lead to chronic inflammation and immune cell dysfunction. Proper care and maintenance of vascular access sites, minimizing trauma, and adhering to aseptic techniques during access procedures can help reduce inflammation and improve immune cell function.

Nutritional Support: Providing adequate nutrition, including sufficient protein and calorie intake, is crucial for supporting immune cell function. Malnutrition and protein-energy wasting are common in HD patients and can contribute to immune dysfunction. Individualized dietary counseling and support can help optimize nutrition and improve immune cell health.

Control of Infections: Prompt identification, treatment, and prevention of infections are vital in managing immune cell dysfunction. Effective infection control measures, including proper hand hygiene, catheter care, and adherence to infection prevention protocols, can help reduce the burden of infections and limit their impact on immune function.

Cytokines levels during hemodialysis

Cytokine levels in HD patients can be affected by various factors, including the underlying inflammatory state, comorbidities, dialysis-related factors, and the presence of vascular access-related infections. Monitoring cytokine levels in HD patients can provide insights into the inflammatory state and help guide treatment strategies. However, it's important to note that cytokine levels can vary among individuals and are influenced by various factors. Assessing individual patients' cytokine profiles and integrating clinical evaluations can aid in optimizing patient care and addressing the underlying inflammatory burden in HD.

Pro-inflammatory Cytokines: HD patients often exhibit higher levels of pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and interleukin-18 (IL-18). The accumulation of uremic toxins, chronic inflammation, and dialysis-related factors contribute to the release of these pro-inflammatory cytokines.

Interleukin-6 (IL-6): IL-6 is a major pro-inflammatory cytokine that is frequently elevated in HD patients. It plays a crucial role in immune responses and inflammation. Increased IL-6 levels in HD patients have been associated with systemic inflammation, cardiovascular disease, and malnutrition.

Tumor Necrosis Factor-alpha (TNF-alpha): TNF-alpha is another important pro-inflammatory cytokine that is elevated in HD patients. It is involved in the regulation of inflammatory responses and immune cell function. Elevated TNF-alpha levels have been linked to the development of cardiovascular disease, malnutrition, and insulin resistance in this population.

Interleukin-1 (IL-1): IL-1 is a pro-inflammatory cytokine that is increased in HD patients. Elevated IL-1 levels have been associated with systemic inflammation, endothelial dysfunction, and vascular calcification.

Anti-inflammatory Cytokines: In contrast to the elevated levels of pro-inflammatory cytokines, HD patients often exhibit lower levels of anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-beta). The imbalance between pro-inflammatory and anti-inflammatory cytokines contributes to a persistent inflammatory state.

Dialysis-related Factors: HD itself can impact cytokine levels. The contact of blood with artificial surfaces, the release of endotoxins, and the process of ischemia-reperfusion during dialysis sessions can stimulate immune cells and trigger the release of pro-inflammatory cytokines.

Alterations in T-cell activity during hemodialysis

T-cell activity during HD plays a significant role in the immune response and overall health of patients undergoing this renal replacement therapy. The altered T-cell activity observed in HD patients has important clinical implications, including increased infection risk, reduced vaccine response, and higher cardiovascular risk. Careful management and immunization, control of inflammation, and monitoring of T-cell health are key strategies to address these challenges and improve patient outcomes.

Immune Dysregulation: HD can lead to immune dysregulation, which includes changes in the function and distribution of T-cells. It can result in a shift from a T-helper 1 (Th1) response to a Th2 response, potentially increasing susceptibility to infections.

T-Cell Senescence: CKD and HD can accelerate T-cell senescence, a state of cell aging that impairs the immune response. Senescent T-cells have reduced proliferative capacity and cytokine production.
T-Cell Activation: HD can lead to T-cell activation and increased expression of activation markers. This activation may be related to the chronic inflammation often seen in HD patients.

T-Cell Subpopulations: HD patients may exhibit changes in T-cell subpopulations. For example, a decrease in regulatory T-cells (Tregs) can contribute to inflammation and immune dysfunction.

Clinical Implications of Altered T-Cell Activity:

Infection Susceptibility: Immune dysregulation and T-cell senescence can increase the risk of infections in HD patients. This includes susceptibility to bacterial, viral, and fungal infections.

Vaccine Response: Altered T-cell activity can impair the response to vaccinations. HD patients may have reduced vaccine efficacy, which is a concern given the importance of vaccination in preventing infections.

Inflammation and Cardiovascular Risk: Chronic inflammation and immune dysregulation in HD patients are associated with an increased risk of cardiovascular disease. T-cell activation and cytokine release can contribute to atherosclerosis and vascular damage.

Allograft Rejection: In HD patients who have received kidney transplants, T-cell dysregulation can increase the risk of allograft rejection. Close monitoring and immunosuppressive management are crucial in such cases.

16. STUDY RATIONALE

Taking into account the inflammatory substrate, the reduced state of immunotolerance, and the tendency and predisposition to infections that dialysis patients exhibit, it is clear how essential it is to minimize infectious risks by implementing tailored strategies and quickly recognizing the types of infections that may develop our dialysis' patients.
With these concepts in mind, we decided to conduct our study on a large cohort of dialysis patients to determine whether the same principles of management and antibiotic therapy that apply to other highly immunosuppressed patients also hold true for this unique patient cohort.
Considering the immunosuppression, dialysis patients constitute a highly immunocompromised population and represent a unique setting similar to intensive care, hematology, or transplant recipients. As we have observed, numerous studies have already been conducted in these populations, demonstrating the high infectious risk, increased mortality and morbidity burdened by CPE. Recent studies have questioned whether it is worthwhile to treat fever occurring in highly immunosuppressed patients with rectal colonization by CPE as CPE-related sepsis.
Our study aims to analyze a large dialysis population and assess if there is an increased risk of mortality, sepsis, and/or hospitalizations within this cohort. Considering the frequency of evaluations for these patients, we have also decided to evaluate the treatments to which these patients are subjected, their antibiotic exposure, the number of hospitalizations and specific infections, while seeking predisposing or protective factors within this specific patient group.

Matherials and methods:

Our work is a retrospective, single-center cohort study that analyzes patients on HD and PD who were followed by the Nephrology and Dialysis Department of the Ravenna Hospital from 01/01/2015 to 31/12/2021.

Inclusion criterias:

- Age>18 years
- HD or PD for at least 3 months

Exclusion criterias:

- Patients undergoing chemotherapy or immunotherapy for a duration longer than 3 months during the observation period.
- Patients with stomas (ureteral, nephro, colon, ileo)

The patients included in the study were assessed for:

- demographic data (Date of birth, gender, blood type)
- comorbidities (Diabetes, hypertension)
- dialysis data (type of dialysis technique, dialytic age)
- blood tests (hemoglobin, white blood cells, platelets, albumin, ALT, lipase, C-reactive protein)
- serology and markers (HBV, HCV, HIV, EBV, CMV, Quantiferon, Helicobacter pylori colonization, SARS-COV2)
- therapies (antihypertensives, proton pump inhibitors, calcium-based phosphorus binders, non-calcium-based phosphorus binders, potassium binders, iron therapy, erythropoietin-stimulating agents)
- antibiotics (cephalosporins, penicillins, fluoroquinolones, macrolides, aminoglycosides, carbapenems, tetracyclines, vancomycin, linezolid, cefepime/avibactam)
- hospitalizations
- colonization or lack thereof by CPE bacteria
- infections (urinary tract infections and pneumonias) or sepsis and responsible microorganisms
- survival

Data were taken from the digital medical records and was imported to create a database specifically for the study. The study was approved by the Local Ethical Committee of Romagna (CMS Board N.2764).

Statistical analysis

The aim of statistical analysis in this observational study was to observe whether presenting CPE colonization was linked with an increased number of sepsis, a higher mortality or an higher number of hospitalization. The proper test of statistical significance depends on the nature of the examined variables. Differences among means in different groups were analyzed using the t-test or the ANOVA. Categorical data were analyzed using contingency tables and $\chi 2$. Survival analysis were used to determine the hazard ratio of comorbidities, performed via Cox regression, illustrated by Kaplan-Meier estimate and compared by a log-rank test. Continuous variables are presented as mean ± Standard Deviation or as median and Interquartile interval (IQR) when appropriated. All statistical tests were two-tailed, and we used SPSS Statistics fo Windows, Version 25.0 (Armonk NY: IBM Corp. Released in 2017 by IBM) for data management and analysis. A p-value < 0.05 was considered statistically significant.

Results

We analyzed 674 patients with ESRD treated with HD or PD in the considered period. Of those, 69 patients didn't perform CPE swab, 30 had stomies and 47 underwent immunosuppression therapy for disease or malignancy. Overall we considered for our analysis 528 patients. Baseline demographic characteristics and comorbidities are shown in Table 1. Considering the whole population, 51 patients presented CPE positive rectal swab during the period of observation. No differences were noted between the two groups. We analyzed baseline nephropathy, thinking that it could be a factor linked to the development of CPE rectal swab, thus we didn't observe any difference between the two groups.

	Population (528)	**Positives (51)**	**Negatives (477)**	**p - value**
Age (years)	71.2 ± 13.6	68.8 ± 12.6	71.5 ± 13.7	0.19
Sex M/F (%)	343 (64.9)/ 185 (35.1)	34 (66.7)/ 17 (33.3)	309 (64.8)/168 (35.2)	0.77
Dialysis time (days)	2346 ± 2440	2513 ± 2650	2328 ± 2419	0.60
Hypertension (%)	428 (81.1)	42 (82.3)	386 (80.9)	0.08
Diabetes (%)	123 (23.3)	12 (23.5)	111 (23.3)	0.56
Nephropathy (%) - CKD unknown origin - Hypertensive - Diabetic nephropathy - ADPKD - Glomerulonephritis - Vasculitis - Tubulo-interstitial nephritis - Malignancies - Other	 104 (19.7) 184 (34.8) 43 (8.1) 44 (8.3) 65 (12.3) 12 (2.3) 42 (7.9) 13 (22.4) 20 (3.8)	 11 (21.6) 18 (35.3) 4 (7.8) 9 (17.6) 2 (3.9) 3 (5.9) 2 (3.9) 0 (0) 2 (3.9)	 93 (19.5) 166 (34.8) 39 (8.2) 35 (7.3) 63 (13.2) 9 (1.9) 40 (8.4) 13 (2.7) 18 (3.8)	0.06

Table 1. Demographic characteristics of the population. CKD: Chronic kidney disease. ADPKD: Autosomic Dominant Polycystic Kidney Disease.

Then we observed the closest blood examination to the time of CPE rectal swab performed and we observed that the those who went positives presented more frequently anemia (figure 9), had higher platelets (Figure 10) and presented hypoalbuminemia (Figure 11). Concerning other examinations we didn't noticed any difference. Results are expressed in Table 2.

	Population	Positives	Negatives	P- value
Hb (g/dl)	11.34	9.92	11.47	0.035
WBC (number/ul)	8578	8428	8601	0.929
Platelets (number/ul)	207	242	202	0.005
Albumin (g/l)	37.9	34.9	38.2	0.014
CRP (mg/l)	78.0	108.2	73.2	0.103
ALT (U/l)	15.7	20.7	15	0.213
Lipase (U/l)	133.6	61.4	141.2	0.775

Table 2. Blood examinations at the time of Enterobacter Producing Carbapenemase rectal swab. Hb: Hemoglobin. WBC: White blood cells. CRP: C-reactive protein. ALT: Alanine aminotransferase.

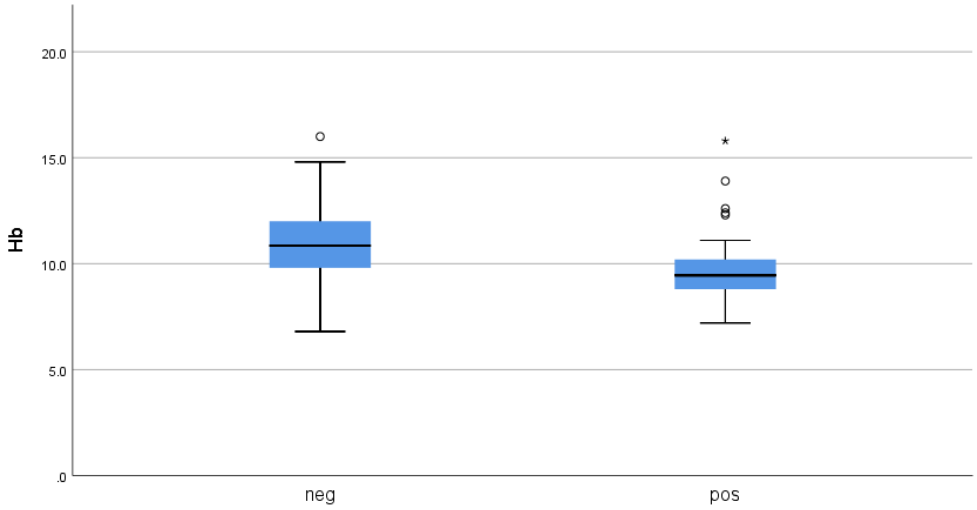


Figure 9. Box blot of hemoglobin according to CPE rectal swab. Positives presented lower hemoglobin compared to those who remained negative.

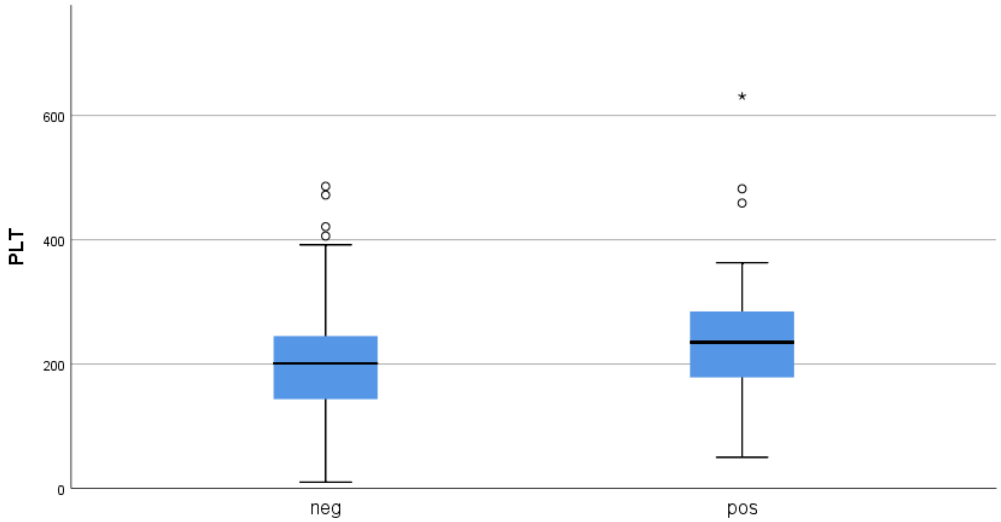


Figure 10. Box blot of platelets according to CPE rectal swab. Positives presented higher platelets compared to those who remained negative.

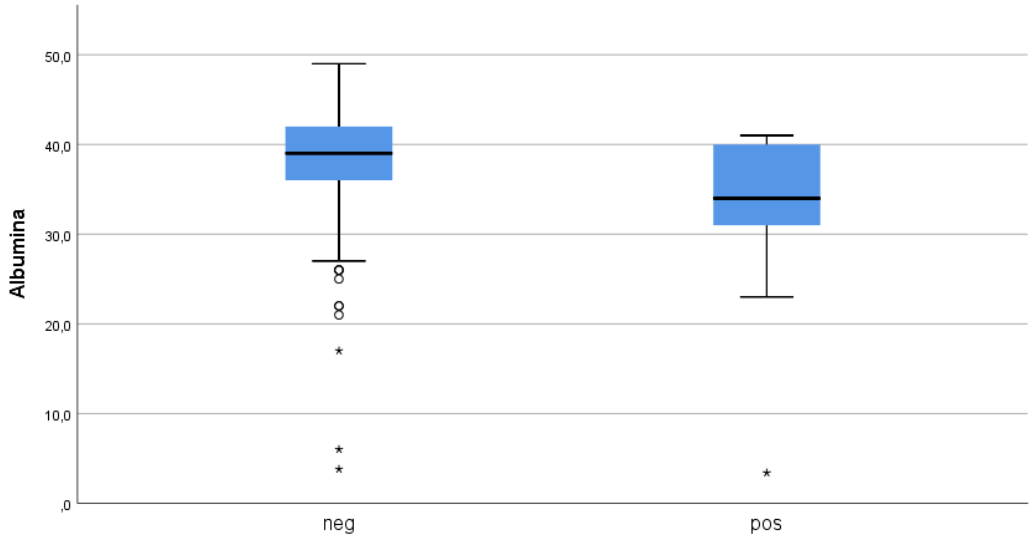


Figure 11. Box blot of albumin levels according to CPE rectal swab. Positives presented lower albumin compared to those who remained negative.

Then we considered renal replacement therapy, observing if the RRT technique could have an impact on the development of CPE positivization. In our population we evidenced some differences but no significancy was reached. Those who underwent Acetate free biofiltration are more prone to develop CPE colonization compared to the other groups. Even those who underwent transplantation but who weren't receiving immunosuppressive therapy since long time are more prone to develop CPE colonization. Results are expressed in Table 3.

	Totali	**Positivi**	**Negativi**
AFB	23 (4.5)	4 (17.4)	19 (82.6)
PD	21 (4.2)	0 (0)	22 (100)

HD	401 (76.4)	33 (8.2)	368 (91.8)
HFR	11 (2.1)	1 (9.1)	10 (90.9)
Transplant	60 (11.3)	8 (13.3)	52 (86.7)
Conservative therapy	4 (0.8)	0 (100)	4(100)
Lost at follow-up	4 (0.8)	0 (100)	4 (100)

Table 3. CPE colonization according to dialysis technique. AFB: Acetate free biophyltration. PD: Peritoneal dialysis. HD: Hemodialysis and Hemodiaphyltration. HRF: Hemodialysis with endogenous reinfusion.

Cox analysis was performed but we evidenced no differences in terms CPE colonization (Figure 12).

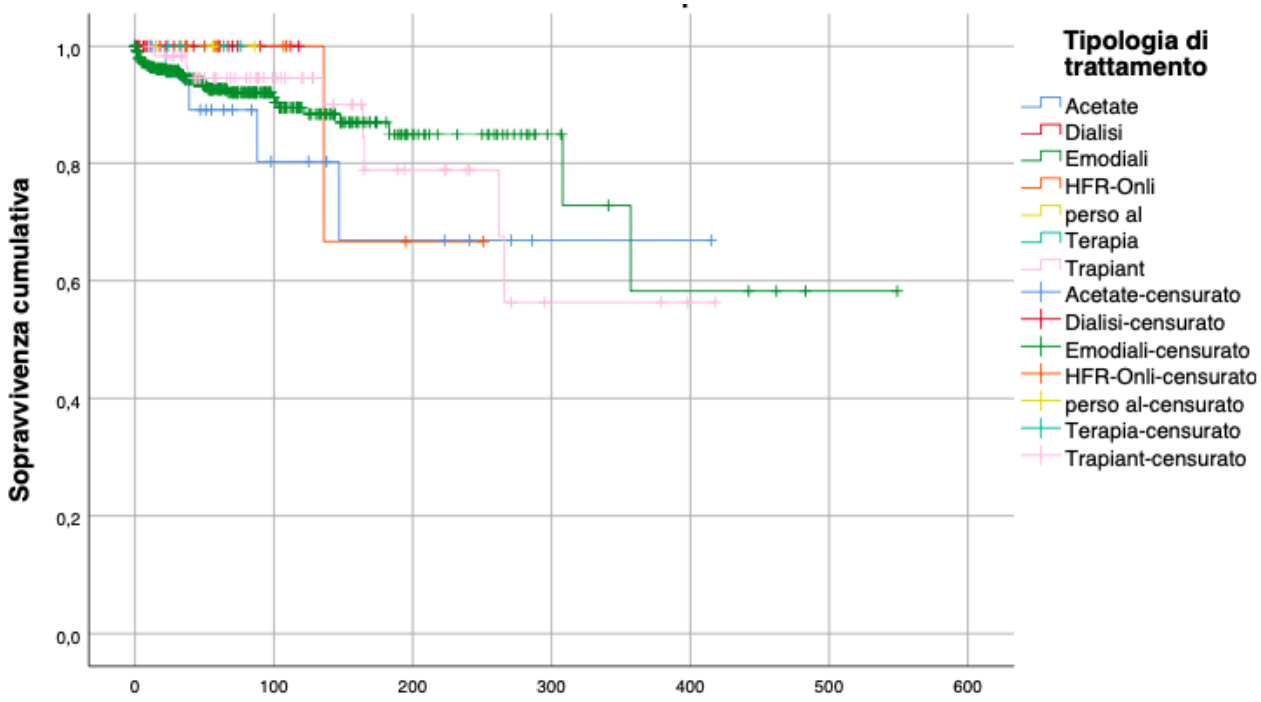


Figure 12. Cox analysis that shows the slope of survival in time of different types of dialysis according to CPE colonization. No differences were noted among groups. HFR: Hemophyltration with endogenous reinfusion.

Then we considered all the main treatments performed for each patients in the time lapse. Specifically we observed anti-hypertensive agents (resumed in Table 4), PPI, phosphorus binders, potassium binders, erytrophoietin stimulating agents, iron therapy (resumed in Table 5).
In the following Figures (Figure 13-17) we show the Kaplan Meier curves concerning main treatment. We evidence a correlation with the use of ACE/ARB agents that seemed to prevent the development of CPE colonization, but these data were not confirmed by Cox analysis.

	Population	Positives	Negatives	LogRank (p-value)
ACEi/ARB	172	22	150	1.755 (0.185)
Calcium channel blockers	324	35	289	0.366 (0.545)
Beta blockers	304	36	268	1.228 (0.268)
Alfa 1 antagonist	159	17	142	0.282 (0.595)
Alfa 2 agonist	27	3	24	0.003 (0.960)

Table 4. Frequencies of anti hypertensive agents in dialysis population. Considering all the treatments we evidenced no significativity between CPE colonized and non colonized. ACEi/ARB: angiotensin converting enzyme inhibitors/angiotensin receptor blockers. CPE: carbapenemase producing enterobacteriacae.

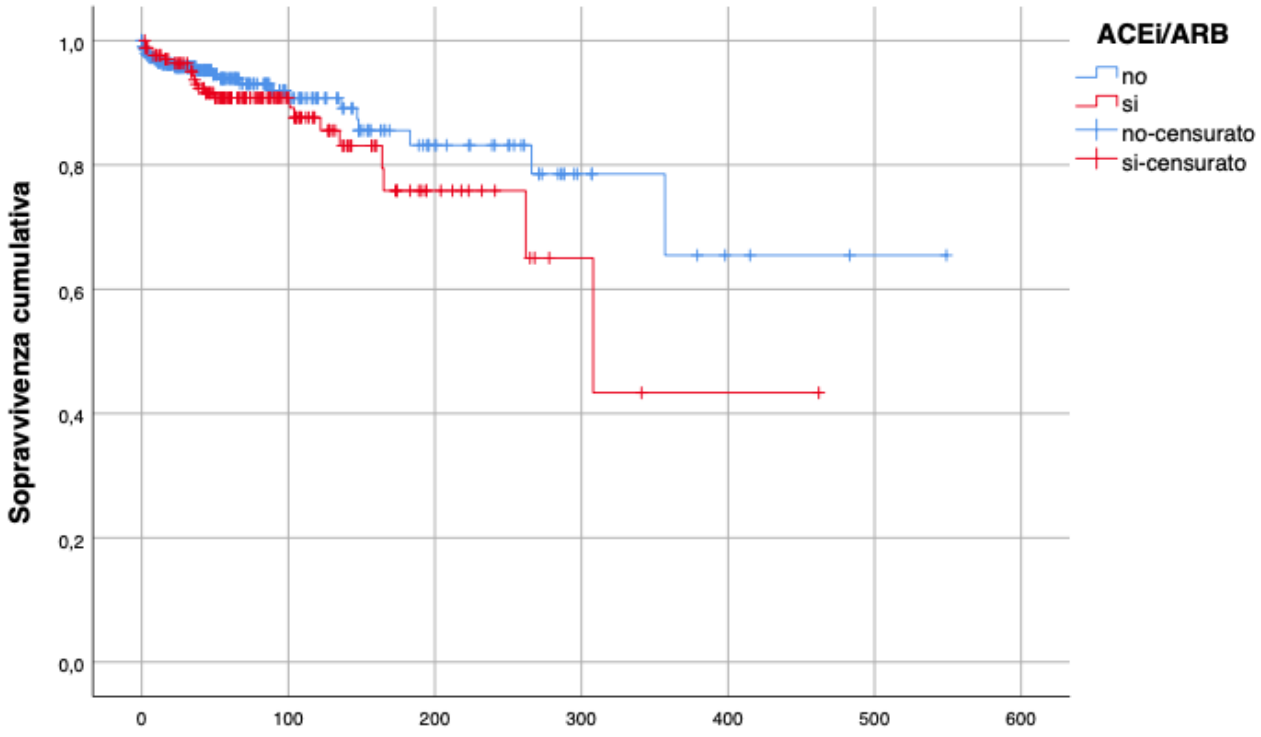


Figure 13. Kaplan Meier for ACEi or ARB treatment according to CPE colonization.

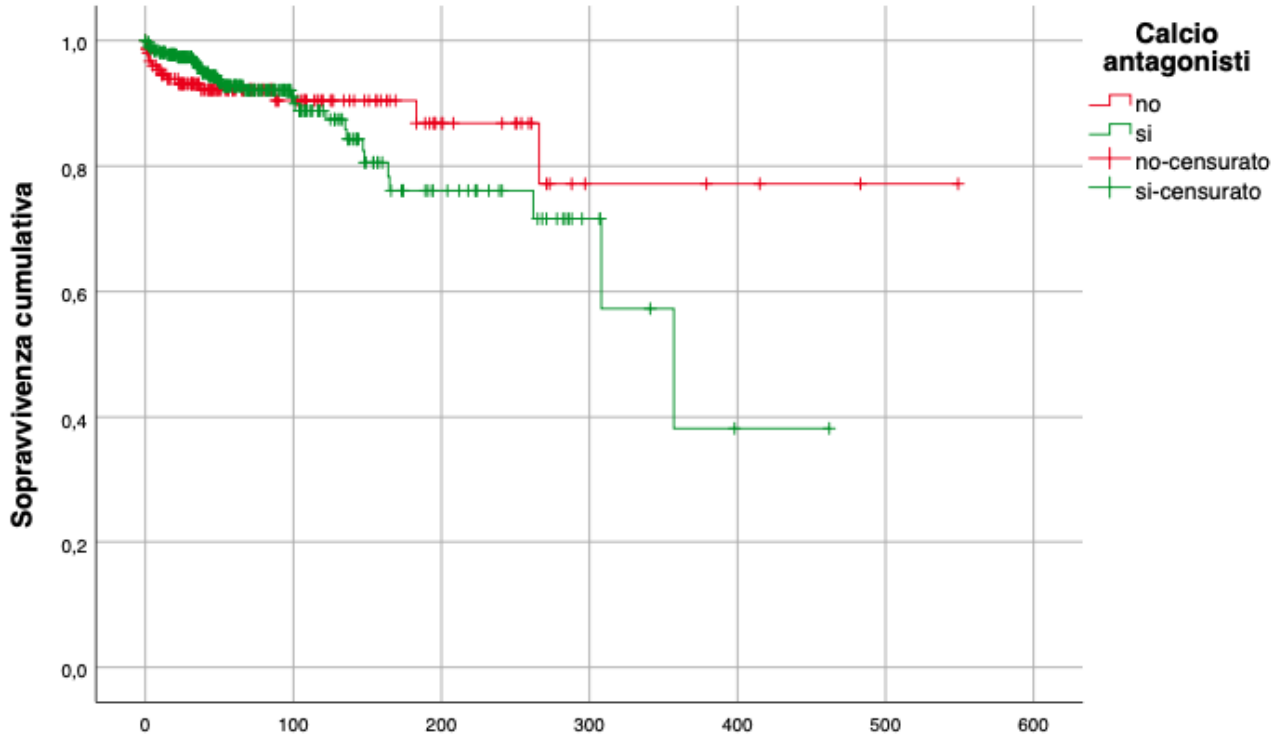


Figure 14. Kaplan Meier for Calcium channel blockers treatment according to CPE colonization

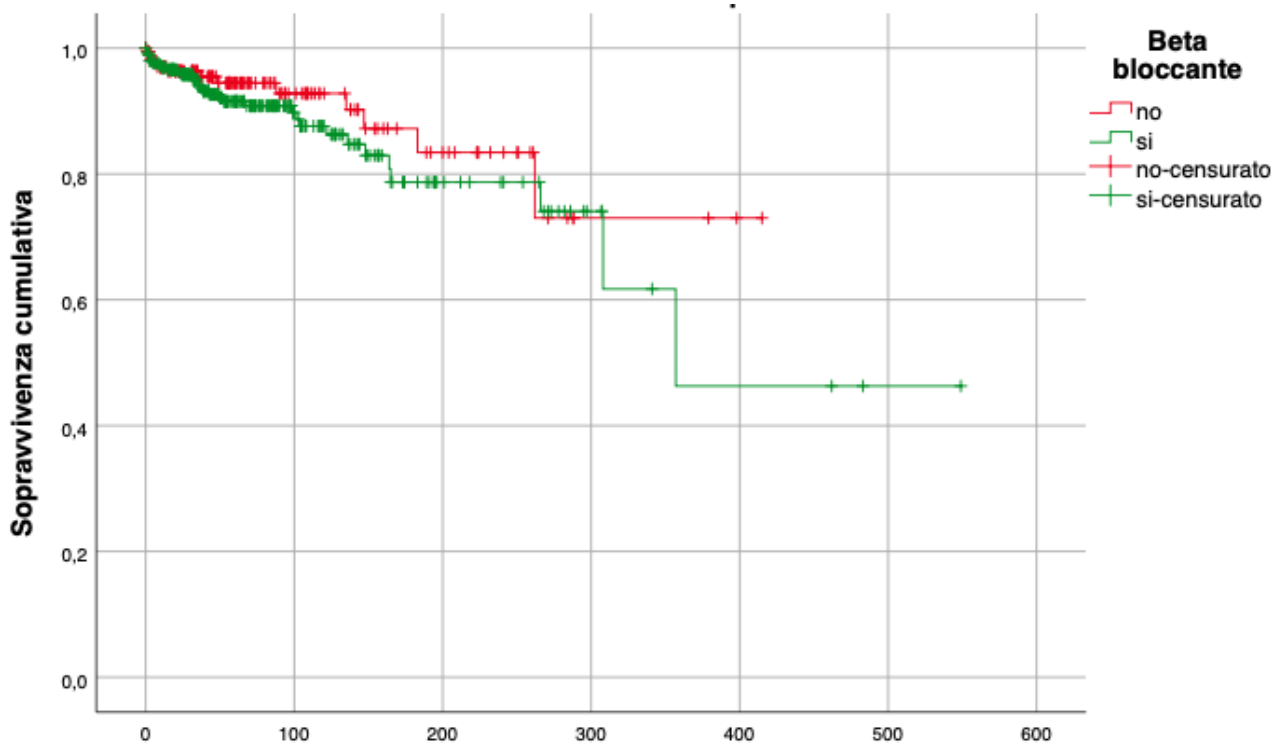


Figure 15. Kaplan Meier for Beta blockers treatment according to CPE colonization

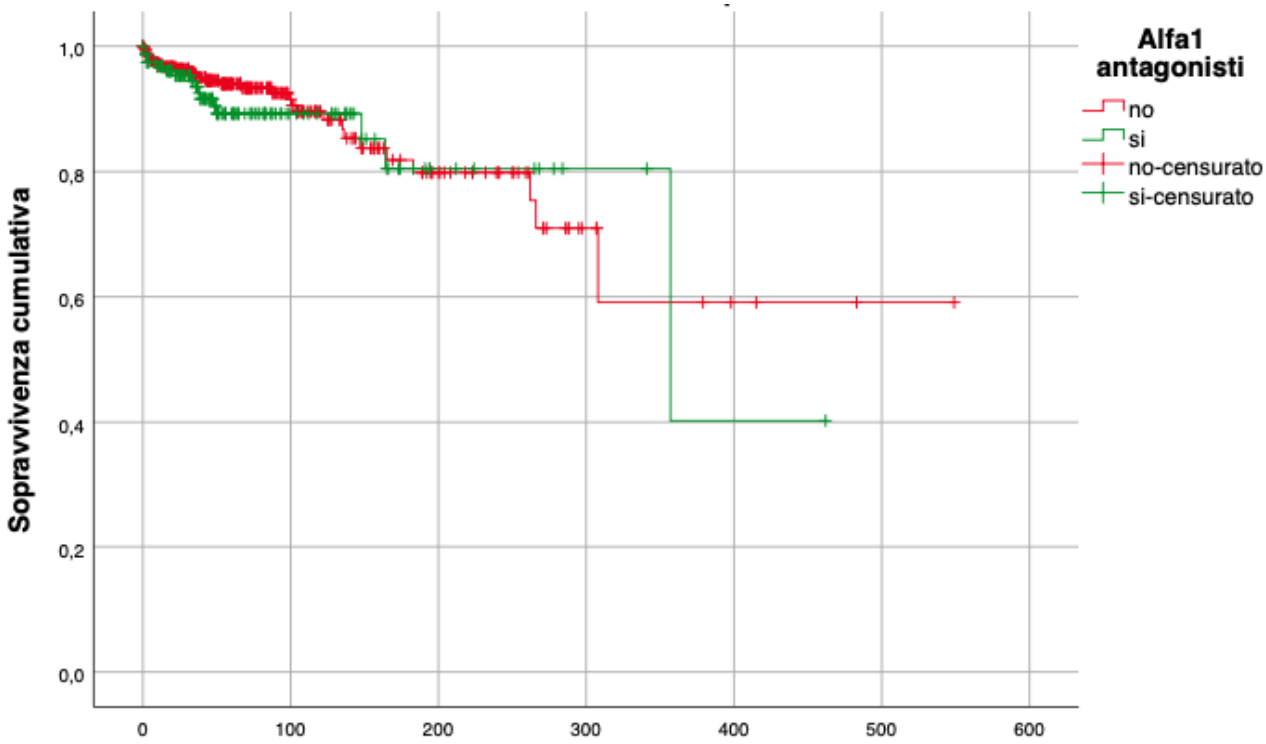


Figure 16. Kaplan Meier for Alfa 1 antagonist treatment according to CPE colonization

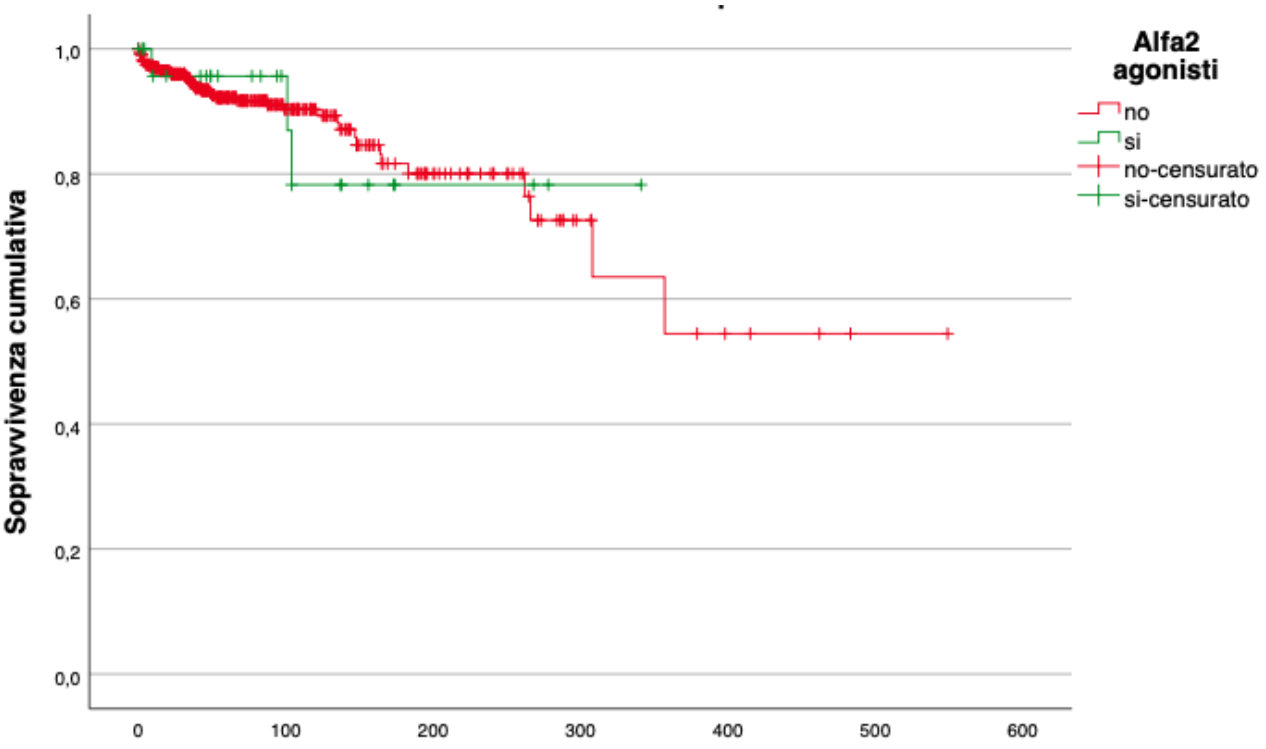


Figure 17. Kaplan Meier for Alfa 2 agonist treatment according to CPE colonization

Concerning specific CKD therapy, we evidenced no differences for phosphorus binders (Figure 18 and 19) both for calcium based and non calcium based, but we evidenced that patients treated with potassium binders seemed to be protected from developing CPE colonization (HR 0.5, p-value 0.034, Figure 20). Data are resumed in Table 5 and other treatment's slopes are shown in Figures 21 to 23.

	Population	Positives	Negatives	Log Rank (p value)
Phosphorus binders calcium-based	352	40	312	0.9 (0.933) HR
Phosphorus binders non calcium-based	207	21	186	1.2 (0.121) HR
Potassium binders	174	14	160	0.5 (0.034) HR
PPI	406	41	365	1.547 (0.214)
Iron	341	37	304	0.054 (0.816)
ESAs	441	48	393	0.101 (0.751)

Table 5. Specific CKD therapy. Data show that potassium binders seemed to be protective in the development of CPE colonization. PPI: Proton Pump Inhibitors. ESA: Erythropoietin stimulating agents. HR: Hazard ratio.

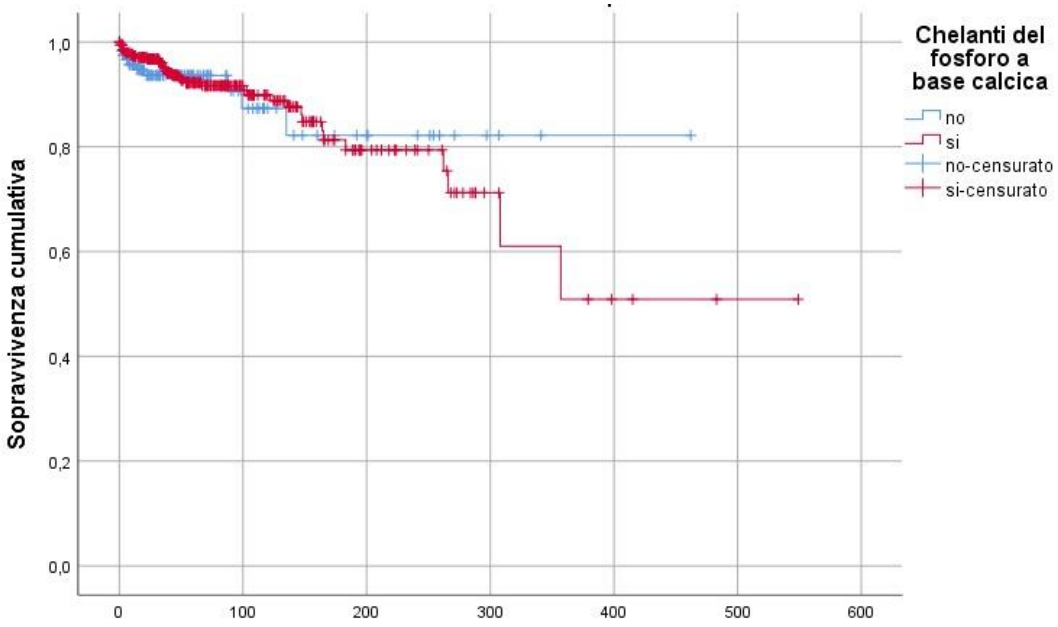


Figure 18. Kaplan Meier for calcium based phosphorus binders according to CPE colonization

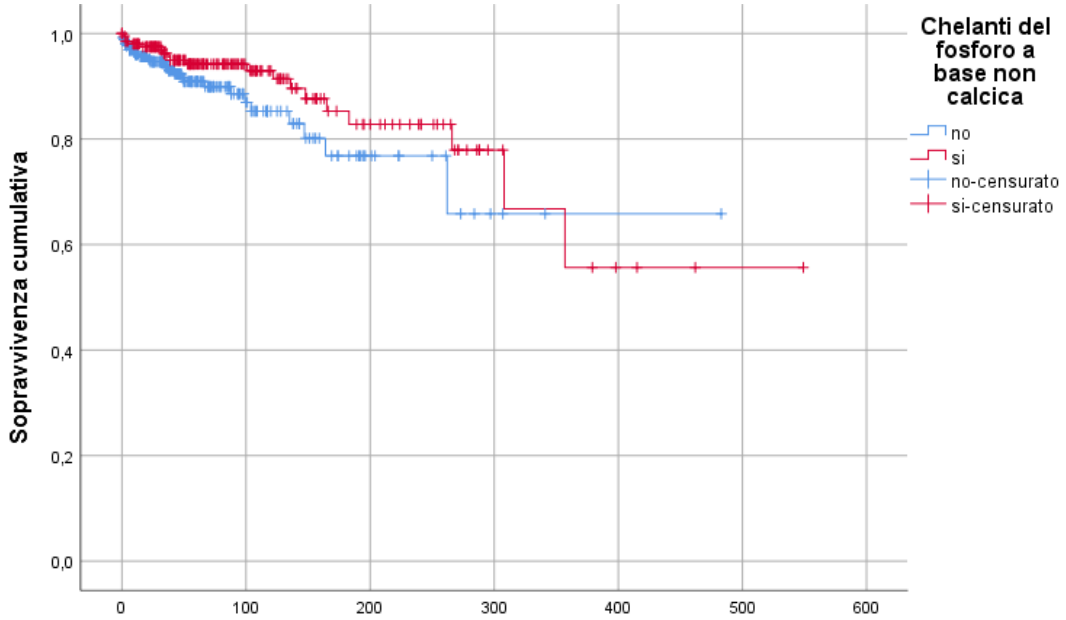


Figure 19. Kaplan Meier for non calcium based phosphorus binders according to CPE colonization

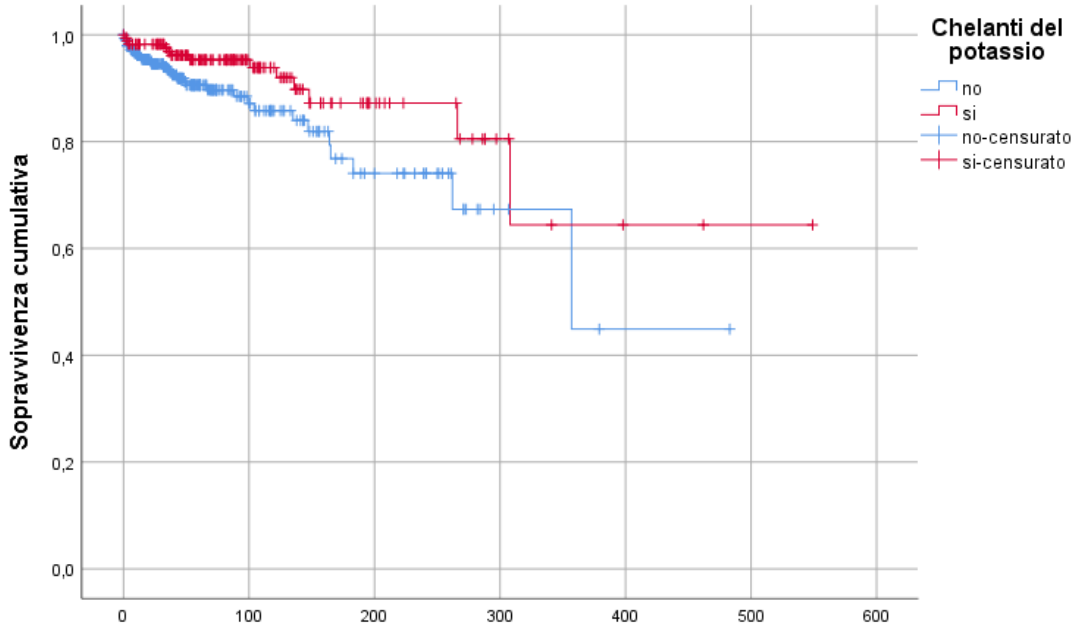


Figure 20. Kaplan Meier for potassium binders according to CPE colonization. The treatment seems to be protective.

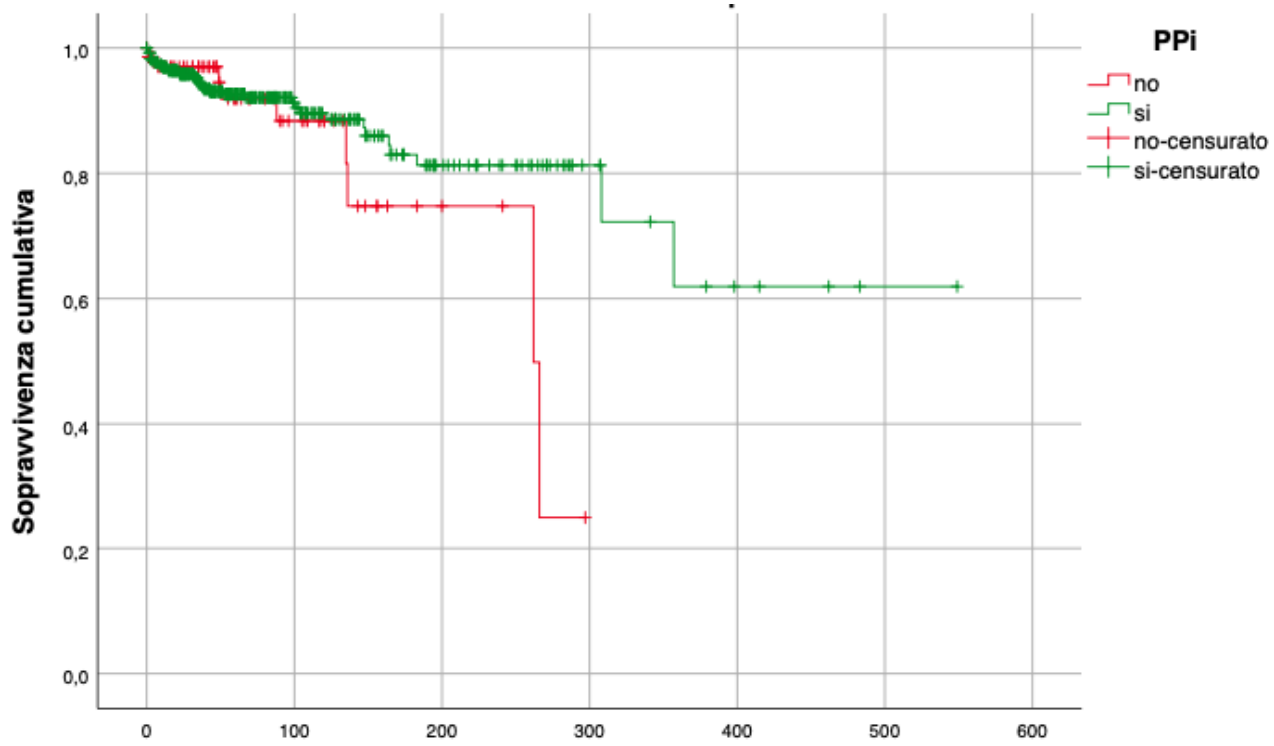


Figure 21. Kaplan Meier for Proton Pump Inhibitors (PPIs) according to CPE colonization

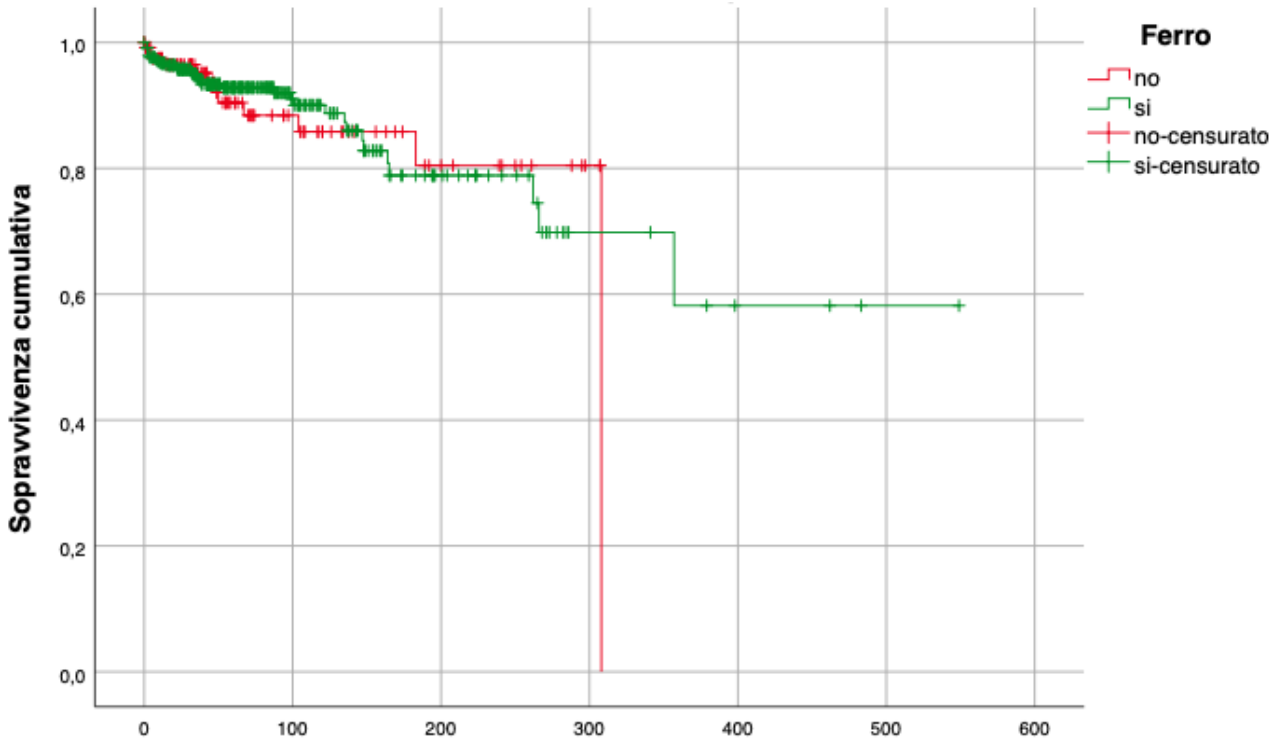


Figure 22. Kaplan Meier for Iron therapy according to CPE colonization

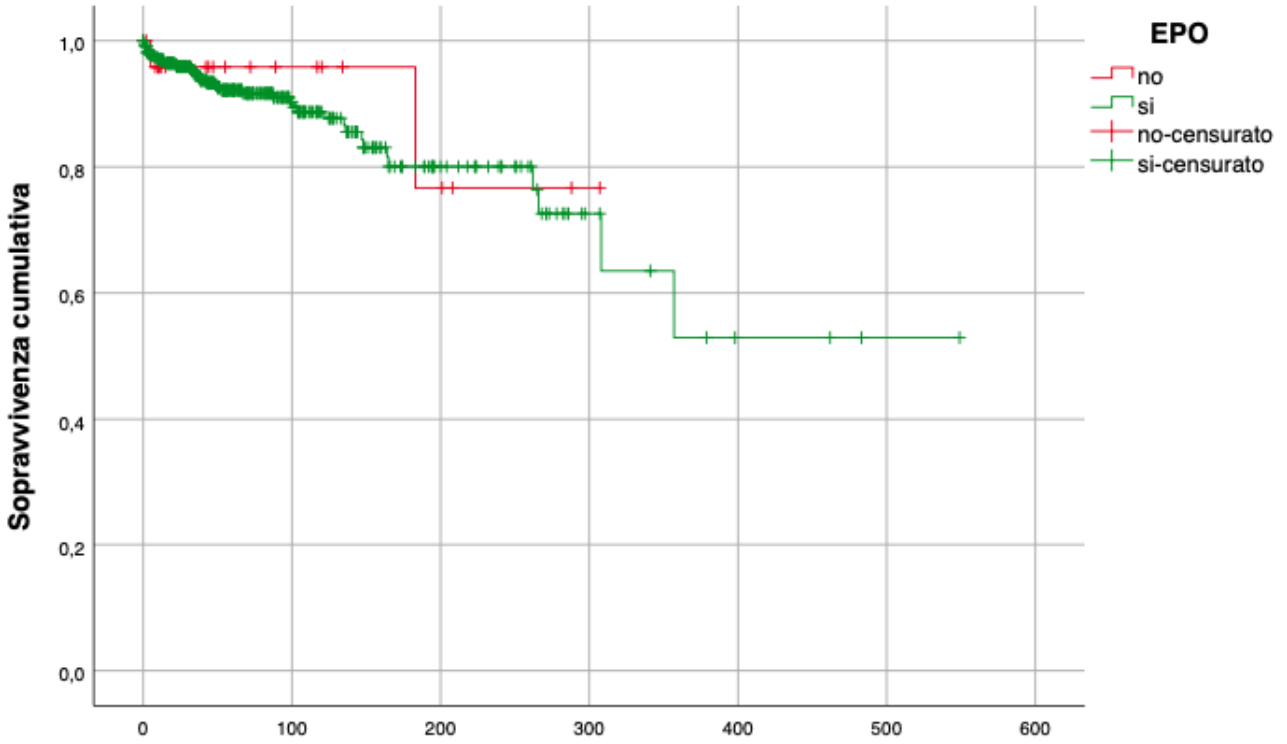


Figure 23. Kaplan Meier for Erythropoiesis stimulating agents according to CPE colonization

Then we describe antibiotics that patients received in the considered period. We described Cephalosporines, Penicillines, Fluoroquinolones, Aminoglicosides, Carbapenems, Vancomycin, Tetracyclines, Linezolid and Ceftazidime/Avibactam. Results are resumed in Table 6 (Figures 24 to 32). Considering the type of antibiotics used, we evidence that our population used mean ± SD, 1.67 ± 1.66, median [IC], 1 [1-3] with a significative difference between the two groups (positives mean ± SD 2.49 ± 2.21, median [IC] 2 [0.5-4], negatives mean ± SD 1.59 ± 1.57, median [IC] [0-3]), p-value = 0.0002. We found some statistical evidence with Linezolid (HR 4.1, p=0.008) and with Ceftazidime/Avibactam (HR 9.3, p=0.003) but we have only few data so that they have to be interpreted with several other studies.

	Population (%)	**Positives (%)**	**Negatives (%)**	**Hazard ratio (p value)**
Cephalosporines	206 (39)	26 (51)	180 (37.7)	0.9 (0.863)
Penicillines	253 (47.9)	31 (60.8)	222 (46.5)	1.2 (0.469)
Fluoroquinolones	154 (29.2)	18 (35.3)	136 (28.5)	0.7 (0.369)
Aminoglicosids	13 (2.5)	3 (5.9)	10 (2.1)	1.9 (0.378)
Carbapenems	24 (4.5)	5 (9.8)	19 (4)	1.9 (0.208)
Vancomycine	108 (20.4)	17 (33.3)	91 (19.1)	1.7 (0.104)

Tetracyclines	6 (1.1)	2 (3.9)	4 (0.8)	0.5 (0.052)
Linezolid	18 (3.4)	4 (7.8)	14 (2.9)	4.1 (0.008)
Ceftazidime-Avibactam	9 (1.7)	5 (9.8)	4 (0.8)	9.3 (0.003)

Table 6. Antibiotics used in the considered time lapse. In the columns are indicated the number of subjects treated with the specific antibiotic.

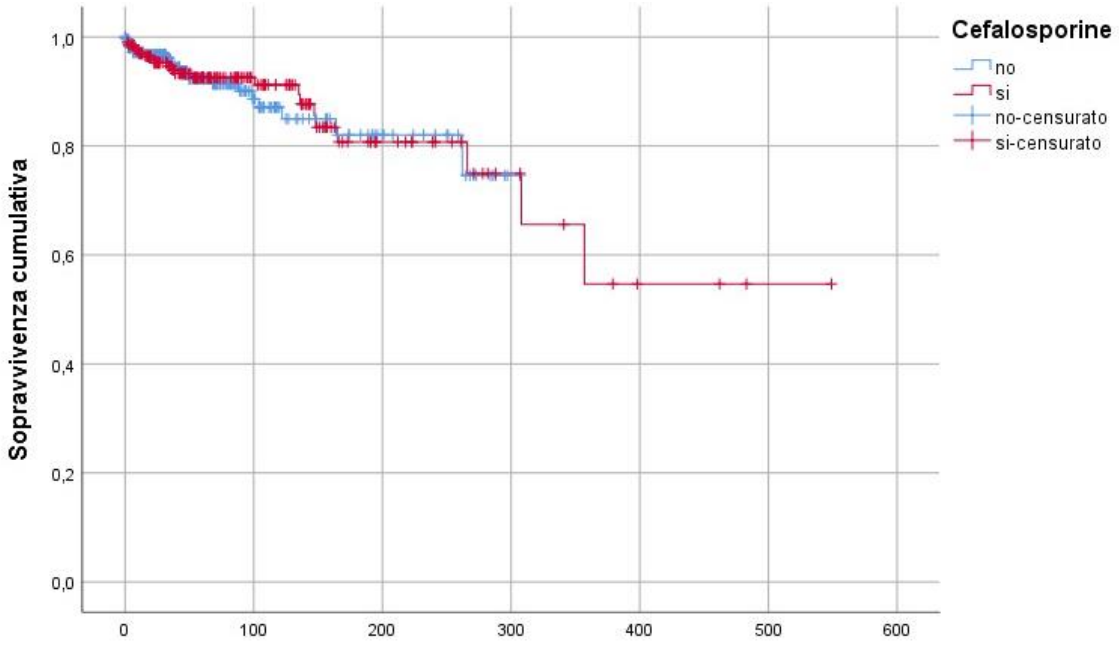


Figure 24. Kaplan Meier for cephalosporines according to CPE colonization

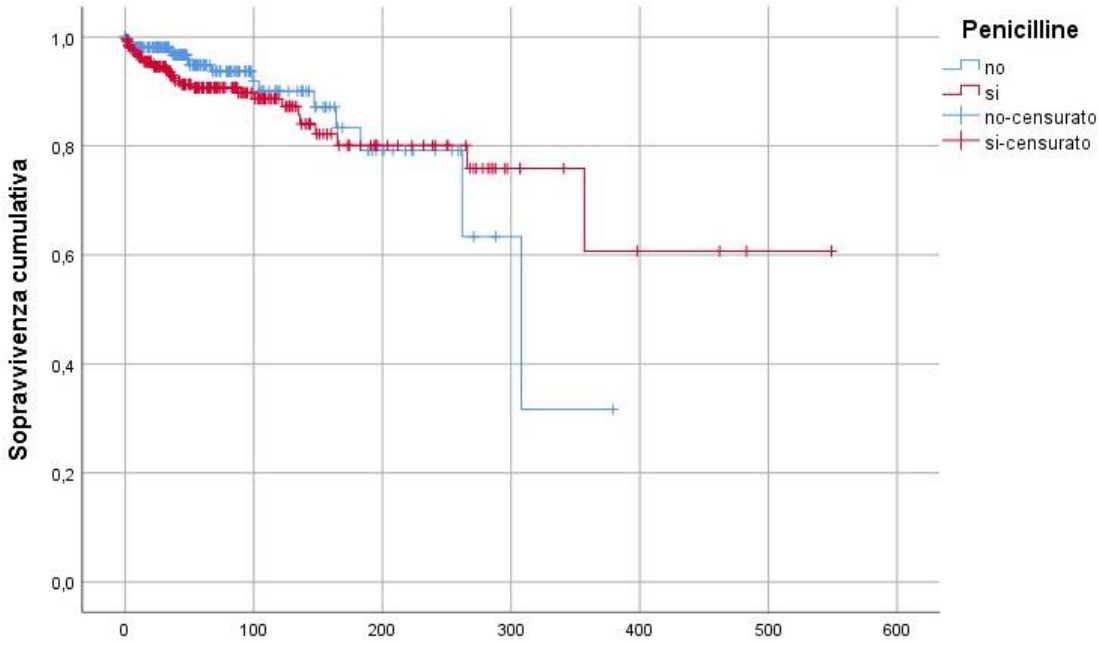


Figure 25. Kaplan Meier for penicillines according to CPE colonization

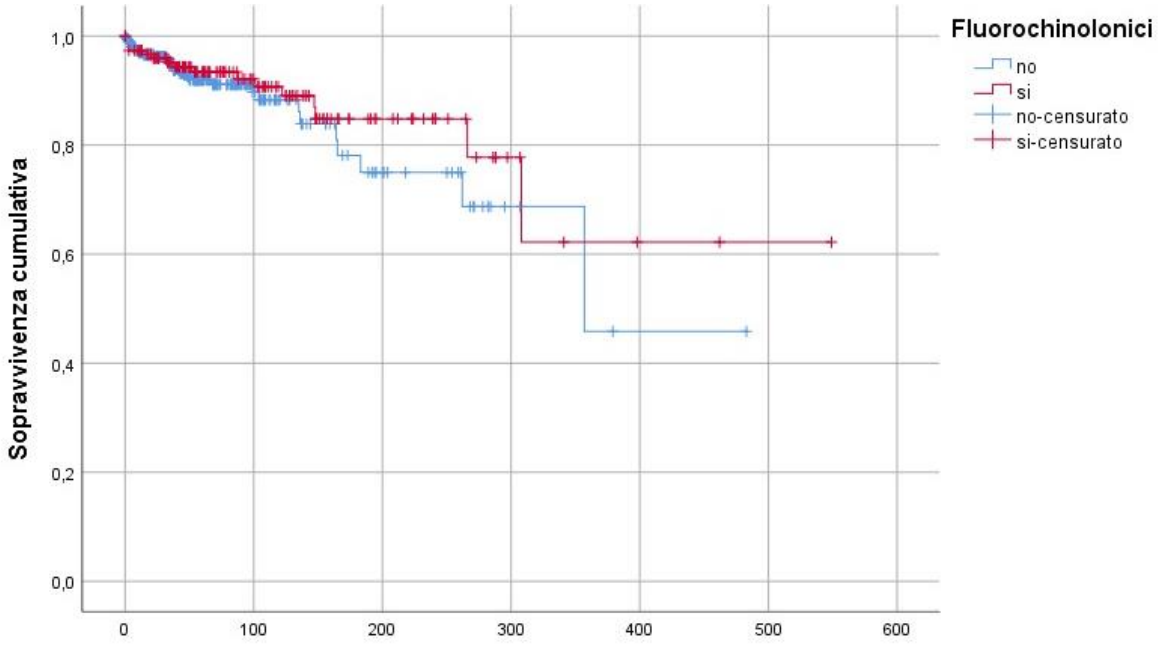


Figure 26. Kaplan Meier for Fluoroquinolones according to CPE colonization

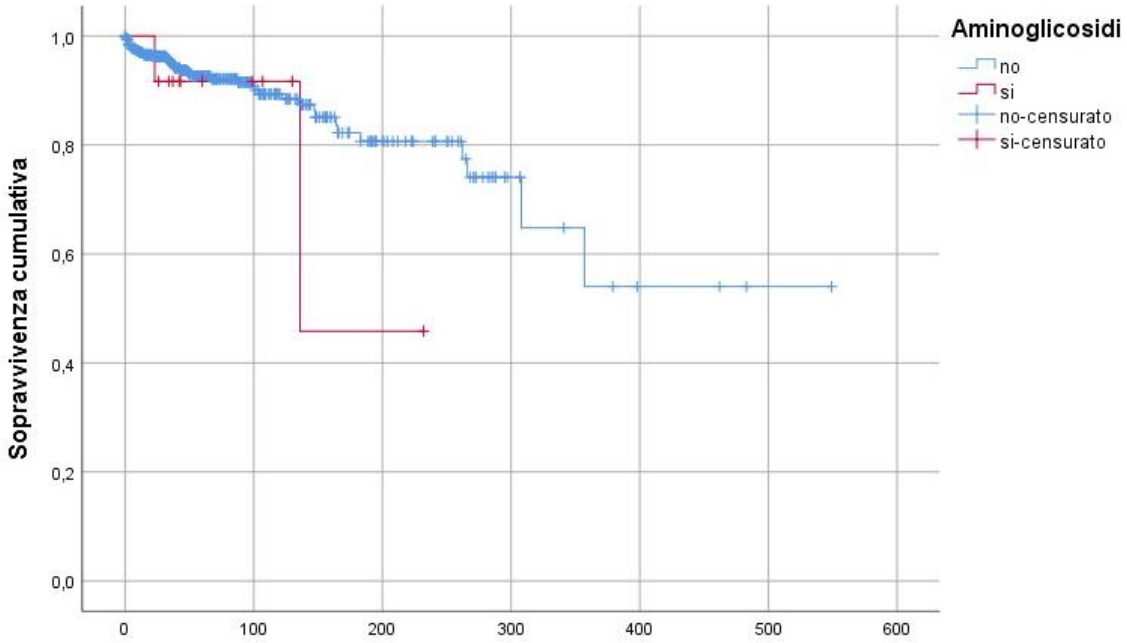


Figure 27. Kaplan Meier for Aminoglicosids according to CPE colonization

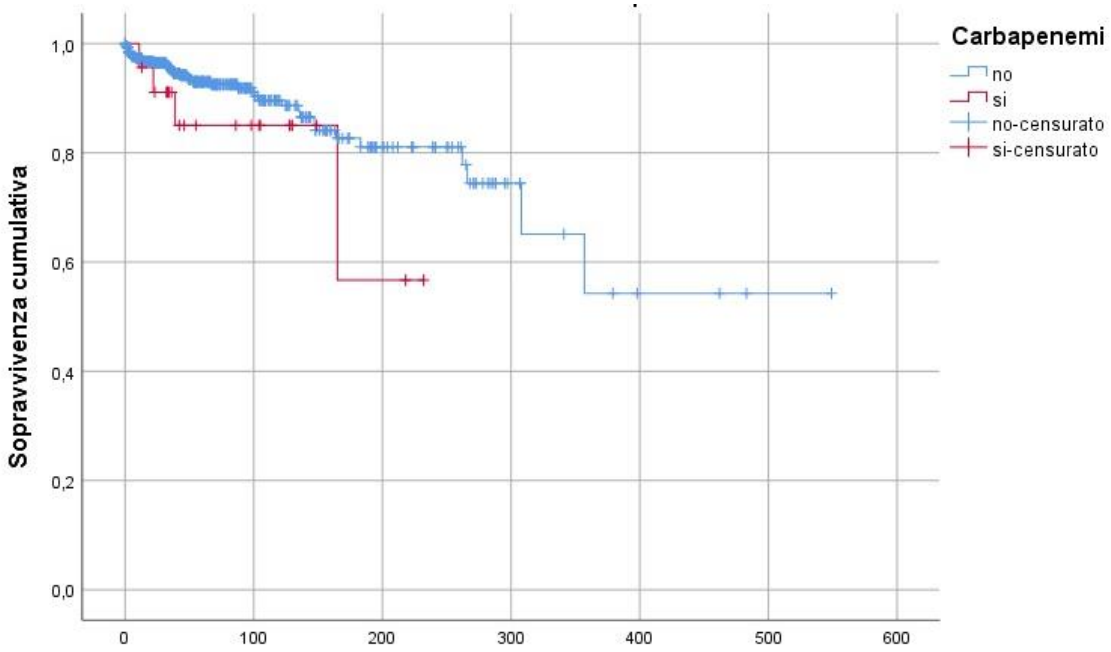


Figure 28. Kaplan Meier for Carbapenems according to CPE colonization

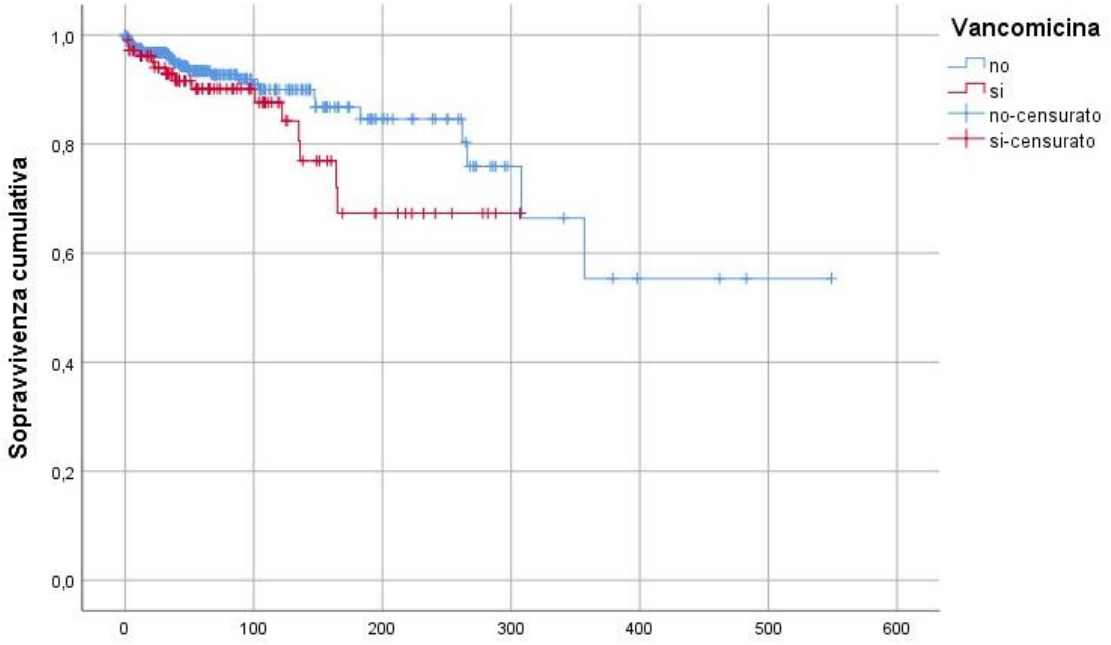


Figure 29. Kaplan Meier for Vancomycine according to CPE colonization

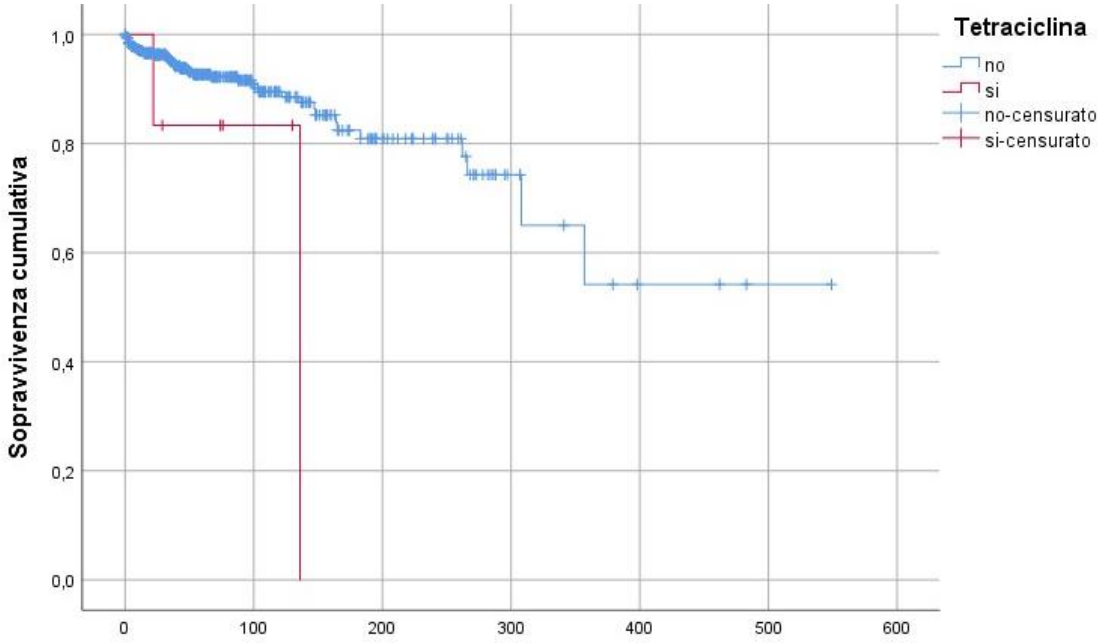


Figure 30. Kaplan Meier for Tetracyclines according to CPE colonization

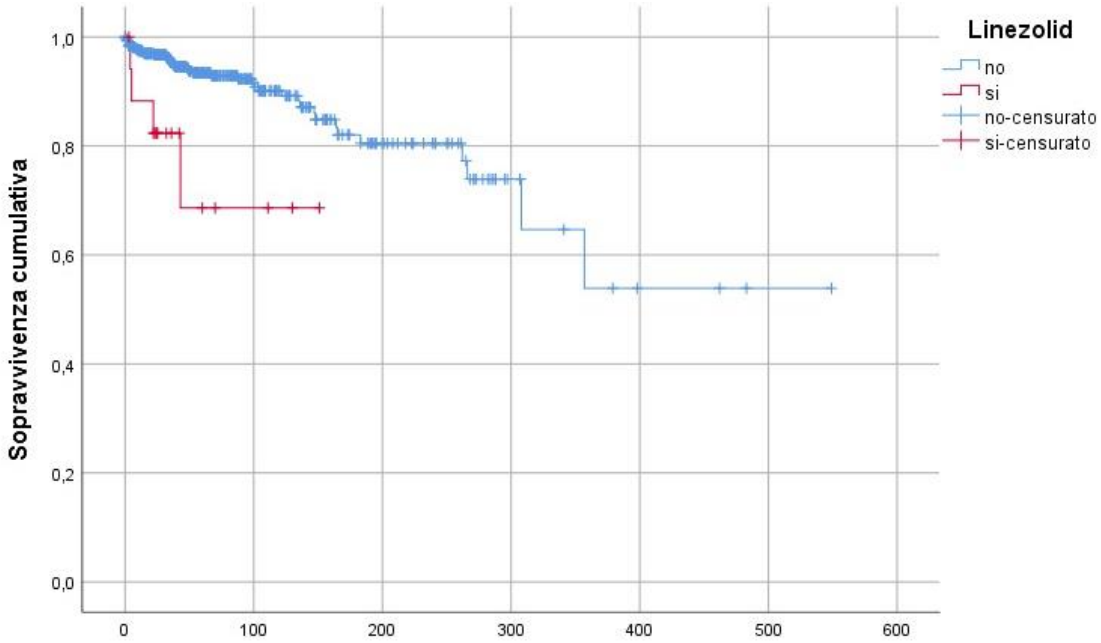


Figure 31. Kaplan Meier for Linezolid according to CPE colonization. Nonetheless we have only few data, HR 4.1, p-value 0.008.

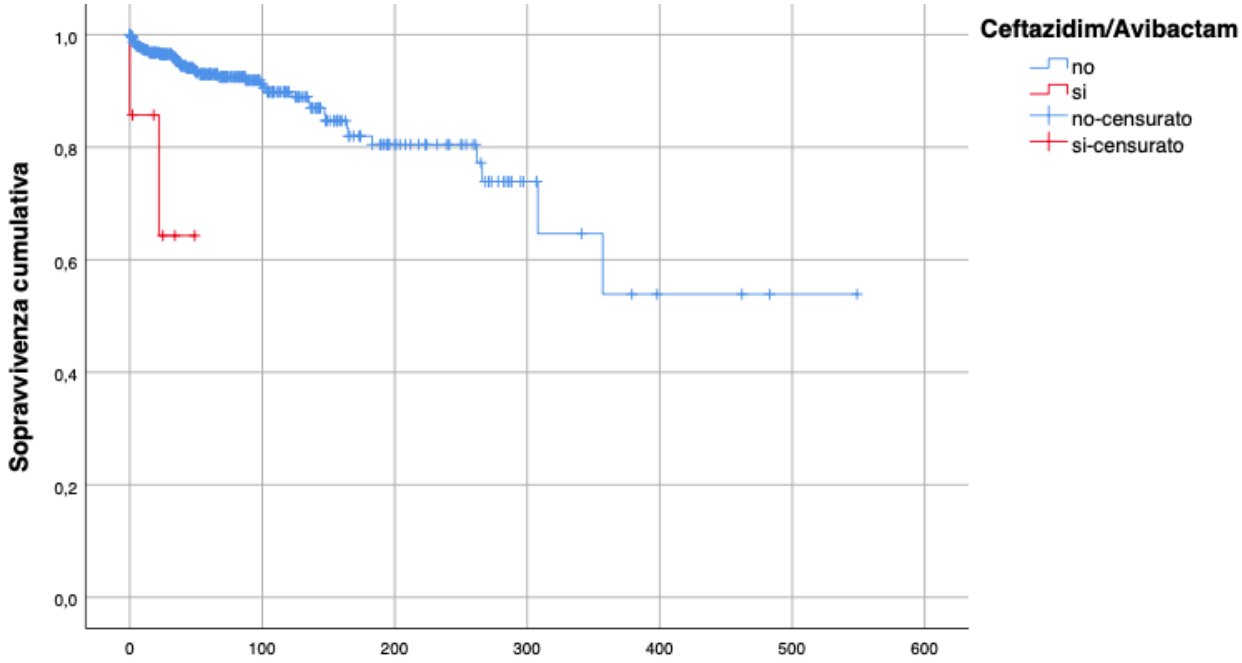


Figure 32. Kaplan Meier for Ceftazidime/Avibactam according to CPE colonization. Nonetheless we have only few data, HR 9,3, p-value 0.003.

After considering all the treatments, we observe the incidence of main infections in our cohort, described in Table 7. Considering urinary tract infections (UTIs), we evidenced no differences in patients with CPE colonization. (Figure 33 and Figure 34). We pointed out 90 UTIs, most of them were caused by Escherichia Coli in both our groups. Data concerning responsible pathogens are resumed in Table 8.

	Population	**Positives**	**Negatives**	**HR (p-value)**
Pneumonias	147 (27.8)	25 (49)	122 (25.6)	2.048 (0.003)
Urinary tract infections	88 (16.7)	13 (25.5)	75 (15.7)	1.612 (0.166)

Table 7. Number of main infections (pneumonias and urinary tract infections) in our population. As expected we presented a higher number of pneumonias and a reduced number of urinary tract infections. In our population, presenting CPE colonization is linked to a higher risk of developing pneumonias. HR: Hazard ratio.

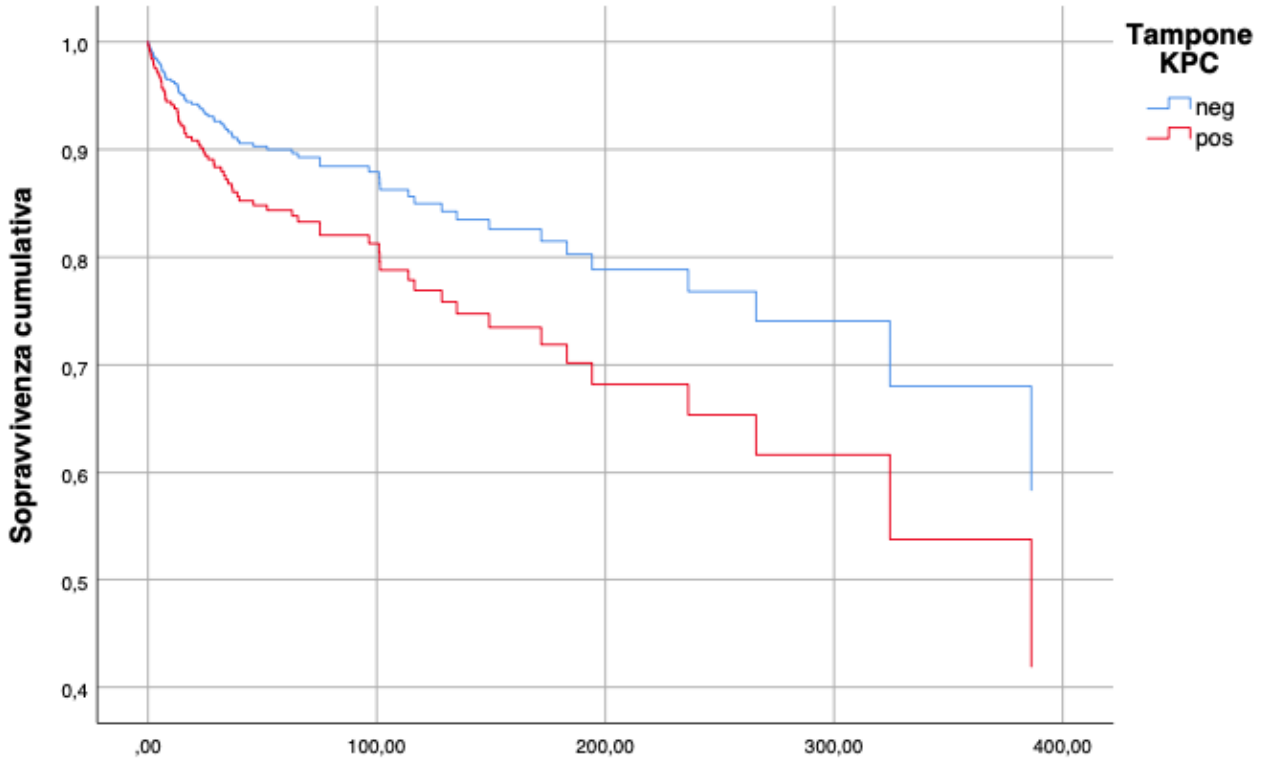


Figure 33. Kaplan Meier for Urinary tract infections according to CPE colonization. Providing Cox analysis we evidenced that there wasn't statistical difference between the two groups (HR 1.612, p=0.166).

	Population 90 (%)	**Positives 16 (%)**	**Negatives 74 (%)**
Staphylococcus Aureus	1 (1.1)	0	1 (1.3)
Staphylococcus epidermidis	2 (2.2)	1 (6.2)	1 (1.3)
Other staphylococci	4 (4.4)	0	4 (5.4)
Escherichia Coli	37 (41.1)	5 (31.2)	32 (43.2)
Klebsiella Pneumoniae	9 (10)	5 (31.2)	4 (5.4)
Other Klebsiellas	4 (4.4)	1 (6.2)	3 (4)
Pseudomonas Aeruginosa	10 (11.1)	2 (12.5)	8 (10.8)
Enterococcus Faecalis	6 (6.7)	1 (6.2)	5 (6.7)

Enterococcus Faecium	0	0	0
Proteus Mirabilis	8 (8.9)	1 (6.2)	7 (9.5)
Other	9 (10)	0	9 (12.2)

Table 8. Microorganisms responsible for urinary tract infections in the considered period.

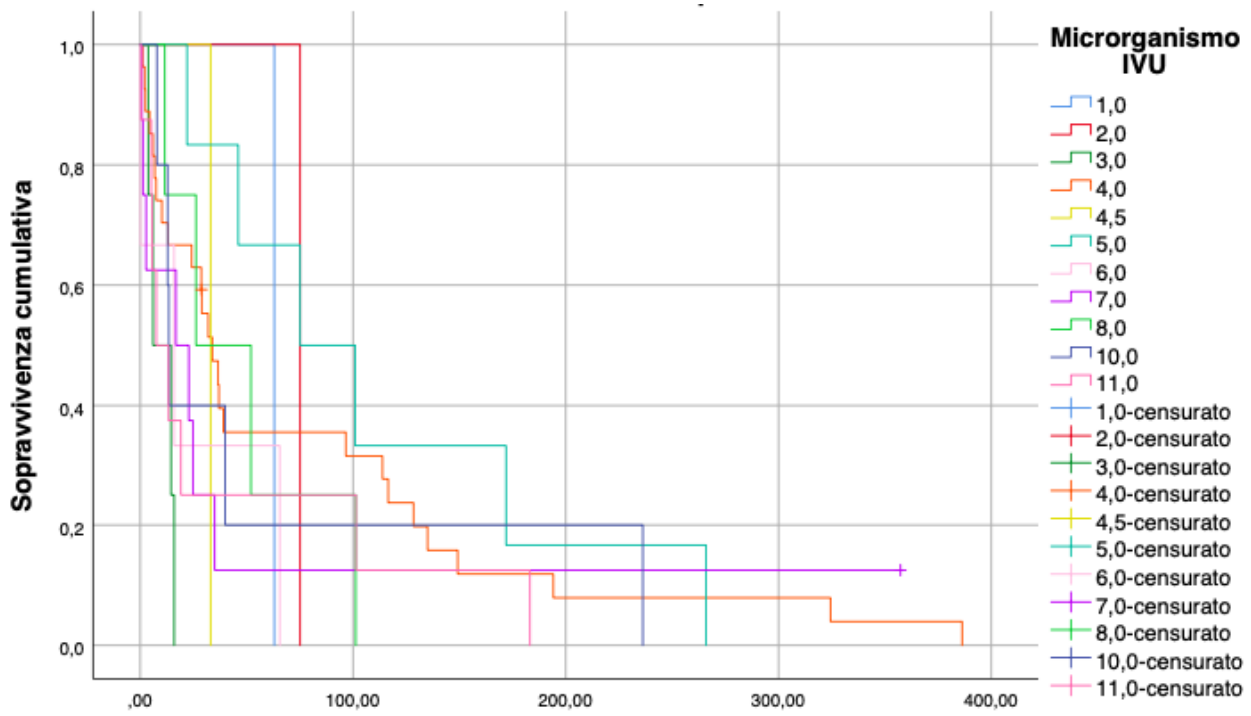


Figure 34. Kaplan Meier for urinary tract infection according to the type of bacterial. The Log Rank (Mantel-Cox) analysis shown no significativity among different bacterials in both groups.

As another main infection we considered pneumonias. In our population we evidenced 147 pneumonias, with a significative difference between the two group. In our population CPE colonized patients present a higher risk to develop pneumonias (HR 2.048, p=0.003). Results are expressed in Table 7 and in Figure 35.

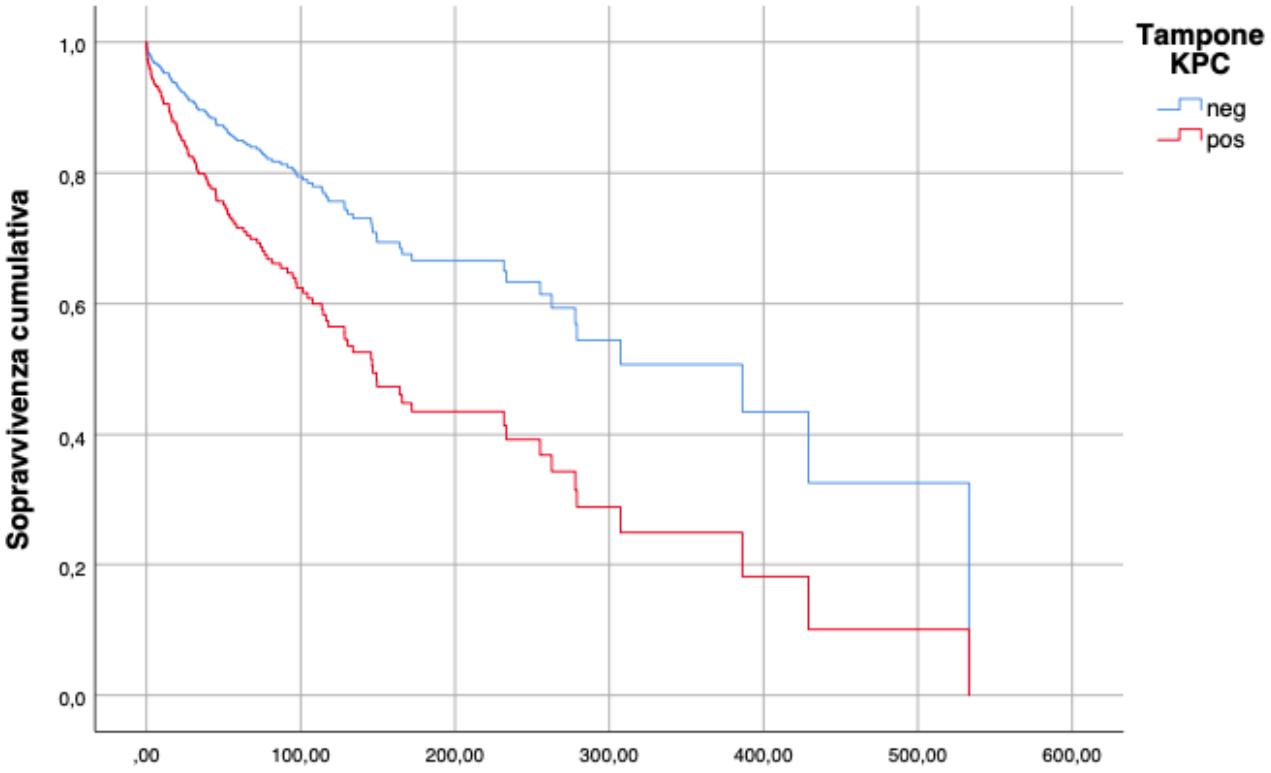


Figure 35. Kaplan Meier for pneumonias according to CPE colonization. Providing Cox analysis we evidenced a statistical difference between the two groups (HR 2.048, p=0.003) confirming that presenting CPE colonization expose to a higher risk of developing pneumonias.

One of our goals was to determine whether presenting CPE colonization expose to a higher number of episodes of sepsis or hospedalizations. Of the considered population, 178 patients presented 497 episodes of sepsis, 451 episodes in the non CPE colonized and 46 episodes in the CPE colonized (HR 2.057, p-value 0.001). Differences are shown in Figure 36. Data concerning bacterials that determined sepsis are resumed in Table 9 and shown in Figure 37. We evidenced no differences among pathogens, nonetheless the most frequent were Staphylococcus Aureus, Staphylococcus Epidermidis and Escherichia Coli.
Considering CPE-induced sepsis, a substantial difference was observed between the two groups. The colonized patient group had 7 (15.2%) cases of CPE sepsis, while the non-colonized group did not have any (0%). The result shows statistical significance, although the limited number of infections does not allow for definitive conclusions on this matter. Results are shown in Figure 38.

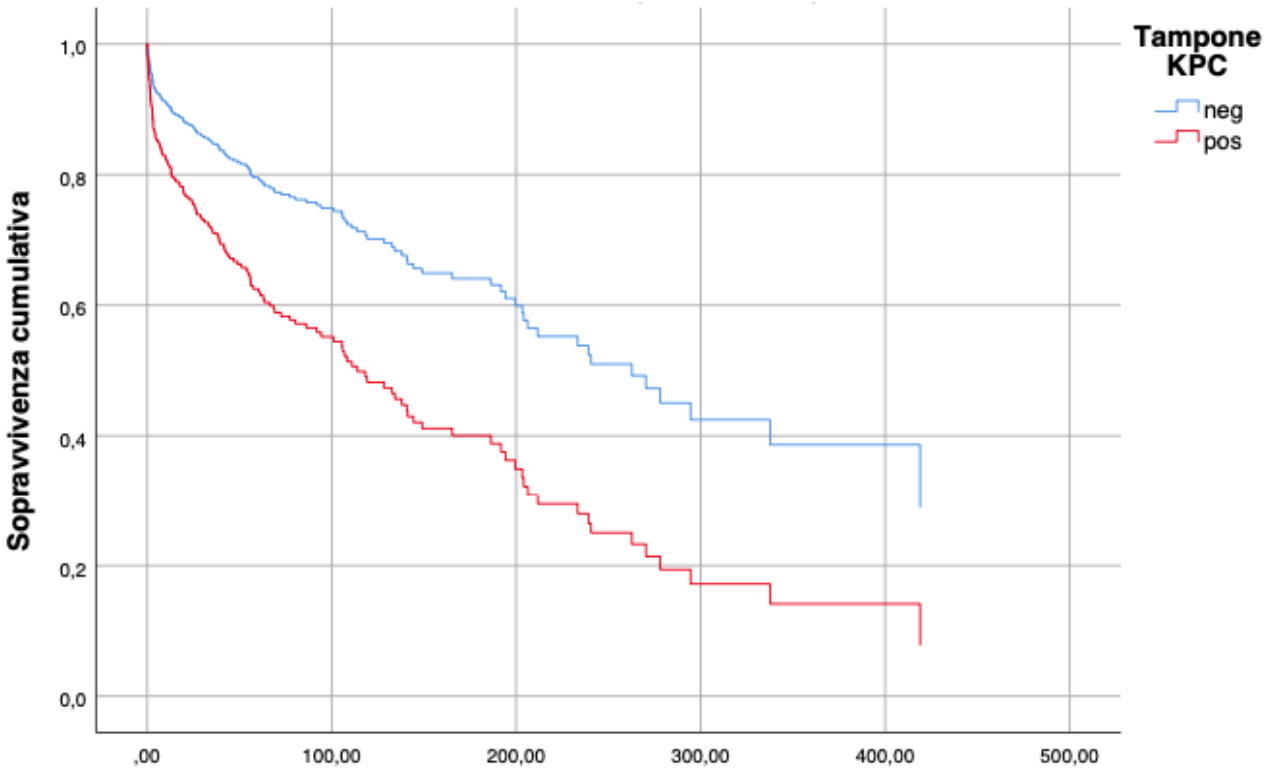


Figure 36. Kaplan Meier for sepsis according to CPE colonization. Providing Cox analysis we evidenced a statistical difference between the two groups (HR 2.057, p=0.0001) confirming that presenting CPE colonization expose to a higher risk of developing sepsis.

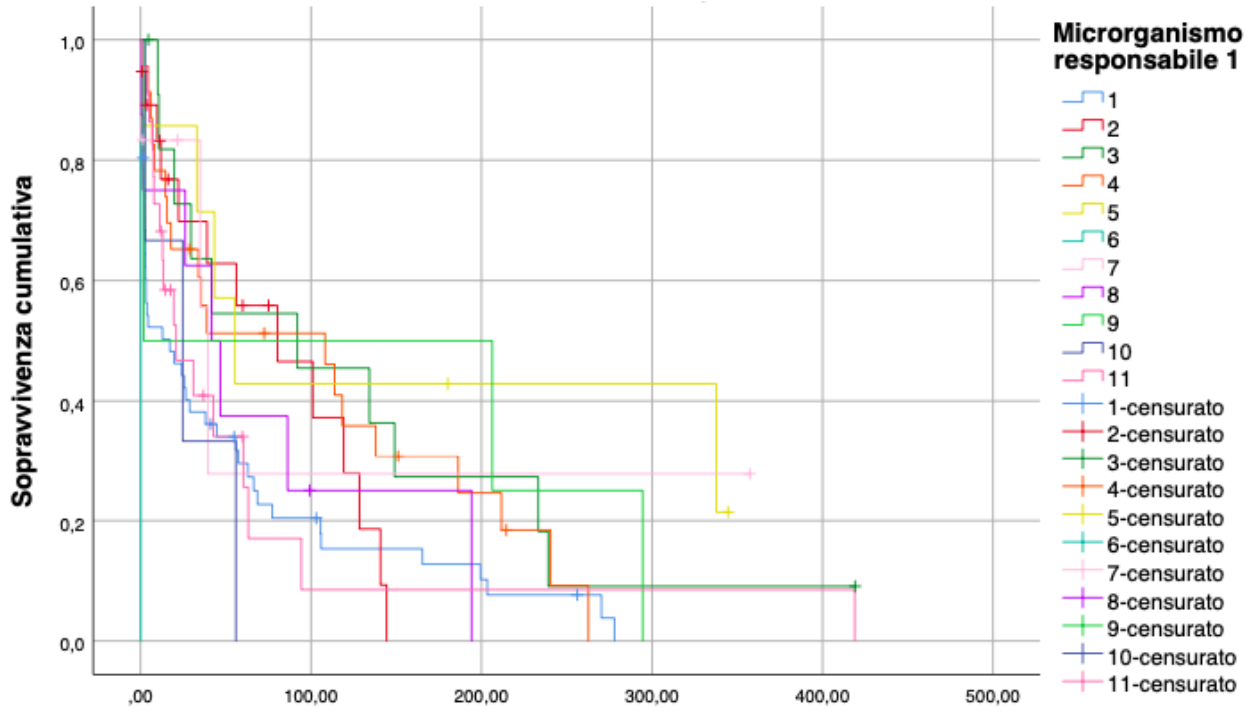


Figure 37. Kaplan Meier of different kind of bacterials responsible for sepsis. Performing Log Rank analysis (Mantel-Cox test) we evidenced no difference in our cohort among pathogens.

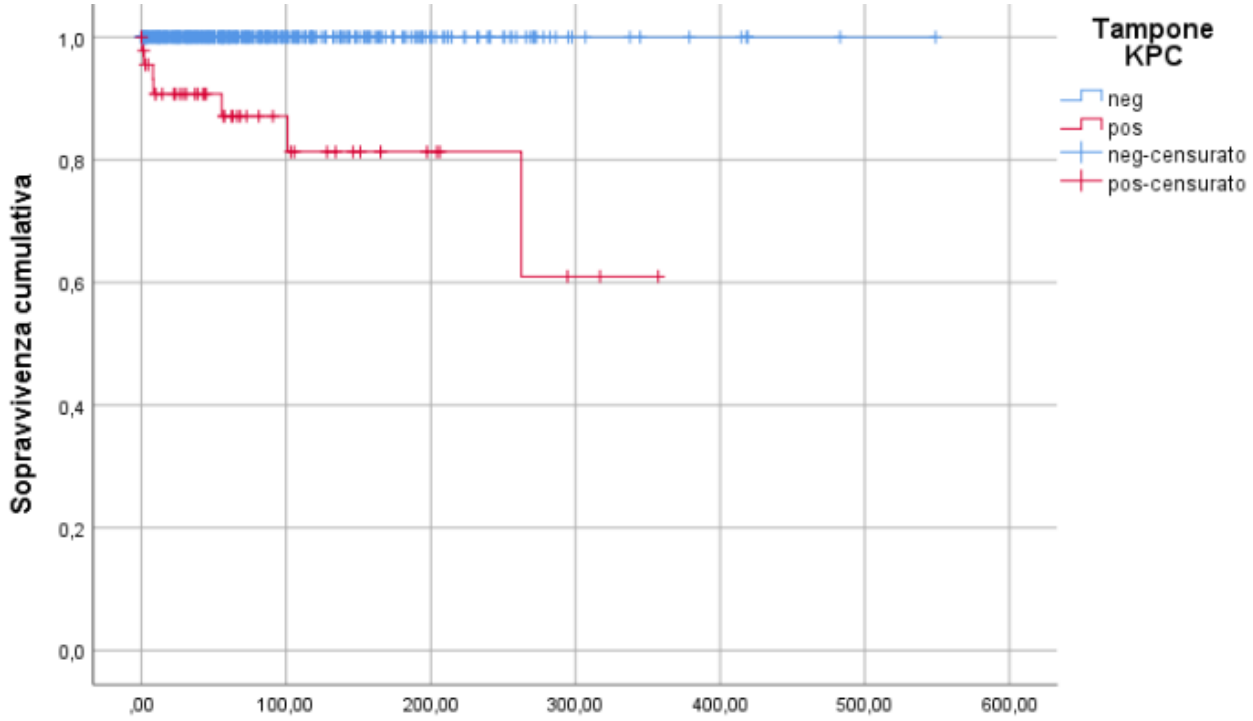


Figure 38. Kaplan Meier for CPE sepsis according to CPE colonization. Log-Rank (Mantel-Cox) 60.229, p=0.000. Due to the low number of infections it's difficult to drive conclusion on the matter.

Evaluating the hospitalization rate, the difference between the two groups was substantial but the curves didn't reach significance (Figure 39). Nevertheless, we examinated the re-hospedalization rate, that shown a significative difference between the two groups, displaying an involvement in CPE colonization in re-hospedalization rate (Figure 40). Results are expressed in table 10.

	Population	**Positives**	**Negatives**	**HR (p-value)**
Number of hospitalizations (mean ± SD)	3.71 ± 3.15	5.45 ± 3.8	3.53 ± 3.02	1.130 (0.505)
Re-hospitalizations (month, mean ± SD)	6.17 ± 0.95	3.78 ± 0.87	7.26 ± 1.02	1.367 (0.038)

Table 10. Number of hospitalization differs between the two groups without reaching a statistical significance, but re-hospitalization rate differs significantly. SD: Standard deviation, HR: Hazard ratio.

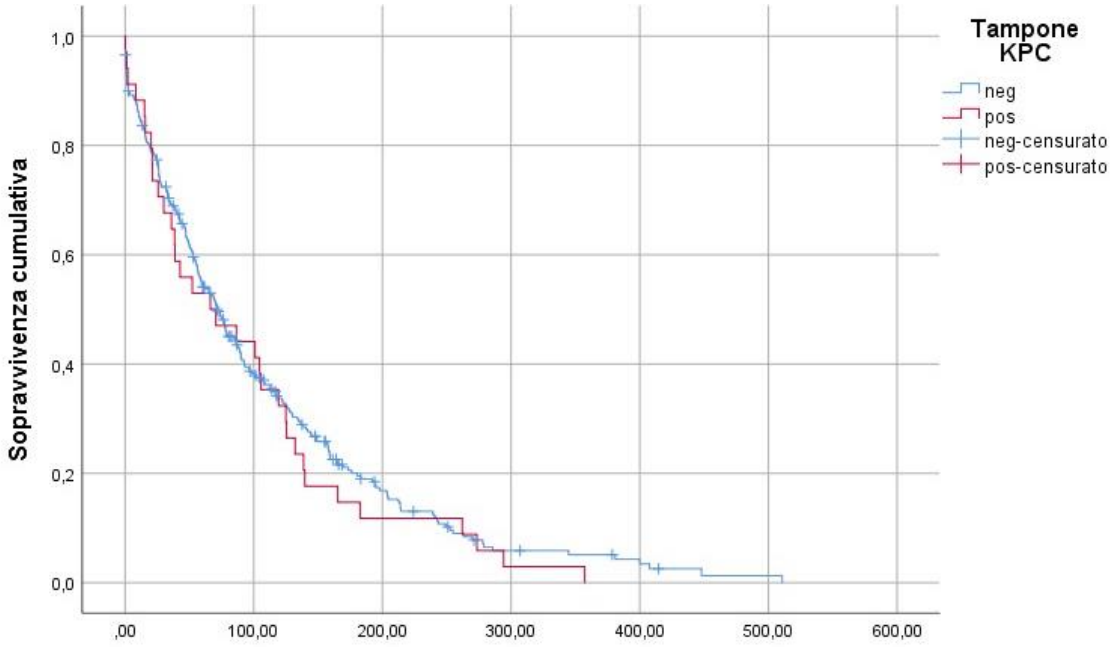


Figure 39. Kaplan Meier for hospitalizations according to CPE colonization. We observe no significative difference between the two slopes.

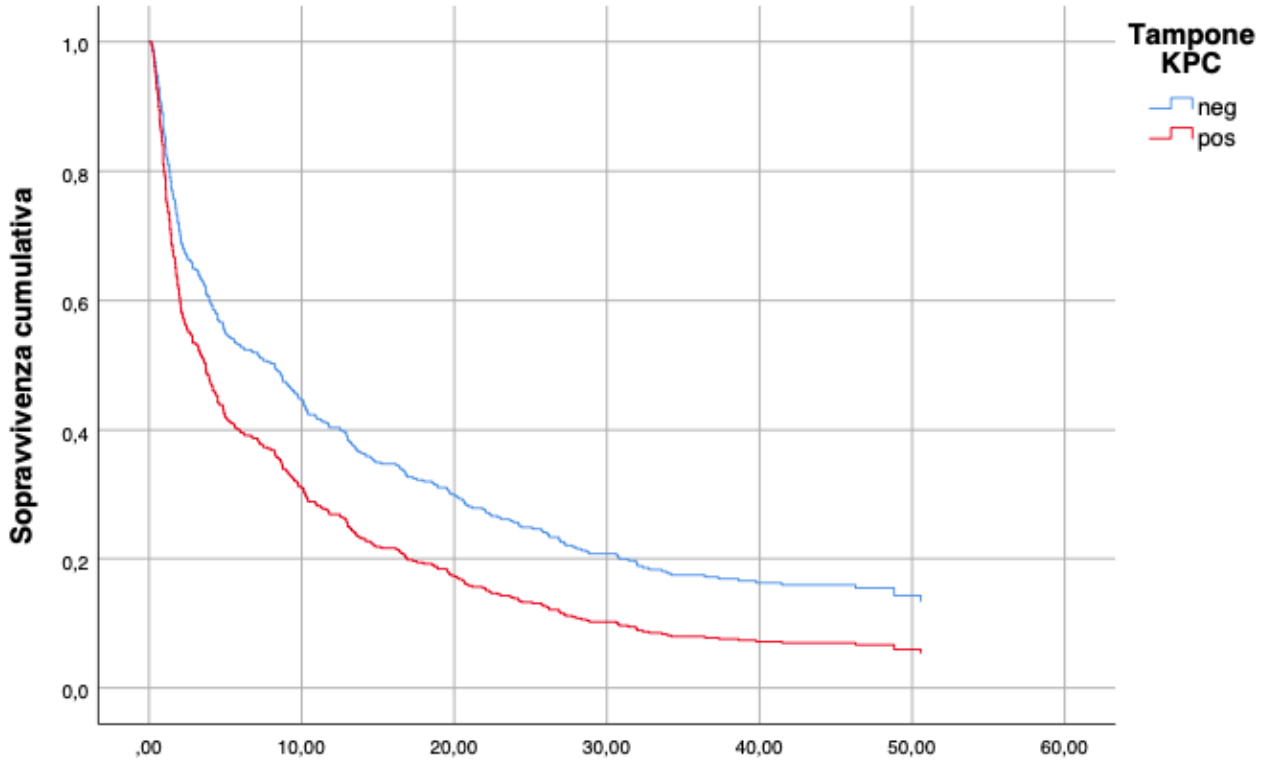


Figure 40. Kaplan Meier for re-hospitalizations according to CPE colonization. We observe a significative difference between the two slopes since the beginning.

As regarding survival, that was one primary end-point, our groups shown different survival since the beginning of dialysis but we didn't evidence a significative difference. Results are expressed in Table 11 and Figure 41.

	Mean ± SD	Median [IC]	HR (p-value)
Population	115.63 ± 8.26	74.12 [61.06 – 87.18]	
Positives	92.98 ± 18.36	50.59 [23.78 – 77.41]	0.643 (0.422)
Negatives	117.33 ± 8.80	75.50 [61.40 – 89.60]	0.643 (0.422)

Table 11. The table shows different survival time, expressed in months, since the beginning of dialysis. Nevertheless the values are different, we evidence no significativity in survival between the two groups.

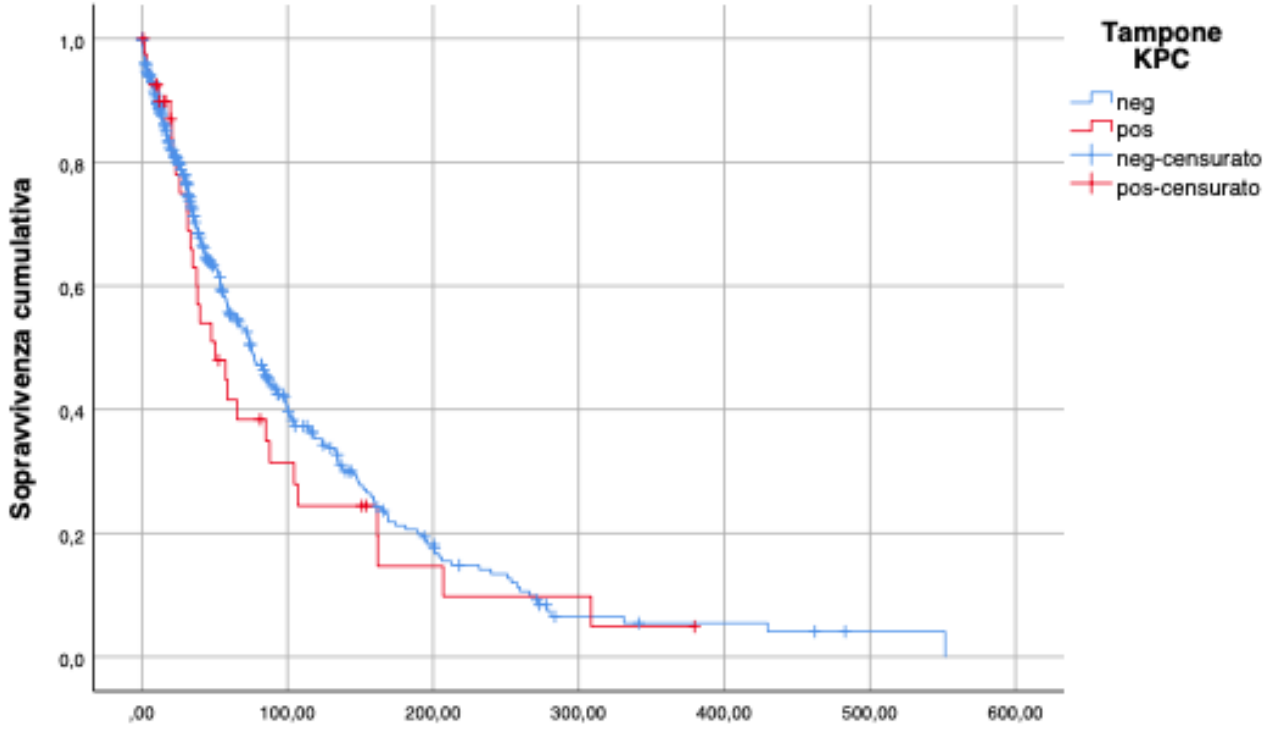


Figure 41. Kaplan Meier for survival according to CPE colonization. We didn't observe a significative difference between the two slopes.

Discussion

The global dissemination of CPE poses a major threat to the health care systems around the world, especially in fragile patients [49]. With the worldwide dissemination of CPE, many hospitals have introduced infection control programmes to limit CPE spread, infection rate and attributable morbidity and mortality [50]. Active surveillance is strongly recommended for timely detection of carriers, separation of carriers from non-carriers, and activation of contact precautions. Information concerning carrier or colonization status may be also useful to guide antibiotic treatment upon suspicion of infection. However, data concerning the incidence of and risk factors for invasive infections in CPE carriers are limited. In our retrospective observational study on a large dialysis population, we found that presenting a CPE colonization exposed to a higher risk of sepsis ($p=0.0001$), of sepsis induced by CPE ($p=0.0000$), of re-hospedalization ($p=0.0038$) and of pneumonias ($p=0.003$). In our cohort these evidences are not accompanied by an increased mortality or by an increased number of UTI.
In literature several studies tried to identify risk factors for CPE infections. The study conducted by Borer and collegues conducted on 464 patients hospitalized at a large hospital in Israel, shown that 9% of carriers developed infection and that independent risk factors for infection were previous invasive procedures, diabetes mellitus, solid tumor, tracheostomy, urinary catheterization and prior use of anti-pseudomonas penicillins [51]. In the same study the authors described that the overall incidence density of carbapenem-resistant Klebsiella pneumoniae infections (CRKP-IN) was 1.57 per 10,000 patient-days. These infections originated primarily from urinary tract infections (57.5%), followed by ventilator-associated pneumonia (30%), and surgical site infections (12.5%). Bacteremia was additionally identified in 8 out of the 42 patients in the CRKP-IN group, accounting for 19% of the cases. In another study conducted by Schechner et al 44 (8.8%) of 502 CPE carriers developed subsequent positive clinical cultures with CPE. In that population, risk factors for subsequent positive clinical samples were admission to the intensive care unit, having a central venous catheter, antibiotics exposure and diabetes mellitus [52]. Data were in part confirmed by a prospective study that demonstrated that 74.5% of patients were ultimately colonized by CPE during their ICU stay, invasive medical procedures and monitoring devices are pivotal in elevating patients' vulnerability to colonization by offering a gateway for multidrug-resistant pathogens to enter the body [53]. This issue is further aggravated by inadequate adherence to proper hand hygiene practices among nursing and medical personnel, which facilitates the transmission of CPE and other bacterias within the ICU. As in our study, the authors conclude that CPE colonization did not increase the overall mortality and that was significantly associated with CPE infection.
In line with data already described in literature, even in our population, the risk of a BSI from CPE bacterials in CPE carriers was higher (7 cases vs 0 cases) compared to non carriers. In a large cohort of CPE rectal carriers, the rate of sepsis from CPE bacterials was 7.8%, not so low that upon suspicion of infection the need for CPE coverage would be considered to be negligible, nor so high that antibiotic coverage should considered to be always necessary in stable carriers with symptoms of infection. [54] In 2017 Tischendorf et al performed a systematic review of the literature on patients colonized by CPE showing an overall 16.5% risk of infection with CPE amongs patients colonized. Of course data come from different studies, that means different rates of infection, type of organism, patient population, clinical setting, microbiologic methods and it’s very difficult to drive conclusions. Nonetheless the authors conclude that eradication of colonization with CRE should be considered in selected situations such as outbreaks [55] A study conducted by [56]. had the purpose to investigate the prevalence, risk factors of intestinal CRE colonization and BSI caused by CRE in allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients. The authors found thant allo-HSCT patients with CRE-induced BSI have poor prognosis, and CR-KP rectal colonization is an independent risk factor for CRE-related BSI. Rectal swab screening during allo-HSCT could provide early warning for later CRE-induced BSI.

According to the latest report from the European Centre for Disease Prevention and Control (ECDC), the most prevalent antibiotic-resistant bacteria were E. coli, followed by S. aureus, K. pneumoniae, E. faecalis, and P. aeruginosa. E. coli was also the most frequently reported drug-resistant pathogen responsible for BSIs worldwide, with 40% of isolates showing resistance to third-generation cephalosporins (ESBL-E. coli). Resistance rates in the case of urinary tract infections were similar, with only a few specimens demonstrating resistance to carbapenems. Conversely, resistance against carbapenems was notably high for K. pneumoniae (7.9%), P. aeruginosa (16.5%), and Acinetobacter species (32%). [57,58,4]. Methicillin-resistant Staphylococcus aureus (MRSA) is the most commonly reported gram-positive bacterium in surveillance systems across Europe. Both MRSA and ESBL-E. coli are used as antimicrobial resistance (AMR) indicators for the Sustainable Development Goals established by the World Health Organization. Recent data suggests a decreasing trend in the incidence of MRSA infections over the past decades. [59] However, in contrast to MRSA, infections caused by other resistant gram-positive bacteria, such as vancomycin-resistant enterococci (specifically, vancomycin-resistant Enterococcus faecium), are on the rise in certain European countries like Portugal, Spain, Italy, and Greece, as well as in various regions of South America. This increase in vancomycin-resistant enterococci infections is a cause for concern. [60, 61] Our resuts are completely in line with literature, the most representing pathogen was E.Coli for UTIs, and Staphylococcus Aureus for sepsis.

According to a recent review on CRE infections during the COVID-19 pandemic, with a particular focus on secondary infections caused by Carbapenemase-Producing Enterobacteriaceae (CPE), the prevalence of coinfections in COVID-19 patients varied significantly, ranging from 0.35% to 53%. It's noteworthy that the most commonly detected resistance gene was KPC, followed by OXA-48 and NDM. The primary sources of infection were pneumonia and BSI. Conversely, urinary tract, intra-abdominal, and skin and soft tissue infections were relatively rare, likely due to mechanical ventilation and central catheters being identified as the primary drivers of CPE dissemination. [62]. Lemenand and collegues showed a decrease in the proportion of extended-spectrum beta- lactamase among E. coli infections in primary care and nursing home during the first year of the coronavirus disease 2019 (COVID- 19) pandemic in France. [63] Various factors may have played a role in the reduction of antimicrobial resistance during the COVID-19 pandemic, including the reinforcement of hygiene practices in the general population (such as hand hygiene and limiting gatherings during lockdowns), a decrease in antibiotic consumption in primary care, restrictions on international travel, and increased awareness and compliance with infection control measures and hand hygiene among healthcare workers. Conversely, within hospital settings, some factors could have contributed to the exacerbation of antibiotic resistance during the pandemic. These include an increase in bacterial infections, higher antibiotic use within hospitals, a larger proportion of patients requiring intensive care, hospital overcrowding, and the fatigue experienced by healthcare workers after months of dealing with the COVID-19 surge. An interesting study conduced by Duverger and collegues showed a decrease in the incidence of CPE cases in a great public institution in France during the COVID-19 pandemic era. The authors suggested that this decrease is linked to a decrease in international exchanges, underlining that to be effective, the fight against antimicrobial resistance will have to be considered at an international level. [64] These findings underscore the necessity for heightened vigilance regarding CPE infections, which are the dominant healthcare-associated pathogens among CRE, especially in patients with critical COVID-19, where the immune system undergoes a complex pattern of immune dysregulation. [65] Concerning COVID-19 infection, our cohort presented 61 cases, 9 cases (18%) occourred in the CPE colonized group, while 52 (10.9%) occourred in CPE non colonized group without reaching a statistical significance (p-value 0.07). Our results are in line with literature.

The high mortality rate associated with CRE infections worldwide is a significant concern. Studies have reported unadjusted 30-day mortality rates of 34% for patients with CRKP bacteremia collected from 71 hospitals worldwide during 2017–2018, a 43% mortality rate for patients with

CRE bacteremia in European hospitals from 2004–2013, and a 38% inpatient mortality rate for CRE bacteremia in South Africa from 2015–2018 [66-68]. During the observation period, the study population exhibited an exceptionally high mortality rate (26.5% deaths per year), and within our population, a substantial difference in terms of mortality between the two groups was observed, but the result did not achieve statistical significance.

We ask whether there were treatment associated with the development of CPE colonization. Therefore we analyzed a huge amount of informations concerning common treatments such as antihypertensive agents, PPI or iron therapy, and very specific therapies for CKD such as phosphorus and potassium binders or ESAs. Phosphate binders act by decreasing the absorbtion of ingested phosphate and converting it into an unsoluble form that is excreted in the stools. Basically, there are two categories: calcium based (calcium carbonate, calcium acetate and calcium acetate/magnesium carbonate) and non calcium base (sevelamer, lanthanum and iron based binders). Although the two categories are equally effective in lowering serum phosphate concentrations when well titrated, each has various drawbacks, and one of the most important is gastrointestinal side effects. [69-73]. Most of the studies demonstrated that reducing phosphate though a phosphate binders reduce the risk of all-cause mortality and cardiovascular disease among dialysis patients, but a few data are reported concerning the risk of infections. [74] Given that phosphorus is a crucial nutrient essential for the growth and replication of bacteria, alterations in its levels in the intestines can significantly affect the activity and makeup of the gut microbiota. Consequently, dietary restrictions can lead to changes in the composition of the gut microbiome. Therefore, it is important to note that the gut microbiome is influenced not just by CKD but also by the use of phosphate binders [75-77]. The influence of phosphate binders on the gut microbiota may have the potential to alter the production of uremic toxins originating in the gut (indole, p-cresol and Indole-3 acetic acid). These binders have the capacity to hinder the absorption of both phosphate and gut-derived uremic toxins. It's worth noting that there is a limited number of published in vitro studies examining the effects of phosphate binders on precursor substances or uremic toxins found in the intestinal environment. A study conducted by Yamada et al. on patients undergoing HD and receiving phosphate binders revealed a noteworthy finding: the occurrence of infection-related fatalities was markedly reduced among patients using phosphate binders when compared to those who did not use them. The hazard ratio for infection-related mortality was 0.63 (with a 95% confidence interval ranging from 0.40 to 0.99). Importantly, these results retained their significance even after employing four distinct propensity score-based analyses. It's worth highlighting that the utilization of phosphate binders was linked to a reduced risk of mortality from any cause. In our population we observed no differences between the two groups (HR 0.9, p=0.933 for calcium based phosphate binders and HR 1.2, p=0.121 for non calcium based phosphate binders), therefore it seems that both treatments have no impact in our population on the risk of development of CPE colonization [78].

Potassium binders are a class of drugs used to treat hyperkaliemia, a frequent event in dialysis patients. Since 2021, in Italy, only sodium polystyrene sulfate (SPS) was used to treat hyperkaliemia, nowadays a couple of new binders are available and safer (Patiromer and Sodium Zirconium Cyclosilicate) but no patients of our cohort received those medications. SPS is a non-selective potassium binders, that act in gastrointestinal tract and facilitates excretion in the feces. The use of SPS has also been associated with rare GI effects, such as mucosal damage and intestinal necrosis. While serious adverse effects are rare, nausea, vomiting, and diarrhea occur frequently and limit the long-term tolerability of the medication. [79-81] To our knowledge, only one case report has documented an infection caused by MDR bacterials (Enterococcus Faecium in the specific case) following the ingestion of SPS, and there are no studies reporting associations between the use of SPS and the development of CPE infections [82]. To the best of our knowledge, this is the first study that investigates the relationship between SPS and colonization by CPE. In our patient cohort, those who received SPS therapy were less frequently colonized by CPE bacteria (HR 0.5, p=0.034). The reason for this finding is not clear, and we can only speculate that SPS induces modifications in

the intestinal microbiota that favor the growth of other types of bacteria. However, this observation should be confirmed through in vitro studies and subsequently through prospective studies involving larger populations.

Several studies has quested whether antibiotics could induced outgrowth of CPE in the bowel. The utilization of carbapenems in managing infections caused by multi-drug resistant bacteria has exerted selective pressure within the clinical environment. This pressure has subsequently given rise to the development of resistance against carbapenems [83]. The primary mechanism underlying carbapenem resistance is associated with carbapenemase genes, which are typically situated on mobile genetic elements like plasmids, integrons, insertion sequences, and transposons. This genetic arrangement facilitates the horizontal transfer of resistance genes across various bacterial species, both within and between them [84-88]. In a mouse model conducted by Sim et al. the authors demonstrated that clinically relevant antibiotics such as ampicillin, vancomycin, and azithromycin induce outgrowth of K. pneumoniae [89]. A brilliant study on mouse models conducted by Yip AYG et al. demonstrated that broad-spectrum antibiotics (carbapenems, fluoroquinolones, penicillin/beta lactamase inhibitor and cephalosporines), enriched for a wide range of nutrients in the intestine, generating a nutrient-enriched niche that supported CRE growth. The authors demonstrated that CRE can utilize these increased nutrient levels as sources of carbon and nitrogen to facilitate their growth, exhibiting a discernible preference for certain nutrients. It was observed that CRE's growth was more pronounced in an oxygen-rich environment, often linked to antibiotic treatment while particular microbial metabolites, which declined in the presence of antibiotics, possessed the capability to hinder the growth of CRE [90]. Even Schechner et al. described that exposure to fluoroquinolones and to metronidazole were associated with subsequent CRE clinical infection [52]. To our knowledge no previous studies have investigated this feature in a dialysis population. In our cohort we evidenced that no antibiotic was linked to the outgrowth of CPE. Nevertheless we had only few data, we observed that CPE colonization is more frequent in those who attended Linezolid or Ceftazidime/Avibactam.

Linezolid (Zyvoxid) is an antibiotic belonging to the oxazolidinone class, with a primarily narrow spectrum of antibacterial activity targeting Gram-positives, including cocci resistant to penicillin, cephalosporins, oxacillin, and vancomycin. It exhibits excellent penetration of the skin, soft tissues, and respiratory tract and is well-absorbed orally, requires no adjusted dose for renal function, making it an ideal choice for patients with limited fluid intake, such as those undergoing dialysis. Its use is reserved for complicated Gram-positive infections in patients with risk factors for multidrug resistance (MDR) [91] One of the most common side effects is myelosuppression, which, on the other hand, may predispose to Gram-negative bacterial infections. [92] The higher rate of CPE colonization in patients who received Linezolid may be explained in this way even in our cohort, identifying a class of patients that are even more fragile.

Ceftazidime-avibactam, marketed under the name Zavicefta, is an intravenously administered combination of the third-generation cephalosporin ceftazidime and the innovative non-beta-lactam beta-lactamase inhibitor avibactam. In the European Union (EU), ceftazidime-avibactam is authorized for the treatment of adults with complex urinary tract infections (cUTIs), including pyelonephritis, complex intra-abdominal infections (cIAIs), hospital-acquired pneumonia (HAP), which includes ventilator-associated pneumonia (VAP), as well as other infections caused by aerobic Gram-negative microorganisms in patients with limited treatment alternatives. [93, 94]. The primary mechanism of acquired resistance to ceftazidime-avibactam in clinically significant Gram-negative pathogens involves the production of beta-lactamases that are impervious to avibactam's inhibitory effects. This includes enzymes such as class B beta-lactamases (metallo-beta-lactamases) and many class D enzymes. Additionally, resistance to ceftazidime-avibactam has been observed in bacterial strains with mutations in AmpC or carbapenemase enzymes. In some instances, resistance to ceftazidime-avibactam has been associated with mutations in plasmid-borne KPC-3 enzymes. Interestingly, it was noted that certain KPC-3 mutations conferring resistance to ceftazidime-avibactam were also linked to reductions in the minimum inhibitory concentrations (MICs) for

carbapenems and other beta-lactam antibiotics [95-97]. In our cohort only some patients underwent a treatment with ceftazidime-avibactam, with a significative difference between the two groups. The explanation could be that these patients are frequently hardly immunocompromised, presented several comorbidities and hospitalizations, attested for a even more fragile kind of patients. Considering all these findings, it would be really interesting studying the microbiome of CKD and dialysis patients, looking for specific bacterials populations that could be provided to patients who are at major risk of developing BSI by CPE.

Study limitations:

- The retrospective nature of the study
- The impossibility of accurately determining the vascular access of HD patients. During the observed period, several patients underwent changes in their vascular access, and it was not possible to track all the variations in access within the cohort. This occurred because, in the past, changes in vascular access were not systematically recorded, as has been the case in recent years. This is a clear limitation since having an intravascular device (temporary or permanent central venous catheter, prosthetic arteriovenous fistula) is associated with a higher risk of sepsis and infections compared to those undergoing HD with a native arteriovenous fistula.
- The lack of certain data, particularly concerning patients from peripheral centers (specifically, blood tests and unreported antibiotic usage)
- The absence of data related to the new potassium binders (such as Patiromer) since they became available only from 2021, and only a few patients had the opportunity to use them before the study concluded

Points of strength:

- The high number of patients of such a specific population
- The completeness of data concerning both anti-hypertensive treatments, treatments specific of CKD, and antibiotic therapies
- The reliability and quantity of data regarding hospitalizations, blood cultures, and urine cultures analyzed
- To our knowledge, this is the first study that analyzes the colonization factor of CPE and its impact on health in a specific population of dialysis patients
- As far as we know, this is the first study to examine the relationship between colonization by CPE and the use of intestinal phosphate or potassium binders
- To the best of our knowledge, no studies have previously investigated the impact of the type of dialysis on the risk of colonization by CPE.

Conclusions:

In a large dialysis population, our study highlighted that a colonization by CPE lead to a greater number of episodes of all bacterial sepsis, of CPE sepsis, of pneumonias and of re-hospitalizations. Therefore, to reduce the morbidity of our patients, CPE eradication has to be one of our primary goals.
In our cohort mortality didn't differ between the two groups, as the number of UTI.
Colonization by CPE conferm an increased risk of CPE sepsis, and therefore, future research should focus on determining whether it is worthwhile to treat hyperpyrexia in colonized CPE dialysis patients as if it were CPE-related sepsis. This is a significant question that can guide our clinical practice.
Some antibiotics and some treatments specifics for CKD has an impact on colonization in our cohort but several studies has to be performed to better understand the risk factors and the best management of CPE colonized patients in dialysis setting.

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