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**TESI DI LAUREA**

# APPLICATION OF WISCA (WEIGHTED-INCIDENCE SYNDROMIC COMBINATION ANTIBIOGRAM) TO GUIDE THE CHOICE OF EMPIRIC ANTIBIOTIC TREATMENT IN ONCOLOGICAL PAEDIATRIC PATIENTS WITH FEBRILE NEUTROPENIA: AN ITALIAN MULTICENTER STUDY


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# SUMMARY

# ABSTRACT

**Background**: In oncological patients, febrile neutropenia (FN) is the hallmark of bacterial and fungal infections, the most frequent and severe complications of cancer chemotherapy, correlated to high morbidity and mortality. The causative pathogen is unknown at the onset of fever, as many bacteria may cause the same clinical infection syndrome; therefore, FN should be promptly treated with broad-spectrum empirical antibiotic therapy. International and national guidelines recommend an anti-pseudomonal beta-lactam in monotherapy - cefepime, piperacillin-tazobactam (PI-TZ) or meropenem - as empiric treatment. However, with the increasing number of multidrug-resistant organisms, the best empiric therapy should be driven by local epidemiology, usually described by cumulative hospital antibiograms, which provide general information on the sensitivity of individual bacterial species or genera to certain antibiotics with no further stratifications. The Weighted-Incidence Syndromic Combination Antibiogram (WISCA) is a syndrome-specific tool that attempts to satisfy the unmet need to obtain syndrome-specific local susceptibility data to guide empirical antibiotic prescribing, providing estimates for several treatment regimens as a weighted average of pathogens' susceptibilities.

**Aim of the study**: This study aims to develop a WISCA model to inform empirical antibiotic regimens selection for FN in children using data from the Paediatric Onco-haematological wards of the Department for Women's and Children's Health in Padua and the Gaslini Hospital in Genoa. The second aim is to identify which combination of antimicrobials is more effective in the coverage of the main bacteria causing bloodstream infections (BSIs) in paediatric patients with cancer or undergoing haematopoietic stem-cell transplantation (HSCT) presenting with FN.

**Materials and methods:** The study cohort included blood cultures of patients with a microbiological diagnosis of BSI and neutropenia admitted to the Paediatric Onco-haematological ward, or Bone Marrow Transplant Unit, of the Department for Women's and Children's Health at University of Padua and the Onco-haematological ward of the Paediatric Hospital Gaslini in Genoa from January 1st, 2016, to December 31st, 2021. WISCAs were developed by estimating the coverage of 20 antibiotics as monotherapy and of 21 combined antibiotics regimens, using a

*Bayesian* model stratified by centre, age group, underlying pathology and HSCT. Moreover, a second model considering only gram-negative bacteria was created.

**Results:** We collected 350 blood cultures (196 gram-negative and 154 gram-positive bacteria) from patients with a median age at the time of the infectious episode of 8,6 years. In both centres, most BSIs (28%) occurred at age 9-14. The two populations of Padua and Genoa turned out homogeneous, with statistically significant differences concerning sex (males more frequent in Genoa) and underlying pathology (leukaemia more frequent in Padua, solid tumours in Genoa). Considering most used antibiotic combinations such as PI-TZ plus amikacin, the median coverage was 78% (*Bayesian Uncertainty Interval*-BUI 11-95%). When adding a glycopeptide, the median coverage further increased to 89%, while the replacement of PI-TZ with meropenem, maintaining the association with amikacin, did not provide benefits. When considering only gram-negative bacteria, monotherapy with PI-TZ showed a slightly inferior coverage compared with meropenem; however, when combined with amikacin, both reached the same coverage level. This second model has been developed considering the low mortality rate associated with gram-positive bacterial infections (mainly *Coagulase-negative staphylococci*) and the possibility of targeting them when cultures turn back positive. WISCA tool applied to blood cultures showed how monotherapy did not offer an adequate coverage rate for the identified pathogens and confirmed the validity of the empirical therapeutic regimens used in both centres (PI-TZ, amikacin and teicoplanin for Padua and PI-TZ and amikacin for Genoa). Albeit encouraging data, the statistical significance was not reached because of the small sample size, being BSIs an uncommon event in the paediatric setting.

**Conclusions:** The Bayesian WISCA provides an innovative approach to pool information from different sources, guiding the choice of empirical antibiotic treatment in oncological paediatric patients with FN. Moreover, the application of WISCA in a multicenter study offers the possibility of maximizing the clinical utility of microbiological surveillance data derived from larger hospitals to inform the selection of the most appropriate empiric therapy also for other minor hospital settings in the same area while contributing to spare broad-spectrum antibiotics and increasing confidence in the selection of narrow-spectrum regimens.

# RIASSUNTO

**Introduzione:** Nei pazienti oncologici, la neutropenia febbrile (NF) è associata alla presenza di infezioni batteriche e fungine, le più frequenti e gravi complicanze della chemioterapia antitumorale, correlate ad alta morbilità e mortalità. Il patogeno responsabile non è noto all'esordio della febbre, pertanto, la NF deve essere tempestivamente trattata con una terapia antibiotica empirica ad ampio spettro. Le linee guida nazionali e internazionali raccomandano un beta-lattamico anti-pseudomonas in monoterapia - cefepime, piperacillina-tazobactam (PI-TZ) o meropenem. Tuttavia, con l'aumento dell'incidenza di germi multiresistenti, la scelta della terapia empirica dovrebbe essere guidata dall'epidemiologia locale, solitamente descritta dagli antibiogrammi cumulativi ospedalieri, che forniscono informazioni generali sulla sensibilità di singole specie batteriche a determinati antibiotici, senza ulteriori stratificazioni. WISCA (Weighted-Incidence Syndromic Combination Antibiogram) è uno strumento sindrome-specifico che tenta di soddisfare la necessità di ottenere dati sulla sensibilità locale sindrome-specifici con il fine di guidare la prescrizione empirica di antibiotici, fornendo stime per una serie di regimi di trattamento come media ponderata delle sensibilità dei patogeni.

**Obiettivi:** Questo studio mira a sviluppare un modello WISCA per informare la selezione di regimi antibiotici empirici per la NF nei bambini, utilizzando i dati dei reparti di Oncoematologia Pediatrica del Dipartimento di Salute della Donna e del Bambino di Padova e dell'Ospedale Gaslini di Genova. L'obiettivo secondario è identificare quale combinazione di antimicrobici sia più efficace nella copertura dei principali patogeni che causano batteriemie in pazienti pediatrici oncologici o sottoposti a trapianto di cellule staminali ematopoietiche (TCSE), che si presentano con NF.

**Materiali e metodi**: In questo studio sono state incluse le emocolture dei pazienti con diagnosi microbiologica di batteriemia in concomitante neutropenia, ricoverati presso il reparto ordinario di Oncoematologia Pediatrica, o presso la sezione Trapianto di Cellule Staminali Ematopoietiche, del Dipartimento di Salute della Donna e del Bambino dell'Università di Padova e nel reparto di Oncoematologia dell'Ospedale Pediatrico Gaslini di Genova dal 1° gennaio 2016 al 31° dicembre 2021. I modelli WISCA sono stati sviluppati stimando la copertura di 20 antibiotici in monoterapia e di 21 regimi antibiotici combinati, utilizzando un modello

*Bayesiano* stratificato per centro di appartenenza, fascia d'età, patologia di base e TCSE. Inoltre, è stato creato un secondo modello considerando solo le batteriemie da gram-negativi.

**Risultati:** Sono state raccolte 350 emocolture (196 batteri gram-negativi e 154 gram-positivi) da pazienti con un'età mediana al momento dell'episodio infettivo di 8,6 anni. In entrambi i centri, la maggior parte degli episodi infettivi (28%) si è verificata nella fascia d'età 9-14 anni. Le due popolazioni di Padova e Genova si sono rivelate omogenee, con tuttavia differenze statisticamente significative riguardanti il genere (sesso maschile più frequente a Genova) e la patologia di base (leucemie più frequenti a Padova, tumori solidi a Genova). Considerando le combinazioni antibiotiche più utilizzate come PI-TZ ed amikacina, la copertura mediana è del 78% (*Bayesian Uncertainty Interval*-BUI 11-95%). Con l'aggiunta del glicopeptide, la copertura mediana è aumentata ulteriormente all'89%, mentre la sostituzione di PI-TZ con meropenem, mantenendo l'associazione con l'amikacina, non ha fornito benefici. Considerando solo i batteri gram-negativi, la monoterapia con PI-TZ ha dimostrato un'efficacia leggermente inferiore rispetto a meropenem; tuttavia, se combinati con amikacina, entrambi hanno raggiunto lo stesso livello di copertura. Questo secondo modello è stato sviluppato considerando il basso tasso di mortalità associato alle infezioni da gram-positivi (principalmente *Stafilococchi coagulasi-negativi*) e la possibilità, quindi, di mirare la terapia in un secondo momento con l'esito positivo delle emocolture. Lo strumento WISCA applicato alle emocolture ha dimostrato come la monoterapia non offra un adeguato tasso di copertura, e ha confermato la validità dei regimi terapeutici empirici utilizzati in entrambi i centri (PI-TZ, amikacina e teicoplanina per Padova e PI-TZ e amikacina per Genova). Pur essendo dati incoraggianti, la significatività statistica non è stata raggiunta a causa della ristrettezza campionaria, essendo l'infezione batterica invasiva un evento comunque non comune in ambito pediatrico.

**Conclusioni**: Il WISCA Bayesiano fornisce un approccio innovativo per orientare la scelta del trattamento antibiotico empirico nei pazienti oncologici pediatrici con NF. Inoltre, l'applicazione di WISCA in uno studio multicentrico offre la possibilità di massimizzare l'utilità clinica dei dati di sorveglianza microbiologica derivati da centri maggiori per informare la terapia empirica anche per altri ospedali minori presenti nel territorio, contribuendo al risparmio degli antibiotici ad ampio spettro e aumentando la fiducia nella selezione di regimi a spettro ristretto.

# 1. INTRODUCTION

## 1.1 Febrile neutropenia

### 1.1.1 Definition

In oncological patients, febrile neutropenia (FN) is the hallmark of bacterial and fungal infections, the most frequent and severe complications of cancer chemotherapy, leading to delays in treatment and necessary dose reductions compromising its efficacy. It is correlated with high morbidity and mortality, as well as significant additional management costs for National Health Service (SSN).[1]

There is no international uniformly agreed definition of FN. The United Kingdom (UK) National Institute for Health and Care Excellence (NICE) defines FN in adult and paediatric patients as a temperature above or equal to 38°C with an absolute neutrophil count (ANC) of less than 500 cells/microlitre (less than 0.5 x $10^9$/L). The cut-off mentioned above was chosen because the risk of bloodstream infection (BSI) and overwhelming sepsis increases as the ANC drop below 0.5 x $10^9$/L. Several guidelines propose different definitions, for example, a single fever spike above or equal to 38.3°C; a temperature above or equal to 38°C for more than one hour or two episodes of fever of more than 38°C within 12 hours. The ANC cut-off can also vary between 1.0 x $10^9$/L and 0.1 x $10^9$/L.[2,3]

**Table I – Published definition of fever neutropenia**[3]

| Source | Fever (°C) | Neutropenia (x $10^9$ cells/L) |
|---|---|---|
| *Bugs & Drugs, 2012*[4] | ≥38.3 oral temp. or ≥38.0 over 1 hour | ANC <0.5 x $10^9$/L |
| *Infectious Diseases Society of America, 2011*[5] | ≥38.3 oral temp. or ≥38.0 over 1 hour | ANC <0.5 or predicted decline to <0.5 over next 48 hours |
| *National Comprehensive Cancer Network, 2011*[6] | ≥38.3 oral temp. or ≥38.0 over 1 hour | ANC <0.5 or <1.0 with predicted decline to ≤0.5 over next 48 hours |
| *European Society of Medical Oncology, 2010*[7] | ≥38.3 oral temp. or 2 consecutive readings of >38.0 for 2 hours | ANC <0.5 or predicted decline to <0.5 |
| *British Columbia Cancer Agency, 2008*[8] | ≥38.3 | ANC <0.5 |
| *Japan Febrile Neutropenia Study Group, 2015*[9,10] | ≥38.0 single oral or ear probe temp. or ≥37.5 single axillary temp. | ANC <0.5 or <1.0 in subjects with predictably deteriorating clinical condition |

Prolonged FN is an episode of neutropenia with co-existent fever lasting more than five days, increasing the risk of invasive fungal infections.[2]

Thus, FN related to BSI is one of the major causes of morbidity, increased cost, prolonged hospitalization, and mortality in oncological paediatric patients. Identifying the predominant microorganisms and antimicrobial susceptibilities in centres helps select effective empirical antimicrobials therapy to improve clinical outcomes.[11] Nevertheless, the emergence of antibiotic resistance makes FN initial management exceptionally challenging.[2]

## 1.1.2 Epidemiology

FN is the most encountered complication of childhood cancer treatment, and the mortality of untreated cases is between 2 and 21%. Its incidence is variable according to different factors, such as the type and cycle of therapy, the type of cancer, and related conditions of patients. Most standard chemotherapy regimens

used in managing childhood cancer cause periods of myelosuppression, with neutropenia often lasting more than seven days.[2,12]

BSIs in paediatric oncological patients can exceed 50%, with an overall mortality of 6% or higher. Similarly to what happens with adults, resistance to antibacterial agents in paediatric patients is increasing but varies widely across institutions and countries.[13]

Paediatric patients with a higher risk of severe bacterial infections are those with leukaemia and, above all, those undergoing haematopoietic stem-cell transplantation (HSCT) or who develop graft-versus-host disease (GvHD) because they need a higher immunosuppressive therapy. Overall, immunosuppression is due to lymphoblastic diseases (bone marrow infiltration by blasts) and therapies (cytotoxic drugs, radiotherapy).[14] HSCT refers to the transplantation of stem cells from various sources (bone marrow, growth factor-stimulated peripheral blood and umbilical cord) for the treatment of some malignant and non-malignant haematological, autoimmune, and genetic diseases. The main indication for HSCT is the treatment of haematological malignancies and solid tumours. Despite advances in HSCT, transplant recipients remain at high-risk for severe and fatal complications, primarily due to immunosuppressive therapy, which is fundamental to preventing or treating graft-versus-host disease (GvHD).[15] GvHD is a systemic disorder that occurs when the graft's immune cells recognize the host as foreign and attack the recipient’s body cells: it is a common complication after allogenic HSCT. GvHD disease can occur within the first few months after transplant (acute) or much later (chronic).[16]

Other determinant factors are the underlying disease (for example, the incidence of bacterial infections is higher in patients with acute myeloid leukaemia than in patients with acute lymphoblastic leukaemia) or the phase of treatment (for example, the incidence of bacterial infections is higher in the induction therapy phase than in the consolidation phase).[14] In particular, FN usually occurs within seven to twelve days following cancer chemotherapy.[1]

### 1.1.3 Aetiology

Any community-acquired pathogen can cause FN, but opportunistic infections must also be considered.[2] Bacteria, including gram-positive (currently dominating) and gram-negative (dominant in the 1970s), are the most common causative agents for FN and can cause life-threatening infections leading to significant morbidities and possible mortality in the immune-deficient neutropenic individuals.[12,17] Viral infections are also common in children; fungal infections should be remembered in prolonged FN. It is also important to remember the possibility of other non-infectious causes of fever in patients who fail to improve on antimicrobial therapy.[2]

Thus, gram-positive cocci are the most common pathogen found in FN, especially skin commensals secondary to increased use of central venous lines. *Coagulase-negative Staphylococci* (especially *Staphylococcus epidermidis*), *Staphylococcus aureus* and *Streptococcal species* account for 50-67% of causative organisms. Gram-negative organisms, such as *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae,* are less common but may lead to a more severe clinical course due to endotoxin and other virulence factors. Instead, among the gram-positive organisms, pathogens known to cause the most severe infections are *Staphylococcus aureus, Enterococcus species* (especially *Vancomycin-resistant Enterococcus/VRE*) and *Streptococcus viridans*.[2]

Multi-Drug Resistance (MDR) is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Therefore, MDROs are defined as microorganisms, predominantly bacteria, that are resistant to one or more classes of antimicrobial agents.[18] Multidrug-resistant gram-negative bacteria have emerged as a significant threat in the management of neutropenic patients worldwide. Some pathogens, such as Enterobacteriaceae (mainly *Escherichia coli* and *Klebsiella species*), *Pseudomonas aeruginosa*, and less frequently *Acinetobacter spp*. and *Stenotrophomonas maltophilia,* have gained multiple mechanisms of resistance to escape antimicrobial pressure, and have become the causative agents of an increasing number of infections.[19,20] The same applies to resistant gram-positive cocci, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant enterococci (VRE). Their prevalence has also increased significantly, becoming the most isolated resistant pathogens in several centres.[19] Even polymicrobial infections are increasingly reported as increased.[2]

Other pathogens responsible for FN are fungi, of which the most documented infections are *Candida spp*. (*C. Albicans* and *C. Parapsilosis*) and *Aspergillus spp*. The occurrence of immunosuppression and the placement of a central catheter facilitate these yeasts to become invasive pathogens. The three major contributors to the development of invasive fungal disease (IFD) are a breakdown in natural barriers (such as indwelling catheter and mucositis) and defects in cell-mediated immunity (lymphopenia from corticosteroids), and another anti-T-cell cytotoxic. Dissemination to secondary sites is reported in 10-20% of paediatric patients with candidemia, and severe sepsis or septic shock occurs in about 30%. Rare yeasts and cryptococcosis are sporadic causes of IFDs. The prognosis of IFD depends on organ involvement and is more severe in disseminated and central nervous system disease, with mortality reported between 20% and 70%. Aspergillosis has an 80% case-fatality rate in haematopoietic stem-cell transplantation.[21]

### 1.1.4 Risk factors

Paediatric patients, in comparison to adult ones, are more likely to have an undetected infectious focus, with mortality rates of up to 80% in gram-negative infections.[22]

The pathogenesis of FN is multifactorial. Contributing factors are an uncontrolled disease, comorbidities and organ involvement, low body surface area/body mass index, treatment with myelosuppressive chemotherapies, type of chemotherapy, specific genetic polymorphisms, HSCT and GvHD.[17] In particular, children and young people who have undergone HSCT are at risk for especially fungal infection given the prolonged neutropenia.[2]

The main contributory factors include:

- pancytopenia,
- marrow replacement,
- humoral and cellular immunity qualitative defects,
- mucositis,
- central venous catheter (CVC) infection.[2]

Pancytopenia can be caused by the administration of cytotoxic drugs or the direct malignant invasion of bone marrow with acquired bone marrow failure. While

anaemia and thrombocytopenia can be corrected with transfusion, neutropenia involves significant danger to the patient, increasing the risk of severe infections and sepsis. In addition to direct marrow invasion, the underlying malignancy can also cause chemotactic and phagocytic defects in neutrophils, impairing their ability to reach the site of infection and contain it. This is especially true for haematological malignancies.[2]

Moreover, chemotherapy-induced mucositis causes the breakdown of usual mucosal barriers in the gastrointestinal system. This allows translocation of commensal gastrointestinal tract bacteria and fungi into the bloodstream, which is thought to be a major causative factor in FN caused by gram-negative organisms.[2]

CVCs become colonized with skin commensal bacteria leading to invasive infections. Poor CVC hygiene can also lead to infection with gram-negative bacteria, and polymicrobial infections are not uncommon.[2]

The administration of broad-spectrum antibacterial agents, prolonged or repeated hospitalization, intensive care unit stay, the severity of illness, healthcare-associated infection, and the presence of a urinary catheter are considered to be significant risk factors for resistant bacterial infections.[19]

### 1.1.5 Risk stratification

Patients presenting with FN should undergo an initial risk assessment of severe infection complications to determine the appropriate treatment, and a validated risk stratification strategy should be incorporated into routine clinical management.[1,23]

The Infectious Diseases Society of America (IDSA), National Comprehensive Cancer Network (NCCN) and European Society of Medical Oncology (ESMO) have outlined the classification of risk for adults with FN. They encourage using the Multinational Association of Supportive Care in Cancer (MASCC) index, a formal method for defining risk stratification. The MASCC index assigns values to patient age, history, outpatient or inpatient status, clinical signs, severity and duration of fever and neutropenia (ANC measure), and presence of medical comorbidities (e.g., renal and hepatic impairment). The summation of those values determines risk classification.[1,3] Thus, patients who develop neutropenia can be stratified into low or high-risk of complications; depending on the level of risk

determined, management of patients may vary in the administration of treatment (oral or intravenous), duration of therapy and treatment setting (outpatient or hospital).[1,2] Patients with FN are stratified into a low-risk category (MASCC Risk Index score of at least 21) if the patient has good performance status and few medical conditions, presents with adequate hepatic function and renal function, and the neutropenia's duration is expected to be less than seven days. These patients are initially treated with oral or intravenous empiric therapy. Instead, patients with FN are classified as having a high-risk of complications if they have severe neutropenia marked by an ANC of less than 100 cells per microliter following chemotherapy and if the duration of neutropenia is anticipated to last longer than seven days. In addition, high-risk patients may have clinically relevant comorbidities such as hypotension, pneumonia, new onset of abdominal pain, renal or hepatic insufficiency, or neurological changes; they are also stratified into the high-risk category if they present with a MASCC Risk Index score of less than 21. These patients are treated with empiric antibiotics administered intravenously in the inpatient setting.[1]

For paediatric patients, it is difficult to recommend any single prediction rule, especially in the presence of geographical and temporal variations between different paediatric populations. In a recent international meta-analysis involving ten countries, evidence-based guidelines for the empirical treatment of febrile neutropenia in children were developed. These guidelines referenced six validated risk stratification protocols for Paediatrics, namely: Rackoff 1996, Santolaya 2001, Alexander 2002, Ammann 2003, Rondinelli 2006 and Ammann 2010. The evaluation of these studies does not allow a single low-risk prediction scheme to be recommended as more efficient than the others, nor does it allow safely recommending different protocols to predict specific outcomes. Geographic and temporal variations require a local validation to be used as a routing protocol.[22,23]

### 1.1.6 Symptoms

FN is characterized, in addition to fever, by the other clinical signs of infection, such as chills and hypotension.[11] Nevertheless, because of the reduced immune system in oncological paediatric patients and neutropenia, typical signs and symptoms of infection may be absent (e.g., lung consolidation or localizing other

signs of infection), and fever usually is the only alarm symptom. Furthermore, due to the low effectiveness of their defense mechanisms, they can present a serious infection up to the development of septic shock, which significantly impacts treatment choice.[1] The International Pediatric Sepsis Consensus Conference defines septic shock in children as severe infection leading to cardiovascular dysfunction (including hypotension, need for treatment with a vasoactive medications, or impaired perfusion) and "sepsis-associated organ dysfunction" in children as severe infection leading to cardiovascular and/or non-cardiovascular organ dysfunction.[24] In paediatric patients with FN, the presence of septic shock is associated with increased mortality.[25]

### 1.1.7 Diagnosis

Body temperature and blood count are routinely screened in cancer patients undergoing treatment such as chemo/radiotherapy.[12] FN is diagnosed with a blood test that confirms an absolute neutrophil count (ANC) of less than 500/mm$^3$ associated with the clinical presentation of fever.[1]

For microbiological diagnosis of BSI, simultaneously, at least two sets of blood cultures must be obtained, usually taken from a peripheral vein and catheter line (or lines) during the febrile period. In the absence of a central venous catheter (CVC), two blood culture sets should be obtained from separate venepunctures. A differential time to positivity of 120 minutes between cultures drawn simultaneously through a CVC and peripheral vein site suggests central line-associated bloodstream infection (CLABSI).[19] Diagnosis of catheter-related bloodstream infection (CRBSI) is based on the IDSA clinical and practice guidelines for diagnosing and managing intravascular catheter-related infections-2009 update.[11,26] In the case of any other suspicious foci of infection, appropriate clinical specimens must be examined for microbiological diagnosis.[11,19]

Additional blood tests to assess the severity of the infection and possible organ involvement should include:

- renal and liver function tests;
- markers of inflammation such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and/or procalcitonin (PCT);

- a blood gas for venous lactate can be considered if the patient is unwell to assess for sepsis;[2]
- detection of fungal antigens in body fluids, including cryptococcus capsular polysaccharide, histoplasma antigen, galactomannan, and β-D-glucan, is considered clinically useful for at least the presumptive diagnosis of invasive fungal infections. β-D-Glucan is found in a broad range of fungal agents, including the commonly encountered agents *Candida* spp. and *Aspergillus* spp. Fungal antigens are constantly monitored over time for early detection of possible fungal infections;[27]
- urinalysis and urine culture should be performed in patients in whom a clean catch, midstream specimen is promptly available.[23]

Besides blood cultures, microbiological investigations should be expanded depending on presenting symptoms. In the case of diarrhoea, bacterial and viral research on faeces is warranted, along with the search for *Clostridium difficile* toxin, which is another possible bacterial infection, especially after prolonged antibiotic courses. Finally, if a localized infection is diagnosed, cultures of the infection site should be performed whenever possible, according to the patient's conditions. Routinary procedures in adults (e.g., bronchoscopy) may be uneasy to perform in paediatrics.

Viral infections are a possible cause of fever, especially in children with HSCT. According to local practice, viral research with nucleic acid amplification tests on blood, including EBV, CMV, Adenovirus, Herpes viruses and others, should be performed in the case of persistent fever despite antibiotic therapy and negative blood cultures.

Imaging for asymptomatic and stable patients with FN is usually not indicated at the beginning of fever. On the other hand, if respiratory signs or symptoms are present, the evaluation of chest radiography (CXR) is recommended.[23] In the same way, other imaging examinations should be tailored to presented symptoms, such as abdomen ultrasound (US) scan to investigate abdominal pain or computerized brain tomography (CT) scan/magnetic resonance imaging (MRI) if neurological signs develop. Further, in the case of persistent fever in otherwise asymptomatic patients, imaging should be considered to look for infective localizations (e.g., chest and abdomen CT or US, sinus scan).[2,19]

## 1.2 Antibiotic prophylaxis

One approach to reducing bacterial infections and their negative consequences is to use antibiotics as prophylaxis in some specific situations. The use of these agents is potentially associated with drug toxicity and the emergence of antibiotic resistance. Therefore, antibiotic efficacy, measured by the reduction of overall and infection-related mortality, bacterial bloodstream infections and FN, should be weighed against the potential negative consequences of antibiotic use.[14]

For this reason, the 8th European Conference on Infections in Leukaemia (ECIL-8) and the IDSA guidelines do not recommend routine antibacterial prophylaxis for paediatric patients with lymphoma, acute leukaemia, relapsed acute leukaemia, or patients with neutropenia during the pre-engraftment stage of HSCT (grade D recommendation, level of evidence III). It is due to the fact that antibacterial prophylaxis does not decrease the mortality rate or the incidence of BSIs. This recommendation is based on data from randomised trials and meta-analyses, information from long-term observational studies on resistance, and European Medicines Agency recommendations.[1,14]

Some randomized trials suggest fluoroquinolone prophylaxis as initial treatment in patients considered high-risk for complications of FN and expected to have an extended period of profound neutropenia lasting longer than seven days, defined by no more than 100 cells per microliter.[1]

Granulocyte colony-stimulating factors (G-CSF) are used as a supportive treatment to reduce neutropenia duration after chemotherapies and the probability of infection in patients with non-myeloid malignancies. Risk assessment also plays a key role in determining whether G-CSF should be initiated for primary prophylaxis.[1]

## 1.3 Antibiotic resistance

Significantly, antibiotic resistance harmfully affects the survival of patients with haematological malignancies and after HSCT.[13] In parallel to what has been observed in the adult population, a progressive increase in the incidence of infections due to multidrug-resistant organisms (MDROs) has occurred in children.[28] The emergence of MDRs and their rapid worldwide spread are closely associated with inappropriate use of antimicrobials, which are the main prescribed and administered drugs in the outpatient and hospital settings, especially among paediatric patients.[18,29]

Data from 39 European haematology centres showed infection incidence rates of 15–24% for extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E), 5–14% for aminoglycoside-resistant gram-negative bacteria and 5–14% for carbapenem-resistant *Pseudomonas aeruginosa* in adult and paediatric patients.[13,30]

The last annual report of 2020 by the European Centre for Disease Prevention and Control (ECDC) presented an increase, up to 50%, for third-generation cephalosporin resistance in *E. coli*. *Carbapenem resistance* in *Enterobacteriaceae* (CRE) and MDR *Pseudomonas spp.* were reported at 25%-50%. The percentages of carbapenem-resistant *Acinetobacter spp*. varied widely from below 1% to equal to or above 50% in 21 countries of Europe.[30]

As a result, the high incidence of bacterial infections in patients with neutropenia and the emergence of antibiotic resistance has led to increased use of broad-spectrum antibiotics, including carbapenems, either as monotherapy or combination therapy. Overall, antibiotics consumption increased worldwide by 65% between 2000 and 2015.[30] Since the increasing exposure to certain classes of drugs leads to a significant increase in the development of antibiotic resistance, the choice of the empiric antibiotic treatment in a patient with FN should be tailored to local ecology and epidemiology.[2,31]

## 1.4 Empiric therapy

Empirical antibacterial therapy is a long-standing standard of care for children and adults with neutropenia at the onset of fever or any other sign or symptom of a possible infection. FN in paediatric onco-haematological patients represents a medical urgency because it must be considered until proven otherwise a sign of infection, and it must therefore be promptly treated with broad-spectrum empiric antibiotic therapy. That is why it is essential not to leave the patient without antibiotic coverage during the waiting period for blood culture results.[14]

Due to empiric therapy, mortality rates due to FN have decreased. Nevertheless, possible additional drug interactions and toxicity could be a limiting factor.[19] Moreover, studies in adults have shown that, compared with patients without multidrug-resistant gram-negative bacteria, patients with cancer and infected with multidrug-resistant gram-negative bacteria more frequently received inadequate empirical antibacterial therapy, which was associated with a poorer outcome. Thus, to optimise the use of antibiotics, evidence-based guidelines (which need a regular update) have been developed for patients with malignancy who are immunocompromised or have undergone HSCT. Unfortunately, these guidelines are not specific for children and adolescents, who can differ from adult patients in several aspects.[14] Accordingly, homogeneous recommendations for empirical treatment are lacking in paediatric, and it is because guidelines should take into account the local epidemiology, individual risk factors (e.g., underlying disease, comorbidities, previous infections, eventual bacterial colonisation, medication usage) and the clinical presentation (e.g., clinical instability, hypotension, duration of neutropenia, absolute neutrophil count measure, renal and hepatic failure). Other critical issues to consider are the continuous increase in MDROs and, on the other hand, the selective pressure that a broader spectrum of empiric coverage may further exert on resistant bacteria.

Depending on the level of risk determined (low or high-risk), management of patients may vary in the administration of treatment (oral or intravenous), duration of therapy, and treatment setting (outpatient or hospital).[1]

Proposed antimicrobial empiric regimens were outlined by Lehrnbecher et al. in 2017 (Guidelines for the Management of Fever and Neutropenia in Children with

Cancer and Haematopoietic Stem-Cell Transplantation Recipients: 2017 Update) and modified in the ECIL-8 guidelines:[14,23]

- In clinically stable patients at low risk of resistant infections are recommended: monotherapy with an antipseudomonal non-carbapenem β-lactam plus β-lactamase inhibitor (piperacillin-tazobactam) combination or fourth-generation cephalosporin. This group includes patients without colonisation or previous infections with resistant bacteria or patients treated in institutions with a low rate of resistant pathogens. Because of the risk of adverse events (e.g., pseudomembranous colitis) and resistance development associated with carbapenem use, the panel does not recommend carbapenems as empirical therapy for clinically stable patients.[14]
- In clinically unstable patients, with signs of sepsis or septic shock, independently of risk of resistant infections, are recommended: Carbapenem, with or without a second anti-gram-negative agent, with or without a glycopeptide.[14]
- Empirical treatment should be adjusted based on the results of resistance testing for patients who are colonised or were previously infected with resistant gram-negative bacteria or in centres with a high rate of resistant pathogens.[14]
- Adding glycopeptides such as vancomycin or teicoplanin is considered in high-risk patients if they have a central venous catheter or for individuals from areas with endemic antibiotic-resistant gram-positive (MRSA). Teicoplanin has some advantages over vancomycin (for example, it induces fewer allergic reactions and nephrotoxic events), so it is usually preferred.[14,32]

Once the pathogen has been identified, a specific treatment will start, using narrower-spectrum antibiotics guided by in-vitro tests. Unfortunately, in only 44% of the case, it is possible to have a proven etiological diagnosis.[32]

## 1.5 Antibiogram and Weighted-Incidence Syndromic Combination Antibiogram (WISCA)

The main aim of antimicrobial stewardship is to optimise clinical outcomes by minimising unwanted consequences of antibiotic use, such as toxicity and MDR organisms' selection. Recent studies have shown that a wrong and delayed treatment choice can be harmful to the patient, while the inappropriate use of broad-spectrum antibiotics represents a potential means of resistance and increases health costs.[33]

A traditional antibiogram is widely used in clinical practice to guide antibiotic therapy. It reflects the local resistance pattern, indicating which therapeutic regimen will most likely cover each isolated pathogen.[34] However, this type of antibiogram has several limitations. First, it is not syndrome-specific but germ-specific, therefore focused on the organism-drug combination; moreover, in addition to information on the percentage of resistance, it does not provide the representation of the distribution and the frequency of a certain pathogen in the context of a specific infection. Presenting only a slight possibility of stratification, it has a limited value in the context of polymicrobial infections and treatments conducted with a combined antibiotics regimen.[33,35]

Another useful tool is the combination antibiogram, which indicates the probability that at least one drug will act on a given pathogen. This type of antibiogram is proper when the causative germ is identified, but its susceptibility is not well known. As with traditional antibiogram, even with the combination antibiogram, there is no specificity for the syndrome investigated; furthermore, it is not informative on the actual coverage of all the organisms that could be present in the infection.[36] In different contexts, data on antimicrobial resistances are reported as cumulative antibiograms that aggregate hospital data. With this method, differences between patient populations (e.g., paediatrics, adults, residents of long-term care facilities), operating units or anatomical sites involved (e.g., blood, urine, respiratory system) are masked, resulting in a greater risk of overestimating or underestimating data on the resistance of isolated germs.[37]

Thus, healthcare providers need a better empiric antibiotic prescribing aid than the hospital's traditional antibiogram, which supplies no information on the relative frequency of organisms recovered in a given infection and is uninformative in situations where multiple antimicrobials are used, or multiple organisms are anticipated. For this purpose, Hebert et al. developed Weighted-Incidence Syndromic Combination Antibiogram (WISCA), an innovative empiric prescribing decision aid: it combines simple diagnosis and microbiology data from the electronic health record (EHR) to classify patients by syndrome and determine, for each patient with a given syndrome, whether a given regimen (one or more agents) would have covered all the organisms recovered for their infection.

WISCA is a syndrome-specific tool that attempts to satisfy the unmet need to obtain syndrome-specific local susceptibility data to guide empirical antibiotic prescribing, providing estimates for several treatment regimens as a weighted average of pathogens' susceptibilities. This allows data to be presented such that clinicians can see the likelihood that each antibiotic regimen will treat all relevant organisms for different infectious syndrome based on the frequency of the causative pathogen sensitivity. Less frequent pathogens have less weight on the overall coverage estimate for the same infection syndrome. In this way, together with the ability to manage polymicrobial infections and antibiotics combinations and information on the relative frequency of specific bacteria in an infectious syndrome, WISCA guarantees the possibility of analysing different clinical and epidemiological aspects.[35]

While in the traditional antibiogram, the unit of analysis is the pathogen, and the information sought is its antibiotic susceptibility, with the WISCA antibiogram, the patient becomes the subject of study, and the probability of coverage using a certain antibiotics regimen represents the outcome of the greatest interest. Furthermore, by leading the clinician towards the choice of adequate empirical therapy, WISCA could contribute to reducing antibiotic resistance.

This method of displaying microbiology data is mainly used for bacterial bloodstream infections and urinary tract infections (UTI).[35]

A study by Randhawa et al. found that WISCA had the potential to more than double the likelihood of adequate empiric antibiotic coverage among patients

admitted to the intensive care unit with ventilator-associated pneumonia and catheter-associated bloodstream infection.[38]

Another study from the University of Padua created a WISCA model to define the empiric antibiotic treatment for UTIs in paediatric patients, demonstrating that the developed WISCAs provide highly informative estimates on coverage patterns overcoming the limitation of combination antibiograms and expanding the framework of previous Bayesian WISCA algorithm. Moreover, it represents a valid tool for monitoring antibiotics resistance data and may help re-evaluate the first-line treatment for local guidelines or clinical pathways.[39]

However, as previously noted in other studies, there are still analytical challenges in developing WISCAs for the paediatric population, especially the scarcity of data in the different layers of models that can be overcome using Bayesian methods.[39]

# 2. AIM OF THE STUDY

This study aims to develop a WISCA model to inform empirical antibiotic regimens selection for febrile neutropenia (FN) in paediatric oncological patients using data from the Paediatric Onco-haematological wards of the Department for Women's and Children's Health in Padua and the Gaslini Hospital in Genoa.

The second aim is to identify which combination of antimicrobials is more effective in the coverage of the main bacteria causing bloodstream infections (BSIs) in paediatric patients with cancer or undergoing haematopoietic stem-cell transplantation (HSCT) presenting with FN.

# 3. MATERIALS AND METHODS

## 3.1 Study design

This is a multicentric, observational, retrospective study conducted in the Paediatric Onco-haematological ward of the Department for Women's and Children's Health at the University of Padua and the Onco-haematological ward of the Paediatric Hospital Gaslini in Genoa (GE).

### 3.1.1 Setting

The Paediatric Onco-haematological ward of the Department for Women's and Children's Health at the University of Padua is a complex operative unit of the Paediatric Hospital of Padua, with a 19 beds ordinary ward and six beds in the bone marrow transplant unit. It accounts for about an average of 780 admissions per year.

The Onco-haematological ward of the Paediatric Hospital Gaslini in Genoa has 18 beds ordinary ward, five beds in the bone marrow transplant unit and ten beds in the Day Hospital unit. It accounts for about an average of 640 admissions per year.

### 3.1.2 Population

The study cohort included patients admitted to the Paediatric Onco-haematological ward, or Bone Marrow Transplant (BMT) Unit, with a microbiological diagnosis of bloodstream infection (BSI) and neutropenia. The study period ranged from January 1$^{st}$, 2016, to December 31$^{st}$, 2021.

### 3.1.3 Outcome

The primary outcome was the definition of the most appropriate antibiotics or combinations to empirically treat neutropenic patients presenting with fever according to the local ecology of BSI, applying a stratified WISCA model.

### 3.1.4 Inclusion criteria

For the retrospective collection of data about BSI episodes in neutropenic patients, both centres applied the following criteria:

- A BSI episode was defined by the microbiological isolation of a pathogen in blood cultures.
- Only the first culture was considered if a pathogen was isolated in repeated cultures within the same infectious episode.
- In the case of isolating coagulase-negative staphylococci (CoNS) or other pathogens considered possible contaminants, the episode was included only if at least two separated blood cultures resulted positive for the same microorganism. If these organisms grew together with other bacteria considered pathogens in blood culture, it was considered a poly-microbial infection only if the suspected contaminant was isolated more than once.
- If the same patient presented with two or more different episodes of BSI, it was taken into account more than once.
- The isolation of the same pathogen from blood cultures for a patient was considered within the same episode if there were less than 20 days between cultures. If cultures remained negative for more than 20 days and the same pathogen was isolated, it was considered a second episode.
- Blood cultures with identified pathogens for which an antibiogram was not available were excluded.

Neutropenia was considered an absolute neutrophil count (ANC) inferior to 500/mm$^3$. However, patients presenting with an uncontrolled or relapsed disease with blood prevalence of blasts were considered functionally neutropenic and then included.

A contaminant is defined as a microorganism that is supposed to be introduced into the culture during either specimen collection or processing, and that is not pathogenic for the patient. The most frequently isolated microorganisms are CoNS in 75% to 88% of contaminated blood cultures, followed by *Bacillus spp*., Viridans group streptococci, *Corynebacterium spp., Propionibacterium spp*., Micrococcus *spp.* and *Clostridium perfringens*. Differentiating a contaminant from a true pathogen is challenging because some of these microorganisms are an increasing

source of true bacteraemia, especially in patients with prosthetic devices or catheters.[40]

## 3.2 Data collection

We retrospectively reviewed the clinical documentation of patients identified by the positive blood cultures from the wards of interest in the study period, provided by the microbiology laboratory or captured by the hospital electronic medical records (EMRs). Data were stored in the secured server at the University of Padova. Each patient was assigned a progressive numeric code to ensure anonymity.

The following data were obtained from the identified BSI episodes:

- Date of birth
- Sex
- Age at the time of the episode
- Date of positive blood culture
- Admitting hospital (Padua or Genoa)
- Diagnosed haematological and/or oncological disease
- Previous haematopoietic stem-cell transplantation (HSCT)
- Previous graft-versus-host disease (GvHD)
- Blood culture: type of isolated pathogen (gram classification and pathogen species) and susceptibility test results.

For both centres, bacteria isolates were identified by standard criteria, and antibiotic sensitivity was studied with the VITEK 2 system by Biomerieux (Marcy l'Etoile, France) using appropriate panels or a disc diffusion method following European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and breakpoints according to the centres' standard procedures.

For the purpose of our analysis, antibiotics in the antibiogram were classified as Susceptible ("S") or Resistant ("R") to investigate the coverage of an empiric antimicrobial association. All the Intermediate ("I") results were classified as susceptible, according to the EUCAST rules and new definitions of 2019. From 2019, a microorganism is categorised as "Susceptible, standard dosing regimen" (S) when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent. A microorganism is categorised as "Susceptible, Increased exposure" (I) when there is a high likelihood of therapeutic success because exposure to the agent can be increased at the site of infection by adjusting the dosing

regimen, mode administration or because the concentration is naturally high at the site of infection. A microorganism is categorised as "Resistant" (R) when there is a high likelihood of therapeutic failure, even when there is increased exposure. Exposure is a function of how the mode of administration, dose, dosing interval, infusion time, as well as distribution and excretion of the antimicrobial agent will influence the infecting organism at the site of infection. So, the only difference between “S” and “I” is the amount of drug at the site of the infection necessary to achieve an adequate clinical response, but in both cases, the isolate is susceptible.

Tested antimicrobials were different between the years and between the two centres. When, in an antibiogram, a specific antibiotic was not tested, it was classified as not available and consequently did not weigh on the analysis. However, to include as many antibiotics combination strategies as possible, we extended the susceptibility results to other untested drugs, according to the following considerations:

1. Intrinsic resistance:
    a. all gram-positive bacteria were considered intrinsically resistant to amikacin;
    b. all gram-negative bacteria were considered resistant to glycopeptides (teicoplanin and vancomycin), daptomycin and linezolid;
    c. all enterococci were considered resistant to cephalosporins;
    d. Pseudomonas aeruginosa was considered resistant to ceftriaxone and cefotaxime;
    e. Staphylococcus spp. were considered resistant to ceftazidime;
    f. Stenotrophomonas maltophilia was considered resistant to all antibiotics except for cotrimoxazole.
2. Inferable susceptibilities and resistances:
    **a.** Staphylococci spp.: when susceptibility tests available were limited to oxacillin or cefoxitin, the result was transferred to other not tested antibiotics, namely: cefepime, piperacillin-tazobactam, meropenem. Hence, all methicillin-susceptible strains were considered susceptible to the drugs mentioned above.

## 3.3 WISCA model

WISCA model was developed as a decision tree (Figure 1) based on data concerning blood cultures of evaluated paediatric patients. As a result of the suspicion of febrile neutropenia due to bloodstream infection (BSI), the first node (circle) represents the clinical decision to initiate empiric treatment. The second node represents all the possible bacteria causing the infection, and the third node represents the possible susceptibility profile of bacteria. For each antibiotic regimen, the probability of expected coverage was then calculated considering the sensitivity and resistance characteristics of the main pathogens identified as causing paediatric BSI in neutropenic patients. WISCA model thus structured allows us to obtain the weighted probability of sensitivity to multiple empirical treatments regardless of the pathogen.

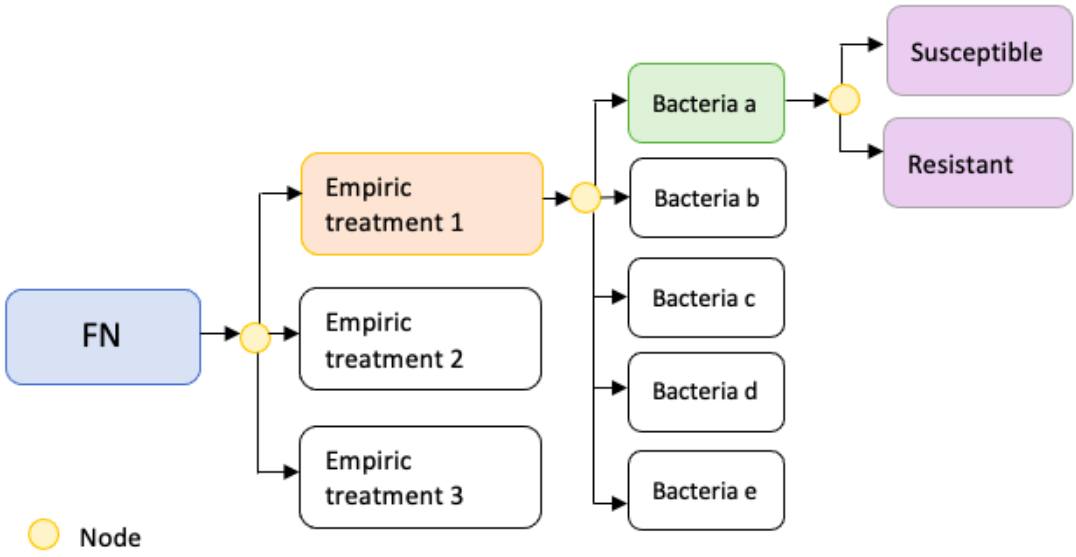



**Figure 1 – Decisional tree of WISCA model (adapted from Bielicki et al.)**[41]

We studied the antibiotic agents available in the centres and for which automated sensitivity testing is routinely performed. In particular, WISCAs were developed by estimating the coverage of 20 antibiotics as monotherapy and of 21 combined antibiotics regimens based on the centre/national guidelines.

The 20 mono-treatments are:

- amikacin
- ampicillin
- cefepime
- cefotaxime-tazobactam
- ceftazidime
- ceftazidime-avibactam
- ceftolozano-tazobactam
- ciprofloxacin
- clindamycin
- erythromycin
- gentamycin
- levofloxacin
- linezolid
- meropenem
- penicillin
- piperacillin-tazobactam
- teicoplanin
- trimethoprim-sulfamethoxazole
- tigecycline
- vancomycin.

The 21 combine regimens are:

- ceftazidime-amikacin
- ceftriaxone-amikacin
- ciprofloxacin-amikacin
- piperacillin_tazobactam-amikacin
- ceftriaxone-teicoplanin
- meropenem-amikacin
- cefotaxime/ceftriaxone-vancomycin
- ceftriaxone-amikacin-teicoplanin
- piperacillin_tazobactam-teicoplanin
- ceftazidime-amikacin-teicoplanin
- piperacillin_tazobactam-amikacin-teicoplanin
- meropenem-teicoplanin

- ceftriaxone-amikacin-vancomycin
- meropenem-amikacin-teicoplanin
- piperacillin_tazobactam-vancomycin
- ceftazidime-amikacin-vancomycin
- piperacillin_tazobactam-amikacin-vancomycin
- meropenem-vancomycin
- meropenem-amikacin-vancomycin
- ciprofloxacin-amikacin-teicoplanin
- ciprofloxacin-amikacin-vancomycin.

To determine the odds of coverage by an antibiotic treatment, it is necessary to refer to a Bayesian logistic regression. In this context, pathogens and antibiotics were included in the model as random effects and age, sex, underlying pathology and haematopoietic stem-cell transplantation (HSCT) occurrence were included in the model as fixed effects

Posterior distributions were summarized using the median and the 95% Highest Density Intervals (HDIs) and the probability that the estimated coverage is at least 85%.

Ultimately, stratified models according to the centre (Padua or Genoa), age group, diagnosed haematological and/or oncological disease, and the presence of HSCT were developed.

Moreover, a second model considering only gram-negative bacteria was created.

## 3.4 Statistical analysis

Categorical variables have been described as frequencies and percentages, while continuous variables have been expressed as median and interquartile range (IQr).

The incidence of each pathogen and its sensitivity for a given antibiotic treatment have been evaluated with an approach based on the WISCA tool.

To test whether there is a difference between the populations of the two centres, we applied a X-squared test or a Fisher exact test depending on the frequencies of the values.

# 4. RESULTS

## 4.1 Characteristics of the population of Padua and Genoa

In the time period from January 1st 2016 to December 31st 2021, 350 positive blood cultures were collected in patients of paediatric age with a febrile episode and a concomitant condition of neutropenia; of these, 92 (26%) blood cultures came from the Onco-haematological ward, or Bone Marrow Transplant Unit, of the Department for Women's and Children's Health at the University of Padua and the other 258 (74%) from the Onco-haematological ward of the Paediatric Hospital Gaslini in Genoa.

For Gaslini Hospital, cultures were provided by a ward registry, while in Padua Hospital, they were retrieved from a ward registry and implemented with the electronic health system tool.

Overall, we excluded a sum of 43 blood cultures from both centres because considered contamination (17/43), collected in other satellite hospitals (14/43), because the pathogen was not identifiable or the antibiogram was not available (12/43). In particular, 32 blood cultures were excluded from Padua's data pool, of which 17 were contaminations, 14 came from satellite hospitals, and in only one case, the antibiogram was not performed. Only 11 blood cultures were excluded from Genoa's data pool because the pathogen was not identifiable or there was no antibiogram.

The difference between centres is due to the fact that the Genoa ward registry provided data in which blood cultures were already screened for possible contamination, while in Padua, contaminations were removed in a second phase.

Excluded and included blood cultures for analysis are reported in Figure 2.

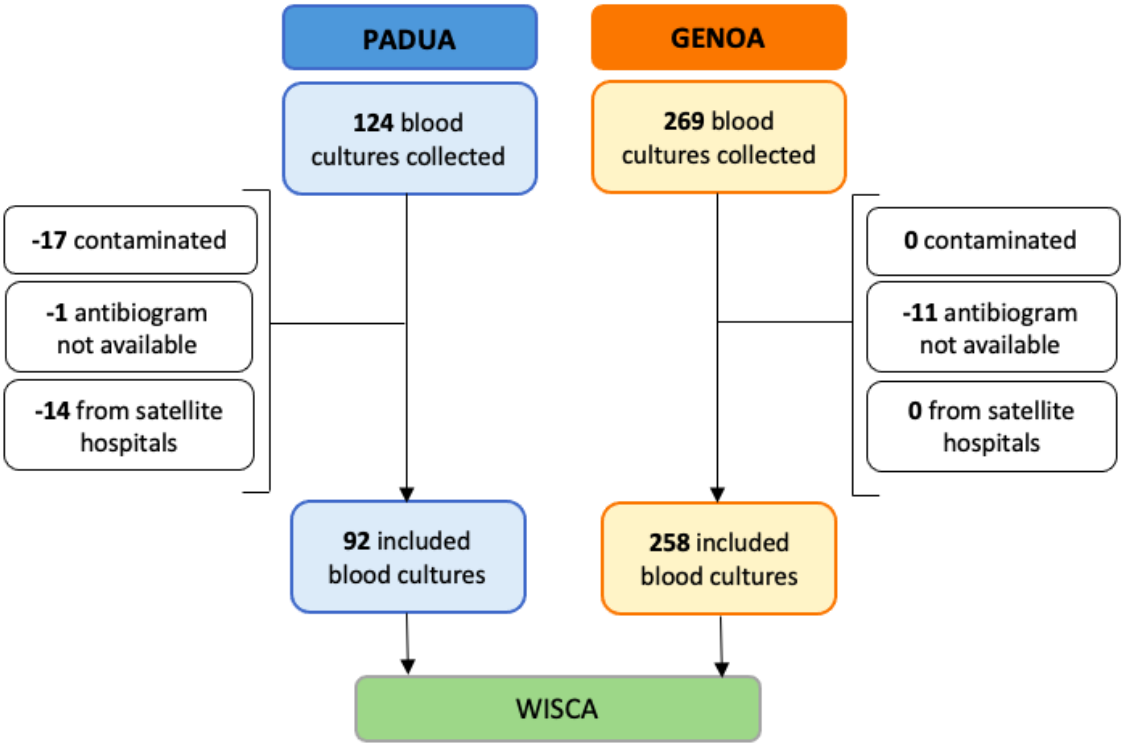



**Figure 2 – Flowchart of inclusion criteria for WISCA model**

### 4.1.1 Demographic characteristics

The demographic and baseline features of patients who tested positive for included blood cultures are shown in Table II.

**Table II – Demographic characteristics of included patients with p-values referred to the overall cohort stratified by centres (Genoa and Padua)**

| Characteristic | N = 350 | GENOA, N = 258 (74%) | PADUA, N = 92 (26%) | p-value |
|---|---|---|---|---|
| ***Age (median (IQr))*** | 8.6 (3.3-14) | 7.9 (2.7-13.1) | 10.3 (5.9-17.7) | |
| ***Age group*** | | | | 0.14 |
| < 3 years | 75 (21%) | 63 (24%) | 12 (13%) | |
| 3 – 5 years | 53 (15%) | 42 (16%) | 11 (12%) | |
| 6 – 8 years | 53 (15%) | 38 (15%) | 15 (16%) | |
| 9 – 14 years | 99 (28%) | 68 (26%) | 31 (34%) | |
| 15 – 19 years | 58 (17%) | 39 (15%) | 19 (21%) | |
| ≥ 20 years | 12 (3.4%) | 8 (3.1%) | 4 (4.3%) | |
| ***Sex*** | | | | **0.003** |
| Female | 130 (37%) | 84 (33%) | 46 (50%) | |
| Male | 220 (63%) | 174 (67%) | 46 (50%) | |
| ***Gram*** | | | | 0.067 |
| Gram - | 196 (56%) | 137 (53%) | 59 (64%) | |
| Gram + | 154 (44%) | 121 (47%) | 33 (36%) | |
| ***TCSE*** | | | | 0.1 |
| No | 226 (65%) | 173 (67%) | 53 (58%) | |
| Yes | 124 (35%) | 85 (33%) | 39 (42%) | |
| ***GvHD*** | | | | >0.9 |
| No | 79 (64%) | 54 (64%) | 25 (64%) | |
| Yes | 45 (36%) | 31 (36%) | 14 (36%) | |
| ***Underlying pathology*** | | | | **<0.001** |
| Other | 26 (7.4%) | 21 (8.1%) | 5 (5.4%) | |
| Aplastic anaemia | 43 (12%) | 32 (12%) | 11 (12%) | |
| Leukaemia | 193 (55%) | 126 (49%) | 67 (73%) | |
| Lymphoma | 17 (4.9%) | 14 (5.4%) | 3 (3.3%) | |
| Solid tumour | 71 (20%) | 65 (25%) | 6 (6.5%) | |

As regards ages, the population was divided into six age groups, with a similar distribution between the two centres: infants and children under three years old (75/350, 21%), children between 3 and 5 years of age (53/350, 15%), children between 6 and 8 years (53/350, 15%), children between 9 and 14 years (99/350, 28%), children between 15 and 19 years (58/350, 17%), children above or equal to 20 years of age (12/350, 3,4%). Most blood cultures (28%, 99/350) belonged to children between 9 and 14 years in both centres. The median age was 8.6 years, with an interquartile range (IQr) of 3.3-14. The median age in Padua was 7.9 (IQr = 2.7-13.1), while in Genoa, it was 10.3 (IQr = 5.9-17.7).

Regarding sex, 63% (220/350) of patients were male, and the other 37% (130/350) were female, with a statistical difference between Padua (M: F ratio 1:1) and Genoa (M: F ratio 2:1).

The underlying disease distribution differed between centres; overall, leukaemia was the most common diagnosis in 55% (193/350) of cases. However, while in Padua, leukaemia represented 73% (67/92) of cases, in Genoa, this percentage dropped to 49% (126/258), with a relative increase in solid tumours, compared to Padua. Children with a solid tumour were 6.5% (6/92) of Padua's population and 25% (65/258) of Genoa's one. Other diagnoses included aplastic anaemia (12%) and lymphoma (4.9%). Uncommon diseases were classified together and occurred in 7.4% of cases: histiocytosis, sickle cell disease, thalassemia, or congenital immunodeficiency syndromes.

Of the total amount of patients, 35% (124/350) underwent hematopoietic stem cell transplantation (HSCT) and, among these, the possible development of graft-versus-host disease (GvHD) has been even evaluated: 45 out of 124 cases (36%) developed acute GvHD of various grades. In particular, among patients investigated in Padua, 39 out of 92 cases (42%) underwent HSCT and 14 out of 39 patients (36%) developed GvHD. In the population of Genoa, 85 out of 258 cases (33%) underwent HSCT, and 36% (31/258) developed GvHD.

To summarize, the two populations of Padua and Genoa turned out homogeneous in age groups, HSCT and GvHD distributions; nevertheless, there were significantly different in sex (males were more frequent in Genoa, $p = 0.003$) and underlying pathology (leukaemia was more frequent in Padua, solid tumours in Genoa, $p < 0.001$).

### 4.1.2 Pathogens distribution

Table III and Figure 3 describe the pathogens found in our collection.

**Table III – Frequency of pathogenic species stratified by centres**

| GRAM | PATHOGEN | N = 350 | % | PADUA, N=92 | GENOA, N=258 |
|---|---|---|---|---|---|
| **Gram -, N=196** | Escherichia coli | 62 | 17.7% | 22 (23.9%) | 40 (15.5%) |
| | Pseudomonas aeruginosa | 33 | 9.4% | 10 (10.9%) | 23 (8.9%) |
| | Klebsiella pneumoniae | 30 | 8.6% | 11 (12%) | 19 (7.4%) |
| | Enterobacter cloacae | 23 | 6.6% | 10 (10.9%) | 13 (5%) |
| | Acinetobacter spp. | 10 | 2.9% | 1 (1.1%) | 9 (3.5%) |
| | Pseudomonas spp. | 8 | 2.3% | 0 (0%) | 8 (3%) |
| | Klebsiella oxytoca | 6 | 1.7% | 0 (0%) | 6 (2.3%) |
| | Stenotrophomonas maltophilia | 5 | 1.4% | 0 (0%) | 5 (1.9%) |
| | Serratia marcescens | 4 | 1.1% | 3 (3.3%) | 1 (0.4%) |
| | Campylobacter jejuni/coli | 2 | 0.6% | 1 (1.1%) | 1 (0.4%) |
| | Citrobacter koseri | 2 | 0.6% | 0 (0%) | 2 (0.8%) |
| | Enterobacter hormaechei | 2 | 0.6% | 0 (0%) | 2 (0.8%) |
| | Moraxella spp. | 2 | 0.6% | 0 (0%) | 2 (0.8%) |
| | Aeromonas sobria | 1 | 0.3% | 1 (1.1%) | 0 (0%) |
| | Capnocytophaga sputigena | 1 | 0.3% | 0 (0%) | 1 (0.4%) |
| | Haemophilus influenzae | 1 | 0.3% | 0 (0%) | 1 (0.4%) |
| | Neisseria mucosa | 1 | 0.3% | 0 (0%) | 1 (0.4%) |
| | Ochrobactrum anthropi | 1 | 0.3% | 0 (0%) | 1 (0.4%) |
| | Proteus spp. | 1 | 0.3% | 0 (0%) | 1 (0.4%) |
| | Salmonella spp. | 1 | 0.3% | 0 (0%) | 1 (0.4%) |
| | | | | | |
| **Gram +, N= 154** | Coagulase-negative Staphylococcus (CoNS) | 72 | 20.6% | 14 (15.2%) | 58 (22.5%) |
| | **Methicillin-Resistant** | 60 (83%) | | | |
| | **Methicillin-Sensible** | 12 (17%) | | | |
| | Staphylococcus aureus | 33 | 9.4% | 5 (5.4%) | 28 (10.9%) |
| | **MRSA** | 1 (3%) | | | |
| | **MSSA** | 32 (97%) | | | |
| | Streptococcus spp. | 20 | 6.0% | 4 (4.3%) | 16 (6.2%) |
| | Enterococcus faecium | 14 | 4.0% | 6 (6.5%) | 8 (3%) |
| | Enterococcus faecalis | 6 | 1.7% | 1 (1.1%) | 5 (1.9%) |
| | Bacillus cereus | 2 | 0.6% | 2 (2.2%) | 0 (0%) |
| | Rothia mucilaginosa | 2 | 0.6% | 0 (0%) | 2 (0.8%) |
| | Brevibacterium casei | 1 | 0.3% | 0 (0%) | 1 (0.4%) |
| | Clostridium tertium | 1 | 0.3% | 0 (0%) | 1 (0.4%) |
| | Corynebacterium aurimucosum | 1 | 0.3% | 0 (0%) | 1 (0.4%) |
| | Micrococcus luteus | 1 | 0.3% | 0 (0%) | 1 (0.4%) |
| | Paenibacillus | 1 | 0.3% | 0 (0%) | 1 (0.4%) |

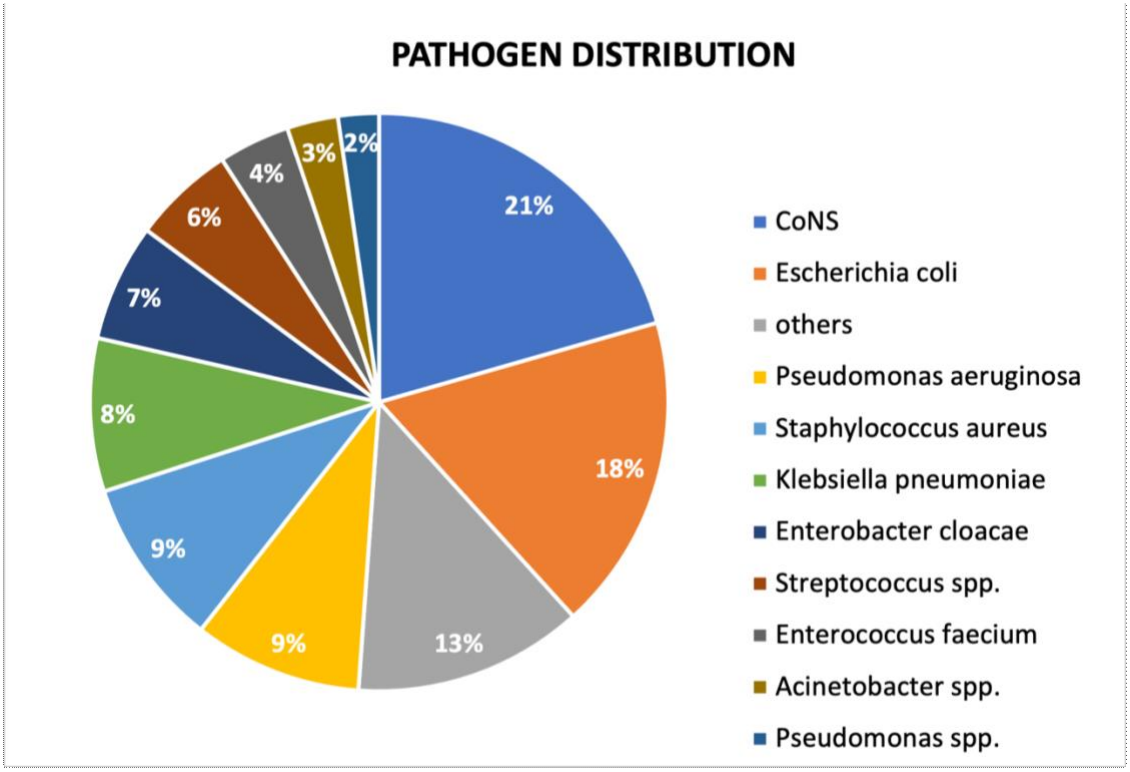



**Figure 3 – Pie chart of frequency of pathogenic species**

The total of gram-negative bacteria was 56% (196/350), while gram-positive were 44% (154/350). In particular, in Padua, 64% (59/92) of isolated pathogens were gram-negative, and the others 36% (33/92), were gram-positive bacteria; in Genoa, 53% (137/258) of microorganisms were gram-negative, and 47% (121/258) were gram-positive.

In both populations, the most frequently isolated pathogens were *Coagulase-negative Staphylococcus (CoNS)*, detected in 20.6% (72/350) of blood cultures, and *Escherichia coli*, detected in 17.7% (62/350) of cases. In particular, in Padua, the presence of *CoNS* was found in 15.2% (14/92) of blood cultures and *E. coli* in 23.9% (22/92) of cases; similarly, in Genoa, *CoNS* were detected in 22.5% (58/258) of blood cultures and *E. coli* in 15.5% (40/258) of cases. They were followed by *Pseudomonas aeruginosa* (in Padua 10/92, in Genoa 23/258) and *Staphylococcus aureus* (in Padua 5/92, in Genoa 28/258), each detected in 9.4% (33/350) of total blood cultures. Regarding *S. aureus*, in only one case (3%), it was a Methicillin-Resistant Staphylococcus aureus (MRSA), while in 32 cases (97%) were Methicillin-Sensible (MSSA). As regards the other *Staphylococcus spp. (S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis),* 83% (60/72) of

pathogens were methicillin-resistant, and the remaining 17% (12/72) were methicillin-sensible. Other relatively frequent pathogens were: *Klebsiella pneumoniae* (8.6%, 30/350), *Enterobacter cloacae* (6.6%, 23/350), *Streptococcus spp.* (6%, 20/350), and *Enterococcus faecium* (4%, 14/350). The other bacteria were isolated in less than 3% of blood cultures.

Table IV shows the population characteristics stratified according to the gram classification. Gram-positive and negative bacteria are similarly distributed between centres. However, gram-positive bacteria were slightly more frequent in the first five years of life than gram-negative.

In the same way, gram-positive bacteria were the most causative pathogens in children suffering from solid tumours compared to other diagnoses (67% in solid tumours, 35% in leukaemia). These results are probably related, as solid tumours have an increased incidence in younger children compared to older ones. In our cohorts, 34% of bacteraemia in solid tumours occurred in children younger than three years, while leukaemia and lymphoma bacteraemia mainly involved children older than six.

**Table IV – Population characteristics stratified by gram classification (gram-negative and gram-positive bacteria)**

| Characteristic | N | Gram -, N = 196 | Gram +, N = 154 |
|---|---|---|---|
| ***Centre*** | 350 | | |
| Genoa (n = 258) | | 137 (53%) | 121 (47%) |
| Padua (n = 92) | | 59 (64%) | 33 (36%) |
| ***Age (median (IQr))*** | | 9.2 (4.3 – 14.1) | 7.8 (2.4 – 13.1) |
| ***Age group*** | 350 | | |
| < 3 years (n = 75) | | 33 (44%) | 42 (56%) |
| 3 – 5 years (n = 53) | | 31 (59%) | 22 (41%) |
| 6 – 8 years (n = 53) | | 32 (60%) | 21 (40%) |
| 9 – 14 years (n = 99) | | 61 (62%) | 38 (38%) |
| 15 – 19 years (n = 58) | | 34 (59%) | 24 (41%) |
| ≥ 20 years (n = 12) | | 5 (42%) | 7 (58%) |
| ***Sex*** | 350 | | |
| Female (n = 130) | | 78 (60%) | 52 (40%) |
| Male (n = 220) | | 118 (54%) | 102 (46%) |
| ***HSCT*** | 350 | | |
| No (n = 226) | | 126 (56%) | 100 (44%) |
| Yes (n = 124) | | 70 (56%) | 54 (44%) |
| ***GvHD*** | 124 | | |
| No (n = 79) | | 48 (61%) | 31 (39%) |
| Yes (n = 45) | | 22 (49%) | 23 (51%) |
| ***Underlying pathology*** | | | |
| Other (n = 26) | | 11 (42%) | 15 (58%) |
| Aplastic anaemia (n = 43) | | 26 (60%) | 17 (40%) |
| Leukaemia (n = 193) | | 126 (65%) | 67 (35%) |
| Lymphoma (n = 17) | | 9 (53%) | 8 (47%) |
| Solid tumour (n = 71) | | 24 (33%) | 47 (67%) |

## 4.2 WISCA results

Table V and Figure 4 describe the results of the 20 antibiotics tested as monotherapy, with relative median values *(median)*, upper and lower *Bayesian Uncertainty Interval (BUI)* and the probability that estimated antibiotic coverage is at least 85% *(p_85)*. No single antibiotic showed a significant probability of providing at least 85% empirical coverage for the identified pathogens.

Piperacillin-tazobactam as monotherapy has a median coverage of 75%, but with an overall 12% probability of covering more than 85% of pathogens (BUI: 28-89%).

Meropenem as monotherapy had a median coverage of 83% and was effective (p_85) in 39% of cases (BUI: 38-93%). These discrepancies between the median coverage value and the p_85 are probably due to the sample narrowness and the not always available susceptibility result for a drug in the antibiograms, explaining the large uncertainty interval and loss of statistical significance.

Other drugs showing an acceptable (greater than 75%) median coverage were ciprofloxacin, gentamycin ceftazidime-avibactam and ceftolozano-tazobactam.

**Table V – Single antibiotics: the probability that the estimated coverage is at least 85% *(p_85)***

| Antibiotics | Median | Lower BUI | Upper BUI | p_85 |
|---|---|---|---|---|
| amikacin | 0.5574 | 0.1595 | 0.7886 | 0.005375 |
| ampicillin | 0.3733 | 0.0229 | 0.7326 | 0.003487 |
| cefepime | 0.6167 | 0.1267 | 0.8697 | 0.05521 |
| cefotaxime/ceftriaxone | 0.5485 | 0.08247 | 0.7337 | 0.002401 |
| ceftazidime | 0.545 | 0.1359 | 0.7674 | 0.002488 |
| **ceftazidime_avibactam** | 0.8417 | 0.5943 | 0.9639 | 0.4655 |
| **ceftolozano_tazobactam** | 0.8442 | 0.5917 | 0.9695 | 0.4757 |
| **ciprofloxacin** | 0.7551 | 0.2496 | 0.8775 | 0.06627 |
| clindamycin | 0.3022 | 0.03242 | 0.888 | 0.06042 |
| erythromycin | 0.1764 | 0.01685 | 0.8099 | 0.01178 |
| **gentamycin** | 0.7771 | 0.3497 | 0.921 | 0.2452 |
| levofloxacin | 0.5533 | 0.0531 | 0.9061 | 0.09461 |
| linezolid | 0.4391 | 0.04009 | 0.7845 | 0.007585 |
| **meropenem** | 0.8284 | 0.3809 | 0.9316 | 0.3886 |
| penicillin | 0.1386 | 0.01672 | 0.9097 | 0.0787 |
| **piperacillin_tazobactam** | 0.7479 | 0.2745 | 0.8939 | 0.1185 |
| teicoplanin | 0.3054 | 0.05206 | 0.5425 | 0.0006582 |
| trimethoprim_sulfamethoxazole | 0.3857 | 0.03755 | 0.6541 | 0.001229 |
| tigecycline | 0.4226 | 0.1639 | 0.7143 | 0.001425 |
| vancomycin | 0.4379 | 0.09448 | 0.6868 | 0.001353 |

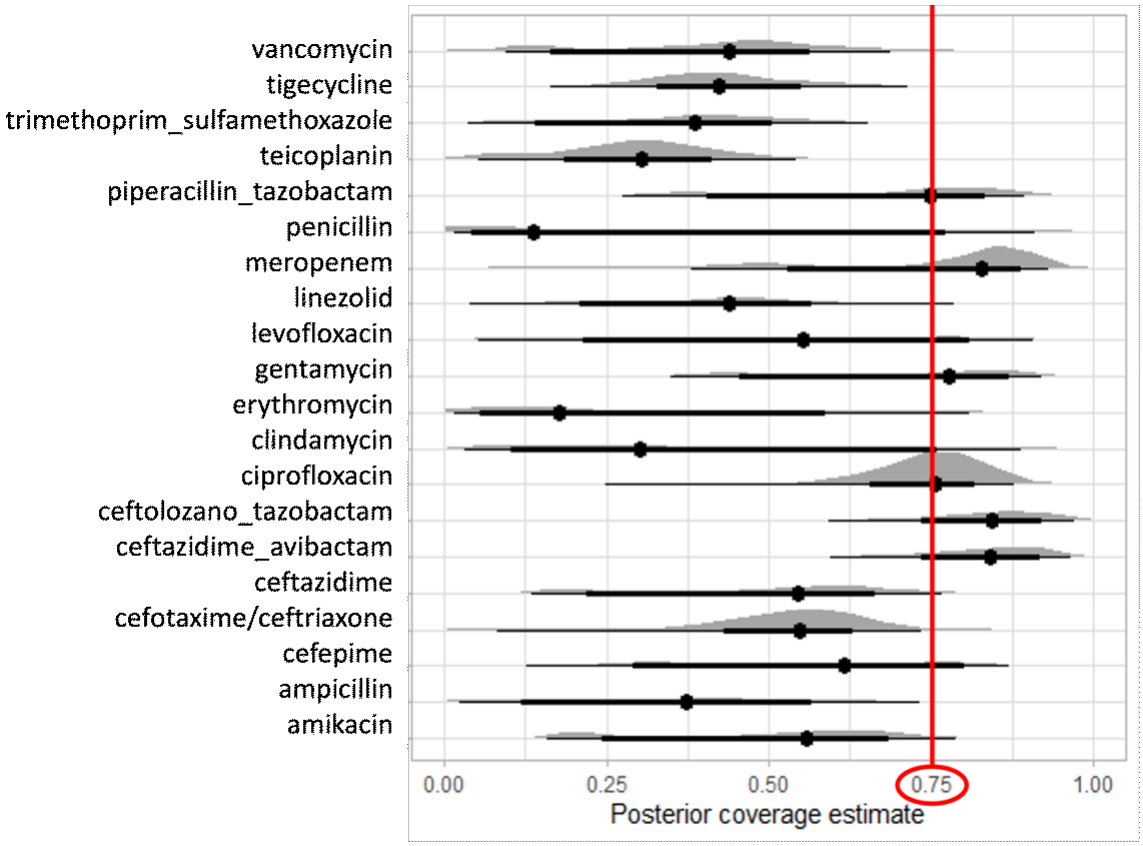



**Figure 4 – WISCA estimated coverage for all the evaluated single antibiotics. Dots represent the median of the posterior distribution and line the associated 95% Highest Density Intervals**

Figure 5 shows the median coverage for the antibiotics as monotherapy stratified for centres. Overall, results overlapped for most molecules, with Padua carrying a larger uncertainty interval due to the inferior blood cultures asset. However, differences were noted for gentamycin, levofloxacin and cefepime, which resulted in more effectiveness in the Padua population than in the Genoa one.

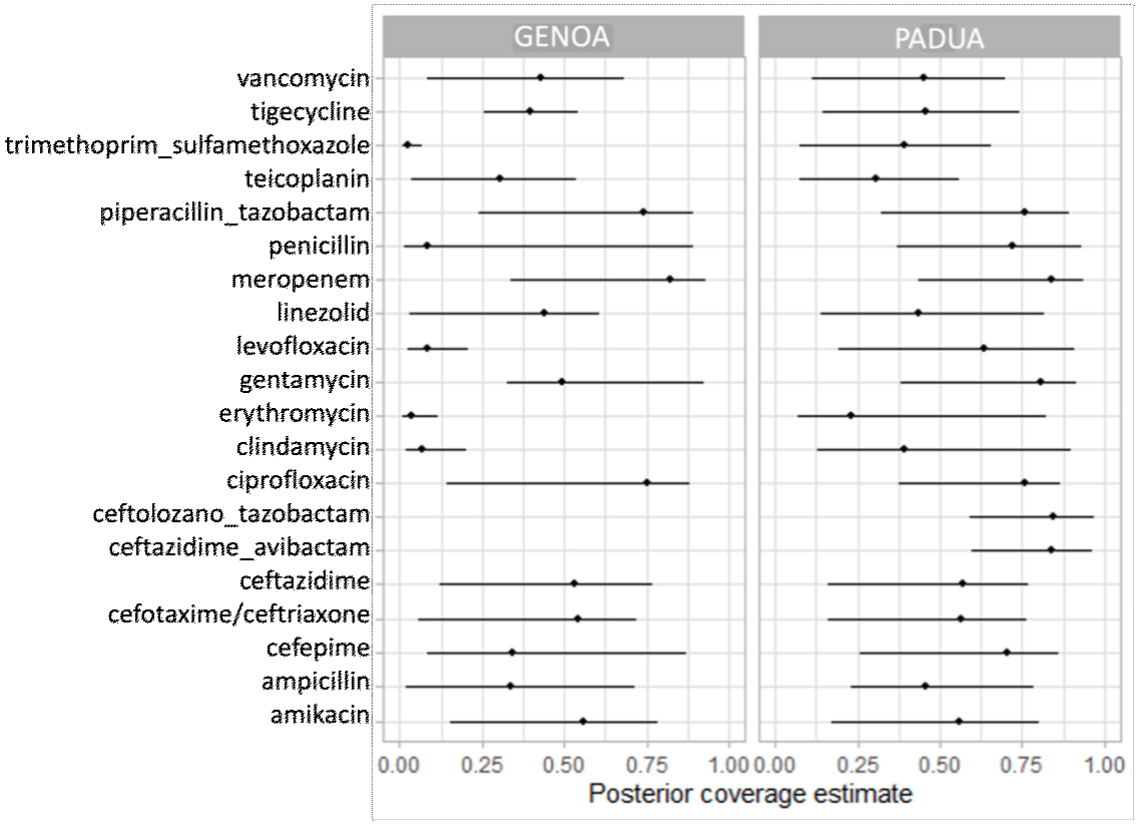



**Figure 5 – WISCA estimated coverage for all the evaluated single antibiotics stratified by centres. Dots represent the median of the posterior distribution and line the associated 95% Highest Density Intervals**

The analysis of the associations between the population variables and the probability of efficacy results for monotherapy is shown in Table VI, reported as odd ratios. Age group, sex and underlying pathology appeared to be independent variables, not influencing the observed monotherapy inadequacy.

**Table VI – Odds ratio (OR) for fixed effects term in the model by exponentiating coefficient**

| Variable | OR | Lower IC | Upper IC |
|---|---|---|---|
| 3 – 5 years | 0.9527 | 0.7172 | 1.266 |
| 6 – 8 years | 1.09 | 0.8029 | 1.463 |
| 9 – 14 years | 1.13 | 0.8734 | 1.469 |
| 15 – 19 years | 1.075 | 0.8042 | 1.44 |
| ≥ 20 years | 0.9916 | 0.6162 | 1.624 |
| Male sex | 1.08 | 0.8967 | 1.293 |
| Other | 0.9509 | 0.6775 | 1.313 |
| Aplastic anaemia | 0.9425 | 0.7134 | 1.245 |
| Lymphoma | 1.028 | 0.6952 | 1.54 |
| Solid tumour | 1.105 | 0.8568 | 1.418 |

Table VII and Figure 6 show results of the 21 analysed combined antibiotics regimens, with relative median values *(median)*, upper and lower *Bayesian Uncertainty Interval (BUI)* and the probability that estimated antibiotic coverage is at least 85% *(p_85)*.

All median coverage values are higher than mono-treatments, and several combined regimens have a significant probability of providing at least 85% empirical antibiotic coverage.

Combination therapies whit a median coverage of at least 75% are described below, from the narrower spectrum molecules to the broader ones.

Ceftriaxone-amikacin-vancomycin coverage was found to be effective in 97% of cases; however, considering the large BUI, the probability of offering adequate coverage in at least 85% (p_85) decreased to 73%.

Ceftazidime-amikacin-vancomycin coverage had a median coverage of 98%, with a p_85 of 81%.

Piperacillin-tazobactam-amikacin combination showed a median coverage rate of 78%. When adding a glycopeptide, the median coverage dramatically increased: up to 89% with teicoplanin and 97% with vancomycin (p_85 respectively 58% and 75%).

Piperacillin-tazobactam-vancomycin coverage was found to be effective in 93% of cases, and the probability of offering adequate coverage in at least 85% of cases was 64%. Ciprofloxacin-amikacin-vancomycin coverage was found to be effective in 98% of cases with a p_85 of 88%.

The combination of meropenem and amikacin performed equally to piperacillin-tazobactam and amikacin with a median coverage of 78%, up to 89% with teicoplanin and 97% with vancomycin (p_85 respectively 59% and 79%). Meropenem without amikacin combined with a glycopeptide had a median coverage overlapping with piperacillin-tazobactam plus amikacin and glycopeptide (median 98%), with a higher p_85, 92%. The discrepancies between p_85 values for meropenem/glycopeptide and meropenem/amikacin/glycopeptide, lower in the second case (contrary to what was expected), are attributable to larger BUI due to fewer antibiograms with combined susceptibility results available for these antibiotics.

**Table VII – Combined antibiotics regimens: the probability that the estimated coverage is at least 85% *(p_85)***

| Antibiotics | Median | Lower BUI | Upper BUI | p_85 |
|---|---|---|---|---|
| cefotaxime/ceftriaxone-vancomycin | 0.8031 | 0.004626 | 0.9496 | 0.389 |
| ceftazidime-amikacin | 0.6487 | 0.05187 | 0.8954 | 0.08699 |
| ceftazidime-amikacin-teicoplanin | 0.8915 | 0.007154 | 0.9756 | 0.587 |
| **ceftazidime-amikacin-vancomycin** | 0.9768 | 0.03665 | 0.9957 | 0.8153 |
| ceftriaxone-amikacin | 0.6361 | 0.06563 | 0.8915 | 0.07167 |
| ceftriaxone-amikacin-teicoplanin | 0.8778 | 0.006318 | 0.9723 | 0.5634 |
| **ceftriaxone-amikacin-vancomycin** | 0.9695 | 0.02766 | 0.994 | 0.7257 |
| ceftriaxone-teicoplanin | 0.6898 | 0.001368 | 0.9036 | 0.0926 |
| ciprofloxacin-amikacin | 0.6866 | 0.01044 | 0.9121 | 0.1586 |
| ciprofloxacin-amikacin-teicoplanin | 0.9002 | 0.0078 | 0.9776 | 0.5981 |
| **ciprofloxacin-amikacin-vancomycin** | 0.9816 | 0.04605 | 0.9967 | 0.8811 |
| meropenem-amikacin | 0.7856 | 0.1305 | 0.9457 | 0.3563 |
| meropenem-amikacin-teicoplanin | 0.8977 | 0.007619 | 0.9773 | 0.5954 |
| **meropenem-amikacin-vancomycin** | 0.9755 | 0.03449 | 0.9953 | 0.7968 |
| meropenem-teicoplanin | 0.9333 | 0.01287 | 0.9846 | 0.6737 |
| meropenem-vancomycin | 0.9858 | 0.07423 | 0.9973 | 0.9288 |
| **piperacillin_tazobactam-amikacin** | 0.781 | 0.1136 | 0.9458 | 0.3453 |
| piperacillin_tazobactam-amikacin-teicoplanin | 0.892 | 0.007197 | 0.9757 | 0.5879 |
| **piperacillin_tazobactam-amikacin-vancomycin** | 0.9718 | 0.03 | 0.9945 | 0.7515 |
| piperacillin_tazobactam-teicoplanin | 0.8399 | 0.005264 | 0.96 | 0.4726 |
| **piperacillin_tazobactam-vancomycin** | 0.9285 | 0.01597 | 0.9843 | 0.6345 |

Figure 6 shows the median sensitivity for all the considered antibiotic associations included between 64% and 99%.

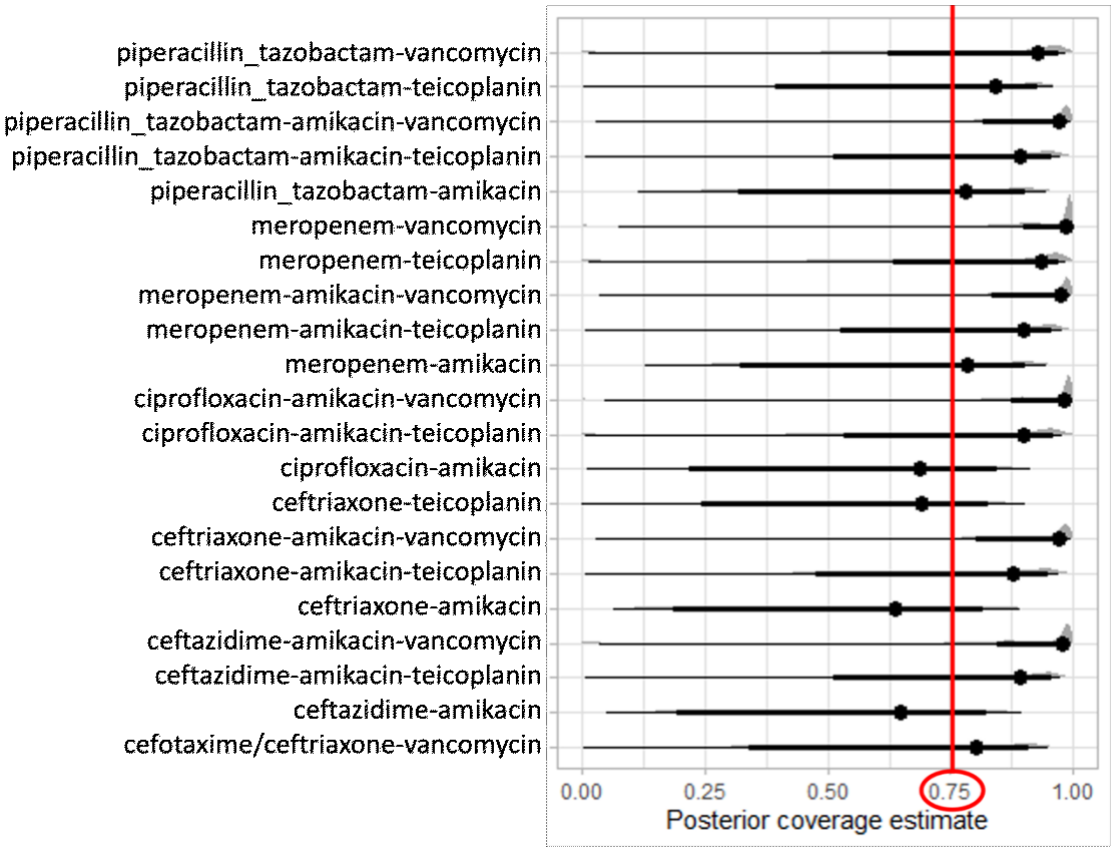
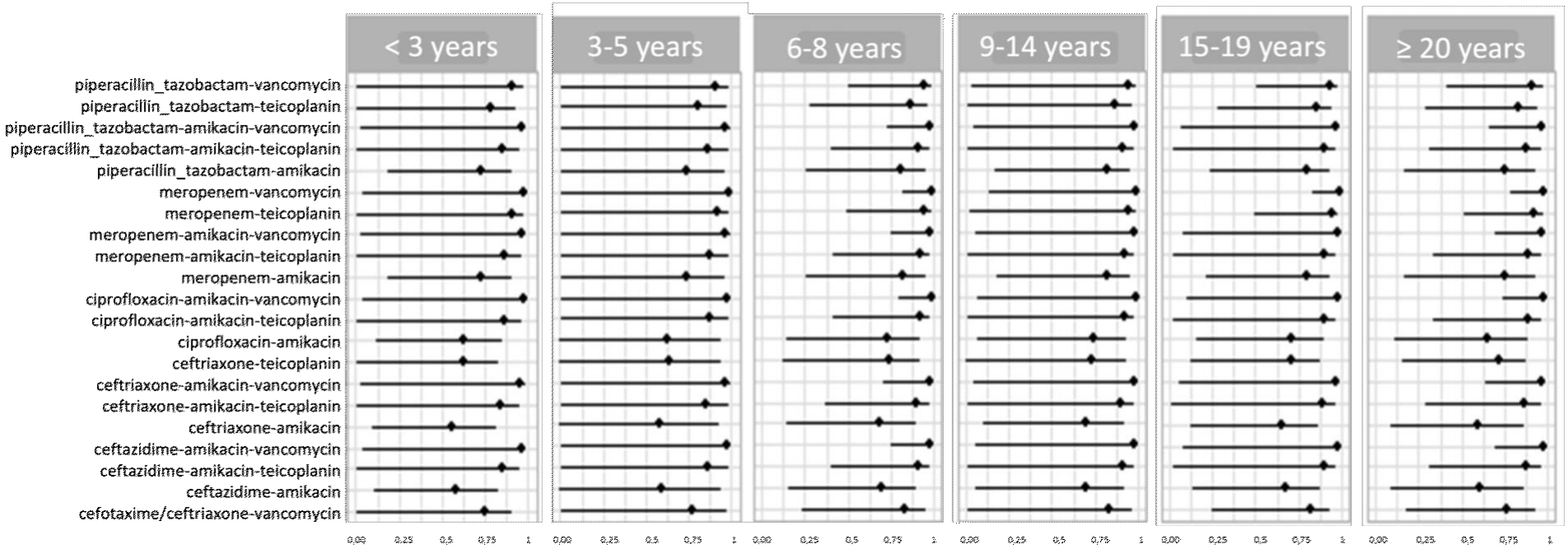



**Figure 6 – WISCA estimated coverage for all the evaluated combined antibiotics regimens. Dots represent the median of the posterior distribution and line the associated 95% Highest Density Intervals**

### 4.2.1 WISCA stratified by age group

In Figure 7, median coverages of combined regimens stratified by age group are represented.

**Figure 7 – WISCA estimated coverage for all the evaluated combined antibiotics regimens stratified by age group. Dots represent the median of the posterior distribution and line the associated 95% Highest Density Intervals**



### 4.2.2 WISCA stratified by centre

In Figure 8 is reported the WISCA referred to each population of Genoa and Padua with the sensitivity prevalence to antibiotics divided into the 21 analysed combined regimens. Stratification by centre did not provide statistically significant differences. The two populations were overlapping, with wider uncertainty intervals in Genoa's. This difference in the intervals, albeit more cultures coming from Genoa, was attributable to lesser antimicrobial available in the antibiograms.

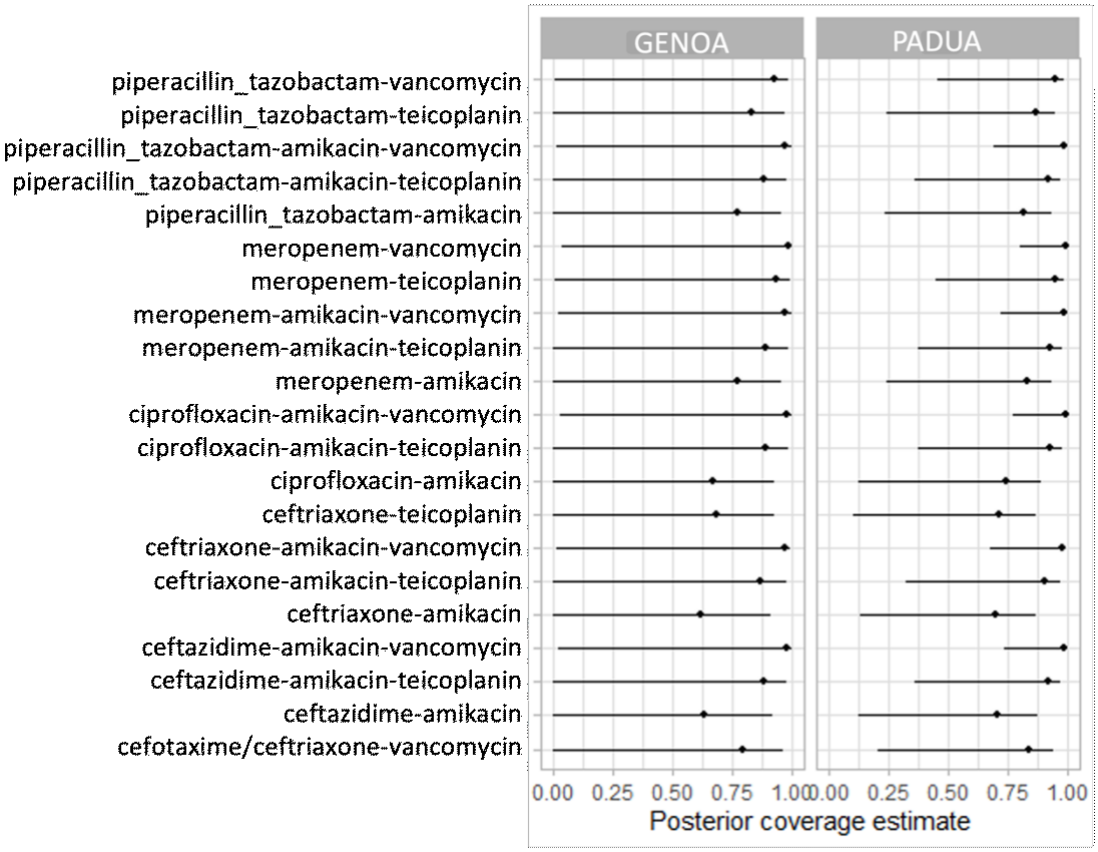



**Figure 8 – WISCA estimated coverage for all the evaluated combined antibiotics regimens stratified by centres. Dots represent the median of the posterior distribution and line the associated 95% Highest Density Intervals**

### 4.2.3 WISCA stratified by haematopoietic stem-cell transplantation

The stratification according to the presence of haematopoietic stem-cell transplantation showed no differences in the coverage rate of the combined therapies analysed (Figure 9).

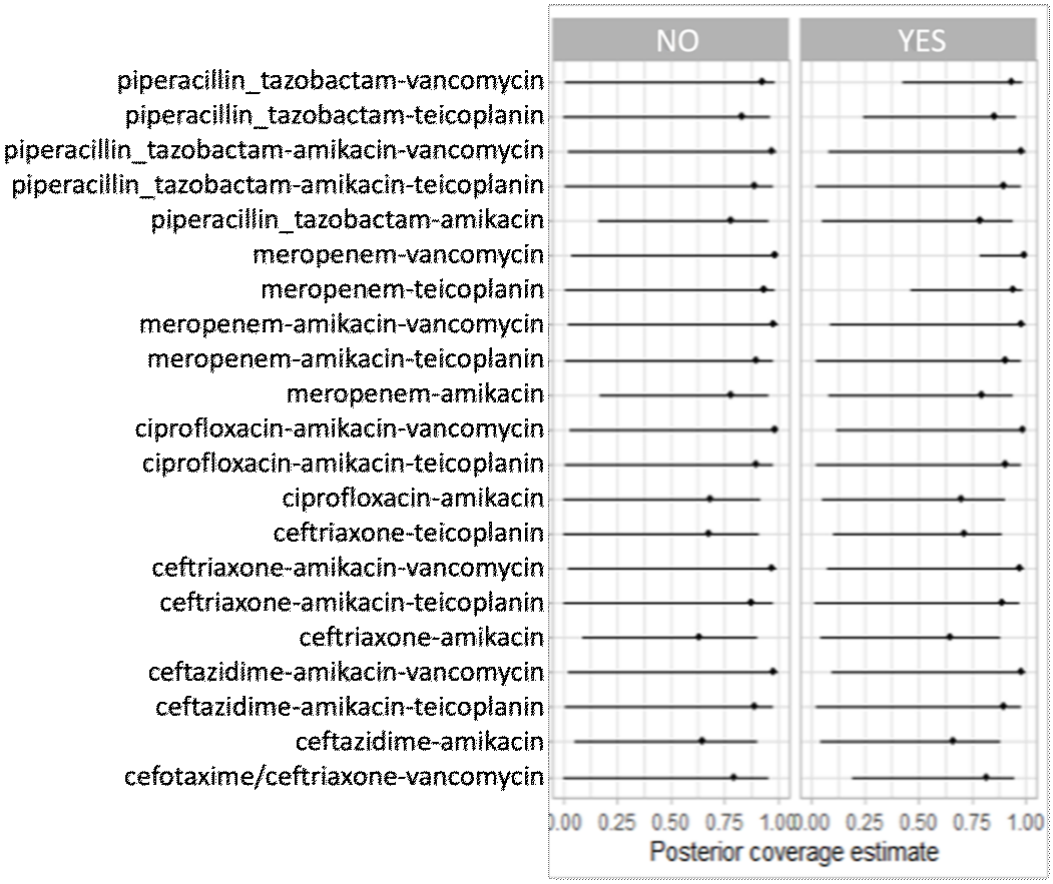
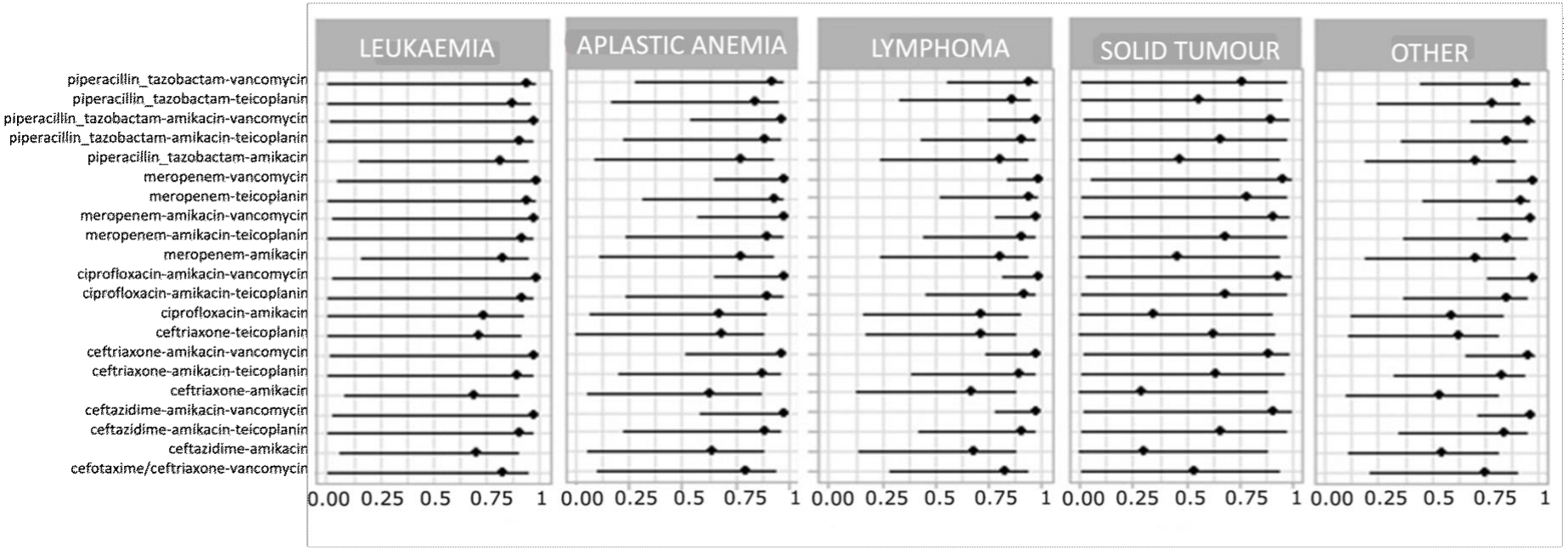



**Figure 9 – WISCA estimated coverage for all the evaluated combined antibiotics regimens stratified by the presence of haematopoietic stem-cell transplantation (yes or no). Dots represent the median of the posterior distribution and line the associated 95% Highest Density Intervals**

### 4.2.4 WISCA stratified by underlying pathology

Figure 10 shows the analysis stratified according to the underlying diagnosis. What is evident is that solid tumours, compared to other diseases, had a slightly different median pattern, with a weaker coverage for combination therapies without glycopeptide (e.g., piperacillin-tazobactam plus amikacin).

**Figure 10 – WISCA estimated coverage for all the evaluated combined antibiotics regimens stratified by underlying pathology. Dots represent the median of the posterior distribution and line the associated 95% Highest Density Intervals**



The analysis of possible variables associated with the probability of efficacy for combined regimens is shown in Table VIII. Patients aged 6-8 years, 9-14 years and 15-19 years have a greater coverage probability with combined regimens in comparison with those under three years of age. On the other side, there was no correlation between sex and underlying disease.

**Table VIII – Odds ratio (OR) for fixed effects term in the model by exponentiating coefficient**

| Variable | OR | Lower IC | Upper IC |
|---|---|---|---|
| 3 – 5 years | 1.11 | 0.8779 | 1.419 |
| **6 – 8 years** | 1.345 | 1.045 | 1.728 |
| **9 – 14 years** | 1.568 | 1.244 | 1.958 |
| **15 – 19 years** | 1.625 | 1.281 | 2.08 |
| ≥ 20 years | 1.279 | 0.8335 | 1.93 |
| Male sex | 1.059 | 0.8931 | 1.256 |
| Other | 0.8607 | 0.652 | 1.144 |
| Aplastic anaemia | 0.9441 | 0.7431 | 1.19 |
| Lymphoma | 1.035 | 0.732 | 1.483 |
| Solid tumour | 1.128 | 0.9266 | 1.381 |

## 4.3 WISCA MODEL with gram-negative bacteria

In this second model, the analysis was performed considering only gram-negative bacteria.

Table IX and Figure 11 describe results for antibiotics in monotherapy. The median coverage values for single drugs significantly increased (compared to the overall cohort) for the following antimicrobials: amikacin (from 56% to 98%), cefepime (from 61% to 86%), ceftazidime-avibactam (from 84% to 99%), ceftolozano-tazobactam (from 84% to 99%), gentamycin (from 78% to 94%), meropenem (from 83% to 98%) and piperacillin-tazobactam (from 75% to 86%). On the other hand, median coverage of vancomycin, teicoplanin, penicillin, linezolid, erythromycin, clindamycin, and ampicillin dramatically decreased because these antibiotics are not efficient versus gram-negative bacteria.

**Table IX – Single antibiotics, gram-negative bacteria: the probability that the estimated coverage is at least 85% *(p_85)***

| Antibiotics | Median | Lower BUI | Upper BUI | p_85 |
|---|---|---|---|---|
| **amikacin** | 0.9828 | 0.8968 | 0.9979 | 0.9841 |
| ampicillin | 0.0009001 | 0.0000006266 | 0.4078 | 0.001249 |
| **cefepime** | 0.856 | 0.03168 | 0.9637 | 0.526 |
| cefotaxime/ceftriaxone | 0.6025 | 0.02217 | 0.8961 | 0.04646 |
| ceftazidime | 0.8282 | 0.09386 | 0.9661 | 0.4134 |
| **ceftazidime_avibactam** | 0.9899 | 0.8098 | 1 | 0.96 |
| **ceftolozano_tazobactam** | 0.9889 | 0.7938 | 1 | 0.9537 |
| ciprofloxacin | 0.7469 | 0.06968 | 0.9466 | 0.1836 |
| clindamycin | 0.002309 | 0.000001333 | 0.1576 | 0.0006246 |
| erythromycin | 0.03917 | 0.001025 | 0.9273 | 0.04841 |
| **gentamycin** | 0.9398 | 0.7507 | 0.9906 | 0.8886 |
| levofloxacin | 0.8155 | 0.0145 | 0.991 | 0.4448 |
| linezolid | 0.003048 | 0.000001903 | 0.06771 | 0.0005908 |
| **meropenem** | 0.9762 | 0.5185 | 0.9966 | 0.9654 |
| penicillin | 0.002638 | 0.000001118 | 0.1928 | 0.0006246 |
| **piperacillin_tazobactam** | 0.8613 | 0.09954 | 0.972 | 0.5466 |
| teicoplanin | 0.0008145 | 0.000001789 | 0.01723 | 0.0006087 |
| trimethoprim_sulfamethoxazole | 0.2955 | 0.003504 | 0.7399 | 0.01221 |
| tigecycline | 0.2507 | 0.08544 | 0.5822 | 0.001152 |
| vancomycin | 0.0008054 | 0.000001805 | 0.01729 | 0.0006087 |

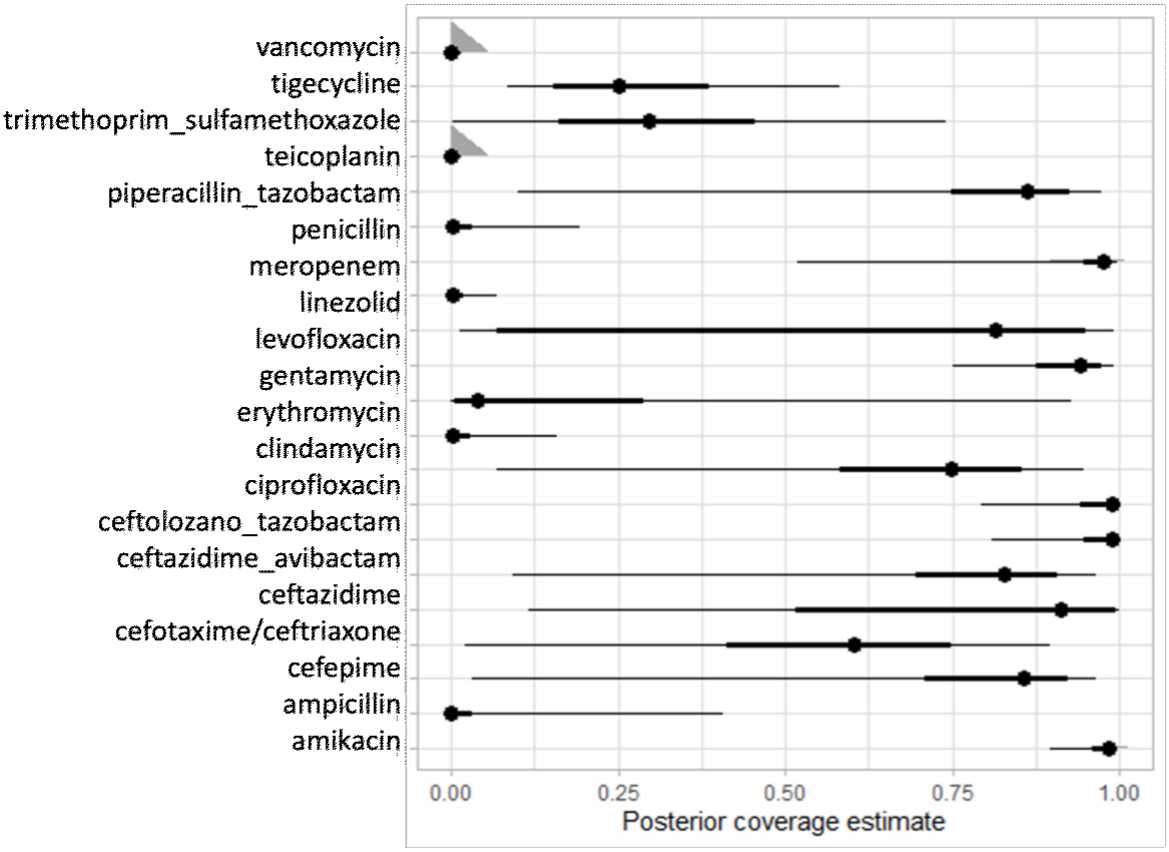



**Figure 11 – WISCA estimated coverage for all the evaluated single antibiotics. Dots represent the median of the posterior distribution and line the associated 95% Highest Density Intervals**

Monotherapies showed an increased probability of success for gram-negative aetiologies in the age groups 9-14 years and 15-19 years compared to children less than three years old (Table X). There was no correlation between the efficacy, the sex, and the underlying disease.

**Table X – Odds ratio (OR) for fixed effects term in the model by exponentiating coefficient**

| Variable | OR | Lower IC | Upper IC |
|---|---|---|---|
| 3 – 5 years | 1.021 | 0.5888 | 1.803 |
| 6 – 8 years | 1.389 | 0.8291 | 2.437 |
| **9 – 14 years** | 1.869 | 1.162 | 3.006 |
| **15 – 19 years** | 2.038 | 1.16 | 3.628 |
| ≥ 20 years | 0.6591 | 0.2917 | 1.476 |
| Male sex | 1.718 | 1.206 | 2.461 |
| Other | 0.8795 | 0.4689 | 1.653 |
| Aplastic anaemia | 0.6905 | 0.4365 | 1.127 |
| Lymphoma | 0.803 | 0.4143 | 1.603 |
| Solid tumour | 0.8904 | 0.5279 | 1.484 |

Table XI indicates the results of the 21 analysed combined antibiotics regimens considering only gram-negative bacteria, with relative median values *(median)*, upper and lower *Bayesian Uncertainty Interval (BUI)* and the probability that estimated antibiotic coverage is at least 85% *(p_85)*.

Compared to the overall pool of blood cultures, the advantage of combining two antibiotics active against gram-negative bacteria is more apparent, as data are cleaned from gram-positive pathogens. Relevant combinations include piperacillin-tazobactam-amikacin, which coverage rate increased from 78% in the overall population to 99% of cases (86% for piperacillin-tazobactam alone in gram-negative series), ceftazidime and amikacin, which reached a similar coverage rate of 99%. Without further advantage compared to meropenem alone, meropenem-amikacin coverage was found to be effective in 99% of cases (meropenem coverage as monotherapy was 98%).

**Table XI – Combined antibiotics regimens, gram-negative bacteria: the probability that the estimated coverage is at least 85% *(p_85)***

| Antibiotics | Median | Lower BUI | Upper BUI | p_85 |
|---|---|---|---|---|
| cefotaxime/ceftriaxone-vancomycin | 0.5019 | 0.00008601 | 0.8604 | 0.02744 |
| **ceftazidime-amikacin** | 0.9889 | 0.03708 | 0.9988 | 0.9635 |
| ceftazidime-amikacin-teicoplanin | 0.9828 | 0.004963 | 0.9977 | 0.9215 |
| ceftazidime-amikacin-vancomycin | 0.9829 | 0.004955 | 0.9978 | 0.9217 |
| ceftriaxone-amikacin | 0.9851 | 0.02472 | 0.9982 | 0.9612 |
| ceftriaxone-amikacin-teicoplanin | 0.9729 | 0.003135 | 0.9962 | 0.9065 |
| ceftriaxone-amikacin-vancomycin | 0.9725 | 0.003052 | 0.9961 | 0.906 |
| ceftriaxone-teicoplanin | 0.5006 | 0.00008575 | 0.8606 | 0.02743 |
| ciprofloxacin-amikacin | 0.9825 | 0.01159 | 0.9978 | 0.9524 |
| ciprofloxacin-amikacin-teicoplanin | 0.9764 | 0.003584 | 0.9966 | 0.9135 |
| ciprofloxacin-amikacin-vancomycin | 0.9765 | 0.003511 | 0.9968 | 0.9125 |
| **meropenem-amikacin** | 0.9942 | 0.06932 | 0.9995 | 0.97 |
| meropenem-amikacin-teicoplanin | 0.9859 | 0.006139 | 0.9982 | 0.925 |
| meropenem-amikacin-vancomycin | 0.9859 | 0.006073 | 0.9982 | 0.925 |
| meropenem-teicoplanin | 0.9763 | 0.003537 | 0.9967 | 0.9128 |
| meropenem-vancomycin | 0.9764 | 0.003571 | 0.9967 | 0.9125 |
| **piperacillin_tazobactam-amikacin** | 0.9874 | 0.02309 | 0.9986 | 0.9665 |
| piperacillin_tazobactam-amikacin-teicoplanin | 0.954 | 0.001777 | 0.993 | 0.8582 |
| piperacillin_tazobactam-amikacin-vancomycin | 0.954 | 0.001788 | 0.9928 | 0.8576 |
| piperacillin_tazobactam-teicoplanin | 0.7877 | 0.0003177 | 0.9584 | 0.3089 |
| piperacillin_tazobactam-vancomycin | 0.7887 | 0.0003204 | 0.9588 | 0.3106 |

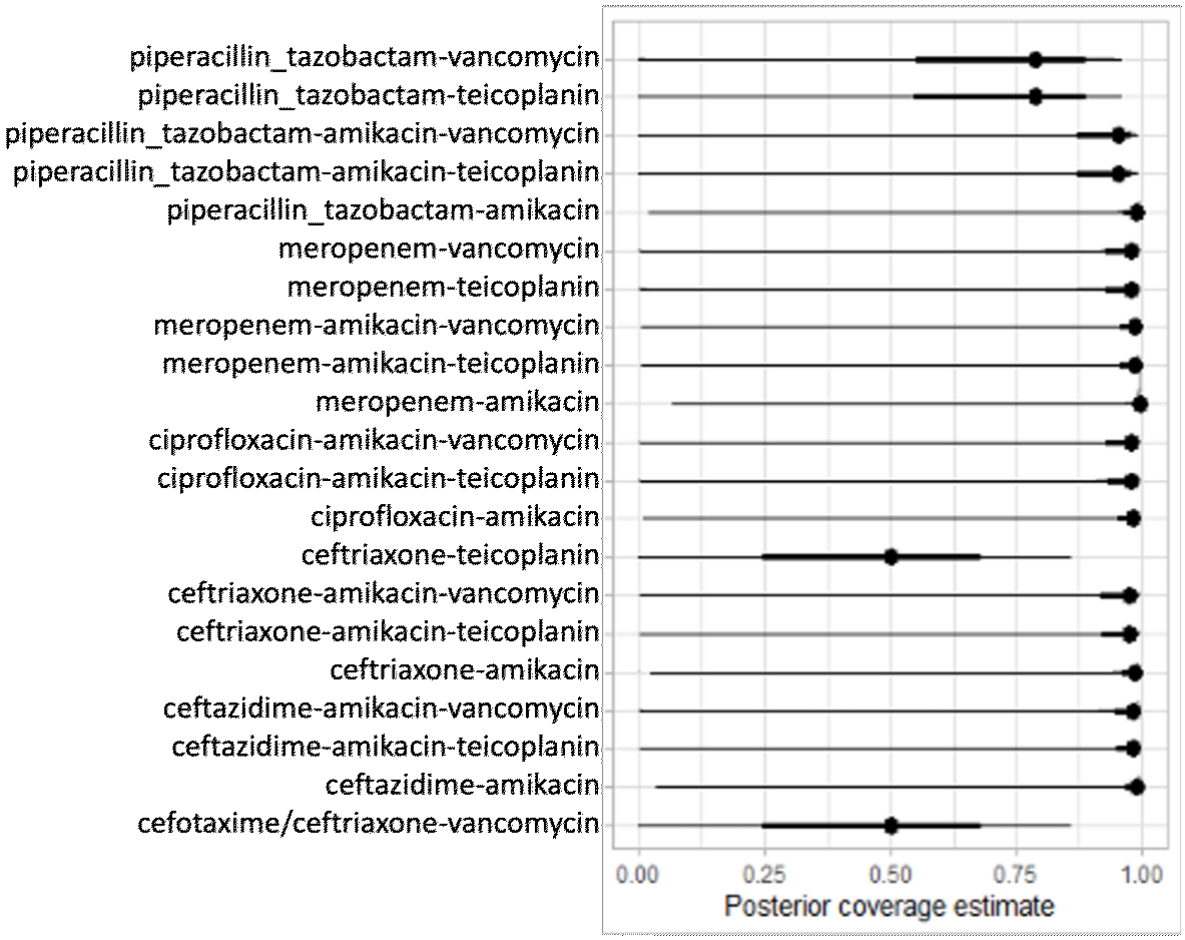



**Figure 12 – WISCA estimated coverage for all the evaluated combined antibiotics regimens. Dots represent the median of the posterior distribution and line the associated 95% Highest Density Intervals**

Analysing the possible influence of population variables (Table XII), patients aged 6-8 years, 9-14 years and 15-19 years have a greater coverage probability with combined regimens in comparison with those under three years of age considering only gram-negative infections. Furthermore, combined therapy showed an increased probability of success for gram-negative aetiologies in patients with solid tumour compared to children of leukaemia group. There was no correlation between the efficacy and the sex.

**Table XII – Odds ratio (OR) for fixed effects term in the model by exponentiating coefficient**

| Variable | OR | Lower IC | Upper IC |
|---|---|---|---|
| 3 – 5 years | 1.4 | 0.8975 | 2.296 |
| **6 – 8 years** | 2.287 | 1.428 | 3.664 |
| **9 – 14 years** | 1.978 | 1.313 | 3.042 |
| **15 – 19 years** | 2.091 | 1.305 | 3.553 |
| ≥ 20 years | 1.613 | 0.7225 | 4.313 |
| Male sex | 1.788 | 1.324 | 2.445 |
| Other | 0.6117 | 0.3714 | 1.037 |
| Aplastic anaemia | 0.7665 | 0.5215 | 1.153 |
| Lymphoma | 1.496 | 0.8071 | 2.967 |
| **Solid tumour** | 1.919 | 1.202 | 3.191 |

# 5. DISCUSSION

To our knowledge, this is the first study developing a WISCA model to guide the empirical choice of the most suitable antibiotic empiric therapy in haemato-oncological paediatric patients.

The presence of immunosuppression (in particular neutropenia) and the high exposure of patients to previous antibiotic treatments pose a challenge for severe infection, possibly due to MDR organisms, making this population a particular epidemiological setting where empirical therapies need to be optimized.

Univocal recommendations for the empirical antibiotic treatment of fever and neutropenia in paediatrics have not been outlined, and guidelines provide general indications without specifying an antibiotic or a combination therapy over another. Proposed antimicrobial empiric regimens for FN for paediatric patients outlined by Lehrnbecher et al. in 2017[23] include in high-risk FN, monotherapy with an antipseudomonal beta-lactam, a fourth-generation cephalosporin, or a carbapenem as empirical therapy. The addition of a second gram-negative agent or a glycopeptide is reserved for clinically unstable patients when a resistant infection is suspected or for centres with a high rate of resistant pathogens.

The 8th European Conference on Infections in Leukaemia (ECIL-8) in 2020[14] underlined the recommendation against using carbapenems as empirical therapy for clinically stable patients.

WISCA has been used in a previous study by Bielicki et al.[41] to evaluate empirical antibiotic regimens for paediatric BSI. Five regimens were evaluated using data on 2000 isolates collected over two years from 19 European hospitals. WISCAs were calculated using first only local data at two exemplar hospitals and in a second model using pooled data from a surveillance network. In the single centre analysis, the best empiric regimen could not be definitively identified because the differences in coverage were not statistically significant. Therefore, combined data from multiple hospitals over a long time period have been adopted to overcome the small local sample size. However, since the pattern of antimicrobial resistance changes in times and across Europe, the overall cohort's regimen susceptibility did not apply to one hospital because of a different pathogen incidence and regimen

susceptibility. Estimating coverage using local data, as widely recommended, may therefore not result in clinically useful information.

A retrospective cohort study by Randhawa et al. based on the application of WISCA to guide empiric treatment of common critical care infections (e. g., ventilator-associated pneumonia and catheter-related bloodstream infections) estimated the potential of the tool to improve time to adequate coverage for intensive care unit (ICU)-acquired infections. The study found that a WISCA can be constructed based on local ecology data for critically ill patients with ICU-acquired infections; furthermore, it demonstrated that the WISCA-derived empiric antimicrobial regimens have the potential to more than double the likelihood of adequate empiric antibiotic coverage and reduce treatment initiation time.[38]

Another retrospective cohort study by Aislinn Cook et al. tried to improve empiric antibiotic prescribing in paediatric bloodstream infections by applying WISCA, using microbiology data from BSI episodes from a global network of paediatric hospitals. The WISCA methodology highlighted the ability to incorporate local data to easily compare different antibiotic regimens for a specific clinical syndrome (i.e., paediatric sepsis). This can aid clinical decision-making, potentially improving outcomes and aiding antimicrobial stewardship efforts. In addition, the application of WISCAs can be incorporated into national surveillance programs as a way to use surveillance data collected in many countries: it may be a way to facilitate pooling and provide enhanced analysis and model adjustments due to expertise beyond what may exist in a single site. Finally, the study concluded that WISCAs provide a clinically relevant way of interpreting local resistance patterns and a set of data-driven tools to guide a more appropriate empiric antibiotic therapy selection in children.[42]

A study from the University of Padua used the WISCA tool to define the empiric antibiotic treatment for suspected paediatric community-acquired urinary tract infections, demonstrating that the developed WISCAs provide highly informative estimates on coverage patterns overcoming the limitation of combination antibiograms and expanding the framework of previous Bayesian WISCA algorithm. As in our study, the cohort included a high-risk population, in this case with renal/urological comorbidities and previous antibiotic treatment.[39]

Our study confirmed the applicability of WISCA as a tool to support and guide the selection of empirical antibiotic regimens for febrile neutropenia in onco-haematological paediatric patients. However, the difference in coverage between regimens was not statistically significant because of the sample narrowness.

Indeed, paediatric BSI is uncommon in the onco-haematological patients, and data are limited even in a six-year, bicentric study. However, median estimated coverage rates confirmed the validity and the rationale of empiric antibiotic regimens used in both centres: piperacillin-tazobactam (PI-TZ), amikacin and teicoplanin for Padua and PI-TZ and amikacin for Genoa.

Padua and Genoa Children Hospitals are two north-Italian reference centres for curing paediatric haemato-oncological diseases. Populations in our analysis turned out homogeneous but with statistically significant differences concerning sex (males more frequent in Genoa) and underlying pathology (leukaemia more frequent in Padua than Genoa, and solid tumours in Genoa). We could not find any explanation why males doubled females in Genoa while they are normally distributed in the Padua cohort.

Overall, pathogens' distribution was similar in the two cohorts, even if Genoa collected many more blood cultures than Padua. The homogeneity in the bacterial ecology and resistance patterns is essential when pooling together data to estimate the appropriateness of empirical antibiotic therapy. Albeit we had no exact data on resistance mechanisms, resistance to third-generation cephalosporins was found to be 20.6% (12/59) in Padua and 34.3% (47/137) in Genoa (the *chi-square* statistic is 3.8238 and the *p-value* is 0.050298; thus, the result is not significant at $p < 0.05$).

Two pathogens tested resistant to meropenem in Padua and one in Genoa. In the same way, methicillin-susceptibility between staphylococci was equally distributed. Gram-negative bacteria were more frequent than gram-positive for all diagnoses except solid tumours, for which gram-positive accounted for 66% of BSI. This is probably because, compared to leukaemia, these patients are less exposed to immunosuppression therapy and suffer more frequently from catheter-related BSI, mainly caused by *coagulase-negative staphylococci* (CoNS).

Our study showed that none of the monotherapy offered an adequate coverage rate for the identified pathogens; both centres are not currently using monotherapies. Piperacillin-tazobactam and meropenem offered 75% and 82% of coverage,

respectively. Median coverage values overlapped among the two centres, age groups, underlying pathologies, and transplanted populations. Therefore, the recommendation to use a monotherapy with an antipseudomonal cephalosporin seems ineffective in our cohort, while we agree, with a carbapenem-sparing policy, to maintain this option for patients presenting with septic shock.

Combination therapies considerably increased the median coverage rates. The key to reaching the optimal coverage rate was the association with a second gram-negative agent as amikacin and a glycopeptide. Ceftazidime performed the same way as piperacillin-tazobactam when associated with amikacin and glycopeptide. However, the utilization of ceftazidime has been abandoned in Padua since its empiric use is associated with the risk of increased extended-spectrum β-lactamases (ESBL)[43] strains. Further, ceftazidime offers no coverage versus gram-positive bacteria, so teicoplanin was preferably maintained when already started empirically, even for methicillin-susceptible gram-positive strains, leading to glycopeptide abuse.

The median coverage of PI-TZ as monotherapy was 75%, while in the association PI-TZ-amikacin, it resulted to 78% and, when adding the glycopeptide, it dramatically increased to 89% with teicoplanin and 97% with vancomycin. Meropenem without amikacin plus vancomycin reached a 98% median coverage, showing the unnecessity of a second gram-negative agent in association with carbapenem due to the very low occurrence of carbapenems resistance.

All the mentioned values for the combined regimens overlapped among the two centres, underlying pathologies, and transplanted population. This is especially relevant for the transplant recipients, who, despite the longer and heavier clinical history, which includes more antibiotic treatments, presented distributions of pathogens' susceptibility like those of non-transplanted. In the same way, age greater than six years appeared to be statistically correlated to the probability of therapy efficacy compared to children younger than three years; we can extrapolate that older children do not necessarily have different resistance patterns.

A second WISCA model including only gram-negative bacteria was developed, considering the low mortality rate associated with gram-positive bacterial infections (mainly *coagulase-negative staphylococci)* and the possibility of targeting them when cultures turn back positive. In this case, monotherapy with PI-

TZ showed a slightly inferior coverage (86%) compared with meropenem (98%); however, when combined with amikacin, both reached the same coverage level (99%). Thus, PI-TZ-amikacin was performing as meropenem in monotherapy, which, in turn, was performing as the meropenem-amikacin combination, proving the low need for adding meropenem to empirical therapy. It would make sense to implement therapy with meropenem only in the case of informative blood culture. Amikacin alone has a median coverage of 98%, but it is never recommended as monotherapy for the high risk of treatment failure in the case of bloodstream infections.

Median coverage rates for only gram-negative bacteria were overlapping when stratified for the population's features, except for age groups and solid tumours.

We could not find a strong explanation for why the occurrence of gram-negative BSI in solid tumours was more associated with the probability of better coverage of combined therapy than leukaemia. However, we can speculate that being this population significantly younger than leukaemia (median age 4 years for solid tumours and 9 for leukaemia), they have been more recently exposed to antibiotics for common infections occurring in the first years of life, that is when "healthy" children are more likely to have an antibiotic prescription. This could lead to increased cephalosporins resistance. Indeed, while resistance to ceftriaxone/cefotaxime in the leukaemia group was found to be 51%, it reached 77% for solid tumours. Finally, solid tumours were more frequent in Genoa (25%) compared to Padua population (6.5%), and Genoa presented a greater percentage of resistance to third-generation cephalosporins (34.3% versus 20.6% in Padua). Thus, we can speculate that younger children may have a greater percentage of resistance to third-generation cephalosporins and this could explain the probability of better coverage of combined therapy in solid tumours than leukaemia.

## 5.1 Limits of the study

Our study presents some limitations. First, the limited number of blood culture isolates did not allow to reach a statistical significance for the regimens analysed. Previous studies have overcome this problem by utilising WISCA models throughout extensive networks in Europe or different world regions. In this way, however, although increasing numbers of isolates, the approach to the local susceptibility patterns may be underestimated, as many countries with different ecology settings and clinical practices are pooled together, and results are generalized.

Our cohort included two similar care centres for geographical setting and overall standard practice of care, for which the afference was supposed to be overlap. However, Genoa provided many more blood culture isolates compared to Padua. The standardization of cultures withdrawal policy should be a priority when aiming to study an infectious syndrome. Many patients have cultures drained after the initiation of antibiotics, limiting the sensitiveness of microbiology to detect the causative agents. Further, Padua receives several oncological patients treated for FN transferred by other departments (emergency department or paediatric intensive care unit) or satellite hospitals where blood cultures have been collected "outside", so we could not collect them.

Another factor limiting the sensitivity of WISCAs in our study may be the large number of pathogens and therapies we considered. Cook et al.[42] applied in their WISCA model for bloodstream infections some restrictive criteria, which probably allowed to minimize the uncertainty of coverage estimates and maximize their discriminatory value: minimum sample size was requested for centre inclusion (at least 100 isolates), only clinically relevant bacteria were considered, and selected antibiotics were studied (non-antipseudomonal third-generation cephalosporins and meropenem). However, we decide to include even once-found bacteria to study the overall possible pathogens and estimate the WISCA applicability to real-setting data.

Another limitation of WISCA application in our study is the lack of correlation between infective pathogens and infection outcomes (e.g., mortality, PICU admission). Since gram-negative bacteria have a more significant clinical impact,

we created the second model excluding gram-positive bacteria. Those data would allow a WISCA calculation to select regimens with expected maximum concordance and, therefore, the greatest potential clinical impact in this high-risk population. This strategy has not yet been adopted in the reported WISCAs study.

To improve the adherence to empirical therapy, in a most extensive and prospective view, the inclusion of an “ecological” outcome, with MDRO colonization status in selected wards should be included in the analysis to assess the effectiveness of controlled empiric therapy to reduce the incidence of AMR.

Lastly, when applying an empiric antibiotic therapy, an “acceptable” cut-off of coverage is usually self-estimated according to clinicians' and microbiologists' experience. Including the clinical outcomes in the WISCA model could further help address this issue. We used an 85% estimated median coverage rate to define a regimen “appropriate”, which overlaps with the study by Randhawa et al.[38] on critical care infections. However, many clinicians could have a preference for antibiotic regimens perceived to have a coverage of 90%.[44]

This issue needs to be addressed in larger and outcomes-driven WISCA models in high-risk populations, such as haemato-oncological paediatric patients.

# 6. CONCLUSIONS

The Bayesian WISCA provides an innovative approach to pool information from different sources about a specific infectious syndrome compared to standard hospital antibiograms. Although WISCAs have already been validated with large datasets, we adopted this model for a specific high-risk population, represented by onco-haematological patients affected by febrile neutropenia. WISCA gave tailored information about the empiric antimicrobial therapies, providing reliable feedback and a guide to support the currently adopted empirical strategies in Haemato-oncological wards of Padua and Genoa Hospitals. Efforts to include more significant numbers of cultures and clinical outcomes may overcome the statistical limitations of this approach.

The application of WISCA in a multicenter study offers the possibility of maximising the clinical utility of microbiological surveillance data derived from larger hospitals to inform the selection of the most appropriate empirical antibiotic therapy also for other minor hospital settings in the same area while contributing to spare broad-spectrum antibiotics and increasing confidence in the selection of narrow-spectrum regimens.

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