Epidemiological Profile and Detection of Resistance Genes in Bloodstream Infection in Cancer Patients: High Occurrence of Metallo- β -lactamases in Enterobacteriales

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Abstract

Bloodstream infections remain one of the most common major complications in cancer patients. The aim of the study was to describe the etiology, phenotypic and molecular epidemiology of ICS isolates from cancer patients. Method: identification and the resistance profile were carried out using the automated biochemical method Vitek 2®. The presence and genes resistant to carbapenemases *bla*_{IMP}, *bla*_{VIM}, *bla*_{GIM}, *bla*_{SIM}, *bla*_{SPM}, *bla*_{KPC}, *bla*_{NDM}, genes oxacillinase *bla*_{OXA-48}, *bla*_{OXA-58}, and the presence of ESBL genes *bla*_{SHV}, *bla*_{CTX}, *bla*_{TEM} for Gram-negatives, as well as, *mecA*, *vanA* and *vanB* for Gram-positives were investigated in blood culture isolates. Result: *Escherichia coli* and *Klebsiella pneumoniae* were the most frequent pathogens. The serine-β-lactamase gene *bla*_{OXA-48} was the most frequent, followed by MβL *bla*_{SIM}, *bla*_{TEM} and *bla*_{CTX} were the most common among ESBL. The *mecA* and *vanA* genes were found in *Staphylococcus* spp. and *Enterococcus faecium*, respectively. *Candida* spp showed high resistance to voriconazole and fluconazole. Conclusion: measures to prevent and control the spread of resistance genes are essential to reduce the risks of morbidity and mortality.

Introduction

Bloodstream infections (ICS) are considered serious complications in cancer patients, especially during chemotherapy. Infections by Gram-negative agents are more frequent in this population, especially when in the course of febrile neutropenia^{1,2,3}.

Gram-negative bacillus (BGN) ICS has been documented in several countries such as India⁴, Australia⁵, Italy⁶, Turkey⁷, China⁸ and Brazil⁹.

Most of these organisms develop resistance to antimicrobial agents, reducing the therapeutic options available and this issue has been considered one of the ten threats to global health¹⁰. *Escherichia coli* and *Klebsiella pneumoniae* have been identified as the main agents of resistant multidrug infections (MDR) in environments related to health care^{7,11}. BGN producers of extended spectrum β-lactamase (ESBL) has emerged and is considered a serious threat to antimicrobial treatment¹², and has been associated with increased mortality in cancer patients^{13,14,15,16,17,18}.

Klebsiella pneumoniae carbapenemases (KPC) and New Delhi metallo-β-lactamases (NDM) represent two important families of carbapenemases, which confer resistance to almost all molecules and have been reported worldwide. Several countries have detected the occurrence of BGN, carrying *bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48-like} or *bla*_{VIM}, genes, and showing high resistance to antibiotics¹⁹.

The aim of the study was to describe the etiology, phenotypic and molecular epidemiology of the ICS isolates from cancer patients.

Materials And Methods

Collection and analysis of clinical isolates

Clinical isolates were obtained from blood samples of patients admitted to the Pernambuco Cancer Hospital, Recife, Brazil, during the period 2019 to 2020. The prospective study included 45 patients aged ≥ 18 years after consent. The study was approved by the Research Ethics Committee of the Pernambuco Cancer Hospital, CAEE (16285219.5.0000.5205).

Blood samples were collected in blood culture flasks which were subsequently incubated by Bactec9095® (Becton Dickinson, USA) with up to 5 days of incubation. The identification and Antimicrobial Susceptibility Test (AST) of bacterial isolates were performed by biochemical tests using the automated system, using the Vitek 2 platform (bioMérieux, Marcy l'Etoile, France) and interpreted according to the Brazilian Committee on Antimicrobial Susceptibility Testing manual (BrCAST). The identification and antifungal susceptibility test (AFST) were performed by biochemical method and microdilution by the minimum antifungal concentration, interpreted according to the CLSI M27-A3/S4 Reference Method for Antifungal Susceptibility Test.

Definition

Resistant multidrug (MDR) was defined as a bacterial isolate, which is resistant to one or more antibiotics in three or more classes of antimicrobial agents.

ICS was defined as the isolation of blood from one (monomicrobial) or more (polymicrobial) organisms.

Broth Microdilution Identification and Antifungal Susceptibility Test (AFST)

The antifungal susceptibility test, in vitro, was performed according to the methods described in documents M27-A3 and M27-S4 of the *Clinical and Laboratory Standards Institute*^{20,21}. For standardization of the experiment, the reference strain ATCC 90028 (*C. albicans*) was used. For the test, the RPMI 1640 medium (Sigma-Aldrich, USA) buffered with morpholino propane sulfonic acid, pH 7.0 ± 0.1 (MOPS; 0.165 mol.L⁻¹; Sigma-Aldrich) was used. Yeasts were evaluated for their susceptibility profile to fluconazole, Amphotericin B, voriconazole, caspofungin, micafungin and anidulafungin.

The yeasts were kept on Sabouraud Dextrose Agar (SDA) and incubated for 24 hours at 37°C. Suspensions of the isolates were prepared in 0.85% saline, with the inoculum concentration adjusted by a spectrophotometer with a wavelength of 530nm, reflecting 90% of the transmittance. Then, the initial suspension was diluted to a concentration of 2-5,103 cells/mL in RPMI 1640. 96-well flat-bottomed micro-titration plates (TPP; Trasadingen, Switzerland) were used. The inoculum was added to the wells containing the drug solution to be tested, and the plates incubated at 37°C for 24-48h. The Minimum Inhibitory Concentrations (MICs) were determined with 100% inhibition for amphotericin B and <50% in relation to the control well for fluconazole and voriconazole.

Extraction of fungal DNA from cultures

The DNA was extracted according to the protocol of Inácio et al., (2016)²², using the extraction buffer (CTAB 2%, NaCl 1.5 M, 100 mM Tris-HCl, 20 mM EDTA, polyvinylpyrrolidone 1%) previously heated to 65 ° C. The cell wall was broken through mechanical agitation (speed 5.5 m / s for 30 seconds) in FastPrep® (BIO 101, Farmingdale, New York, USA) and incubated for 10 minutes at 65°C. The DNA was recovered from a sequential treatment based on alcohol-isoamyl chloroform (24: 1), isopropanol ethanol (70%). Finally, 50µl of MiliQ autoclaved water was added, and the sample was incubated at 37°C for 20min for DNA elution. The extraction products were assembled in parafilm (5µL of the extraction product, 2µL GLB and 2 µL GelRed), using the Lambda DNA marker as standard. The DNA was subjected to electrophoresis on 1.5% agarose gel, in a horizontal electrophoresis tank, containing the Tris-Acetate-EDTA (TAE) buffer, with a voltage of 100v for 20min. Subsequently, the DNA was analyzed for quality and quantity in a transilluminator. The genomic material was kept at 4°C until the time of analysis.

Identification of fungal isolates

The species were identified from the partial sequencing of the D1/D2 domain of the LSU (28S) region of the rDNA using *primers* NL1 (5'-GCATATCAATAAGCGGAGGAAAAG - 3') and NL4 (5'-GGTCCGTGTTTCAAGACGG - 3')²³. The sequencing samples had the PCR products purified (GenJET PCR Purification - Fermentas, UK), and the sequencing was performed on the Sequencing Platform-LABCEN / CCB (UFPE), according to internal protocols of the partner laboratory.

Identification and Antimicrobial Susceptibility Test (TSA)

The identification and antimicrobial susceptibility test (TSA) were carried out by phenotypic methods by automation. The automated identification tests (Vitek®) based on the fermentation of carbohydrates, followed the interpretation of the *Clinical and Laboratory Standards Institute* (CLSI) manual, and the TSA interpretation followed the *Brazilian Committee on Antimicrobial Susceptibility Testing* (BrCAST) protocol for interpretation of the results.

Extraction of plasmid and chromosomal DNA from bacterial isolates

All bacterial isolates were collected from positive blood cultures and subjected to DNA extraction and detection of resistance genes. From the cultures of the isolates, on BHI agar, the DNA was extracted using the PROMEGA ReliaPrep™ kit (Promega®, São Paulo, Brazil) according to the manufacturer's protocol. The DNA was eluted in 50 µL of nuclease-free water. An aliquot of the extracted DNA was quantified in NanoDrop 2000/2000c Spectrophotometer (Thermo Fisher Scientific), in order to verify the efficiency, purity and yield of the extraction. Then it was analyzed by electrophoresis on 1% agarose gel and stained with ethidium bromide.

Detection of resistance genes in Gram-negative bacterial isolates

DNA amplification using the Polymerase Chain Reaction (PCR) technique was performed on BioRad equipment (System, Applied Biosystems®, CA, using 10 µL (1.25U / reaction) TopTaq Master Mix Kit (Qiagen®, USA) using 10 µM of each primer, 200 µM of each dNTP (10mM of each), 1x TopTaq PCR Buffer containing 15mM MgCl₂, 1x coralLoad, ultra-pure water and 2 µL of DNA Thermocycling conditions were established according to the studied resistance genes reference strains such as, *A. baumannii* (ATCC 19606), *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (ATCC 13882), *E. coli* (ATCC 25922), *Serratia marcescens* (ATCC 14756), *Salmonella enterica* (ATCC 35640) were provided by the Department of Microbiology of the Aggeu Magalhães Institute (IAM) - Fundação Oswaldo Cruz (FIOCRUZ).

A conventional PCR assay was performed for 55 isolates recovered from 45 patients to detect three families of ESBL genes. The detection of *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M} resistance genes was performed in all Gram-negative bacterial isolates. And the detection of the *mcr-1* gene was carried out in everyone who was resistant to multiple drugs. PCR for detection of ESBL was performed using the following protocol for amplification; initial denaturation 95°C to 5 minutes, followed by 30 cycles of denaturation 95°C to 1 minute, annealing 60°C to 1 minute and extension 72°C to 1 minute. A final extension step was performed at 72°C at 10 minutes, for each of the studied genes.

And eight families of the metallo-β-lactamase (MβL) genes, (*bla*_{KPC}, *bla*_{GES}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{SPM}, *bla*_{GIM} and *bla*_{SIM}) were investigated for the detection of carbapenemases, performed in all Gram-negative bacterial isolates. Using the following protocol for amplification; initial denaturation 95°C at 5 minutes, followed by 25 cycles of denaturation 95°C at 1 minute, ringing at 1 minute (according to the ringing temperature for each gene) and extension 72°C at 1 min. A final extension step was performed at 72°C at 10 minutes, table 1.

The strains were also subjected to PCR to detect class D carbapenemases, such as oxacillinase (*bla*_{OXA-48}, *bla*_{OXA-58}), using the following protocol for amplification; initial denaturation 94°C at 5 minutes, followed by 30 cycles of denaturation 94°C at 45 seconds, annealing 52°C at 45 seconds and extension 72°C at 1 minutes. A final extension step was performed at 72°C at 6 minutes, as shown in table 1.

PCR conditions for identification of the *mcr-1* gene

The investigation of the *mcr-1* gene was performed using the primers described by Lima et al., (2017)³¹. The amplification reactions were prepared in a volume of 25 µL per tube, comprising: 1µL of genomic DNA, 1.0U of the Taq DNA polymerase enzyme (Promega), 2 mM of each dNTP, 2.5 mM of MgCl₂ and 3.5 pmol of the *primers*. The amplification was performed under the following thermal cycling conditions: 94°C for 10 minutes followed by 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 2 minutes, with a final extension of 72°C for 10 minutes. The PCR products were visualized on a 1% agarose gel stained with ethidium bromide.

Detection of resistance genes in Gram-positive bacterial isolates

All Gram-positive isolates, *mecA*, *vanA*, *vanB* resistance genes were detected using reference strains of organisms, *S. aureus* (ATCC 29213 and 25923), *Streptococcus pneumoniae* (ATCC 6305) *S. epidermidis* (ATCC 12228), *E. faecalis* (ATCC 29212), *E. faecium* (ATCC 19434). The *primers* *mecA*-F (5'-AAAAGTGGTGGTGAAGATATACC-3') and *mecA*-R (5'-GAAAGGATCTGTACTGGGTTAATCAG-3') with a fragment size of 147pb, the *primers* *VanA*-F (5'-CATGACGTAATCGGTAAAATC-3') and *VanA*-R (5'-ACCGGGCAGRGTTATTGAC-3') with fragment size of 732 bp. And the *primers* *VanB*-F (5'-CATGATGTGTCGGTAAAATC-3') and *VanB*-R (5'-ACCGGGCAGRGTTATTGAC-3') with 635 bp fragment size. Using the following protocol for amplification; initial denaturation 95°C to 5 minutes, followed by 30 cycles of denaturation 95°C to 1 minute, annealing 60°C to 1 minute and extension 72°C to 1 minute. A final extension step was performed at 72°C for 10 minutes.

The amplified PCR products were stained with ethidium bromide and visualized by electrophoresis on 1% agarose gels, using the System L-Pix EX photographic documentation system (LoccusBiotechnology, Brazil).

The consensus strings were edited using the Sequencher 4.7 program and then submitted to the BLAST tool from GenBank (National Center of Biotechnology Information, <http://www.ncbi.nlm.nih.gov>) to search for similar strings. The obtained sequences were aligned with confidence sequences deposited in the database and analyzed phylogenetically with the aid of the MEGA-x program.

Results

Detection of pathogens in the bloodstream

Were recovered 55 clinical isolates from blood culture samples from 45 cancer patients, 38 Gram-negative, 13 Gram-positive and four yeast. Of the BGN, the most frequent were *Escherichia coli* (36,8%) and *Klebsiella pneumoniae* (28,9%). Among the Gram-positive, the most frequent were *Staphylococcus aureus* (38,5%) and *Staphylococcus epidermidis* (23%). Four patients had infections due to *Candida* species (graphic 1). Polymicrobial bloodstream infection was identified in three cases. As *Escherichia coli*, *Klebsiella pneumoniae*, *Candida parapsilosis*. Coagulase negative *Staphylococcus* (SCN) and *Candida tropicalis*. And finally, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*.

Antimicrobial susceptibility profile in bacterial isolates

Among gram-negative bacteria, most showed resistance to ampicillin (63,15%), cefepime (39,4%), ciprofloxacin (34,2%) and cefuroxime (29%). It was possible to observe a high percentage of resistance to ampicillin in *Klebsiella* spp 100% (11/11) and *Escherichia coli* 78,6% (11/14) table 2. 4th generation cephalosporin was the class of antibiotics that showed greater resistance in *Acinetobacter baumannii* (66%), *Pseudomonas aeruginosa* (42,8%), *Klebsiella* spp (36,3%) and *Escherichia coli* (28,5%). As cefuroxime, 2nd generation cephalosporins showed resistance in *Serratia marcescens* (100%), *Salmonella* spp (100%), *Klebsiella* spp (36,3%) and *Escherichia coli* (28,5%) table 3. In the fluoroquinolone class, the percentage of resistance to ciprofloxacin ranged from 27,2% *Klebsiella* spp to 66% in *Acinetobacter baumannii* (table 3). The quinolone class nalidixic acid antibiotic showed greater resistance in *Escherichia coli* (42,8%).

In general, when analyzing the percentage of sensitivity of BGN, we observed that 78,9% were sensitive to amikacin and meropenem, and 76,3% to gentamicin and 68,4% to ertapenem.

In gram-positive bacteria the highest resistance rate was to oxacillin 6/13 (46,1%) and erythromycin 5/13 (38,4%), high resistance to penicillin and gentamicin 4/13 was also observed (30,7%) (table 2).

Oxacillin resistance was observed in *Staphylococcus aureus* 3/5 (60%) *Staphylococcus* Coagulase Negative 3/4, the latter being identified as *Staphylococcus epidermidis* (75%). From the macrolides class, resistance to erythromycin was observed in *Staphylococcus epidermidis* 3/3 (100%) and *Staphylococcus aureus* 2/5 (40%). Resistance to penicillin and gentamicin aminoglycoside were 2/5 (40%) and 1/3 *Staphylococcus epidermidis* (33,3%) and 1/1 coagulase negative *Staphylococcus* (100%) table 3.

Resistance to clindamycin, sulfamethoxazole / trimethoprim and levofloxacin were observed in *S. aureus* and *S.epidermidis*. Resistance to amikacin and vancomycin was observed only in *Enterococcus faecium* (table 3).

Of the 55 isolates, 28 were MDR (50,9%), while six (10,9%) bacterial isolates were sensitive to all antibiotics tested and three (5,45%) of the isolated pathogens were resistant to all classes of antibiotics tested.

Detection of bacterial resistance genes

The detection of a broad-spectrum β -lactamases gene was observed in 28/38 (73,7%) gram-negative isolates. The *bla*_{TEM} gene was detected in 22 isolates, including 10 *E. coli*, 5 *K. pneumoniae*, 2 *P. aeruginosa* and 2 *A. baumannii*. The *bla*_{CTX-M} gene was detected in 16 isolates, 7 *K. pneumoniae*, 5 *E. coli* and 2 *P. aeruginosa*. The *bla*_{SHV} gene was detected in only 2 isolates, 1 *E. coli* and 1 *A. baumannii* (table 4).

High frequency of 36/38 metallo- β -lactamases genes (94,7%) were detected in the isolates. The gene most often found in the study was a serine- β -lactamase *bla*_{OXA-48} 31/38 (81,5%), being *E. coli* (12), *K. pneumoniae* (8), *P. aeruginosa* (5), *Serratia marcescens* (2), *A. baumannii* (2), and in the species of *P. alcaligenes* and *Salmonella* spp (1).

Then the *bla*_{SIM} gene 27/38 (71%), *E. coli* (10), *K. pneumoniae* (9), *P. aeruginosa* (6), *P. alcaligenes* (1) and *Serratia marcescens* (1). The detection of the *bla*_{GES} gene was 21/38 (55,2%), *E. coli* (8), *K. pneumoniae* (7), *P. aeruginosa* (3), *A. baumannii* (1), *Serratia marcescens* (1) and *Klebsiella oxytoca* (1). The *bla*_{NDM}

15/38 gene (39,4%) was detected in *E. coli* (7), *K. pneumoniae* (6), *P. aeruginosa* (1) and *Serratia marcescens* (1). The *bla*_{KPC} 11/38 gene (28,9%) was detected in *E. coli* (5), *K. pneumoniae* (3), *P. aeruginosa* (2) and *S. marcescens* (1). The *bla*_{SPM} 7/38 gene (26,3%) was found in 7 isolates, *K. pneumoniae* (2), *P. aeruginosa* (2), *Pseudomonas alcaligenes* (1), *A. baumannii* (1) and *Serratia marcescens* (1). The *bla*_{OXA-58} 10/38 gene (26,3%) was detected in *E. coli* (7), and in the species of *K. pneumoniae*, *A. baumannii* and *Serratia marcescens* (1). The *bla*_{VIM} 7/38 gene (18,4%), *E. coli* (5) and *K. pneumoniae* (2). The *bla*_{IMP} gene was detected in 6 isolates, being *E. coli* (5) and *K. pneumoniae* (1). The same isolate can carry more than one gene (table 4). The *mcr-1* gene was not detected in any of the tested isolates.

Of the Gram-positive bacteria, five were *S. aureus*, three were coagulase-negative *Staphylococcus* (three were *S. epidermidis*, one *S. warneri* and one *Streptococcus salivarius*, an unidentified SCN), and two *Enterococcus* spp. The presence of the *mecA* gene was detected in 6/13 (46,15%) isolates, *Staphylococcus aureus* and coagulase negative *Staphylococcus* (*S. epidermidis*) and *Enterococcus faecalis*. Only one *Enterococcus faecium* isolate had the *vanA* gene (1/13) (7,7%). The *vanB* gene was not detected in any of the studied isolates (table 4).

Detection of fungal ICS

Four species of *Candida* spp. were found in this population. Resistance to fluconazole was observed in 3/4 isolates. One *Candida tropicalis* isolate presented caspofungin as a sensitive dose dependent. All species showed resistance to voriconazole (table 5).

Discussion

The phenotypic and molecular characteristics of 55 clinical isolates rescued from blood cultures of patients with malignant neoplasms, allowed to observe a higher frequency of BGN (69%) greater than CGP (23,63%), in addition to 7,2% of yeasts. BGNs have been implicated as the main cause of ICS in cancer patients^{32, 33}.

The most commonly found BGN were *Escherichia coli* (25,4%), *Klebsiella pneumoniae* (20%) and *Pseudomonas aeruginosa* (12,7%), as well as reported in recent studies^{34, 35, 36, 37}. Evidence has suggested the phenomenon of bacterial translocation, where enterobacteria cross the intestine wall, migrating into the bloodstream, causing bacteremia in patients receiving cancer chemotherapy^{38, 39}, explaining in the greater frequency of this group of bacteria is part of the etiology of bloodstream infection.

The epidemiology of the occurrence of bacteremia in cancer patients has changed over time and has been characterized by a change in the profile from gram-negative bacteria (1960s to 1970s) to gram-positive bacteria (1980s). Gram-negative bacteria have been predominant as the main agents of bloodstream infections in cancer patients in the last two decades on the global stage^{40, 41}.

According to the Antimicrobial Surveillance Program (SENTRY) *S. aureus* and *E. coli* have been identified as the predominant pathogens of ICS in the world for the past 20 years⁴². Our study showed a higher frequency of coagulase-negative *Staphylococcus* and *Staphylococcus aureus* among gram-positive bacteria, corroborating with other studies^{16, 7, 43}.

The occurrence of polymicrobial infections in three cases was associated with neutropenia and the presence of a catheter. In two cases, they were associated with MDR and in one case, an operative procedure was performed. Presence of neutropenia, recent invasive procedures and devices have been identified as risk factors for polymicrobial infection of the bloodstream, highlighting that MDR isolates have been more commonly found in polymicrobial infection^{44, 45}. Polymicrobial infections were responsible for 5,45% (3/55) of these cases, corroborating the study of ICS in cancer patients in India⁴⁶.

The occurrence of β -lactamase resistance genes has been reported in isolates of polymicrobial bloodstream infection from a patient with acute myeloid leukemia in Brazil, including *bla*_{TEM} e *bla*_{SIM} in *P. aeruginosa*, *bla*_{TEM}, *bla*_{CTX} and *bla*_{OXA-48} in *E. coli*, *bla*_{CTX}, *bla*_{KPC}, *bla*_{NDM}, *bla*_{SIM} and *bla*_{OXA-48} in *K. pneumoniae* and *bla*_{OXA-48} in *A. baumannii*⁴⁷.

BGNs showed greater resistance to aminopenicillins, fourth generation cephalosporins, fluoroquinolones, second and first generation cephalosporins and aminoglycosides, in agreement with the results of a study in India carried out with 66 cases of bloodstream infection⁴⁰. Increased resistance to carbapenems, combinations of beta-lactamase inhibitors, beta-lactamase, aminoglycosides, fluoroquinolones and cephalosporins (including high resistance to cefepime) has been observed⁴¹.

Our study showed (46,15%) of MRSA, including *Staphylococcus aureus* and *Staphylococcus* coagulase negative (MRCoNS) species, respectively. Recent studies have observed resistance to methicillin in *Staphylococcus* spp ranging from 38,4% to 93%^{48, 7}. Resistance to methicillin has been more frequent in SCN compared to *S. aureus*^{49, 50}.

And the occurrence of vancomycin-resistant *Enterococcus* was 7,7%, similar to that found by Bhat et al., (2021)⁴⁰. The prevalence of vancomycin-resistant enterococci (VRE) has become stable or decreasing after 2012⁴².

MDR infections are worrisome phenomena, particularly in cancer patients¹⁷, due to low therapeutic options and, consequently, a higher risk of mortality⁵¹. Among the isolated pathogens, the general frequency of MDR was 50,9% (28/55). The main isolated MDR bacteria were *E. coli* and *K. pneumoniae*, which present results in agreement with other studies^{49, 41}.

The detection of ESBL resistance genes was 73,7%, presenting data similar to those found by other studies in this same population of cancer patients. Mexico (72,8%)⁵¹, Korea (55,6%)⁵², Iran (84,9%)⁵⁰ and Brazil, (71,4%)⁴⁸. The main genes detected were *bla*_{TEM} (58%) and *bla*_{CTX} (42,1%), in addition, 18,4% (7/38) co-

housed the *bla*_{TEM} and *bla*_{CTX} genes, corroborating with other studies^{53,36}.

A high prevalence of MBL (94,7%) was detected, which seems to express a much higher frequency than those presented by other studies^{51,54}. Carbapenem-resistant enterobacteriaceae (CRE) has been increasingly prevalent in cancer patients and associated with ineffective empirical therapy⁵⁵. The mechanism of resistance to carbapenems is hydrolysis by carbapenemases that are encoded in plasmids and are highly transmissible. Resistance to carbapenem can also be attributed to mutations or other modifications that alter the level of production or binding affinity of penicillin-binding proteins⁵⁶. This problem reflects the use of different carbapenems in hospitals, in addition to varying according to geographic location⁵⁶.

The most frequently detected carbapenemase was *bla*_{OXA-48} (81,5%). A study from Egypt with cancer patients also found a high prevalence of *bla*_{OXA-48} (68,88%)⁵⁴. Another study involving several European countries showed that *E. coli* and *Klebsiella pneumoniae* were the main producers of carbapenemases (KPC, NDM, OXA-48-like or VIM)¹⁹.

The frequency of the *bla*_{OXA-48} gene was detected in 70,83% and 92% in isolates of *P. aeruginosa* and *A. baumannii*, respectively in Iran⁵⁷.

Other studies have reported the occurrence of genes that encode subgroups of OXA carbapenemases, including the *bla*_{OXA-58} gene^{58,59}. The OXA-48 enzyme leads to resistance to carbapenems, limiting therapeutic options, organisms that produce OXA-48 and are intrinsically resistant to colistin when infecting patients can be fatal, cause high mortality⁶⁰.

We detected a high frequency of *bla*_{SIM} 27/38 (71%) in enterobacteriales. The frequency of *bla*_{SIM-1} in Egypt was 48%⁶¹. And in Brazil, 66.6% were reported in human and animal samples⁶².

We highlight the presence of the *bla*_{SPM} gene in *Pseudomonas* spp isolates, as has already been found²⁸ and in *A. baumannii*⁶³, who described the first report of the *bla*_{SPM-1} gene in *Acinetobacter baumannii* in Brazil.

Although MRSA is frequent in ICS in the hospital population⁶⁴, few studies mention the occurrence of MRSA in ICS of cancer patients⁶⁵. The frequency of the *mecA* gene, in which all showed phenotypic resistance to methicillin, was 46,1% (6/13). However, the prevalence of MRSA in cancer patients in hospitals in Turkey was 50%, harboring the *mecA* genotype⁶⁶. The *vanA* gene was detected in an isolate of *Enterococcus faecium* rescued from the bloodstream of a patient with colon cancer, whose phenomenon of bacterial translocation through the permeability of the injured colon mucosa may have acted as a risk factor for the event⁶⁷.

Candida species were found in four samples (7,2%). *Candida* spp. is the most commonly isolated yeast in ICS⁶⁸. *Candida tropicalis* was the most frequent species in our study, being commonly found in cancer patients⁶⁹. Our study showed two concomitant bloodstream infections involving fungi and bacteria, one being *Klebsiella pneumoniae*, *Escherichia coli* and *Candida parapsilosis*. And another from SCN and *Candida tropicalis*. A fungal monoinfection was registered by *Candida tropicalis*. Bloodstream infections involving both bacteria and yeast have been documented⁷⁰.

Our result showed resistance to voriconazole in all *Candidas* spp. The rate of azole resistance among *C. tropicalis* has been significant compared to rates of resistance to micafungin⁷¹. The antifungal caspofungin showed dose-dependent susceptibility in *Candida tropicalis* (1/4). Caspofungin has been reported to have potent activity against *Candida* spp. resistant to fluconazole⁷².

The growing scenario related to the emergence of *Candida* species resistant to fluconazole and voriconazole is a worrying scenario. A study in Australia warns of the emergence of azole resistance among *C. tropicalis*⁷³. Voriconazole has been reported as an important agent in the treatment of hyaline fungi and effective for infections caused by *Candida* species, including those that are resistant to fluconazole⁷⁴.

Azole resistance can arise through several mechanisms, including overexpression or alteration of the drug target, positive regulation of drug transporters or cellular alterations that reduce drug toxicity or allow tolerance to drug-induced stress. The activation of membrane-associated efflux pumps that modulate resistance through ABC transporters and main facilitator (MFS), allowing resistance to multiple drugs⁷⁵.

Thus, as the emergence of drug-resistant fungi, the combination of antibiotics with antifungals has been explored to enhance the treatment, as colistin could increase the antifungal activity of fluconazole⁷⁶.

The worldwide dissemination of resistant isolates, combined with the few available therapeutic options, has negatively influenced the treatment and prognosis of patients⁷⁷. Species of *Candida albicans*, especially emerging ones, have significantly influenced the clinical course of patients around the world. Currently, the most recent guidelines for the treatment of fungal infections have recommended fluconazole as a primary therapeutic option to combat these infections. However, there have been increasing reports of increased resistance inherent in this drug that can pose a global health threat⁷⁸.

Antimicrobial resistance is of great concern mainly in the population group vulnerable to infections due to chemotherapy itself or due to factors attributed to the history of cancer. Measures to prevent and control the spread of resistance genes are essential to reduce the risks of morbidity and mortality.

Abbreviations

Bloodstream infections (ICS)

Gram-negative bacilli (BGN)

Carbapenem-resistant enterobacteriaceae (CRE)

Coco Gram-positives (CGP)

Extended spectrum β -lactamase (ESBL)

Metallo- β -lactamase (M β L)

Resistant multidrug (MDR)

Klebsiella pneumoniae carbapenemases (KPC)

New Delhi metallo- β -lactamases (NDM)

Polymerase Chain Reaction (PCR)

Staphylococcus aureus resistance to methicillin (MRSA)

Staphylococcus coagulase negative resistance to methicillin (MRCoNS)

Declarations

Statements:

- Financing
- Interest conflicts
- Availability of data and materials
- Code Availability
- Ethics approval
- Consent to participate
- Consent to publication
- Author Contribution Statement
- Acknowledgment

Financing

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Interest conflicts

All authors declare no conflict of interest.

Availability of data and materials

All data generated or analyzed during this study is included in this published article.

Code Availability

Not applicable

Ethics approval

Written informed consent for the publication of their clinical details was obtained from the patient's parents. A copy of the consent form is available for review by the Editor of this magazine. Written informed consent was given and maintained by the authors. This work was approved by the Research Ethics Committee of the Pernambuco Cancer Hospital (HCP) in which the work was carried out (CAAE: 16285219.5.0000.5205).

Consent to participate

Consent to participate was obtained by the patient or guardian in case of death, by signing the Informed Consent Form (ICF) approved by the CEP of the Cancer Hospital of Pernambuco, following resolution 466/12 of the National Health Council.

Consent to publication

Consent for publication was obtained by the patient, signing the Free and Informed Consent Term (ICF) approved by the CEP of the Pernambuco Cancer Hospital, following resolution 466/12 of the National Health Council.

Author Contribution Statement

PSRA and VMS conceived and designed surveys. CRPS, CPI and MJBS conducted experiments. NCL and DEX contributed new reagents or analytical tools, correction. CRPS wrote the manuscript. All authors read and approved the manuscript.

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References

1. Klustersky, J.; Amey, L.; Maertns, J., et al. Bacteremia in febrile neutropenic cancer patients. *Int J. Antimicrob Agent.* 2007;30,51-59.
2. Cattaneo, C. Antoniazzi, F. Casari, S. et al. *P. aeruginosa* blood-stream infection among hematological patients: an old or new question? *Ann Hematol.* 2012;91(8),1299-1304.
3. Aslan, S.; Citak, E. C.; Yis, R.; Degirmenci, S.; Arman, D. Bacterial Spectrum and antimicrobial susceptibility pattern of bloodstream infections in children with febrile neutropenia: experience of single center in southeast of Turkey. *Indian J. Microbiol.* 2012; 52(2),203-208.
4. Mandal, P. K.. Micro-organisms Associated with Febrile Neutropenia in Patients with Haematological Malignancies in a Tertiary Care Hospital in Eastern India. *Indian J Hematol Blood Transfus.* 2015;31(1):46–50.
5. Conn JR, Catchpoole EM, Runnegar N, Mapp SJ, Markey KA. Low rates of antibiotic resistance and infectious mortality in a cohort of high-risk hematology patients: A single center, retrospective analysis of blood stream infection. *PLoS One.* 2017;12(5): e0178059. doi: 10.1371 / journal.pone.0178059.
6. Di Blasi R, Cattaneo C, Lewis RE, Tumbarello M, Angelici L, Dragonetti G, et al. Febrile events in acute lymphoblastic leukemia: a prospective observational multicentric SEIFEM study (SEIFEM-2012/B ALL). *Ann Hematol.* 2018;97(5):791-798. doi: 10.1007/s00277-018-3252-6.
7. Mert D, Ceken S, Iskender G, Iskender D, Merdin A, Duygu F, Ertek M, Altuntas F. Epidemiology and mortality of bacterial bloodstream infections in patients with hematologic diseases. *J Infect Dev Ctries.* 2019;13:727-735. doi: 10.3855 / jidc.11457
8. Jiang AM, Shi X, Liu N, Gao H, Ren MD, Zheng XQ, Fu X, Liang X, Ruan ZP, Yao Y, Tian T. Nosocomial infections due to multidrug-resistant bacteria in cancer patients: a six-year retrospective study of an oncology Center in Western China. *BMC Infect Dis.* 2020;29;20(1):452. doi: 10.1186/s12879-020-05181-6.
9. Garrido MM, Garrido RQ, Cunha TN, Ehrlich S, Martins IS. Comparison of epidemiological, clinical and microbiological characteristics of bloodstream infection in children with solid tumours and haematological malignancies. *EpidemiolInfect.* 2019;8;147:e298. doi: 10.1017/S0950268819001845.
10. OPAS - Organização Pan-Americana da Saúde. Dez ameaças à saúde que a OMS combaterá em 2019. Brasília (DF). 2019. https://www.paho.org/bra/index.php?option=com_content&view=article&id=5848:dez-ameacas-a-saude-que-a-oms-combatera-em-2019&Itemid=875. Accessed 26 dezembro 2020.
11. Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1- and OXA-181- producing Enterobacteriaceae in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program, 2006–2007. *Antimicrob Agents Chemother.* 2011;55:1274–8.
12. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev.* 2005;18(4):657-86. doi: 10.1128/CMR.18.4.657-686.2005.
13. Ceken S, Iskender G, Gedik H, Duygu F, Mert D, Kaya AH, Altuntas F, Ertek M. Risk factors for bloodstream infections due to extended-spectrum β -lactamase producing Enterobacteriaceae in cancer patients. *J Infect Dev Ctries.* 2018;30;12(4):265-272. doi: 10.3855/jidc.9720.
14. Mitsuboshi S, Tsuruma N, Watanabe K, Takahashi S, Nakashita M, Ito A, Kobayashi K, Tsugita M. Does Quick Sepsis-Related Organ Failure Assessment Suggest the Use of Initial Empirical Carbapenem Therapy in Bacteremia Caused by Extended-Spectrum β -Lactamase-Producing Bacteria?: A Multicenter Case-Control Study. *Jpn J Infect Dis.* 2019;25;72(2):124-126. doi: 10.7883/yoken.JJID.2018.272.
15. Tohamy ST, Aboshanab KM, El-Mahallawy HA, El-Ansary MR, Afifi SS. Prevalence of multidrug-resistant Gram-negative pathogens isolated from febrile neutropenic cancer patients with bloodstream infections in Egypt and new synergistic antibiotic combinations. *Infect Drug Resist.* 2018;25;11:791-803. doi: 10.2147/IDR.S163293.
16. Calvo-Lon J, Landaverde DU, Ramos-Esquivel A, Villalobos-Vindas JM. Epidemiology and Outcomes of Bloodstream Infections in Patients With Solid Tumors in a Central American Population at Mexico Hospital, San Jose, Costa Rica. *J Glob Oncol.* 2018;4(4):1-6. doi: 10.1200/JGO.17.00058.
17. Cattaneo C, Di Blasi R, Skert C, et al. Bloodstream infections in haematological cancer patients colonized by multidrug-resistant bacteria. *Ann Hematol.* 2018;97(9):1717-1726. doi:10.1007/s00277-018-3341-6
18. Nham, E., Huh, K., Cho, SY, Chung, DR, Peck, KR, Lee, NY, & Kang, CI. Characteristics and clinical outcomes of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* bacteria in cancer patients. *Infection and chemotherapy.* 2020;52(1),59–69. <https://doi.org/10.3947/ic.2020.52.1.59>

19. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, et al. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *Lancet Infect Dis*. 2017;17(2):153-163. doi: 10.1016/S1473-3099(16)30257-2.
20. Clinical and Laboratory Standards Institute. (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts; Third Edition. 2008, CLSI document M27-A3. Wayne, PA: Clinical and Laboratory Standards Institute.
21. Clinical and Laboratory Standards Institute. (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts; fourth informational supplement. 2012, (Document M27-S4), Wayne: Clinical and Laboratory Standards Institute.
22. Inácio CP, Rocha AP, Barbosa RN, Oliveira NT, Silva JC, de Lima-Neto RG, Macêdo DP, Neves RP. (2016). Experimental white piedra: a robust approach to ultrastructural analysis, scanning electron microscopy and etiological discoveries. *Exp Dermatol*. 2016;25(1):79-81. doi: 10.1111/exd.12884.
23. Kurtzman, CP, & Robnett, CJ (1997). Identification of clinically important ascomycete yeasts based on nucleotide divergence at the 5' end of the large subunit ribosomal DNA (26S) gene. *Journal of Clinical microbiology*. 1997;35(5),1216-1223. <https://doi.org/10.1128/JCM.35.5.1216-1223.1997>
24. Menezes LC, Rocchetti TT, BauabKde C, Cappellano P, Quiles MG, Carlesse F, de Oliveira JS, Pignatari AC. Diagnosis by real-time polymerase chain reaction of pathogens and antimicrobial resistance genes in bone marrow transplant patients with bloodstream infections. *BMC Infect Dis*. 2013;5;13:166. doi: 10.1186/1471-2334-13-166.
25. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex real-time PCR. *J Antimicrob Chemother*. 2012;67(4):906-9. doi: 10.1093/jac/dkr563.
26. Nithia N, Remitha R, Jayasree PR, Faisal M, ManishKumar PR. Analysis of beta-lactamases, blaNDM-1 phylogeny & plasmid replicons in multidrug-resistant *Klebsiella* spp. from a tertiary care centre in south India. *Indian J Med Res*. 2017;146(1):38-45. doi: 10.4103 / ijmr.IJMR_31_16
27. Mendes RE, Kiyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC, Pignatari AC, Tufik S. Rapid detection and identification of metallo-beta-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. *J Clin Microbiol*. 2007;45(2):544-7. doi: 10.1128/JCM.01728-06.
28. Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*. 2004;48(1):15-22. doi: 10.1128/aac.48.1.15-22.2004.
29. Amudhan, S.M., Sekar, U., Arunagiri, K., Sekar, B. (2011). OXA beta-lactamase mediated carbapenem resistance in *Acinetobacter baumannii*. *Indian J Med Microbio*. 2011;29(3),269-274. doi: 10.4103 / 0255-0857.83911.
30. Ambler, R. P. The structure of beta-lactamases. *Philosophical Transactions of the Royal Society Biological Sciences, London*. 1980;289(36),321-331. doi: 10.1098/rstb.1980.0049.
31. Lima Barbieri N, Nielsen DW, Wannemuehler Y, Cavender T, Hussein A, Yan SG, Nolan LK, Logue CM. mcr-1 identified in Avian Pathogenic *Escherichia coli* (APEC). *PLoS One*. 2017;6;12(3):e0172997. doi: 10.1371/journal.pone.0172997.
32. Kara Ö, Zarakolu P, Aşçıoğlu S, Etgül S, Uz B, Büyükaşık Y, Akova M. Epidemiology and emerging resistance in bacterial bloodstream infections in patients with hematologic malignancies. *Infect Dis (Lond)*. 2015;47(10):686-93. doi: 10.3109/23744235.2015.1051105.
33. Gudiol C, Aguado JM, Carratalà J. Bloodstream infections in patients with solid tumors. *Virulence*. 2016;2;7(3):298-308. doi: 10.1080/21505594.2016.1141161.
34. Lubwama M, Phipps W, Najjuka CF, Kajumbula H, Ddungu H, Kambugu JB, Bwanga F. Bacteremia in febrile cancer patients in Uganda. *BMC Res Notes*. 2019;30;12(1):464. doi: 10.1186/s13104-019-4520-9.
35. Liang T, Xu C, Cheng Q, Tang Y, Zeng H, Li X. Epidemiology, Risk Factors, and Clinical Outcomes of Bloodstream Infection due to Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* in Hematologic Malignancy: A Retrospective Study from Central South China. *Microb Drug Resist*. 2021;27(6):800-808. doi: 10.1089/mdr.2020.0033.
36. Abbasi Montazeri E, Khosravi AD, Saki M, Sirous M, Keikhaei B, Seyed-Mohammadi S. Prevalence of Extended-Spectrum Beta-Lactamase-Producing *Enterobacteriaceae* Causing Bloodstream Infections in Cancer Patients from Southwest of Iran. *Infect Drug Resist*. 2020;6;13:1319-1326. doi: 10.2147/IDR.S254357.
37. Paul M, Bhatia M, Rekha US, Diksha 1, Omar BJ, Gupta P. Microbiological Profile of Blood Stream Infections in Febrile Neutropenic Patients at a Tertiary Care Teaching Hospital in Rishikesh, Uttarakhand. *J Lab Physicians*. 2020;12(2):147-153. doi: 10.1055/s-0040-1716661.
38. Patel HG, Tabassum S, Shaikh S. *E. coli* Sepsis: Red Flag for Colon Carcinoma-A Case Report and Review of the Literature. *Case Rep Gastrointest Med*. 2017;2570524. doi: 10.1155/2017/2570524.
39. Ota A, Morita S, Matsuoka A, Shimokata T, Maeda O, Mitsuma A, Yagi T, Asahara T, Ando Y. Detection of bacteria in blood circulation in patients receiving cancer chemotherapy. *Int J Clin Oncol*. 2020;25(1):210-215. doi: 10.1007/s10147-019-01521-y.

40. Bhat S, Muthunatarajan S, Mulki SS, Archana Bhat K, Kotian KH. Bacterial Infection among Cancer Patients: Analysis of Isolates and Antibiotic Sensitivity Pattern. *Int J Microbiol.* 2021;7;2021:8883700. doi: 10.1155/2021/8883700.
41. Ghosh S, Chakraborty M, Samanta S, Sinha N, Saha S, Chattopadhyay A, et al. Analysis of blood stream infections, antibiograms and clinical outcomes in haematological patients with febrile neutropenia: data from a tertiary care haematology institute in India. *Ann Hematol.* 2021;100(2):395-403. doi: 10.1007/s00277-020-04324-8.
42. Diekema DJ, Hsueh PR, Mendes RE, Pfaller MA, Rolston KV, Sader HS, Jones RN. The Microbiology of Bloodstream Infection: 20-Year Trends from the SENTRY Antimicrobial Surveillance Program. *Antimicrob Agents Chemother.* 2019;24;63(7):e00355-19. doi: 10.1128/AAC.00355-19.
43. Sierra J, Díaz MV, de Jesús García M, Finello M, Suasnabar DF, Richetta L, Toranzo A, Hernández D, Cometto MA, Vázquez SM, Caeiro JP, Saad EJ. Infecciones del torrente sanguíneo en pacientes oncológicos [Bloodstream infections in cancer patients]. *Medicina (B Aires).* 2020;80(4):329-338.
44. Royo-Cebrecos C, Gudiol C, Ardanuy C, Pomares H, Calvo M, Carratalà J. A fresh look at polymicrobial bloodstream infection in cancer patients. *PLoS One.* 2017;24;12(10):e0185768. doi: 10.1371/journal.pone.0185768.
45. Goldman S, Itshaki O, Shochat T, Gaftor-Gvili A, Yahav D, Rubinovitch B, Shepshelovich D. Risk Factors and Outcome of Polymicrobial Bacteremia: A Retrospective Cohort Study. *Isr Med Assoc J.* 2020;22(5):279-284.
46. Agrawal SK, Gautam H, Choudhary AH, Das BK, Kumar L, Kapil A. Central Line-associated Bloodstream Infections in Cancer Patients: An Experience from a Tertiary Care Cancer Centre. *Indian J Med Microbiol.* 2019;37(3):376-380. doi: 10.4103/ijmm.IJMM_19_352.
47. Soares, CRP, Magalhães, V., Araújo, PSR. Coexistence of blaTEM, blaCTX, blaKPC, blaNDM, blaSIM e blaOXA-48 in polymicrobial bloodstream isolates from a patient with acute myeloid leukemia. *Research, Society and Development*, 2021;10(5)e39310514985. doi: 10.33448/rsd-v10i5.14985.
48. Carlesse F, Cappellano P, Quiles MG, Menezes LC, Petrilli AS, Pignatari AC. Clinical relevance of molecular identification of microorganisms and detection of antimicrobial resistance genes in bloodstream infections of paediatric cancer patients. *BMC Infect Dis.* 2016;1;16(1):462. doi: 10.1186/s12879-016-1792-8.
49. Fentie A, Wondimeneh Y, Balcha A, Amsalu A, Adankie BT. Bacterial profile, antibiotic resistance pattern and associated factors among cancer patients at University of Gondar Hospital, Northwest Ethiopia. *Infect Drug Resist.* 2018;8;11:2169-2178. doi: 10.2147/IDR.S183283.
50. Vahedian-Ardakani HA, Moghimi M, Shayestehpour M, Doosti M, Amid N. Bacterial Spectrum and Antimicrobial Resistance Pattern in Cancer Patients with Febrile Neutropenia. *Asian Pac J Cancer Prev.* 2019;25;20(5):1471-1474. doi: 10.31557/APJCP.2019.20.5.1471.
51. Islas-Muñoz B, Volkow-Fernández P, Ibanes-Gutiérrez C, Villamar-Ramírez A, Vilar-Compte D, Cornejo-Juárez P. Bloodstream infections in cancer patients. Risk factors associated with mortality. *Int J Infect Dis.* 2018;71:59-64. doi: 10.1016/j.ijid.2018.03.022.
52. Kang CI, Chung DR, Ko KS, Peck KR, Song JH; Korean Network for Study of Infectious Diseases. Risk factors for infection and treatment outcome of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* bacteremia in patients with hematologic malignancy. *Ann Hematol.* 2012;91(1):115-21. doi: 10.1007/s00277-011-1247-7.
53. Zhang Q, Gao HY, Li D, et al. Clinical outcome of bloodstream infection by *Escherichia coli* in cancer patients with / without biofilm formation: a single center retrospective study. *Infect Drug Resist.* 2019;12:359–371. doi: 10.2147 / IDR.S192072
54. Tawfik MM, Alshareef WA, Bendary HA, Elmahalawy H, Abdullal AK. The emergence of carbapenemase bla_{NDM} genotype among carbapenem-resistant Enterobacteriaceae isolates from Egyptian cancer patients. *Eur J Clin Microbiol Infect Dis.* 2020;39(7):1251-1259. doi: 10.1007/s10096-020-03839-2.
55. Satlin MJ, Calfee DP, Chen L, Fauntleroy KA, Wilson SJ, Jenkins SG, Feldman EJ, Roboz GJ, Shore TB, Helfgott DC, Soave R, Kreiswirth BN, Walsh TJ. Emergence of carbapenem-resistant Enterobacteriaceae as causes of bloodstream infections in patients with hematologic malignancies. *Leuk Lymphoma.* 2013;54(4):799-806. doi: 10.3109/10428194.2012.723210.
56. Nordmann P, Poirel L. Epidemiology and Diagnostics of Carbapenem Resistance in Gram-negative Bacteria. *Clin Infect Dis.* 2019;13;69(Suppl 7):S521-S528. doi: 10.1093/cid/ciz824.
57. Tarafdar F, Jafari B, Azimi T. Evaluating the antimicrobial resistance patterns and molecular frequency of bla_{OXA-48} and bla_{GES-2} genes in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains isolated from burn wound infection in Tehran, Iran. *New Microbes New Infect.* 2020;23;37:100686. doi: 10.1016/j.nmni.2020.100686.
58. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, Amyes SG, Livermore DM. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents.* 2006;27(4):351-3. doi: 10.1016/j.ijantimicag.2006.01.004.
59. Hou C, Yang F. Drug-resistant gene of blaOXA-23, blaOXA-24, blaOXA-51 and blaOXA-58 in *Acinetobacter baumannii*. *Int J Clin Exp Med.* 2015;15;8(8):13859-63.
60. Sah R, Khadka S, Shrestha GS, Acharya S, Aryal D, Shrestha P, Kattel HP, Shah NP, Pokhrel BM, Singh YP, Rijal B, Erdem H. Detection of Pan drug resistance OXA-48 producing *Providencia* in an ICU patient for the first time in Nepal. *Antimicrob Resist Infect Control.* 2019;15;8:155. doi: 10.1186/s13756-019-0608-1.

61. Hashem H, Hanora A, Abdalla S, Shaeky A, Saad A. Dissemination of metallo- β -lactamase in *Pseudomonas aeruginosa* isolates in Egypt: mutation in blaVIM-4. *APMIS*. 2017;125(5):499-505. doi: 10.1111/apm.12669.
62. Santos AL, Dos Santos AP, Ito CRM, Queiroz PHP, de Almeida JA, de Carvalho Júnior MAB, de Oliveira CZ, Avelino MAG, Wastowski IJ, Gomes GPLA, Souza ACSE, Vasconcelos LSNOL, Santos MO, da Silva CA, Carneiro LC. Profile of Enterobacteria Resistant to Beta-Lactams. *Antibiotics (Basel)*. 2020;15(9):410. doi: 10.3390/antibiotics9070410.
63. Jácome PRLA, Alves LR, Jácome-Júnior AT, Silva MJBD, Lima JLDC, Araújo PSR, Lopes ACS, Maciel MAV. Detection of blaSPM-1, blaKPC, blaTEM and blaCTX-M genes in isolates of *Pseudomonas aeruginosa*, *Acinetobacter* spp. and *Klebsiella* spp. from cancer patients with healthcare-associated infections. *J Med Microbiol*. 2016;65(7):658-665. doi: 10.1099/jmm.0.000280.
64. Monaco M, Pimentel de Araujo F, Cruciani M, Coccia EM, Pantosti A. Worldwide Epidemiology and Antibiotic Resistance of *Staphylococcus aureus*. *Curr Top Microbiol Immunol*. 2017;409:21-56. doi: 10.1007/82_2016_3.
65. Li Z, Zhuang H, Wang G, Wang H, Dong Y. Prevalence, predictors, and mortality of bloodstream infections due to methicillin-resistant *Staphylococcus aureus* in patients with malignancy: systemic review and meta-analysis. *BMC Infect Dis*. 2021;14;21(1):74. doi: 10.1186/s12879-021-05763-y.
66. Atmaca O, Zarakolu P, Karahan C, Cakir B, Unal S. Hacettepe Üniversitesi Erişkin ve Onkoloji Hastanelerinde yatan hastalarda haftanın metisiline dirençli *Staphylococcus aureus* bakteriyemilerinde risk faktörleri (2004-2011), antibiyotik kullanımı ve izolatların antimikrobiyal duyarlılıkları: Yuvalandırılmış olgu-kontrol çalışması [Risk factors and antibiotic use in methicillin-resistant *Staphylococcus aureus* bacteremia in hospitalized patients at Hacettepe University Adult and Oncology Hospitals (2004-2011) and antimicrobial susceptibilities of the isolates: a nested case-control study]. *Mikrobiyol Bul*. 2014;48(4):523-37. Turkish. doi: 10.5578/mb.8280.
67. Amarnani R, Rapose A. Colon cancer and enterococcus bacteremia co-affection: A dangerous alliance. *J Infect Public Health*. 2017;10(5):681-684. doi: 10.1016/j.jiph.2016.09.009. Epub 2017 Jan 29.
68. Alves J, Palma P, Azevedo D, Rello J. Candidemia in the patient with malignancy. *Hosp Pract (1995)*. 2018;46(5):246-252. doi: 10.1080/21548331.2018.1508290.
69. Wu PF, Liu WL, Hsieh MH, Hii IM, Lee YL, Lin YT, Ho MW, Liu CE, Chen YH, Wang FD. Epidemiology and antifungal susceptibility of candidemia isolates of non-albicans *Candida* species from cancer patients. *Emerg Microbes Infect*. 2017;11;6(10):e87. doi: 10.1038/emi.2017.74.
70. Tang HJ, Liu WL, Lin HL, Lai CC. Epidemiology and prognostic factors of candidemia in cancer patients. *PLoS One*. 2014;5;9(6):e99103. doi: 10.1371/journal.pone.0099103.
71. Guo LN, Yu SY, Xiao M, Yang CX, Bao CM, Yu YH, Ye LY, Yang Y, Zhang G, Liu J, Liang GW, Min R, Zhu Y, Lei H, Liu YL, Liu LJ, Hu YJ, Hsueh PR, Xu YC. Species Distribution and Antifungal Susceptibility of Invasive Candidiasis: A 2016-2017 Multicenter Surveillance Study in Beijing, China. *Infect Drug Resist*. 2020;20;13:2443-2452. doi: 10.2147/IDR.S255843.
72. Shekari Ebrahim Abad H, Zaini F, Kordbacheh P, Mahmoudi M, Safara M, Mortezaee V. In Vitro Activity of Caspofungin Against Fluconazole-Resistant *Candida* Species Isolated From Clinical Samples in Iran. *Jundishapur J Microbiol*. 2015;27;8(6):e18353. doi: 10.5812/jjm.18353v2.
73. Chapman B, Slavin M, Marriott D, Halliday C, Kidd S, Arthur I, Australian and New Zealand Mycoses Interest Group. Changing epidemiology of candidaemia in Australia. *J Antimicrob Chemother*. 2017;1;72(4):1103-1108. doi: 10.1093/jac/dkw422.
74. Malani AN, Kerr LE, Kauffman CA. Voriconazole: How to Use This Antifungal Agent and What to Expect. *Semin Respir Crit Care Med*. 2015;36(5):786-95. doi: 10.1055/s-0035-1562903.
75. Cowen LE, Sanglard D, Howard SJ, Rogers PD, Perlin DS. Mechanisms of Antifungal Drug Resistance. *Cold Spring Harb Perspect Med*. 2014;10;5(7):a019752. doi: 10.1101/cshperspect.a019752.
76. Bibi M, Murphy S, Benhamou RI, Rosenberg A, Ulman A, Bicanic T, Fridman M, Berman J. Combining Colistin and Fluconazole Synergistically Increases Fungal Membrane Permeability and Antifungal Cidality. *ACS Infect Dis*. 2021;20. doi: 10.1021/acsinfecdis.0c00721.
77. Sharafutdinov, I.S. et al. Increasing Susceptibility of Drug-Resistant *Candida albicans* to Fluconazole and Terbinafine by 2(5*H*)-Furanone Derivative. *Molecules*. 2020;25(3):642. doi: 10.3390/molecules25030642
78. Berkow E.L., Lockhart S.R. Fluconazole resistance in *Candida* species: A current perspective. *Infect. Drug Resist*. 2017;10:237–245. doi: 10.2147/IDR.S118892.

Tables

Adjusted according to the classification of β -lactamases by Ambler (1980)³⁰.

Table 2. Profile of antimicrobial resistance in organisms isolated from infection in the bloodstream of cancer patients through automated testing using the vitek 2® platform.

Table 1 - Sequence of primers of the carbapenemase resistance genes with the respective ringing temperatures and amplicon size.

Carbapenemases	Genes of Carbapenemases	Primer Sequence (5'-3')	Amplicon size in pb	Temperature (t) °C	References
A Class	<i>bla</i> _{KPC-F}	ATGTCACTGTATCGCCGTCTAGTTC	800	60°C	Menezes et al, 2013 ²⁴
	<i>bla</i> _{KPC-R}	CAATCCCTCGAGCGCGAGTC			
	<i>bla</i> _{GES-F}	CTATTACTGGCAGGGATCG	594	55°C	Monteiro, 2012 ²⁵
	<i>bla</i> _{GES-R}	CCTCTCAATGGTGTGGGT			
B Class	<i>bla</i> _{NDM-F}	CGGAATGGCTCATCACGATC	580-605	60°C	Nithia et al, 2017 ²⁶
	<i>bla</i> _{NDM-R}	GGTTTGGCGATCTGGTTTTTC			
	<i>bla</i> _{IMP-F}	GAATAG(A\G) (A\G)TGGCTTAA(C\T)TCTC	188	52°C	Mendes, 2007 ²⁷
	<i>bla</i> _{IMP-R}	CAAAC(C\T)ACTA(G\C)GTTATC			
	<i>bla</i> _{VIM-F}	GTTTGGTCGCATATCGCAAC	382	52°C	Mendes, 2007 ²⁷
	<i>bla</i> _{VIM-R}	AATGCGCAGCACCAGGATAG			
	<i>bla</i> _{SPM-F}	CTAAATCGAGAGCCCTGCTTG	798	52°C	Mendes, 2007 ²⁷
	<i>bla</i> _{SPM-R}	CCTTTTCCGCGACCTTGATC			
	<i>bla</i> _{GIM-F}	TCAATTAGCTCTTGGGCTGAC	72	55°C	Mendes, 2007 ²⁷
	<i>bla</i> _{GIM-R}	CGGAACGACCATTGAATGG			
D Class	<i>bla</i> _{SIM-F}	GTACAAGGGATTCGGCATCG	569	55°C	Mendes, 2007 ²⁷
	<i>bla</i> _{SIM-R}	TGGCCTGTTCCCATGTGAG			
	<i>bla</i> _{OXA-48-F}	TTGGTGGCATCGATTATCGG	743	52°C	Poirel et al, 2004 ²⁸
	<i>bla</i> _{OXA-48-R}	GAGCACTTCTTTTGTGATGGC			
	<i>bla</i> _{OXA-58-F}	AAGTATTGGGGCTTGTGCTG	599	52°C	Amudhan et al, 2011 ²⁹
	<i>bla</i> _{OXA-58-R}	CCCCTCTGCGCTCTACATAC			

Resistance profile against antimicrobials	(n°)	(%)
Gram-negatives	38	
Acid. Nalidixic	10	26,3%
Ciprofloxacin	13	34,2%
Norfloxacin	7	18,4%
Amoxicillin/clavulanate	3	7,9%
Ampicilin/subactam	4	10,5%
Piperacilin/tazobactam	8	21%
Cephalothin	10	26,3%
Cefepime	15	39,4%
Ceftriaxone	10	26,3%
Cefuroxime	11	29%
Cefoxitin	1	2,6%
Ceftazidime	5	13%
Amikacin	6	15,7%
Gentamycin	9	23,6%
Meropenem	7	18,4%
Imipenem	4	10,5%
Ertapenem	2	5,2%
Ampicillin	24	63,15%
Tigecycline	4	10,5%
Sulfamethoxazole/trimethoprim	4	10,5%
Gram-positive	13	
Oxacillin	6	46%
Penicillin	4	30,7%
Erythromycin	5	38,4%
Clindamycin	3	23%
Sulfamethoxazole/trimethoprim	3	23%
Gentamycin	4	30,7%
Vancomycin	1	7,7%
Daptomycin	0	
Tigecycline	0	
Linezolid	0	
Ceftaroline	0	

Table 3. Resistance profile against bacterial isolates in cancer patients.

Gram-negatives	(14) <i>Escherichia coli</i>	(11) <i>Klebsiella spp</i>	(7) <i>Pseudomonas spp</i>	(3) <i>Acinetobacter baumannii</i>	(2) <i>Serratia marcescens</i>	(1) <i>Salmonel</i>
Acid. Nalidixic	6 (42,8%)	2 (18,2%)			1 (50%)	
Amikacin			3(42,8%)		2 (100%)	1 (100%)
Amoxicillin/clavulanate		1 (9%)			2 (100%)	
Ampicillin	11 (78,5%)	11 (100%)			2 (100%)	
Ampicilina/sulbactam	2 (14,28)	1 (9%)		1 (33,33%)		
Cephalothin	4 (28,5%)	3 (27,3%)			2 (100%)	1 (100%)
Cefepime	4 (28,5%)	4 (36,3%)	3(42,8%)	2 (66,6%)	2 (100%)	
Ceftriaxone	4 (28,5%)	4 (36,3%)			2 (100%)	
Cefuroxime	4 (28,5%)	4 (36,3%)			2 (100%)	1 (100%)
Cefoxitin		1 (9%)				
Ceftazidime	1 (7,14%)	1 (9%)	2 (28,6%)	1 (33,33%)		
Ciprofloxacin	5 (35,7%)	3 (27,3%)	3(42,8%)	2 (66,6%)		
Ertapenem		1 (9%)			1 (50%)	
Gentamycin	2 (14,28%)	2 (18,2%)	3(42,8%)			1 (100%)
Meropenem		1 (9%)	3 (42,8%)	2 (66,6%)	1 (50%)	
Imipenem		1 (9%)	2 (28,6%)	1 (33,33%)		
Norfloxacin	4 (28,5%)	2 (18,2%)	1 (14,3%)			
Piperacilin/tazobactam		2 (18,2%)	3 (42,8%)	2 (66,6%)	1 (50%)	
Tigecycline			4 (57,1%)			
Sulfametoxazole/trimetoprim	3 (21,4%)	1 (9%)				
Gram-positives	(5) <i>Staphylococcus aureus</i>	(6) <i>Staphylococcus coagulase negativo</i>	(1) <i>Enterococcus faecium</i>	(1) <i>Enterococcus faecalis</i>		
Gentamycin	2 (40%)	2 (33,33%)				
Sulfametoxazole/trimetoprim	2 (40%)	1 (16,6%)				
Clindamycin	2 (40%)	1 (16,6%)				
Erythromycin	2 (40%)	3 (50%)				
Oxacilin	3 (60%)	3 (50%)				
Penicilin	2 (40%)	2 (33,33%)				
Rifampicin	1 (20%)	2 (33,33%)				
Vancomycin			1 (100%)			
Levofloxacin	2 (40%)	1 (16,6%)				
Ampicillin			1 (100%)			

Table 4. Detection of ESBL and M β L resistance genes in bloodstream isolates from cancer patients.

ID	Pathogen	Genes
1HCP334	<i>Escherichia coli</i>	SHV, TEM, KPC, NDM, IMP, GES, SIM, OXA-48 e OXA-58
2HCP1243	<i>Escherichia coli</i>	TEM, KPC, NDM, IMP, VIM, GES, SIM, OXA-48 E OXA-58
2HCP1121		CTX, NDM, SPM, GES, SIM, OXA-48
3HCP291	<i>Escherichia coli</i>	TEM, KPC, NDM, IMP, VIM, GES, SIM, OXA-48 E OXA-58
4HCP649	<i>Escherichia coli</i>	TEM, KPC, IMP, GES, SIM, OXA-48 E OXA-58
5HCP860	<i>Escherichia coli</i>	TEM, KPC, IMP, VIM, GES, SIM, OXA-48 E OXA-58
6HCP868	<i>Klebsiellapneumoniae</i>	TEM, KPC, IMP, VIM, GES, SIM, OXA-48
7HCP1058	<i>Escherichia coli</i>	TEM, CTX, NDM, VIM, GES, SIM, OXA-58
8HCP1112	<i>Klebsiellapneumoniae</i>	TEM, CTX, NDM, VIM, SPM, GES, SIM, OXA-48 E OXA-58
8HCP1112	<i>Escherichia coli</i>	TEM, CTX, NDM, VIM, GES, SIM, OXA-48 E OXA-58
9HCP1118	<i>Pseudomonasaeruginosa</i>	CTX, KPC, NDM, SPM
10HCP1405	<i>Klebsiellapneumoniae</i>	CTX, NDM, GES, SIM, OXA-48
12HCP1060	<i>Staphylococcuscoagulase Negativa</i>	<i>mecA</i>
13HCP1401	<i>Serratiamarcescens</i>	CTX, NDM, GES, SIM, OXA-48
14HCP1257	<i>Klebsiellapneumoniae</i>	TEM, CTX, GES, SIM, OXA-48
15HCP1253	<i>Pseudomonasalcaligenes</i>	SPM, SIM, OXA-48
16HCP1191	<i>Staphylococcusepidermidis</i>	<i>mecA</i>
17HCP1177	<i>Staphylococcus aureus</i>	-
19HCP888	<i>Klebsiellapneumoniae</i>	TEM, CTX, NDM, GES, SIM, OXA-48
20HCP7	<i>Escherichia coli</i>	SIM, OXA-48
21HCP61	<i>Pseudomonasaeruginosa</i>	SIM, OXA-48
22HCP07	<i>Escherichia coli</i>	TEM, CTX, NDM, SIM, OXA-48
23HCP1251	<i>Pseudomonasaeruginosa</i>	CTX, SPM, GES, SIM, OXA-48
24HCP921	<i>Klebsiellaoxytoca</i>	GES
25HCP796	<i>Staphylococcusepidermidis</i>	-
26HCP127	<i>Staphylococcuswarneri</i>	-
27HCP111	<i>Staphylococcus aureus</i>	-
28HCP1168	<i>Acinetobacterbaumannii</i>	SHV, TEM, SPM, GES, OXA-58
29HCP333	<i>Serratiamarcescens</i>	TEM, CTX, KPC, SPM, OXA-48, OXA-58
30HCP263	<i>Klebsiellapneumoniae</i>	CTX, KPC, NDM, GES, SIM
31HCP478	<i>Escherichia coli</i>	TEM, OXA-48
32HCP467	<i>Streptococcussalivarius</i>	-
33HCP604	<i>Enterococcusfaecalis</i>	<i>mecA</i>
34HCP682	<i>Escherichia coli</i>	TEM, OXA-48
35HCP747	<i>Pseudomonasaeruginosa</i>	KPC, SIM, OXA-48
36HCP835	<i>Salmonellagroup</i>	OXA-48
37HCP952	<i>Staphylococcus aureus</i>	<i>mecA</i>
38HCP1326	<i>Acinetobacterbaumannii</i>	TEM, OXA-48
39HCP1336	<i>Klebsiellapneumoniae</i>	TEM
40HCP1220	<i>Pseudomonasaeruginosa</i>	TEM, SIM
40HCP1398	<i>Escherichia coli</i>	TEM, OXA-48

40HCP49	<i>Escherichia coli</i>	TEM, CTX
40HCP263	<i>Klebsiellapneumoniae</i>	CTX, KPC, NDM, SIM, OXA-48
40HCP419	<i>Acinetobacterbaumannii</i>	OXA-48
41HCP4	<i>Enterococcusfaecium</i>	<i>VanA</i>
42HCP223	<i>Staphylococcus aureus</i>	<i>mecA</i>
43HCP109	<i>Staphylococcusepidermidis</i>	<i>mecA</i>
44HCP327	<i>Klebsiellapneumoniae</i>	SIM, OXA-48
45HCP480	<i>Staphylococcus aureus</i>	-
46HCP526	<i>Escherichia coli</i>	TEM, CTX, NDM, GES, SIM OXA-48

Table 5- Antifungal susceptibility test for *Candida* spp. in isolates obtained from cancer patients.

Antifungal treatment / MIC values (µg / ml)								
PT	Id	Strains	AMB	FLZ	VRZ	CPF	MCF	ANF
1	1047	<i>C. tropicalis</i>	0,03	32	16	0,06	0,03	0,03
2	1173	<i>C. albicans</i>	0125	8	16	0,125	0,03	0,03
3	1345	<i>C. tropicalis</i>	1	32	16	0,5	0,03	0,125
4	539	<i>C. parapsilosis</i>	0,5	0,5	16	0,06	0,06	0,06

PT- patients. Id - identification code number. AMB - amphotericin B, FLZ - fluconazole, VRZ - voriconazole, CPF - caspofugin, MCF - micafugin, ANF - anidulafugin.

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