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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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\textit{MP}, bla\textit{VIM}, bla\textit{GAM}, bla\textit{SIM}, bla\textit{SPM}, bla\textit{KPC}, bla\textit{NDM}, genes oxacillinase bla\textit{OXA-48}, bla\textit{OXA-58}, and the presence of ESBL genes bla\textit{SHV}, bla\textit{CTX}, bla\textit{TEM} for Gram-negatives, as well as, mecA, vanA and vanB for Gram-positives were investigated in blood culture isolates. Result: \textit{Escherichia coli} and \textit{Klebsiella pneumoniae} were the most frequent pathogens. The serine-\(\beta\)-lactamase gene bla\textit{OXA-48} was the most frequent, followed by MBL bla\textit{SIM}, bla\textit{TEM} and bla\textit{CTX} were the most common among ESBL. The mecA and vanA genes were found in \textit{Staphylococcus spp} and \textit{Enterococcus faecium}, respectively. \textit{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.

**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 \(\geq 18\) 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’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 \textit{Clinical and Laboratory Standards Institute}. For standardization of the experiment, the reference strain ATCC 90028 (\textit{C.albicans}) was used. For the test, the RPMI 1640 medium (Sigma-Aldrich, USA) buffered with morpholinopropane 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 MilliQ 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'-GCAATTCATAAAGCAGGAAGAAGAAG - 3') and NL4 (5'-GGTCCGTCGTTCTAGACGCG - 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 blaSHV, blaTEMP, blactxM 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 for 10 minutes, for each of the studied genes.

And eight families of the metallo-β-lactamase (MβL) genes, (blaKPC, blAES, blalDM, blalIM, blavIM, blasmP, blasG and blasG]) 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 (blaoXA-48, blaoXASB), 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), E. coli (ATCC 12228), E. faecalis (ATCC 29212), E. faecium (ATCC 19434). The primers mecA-F (5'-AAAACTAGTTGGTGAAGATACCC -3') and mecA-R (5'-GGAAGGACTCTGTCTGGTTTACGACT -3') with a fragment size of 147pb, the primers VanA-F (5'-CATGACGTAATCGTGAAAATC -3') and VanA-R (5'-ACCGGGCAGRTTGGAC -3') with fragment size of 732 bp. And the primers VanB-F (5'-CATGATGTTGCGTAAACTCC -3') and VanB-R (5'-ACCGGGCAGRTTGGAC -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 (LocusBiotechnology, 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%), ceftazidime (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 (66,2%), 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 etapenem.

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-lactamas gene was observed in 28/38 (73,7%) gram-negative isolates. The blaTEM gene was detected in 22 isolates, including 10 E. coli, 5 K. pneumoniae, 2 P. aeruginosa and 2 A. baumannii. The blaCTX-M gene was detected in 16 isolates, 7 K. pneumoniae, 5 E. coli and 2 P. aeruginosa. The blasmH gene was detected in only 2 isolates, 1 E. coli and 1 A. baumannii (table 4).

High frequency of 36/38 metallo-β-lactamas genes (94,7%) were detected in the isolates. The gene most often found in the study was a serine-b-lactamase blaxa48 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 blasm gene 27/38 (71%), E. coli (10), K. pneumoniae (9), P. aeruginosa (6), P. alcaligenes (1) and Serratia marcescens (1). The detection of the blasgE 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 blashv.
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*ESBL* 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*IMP* 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 van*A* gene (1/13) (7.7%). The van*B* 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.34-37 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, 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 years.42 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 India.46

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*ESBL* 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 infection.46 Increased resistance to carbapenems, combinations of beta-lactamase inhibitors, beta-lactamase, aminoglycosides, fluoroquinolones and cephalosporins (including high resistance to cefepime) has been observed.41

Our study showed (46.15%) of MRSA, including *S. aureus* and *Staphylococcus coagulase negative* (MRCoNS) species, respectively. Recent studies have observed resistance to methicillin in *Staphylococcus* spp ranging from 38.4% to 93%.46,7 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 2012.42

MDR infections are worrisome phenomena, particularly in cancer patients, due to low therapeutic options and, consequently, a higher risk of mortality.51 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$^{53,26}$.

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 therapy$^{55}$. 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 proteins$^{56}$. This problem reflects the use of different carbapenems in hospitals, in addition to varying according to geographic location$^{56}$.

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 blaOXA-48 gene was detected in 70.83% and 92% in isolates of $P. aeruginosa$ and $A. baumannii$, respectively in Iran$^{57}$. 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 mortality$^{60}$.

We detected a high frequency of $bla_{SIM}$ 27/38 (71%) in enterobacterales. The frequency of $bla_{SIM}$ in Egypt was 48%$^{61}$. And in Brazil, 66.6% were reported in human and animal samples$^{62}$.

We highlight the presence of the $bla_{SPM}$ gene in Pseudomonas spp isolates, as has already been found$^{28}$ and in $A. baumannii$,$^{63}$ who described the first report of the $bla_{SPM}$ gene in Acinetobacter baumannii in Brazil.

Although MRSA is frequent in ICS in the hospital population$^{64}$, few studies mention the occurrence of MRSA in ICS of cancer patients$^{65}$. 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 genotype$^{66}$. 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 event$^{67}$.

Candida species were found in four samples (7.2%). Candida spp. is the most commonly isolated yeast in ICS$^{58}$. Candida tropicalis was the most frequent species in our study, being commonly found in cancer patients$^{59}$. 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 documented$^{70}$.

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 micafungin$^{71}$. 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 fluconazole$^{72}$.

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 fluconazole$^{74}$.

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 drugs$^{75}$.

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 fluconazole$^{76}$.

The worldwide dissemination of resistant isolates, combined with the few available therapeutic options, has negatively influenced the treatment and prognosis of patients$^{77}$. 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 threat$^{78}$.

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 (MβL)
Resistant multidrug (MDR)
*Klebsiella pneumoniae* carbapenemases (KPC)
New Delhi metalo-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

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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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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) ºC | References       |
|----------------|-------------------------|-------------------------|---------------------|--------------------|------------------|
| A Class        | bla\textsubscript{KPC-F} | ATGTCACTGTATCGCGGTACTGTTC | 800                 | 60ºC               | Menezes et al, 2013\textsuperscript{24} |
|                | bla\textsubscript{KPC-R} | CAATCCCTCGAGCCGAGTC     |                     |                    |                  |
|                | bla\textsubscript{GES-F} | CTATTACTGGCAGGGAGTGCG   | 594                 | 55ºC               | Monteiro, 2012\textsuperscript{25} |
|                | bla\textsubscript{GES-R} | CCTCTCAATGGTTGGGTT      |                     |                    |                  |
| B Class        | bla\textsubscript{NDM-F} | CGGAATGGCTCATCACGATC    | 580-605             | 60ºC               | Nithia et al, 2017\textsuperscript{26} |
|                | bla\textsubscript{NDM-R} | GTTGGGCGATCTGGTTTTC     |                     |                    |                  |
|                | bla\textsubscript{IMP-F} | GAATAG(A\(\text{A\|G}))(A\(\text{G\|T})TGCGTTAA(C\(\text{T\|G}))TCTC | 188                 | 52ºC               | Mendes, 2007\textsuperscript{27} |
|                | bla\textsubscript{IMP-R} | CAAAC(C\(\text{T\|A})ACTA(G\(\text{G\|C}))GTATTC | | | |
|                | bla\textsubscript{VIM-F} | GTTGGTGCTCATACGACACAC   | 382                 | 52ºC               | Mendes, 2007\textsuperscript{27} |
|                | bla\textsubscript{VIM-R} | AATGCGCAGCACCAGGATG     |                     |                    |                  |
|                | bla\textsubscript{SPM-F} | CTAAATCGAGCCCTGCTTG     | 798                 | 52ºC               | Mendes, 2007\textsuperscript{27} |
|                | bla\textsubscript{SPM-R} | CCTTTTCCGCGACCTTGATC    |                     |                    |                  |
|                | bla\textsubscript{SIM-F} | TCAATTAGCTCTTGCGGCTGAC  | 72                  | 55ºC               | Mendes, 2007\textsuperscript{27} |
|                | bla\textsubscript{SIM-R} | CGGAACGACCATTTGAATGG    |                     |                    |                  |
| D Class        | bla\textsubscript{OXA-48-F} | TTGGTGCGATCGATTACGG     | 743                 | 52ºC               | Poirel et al, 2004\textsuperscript{28} |
|                | bla\textsubscript{OXA-48-R} | GAGCCTCTTCTTTGTGATGG    |                     |                    |                  |
|                | bla\textsubscript{OXA-58-F} | AAGTATGGCGCTTGCTG       | 599                 | 52ºC               | Amudhan et al, 2011\textsuperscript{29} |
|                | bla\textsubscript{OXA-58-R} | CCCCTCGGCGCTCATACAC     |                     |                    |                  |
## Resistance profile against antimicrobials

|                      | (n°) | (%)    |
|----------------------|------|--------|
| **Gram-negatives**   |      |        |
| Acid. Nalidixic      | 10   | 26,3%  |
| Ciprofloxacin        | 13   | 34,2%  |
| Norfloxacin          | 7    | 18,4%  |
| Amoxicillin/clavulanate | 3 | 7,9%   |
| Ampicillin/subactam  | 4    | 10,5%  |
| Piperacillin/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**    |      |        |
| 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    |        |
| Gram-negatives | (14) *Escherichia coli* | (11) *Klebsiella spp* | (7) *Pseudomonas spp* | (3) *Acinetobacter baumannii* | (2) *Serratia marcescens* | (1) *Salmonella* |
|---------------|------------------------|-----------------------|-----------------------|-------------------------------|------------------------|-----------------|
| Acid. Nalidixic | 6 (42.8%) | 2 (18.2%) | 1 (50%) | | | |
| Amikacin | 3 (42.8%) | 2 (100%) | 1 (100%) | | | |
| Amoxicillin/clavulanate | 1 (9%) | | | | | |
| Ampicillin | 11 (78.5%) | 11 (100%) | | 2 (100%) | | |
| Ampicillin/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%) | 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 | | | | | | |
| 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%) | | | |
| Piperacillin/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) *Staphylococcus aureus* | (6) *Staphylococcus coagulase negativo* | (1) *Enterococcus faecium* | (1) *Enterococcus faecalis* |
|---------------|---------------------------|-------------------------------|----------------|----------------|
| Gentamycin | 2 (40%) | 2 (33.33%) | | |
| Sulfametoxazole/trimetoprim | 2 (40%) | 1 (16.6%) | | |
| Clindamycin | 2 (40%) | 1 (16.6%) | | |
| Erythromycin | 2 (40%) | 3 (50%) | | |
| Oxacillin | 3 (60%) | 3 (50%) | | |
| Penicillin | 2 (40%) | 2 (33.33%) | | |
| Rifampicin | 1 (20%) | 2 (33.33%) | | |
| Vancomycin | | | 1 (100%) | | |
| Levofoxacin | 2 (40%) | 1 (16.6%) | | |
| Ampicillin | | 1 (100%) | | | |
Table 4. Detection of ESBL and MβL resistance genes in bloodstream isolates from cancer patients.

| ID   | Pathogen             | Genes                              |
|------|----------------------|------------------------------------|
| 1HCP334 | *Escherichia coli*  | SHV, TEM, KPC, NDM, IMP, GES, SIM, OXA-48 e OXA-58 |
| 2HCP1243 | *Escherichia coli*  | TEM, KPC, NDM, IMP, VIM, GES, SIM, OXA-48 e OXA-58 |
| 2HCP1121 |                      | CTX, NDM, SPM, GES, SIM, OXA-48 |
| 3HCP291 | *Escherichia coli*  | TEM, KPC, NDM, IMP, VIM, GES, SIM, OXA-48 E OXA-58 |
| 4HCP649 | *Escherichia coli*  | TEM, KPC, IMP, GES, SIM, OXA-48 E OXA-58 |
| 5HCP860 | *Escherichia coli*  | TEM, KPC, IMP, VIM, GES, SIM, OXA-48 E OXA-58 |
| 6HCP868 | *Klebsiella pneumoniae*  | TEM, KPC, IMP, VIM, GES, SIM, OXA-48 |
| 7HCP1058 | *Escherichia coli*  | TEM, CTX, NDM, VIM, GES, SIM, OXA-58 |
| 8HCP1112 | *Klebsiella pneumoniae*  | TEM, CTX, NDM, VIM, SPM, GES, SIM, OXA-48 E OXA-58 |
| 8HCP1112 | *Escherichia coli*  | TEM, CTX, NDM, VIM, GES, SIM, OXA-48 E OXA-58 |
| 9HCP1118 | *Pseudomonas aeruginosa*  | CTX, KPC, NDM, SPM |
| 10HCP1405 | *Klebsiella pneumoniae*  | CTX, NDM, GES, SIM, OXA-48 |
| 12HCP1060 | *Staphylococcus coagulase Negativa*  | meca |
| 13HCP1401 | *Serratia marcescens*  | CTX, NDM, GES, SIM, OXA-48 |
| 14HCP1257 | *Klebsiella pneumoniae*  | TEM, CTX, GES, SIM, OXA-48 |
| 15HCP1253 | *Pseudomonas alcaligenes*  | SPM, SIM, OXA-48 |
| 16HCP1191 | *Staphylococcus epidermidis*  | meca |
| 17HCP1177 | *Staphylococcus aureus*  | - |
| 19HCP888 | *Klebsiella pneumoniae*  | TEM, CTX, NDM, GES, SIM, OXA-48 |
| 20HCP7 | *Escherichia coli*  | SIM, OXA-48 |
| 21HCP61 | *Pseudomonas aeruginosa*  | SIM, OXA-48 |
| 22HCP07 | *Escherichia coli*  | TEM, CTX, NDM, SIM, OXA-48 |
| 23HCP1251 | *Pseudomonas aeruginosa*  | CTX, SPM, GES, SIM, OXA-48 |
| 24HCP921 | *Klebsiella oxytoca*  | GES |
| 25HCP796 | *Staphylococcus epidermidis*  | - |
| 26HCP127 | *Staphylococcus warneri*  | - |
| 27HCP111 | *Staphylococcus aureus*  | - |
| 28HCP1168 | *Acinetobacter baumannii*  | SHV, TEM, SPM, GES, OXA-58 |
| 29HCP333 | *Serratia marcescens*  | TEM, CTX, KPC, SPM, OXA-48, OXA-58 |
| 30HCP263 | *Klebsiella pneumoniae*  | CTX, KPC, NDM, GES, SIM |
| 31HCP478 | *Escherichia coli*  | TEM, OXA-48 |
| 32HCP467 | *Streptococcus salivarius*  | - |
| 33HCP604 | *Enterococcus faecalis*  | meca |
| 34HCP682 | *Escherichia coli*  | TEM, OXA-48 |
| 35HCP747 | *Pseudomonas aeruginosa*  | KPC, SIM, OXA-48 |
| 36HCP835 | *Salmonella group*  | OXA-48 |
| 37HCP952 | *Staphylococcus aureus*  | meca |
| 38HCP1326 | *Acinetobacter baumannii*  | TEM, OXA-48 |
| 39HCP1336 | *Klebsiella pneumoniae*  | TEM |
| 40HCP1220 | *Pseudomonas aeruginosa*  | TEM, SIM |
| 40HCP1398 | *Escherichia coli*  | TEM, OXA-48 |
| PT  | Id  | Strains           | AMB   | FLZ | VRZ | CPF | MCF | ANF |
|-----|-----|-------------------|-------|-----|-----|-----|-----|-----|
| 1   | 1047 | C. tropicalis     | 0,03  | 32  | 16  | 0,06| 0,03| 0,03|
| 2   | 1173 | C. albicans       | 0,0125| 8   | 16  | 0,125| 0,03| 0,03|
| 3   | 1345 | C. tropicalis     | 1     | 32  | 16  | 0,5 | 0,03| 0,125|
| 4   | 539  | C. parapsilosis   | 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.

**Supplementary Files**

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- [graph1.jpg](#)