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## Original article

# PCR-based rapid identification of methicillin-resistant *Staphylococcus aureus* strains and their antibiotic resistance profiles in febrile neutropenic patients

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## ABSTRACT

**Background:** Bloodstream infections (BSI) in immunocompromised patients suffering from hematological malignancies continue to be an essential cause of morbidity and mortality. Methicillin-resistant *Staphylococcus aureus* (MRSA)-related BSI in patients with febrile neutropenia (FN) is a life-threatening bacterial infection and extremely challenging to treat. **Methods:** Blood samples were collected from febrile neutropenic patients. Conventional blood culture and direct PCR identification of 16S rRNA, *mecA*, *femA*, *nuc*, and *lukS* genes were performed for detection of MRSA. Antibiotic sensitivity profiles of the isolates were investigated using disc diffusion and minimum inhibitory concentration methods. **Results:** Among 24 positive blood cultures isolates, MRSA (12/24, 50%) was the predominant bacteria followed by coagulase negative *staphylococci* (CoNS) (6/24, 33.3%). All MRSA isolates were resistant to ceftazidime (MIC  $\geq 8$   $\mu\text{g/ml}$ ), and oxacillin (MIC  $\geq 4$   $\mu\text{g/ml}$ ) and harbored *mecA* gene. 10/12 MRSA isolates were vancomycin resistant (VRSA) (MIC  $\geq 16$   $\mu\text{g/ml}$ ). PCR for 16S rRNA and *mecA* genes yielded positive results in 14 negative blood culture samples. **Conclusions:** We cannot rely on blood culture as a reliable method for BSI diagnosis in patients with FN. 16S rRNA and characteristic MRSA genes PCR showed important role for diagnosis of culture-negative MRSA BSI particularly in patients with preceding prophylactic or empirical antibiotics.

## Introduction

Febrile neutropenia (FN) in patients suffering from hematological malignancies following intense chemotherapy is an urgent medical state. Gram-positive bacteria are the most prevalent pathogens in this dangerous consequence [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative *staphylococci* (MRCNS)-related bloodstream infection (BSI) in patients with FN have been showing rising prevalence, which may be

contributed to the widespread implementation of central venous catheters [2]. In order to properly handle patients with FN and provide the proper antibiotic treatment, prompt identification of BSI along with knowledge of the prevalent local pathogen in that area and their antibiotic susceptibility profile are crucial [3]. Techniques utilizing Polymerase Chain Reaction (PCR) have proven to provide fast and precise means to detect MRSA promptly. The acquisition of staphylococcal cassette chromosome *mec* (SCC*mec*) via horizontal

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gene transfer encodes a modified penicillin-binding protein (PBP2a) conferring resistance to methicillin and most  $\beta$ -lactam antibiotics. *MecA* gene has been employed to forecast methicillin resistance in both MRSA and MRCNS4. The *femA* gene (factor for expression of methicillin resistance) controls the production of proteins that affect how resistant *S. aureus* are to methicillin [5,6]. The *nuc* gene encodes the production of thermostable nuclease of coagulase-positive *staphylococci* [7,8]. The Panton Valentine Leukocidin (PVL), which is a significant cytotoxin encoded by *lukS-PV* and *lukF-PV* genes, is an established indicator and essential element for pathogenicity of community-acquired MRSA [9]. It binds to leukocytic membrane, creating pores and causes lytic cell death [10].

We aimed to identify the frequency of MRSA bloodstream infection (BSI) among patients with FN and describe their antimicrobial susceptibility profile to direct prompt diagnosis and effective antimicrobial empirical therapy regimens, hoping to implement policies for evidence-based infection control and apply antimicrobial stewardship protocols.

## Methods

The study was carried out among patients with hematological malignancies attending the hematology intensive care unit at Assiut University Hospital.

Patients were identified using the definition of FN provided by the Infectious Diseases Society of America (IDSA): a patient who has both an absolute neutrophil count (ANC) of  $\leq 500$  cells/mm<sup>3</sup> or an ANC that is expected to decrease to  $\leq 500$  cells/mm<sup>3</sup> within 48 h and a fever of  $\geq 38^\circ\text{C}$  at least sustained over an hour or a (single measure) fever of  $\geq 38.3^\circ\text{C}$  determined as a febrile neutropenic [11].

## The sample size was calculated according to the following equation

Sample size (n) =  $[\text{DEFF} * Np(1-p)] / [(d^2 / Z^2 1 - \alpha / 2 * (N-1) + p * (1-p)]$ . Sample size of 31 was determined for 95% confidence level, and we raised it to 39. The population size (for finite population correction factor or fpc) (N): 1000000. Hypothesized % frequency of outcome factor in the population (p): 2% +/-5. Confidence limits as % of 100 (absolute +/-%) (d): 5%. Design effect (for cluster surveys-DEFF): 1. Results from Open Epi, Version 3, open-source calculator, SSPropor.

The blood samples were aseptically collected from 39 patients with febrile neutropenia during the study period. A minimum of two blood culture sets were gained from different venipunctures [12]. To prevent contaminating the samples and culture medium, blood was carefully obtained and dispensed. The site for venipuncture was completely cleaned and sterilized while wearing gloves. A 50 mm-diameter area was cleaned with 70% ethanol and let to air dry. The region starting at the spot in which the needle penetrates the vein has been wiped with 2% iodine tincture using circular motion. A minimum of one minute was given for the iodine to dry. 5 ml of blood were withdrawn and promptly injected into the blood culture container with a sterile syringe [13]. Blood was mixed with 25 ml of Brain Heart Infusion (BHI) broth for each blood culture bottle and was incubated at  $37^\circ\text{C}$ . Daily checks for bacterial growth were done. Then subculture was performed on blood agar, Mannitol salt agar and ORSAB. Suspected staphylococcal colonies were identified according to standard bacteriological methods [14] and Vitek2 automated method.

Antibiotic susceptibility testing by Kirby Bauer's disc diffusion method was performed on Mueller-Hinton agar in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [15]. Antibiotic disks (Mast diagnostics, UK), including amikacin (30 $\mu\text{g}$ ), amoxicillin (30 $\mu\text{g}$ ), augmentin (10 $\mu\text{g}$ ), cefotaxime (30 $\mu\text{g}$ ), ceftriaxone (30 $\mu\text{g}$ ), clindamycin (2 $\mu\text{g}$ ), imipenem (10 $\mu\text{g}$ ), linezolid (10 $\mu\text{g}$ ), chloramphenicol (30 $\mu\text{g}$ ), and vancomycin (30 $\mu\text{g}$ ) were dispensed on inoculated plates using a Mast discs dispenser (Mast diagnostics, UK). These antibiotics were selected with an emphasis on their clinical significance in controlling staphylococcal infection and in accordance with the protocol of the British Columbia Cancer Agency (BCCA) for treatment of FN. Plates were inoculated in duplicates and incubated at  $35^\circ\text{C}$  for 16–18 hours and the diameters of inhibition zones were measured to the nearest millimeter. Each mean reading was interpreted according to CLSI breakpoints [15]. The term multi-drug resistance (MDR) was used to define acquired nonsusceptibility to minimum one antimicrobial agent in three or more distinct antimicrobial classes [3,16].

Broth microdilution assay was performed in accordance with the guidelines of CLSI [15] to

detect minimum inhibitory concentration (MIC) for oxacillin, cefoxitin, vancomycin and linezolid.

DNA was extracted directly from blood samples using Quiagen pathogen mini kit (QIAamp UCP Pathogen Mini Kit, Cat. No. 50214). PCR was used to identify the bacteria molecularly by looking for their 16S rRNA gene. *MecA*, *femA*, *nuc*, and *lukS* genes were investigated to characterize the isolates molecularly [8,17]. The used primers were provided by Quiagen (**table 1**).

All PCR reactions were performed in a 20  $\mu$ L reaction mix, each containing 5  $\mu$ L DNA template, 1  $\mu$ L of each primer, 10  $\mu$ L of PCR master mix (Bio Labs, New England), and 3  $\mu$ L of nuclease-free water (Bio Labs, New England). PCR was done with the following cyclic conditions: one cycle of initial denaturation at 94°C for 3 minutes with all genes, 35 cycles of denaturation at 94°C for 30 seconds and annealing at 55°C (for 16S rRNA, *femA* and *mecA* genes), at 58.5°C (for *lukS* gene) and at 66°C (for *nuc* gene) for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

Statistical analysis was performed using IBM® Statistical Package for Social Sciences (SPSS) Statistics version 25 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 365 (Microsoft Corporation, Redmond, WA). Categorical data was presented as frequencies and percentages, and Chi-square tests were used for comparisons between groups. Continuous data was reported as means  $\pm$  standard deviations and medians (interquartile range), which were tested for normality using the Shapiro-Wilk test.

The study was approved by the "Institutional Review Board" of the Faculty of Medicine, Assiut University, Assiut, Egypt (IRB approval number: 17101806) and was carried out in accordance with the Declaration of Helsinki. Informed written consents were acquired from patients or their concomitants after clarifying the goals of the study.

## Results

This study included 39 patients with FN. All patients had neutropenia with a neutrophil count range between mild ( $1 - 1.5 \times 10^9/L$ ) in 16 (41%)

patients, moderate ( $0.5 - 1 \times 10^9/L$ ) in 5 (12.8%) patients and severe ( $< 0.5 \times 10^9/L$ ) in 18 (46.2%) patients. Fever ranged from high grade fever (38.9 - 40.5°C) in 13 (33.3%) patients to low grade fever (37.8-38.8°C) in 26 (66.7%) patients. The characteristics of the study sample were shown in **table (2)**. Mortality rate was higher than the recovery rate, as 56% of patients died during the study and before blood culture results were released.

Out of 39 specimens from patients with FN, 24 (61.5%) showed bacterial growth. Positive blood cultures were made up of Gram-positive bacteria (48.8%), Gram-negative bacteria (46.1%) and fungal (candida) (5.1%). Of the Gram-positive isolated bacteria, 18/19 (94.7%) were *staphylococci*. Among 24 positive blood cultures isolates, MRSA (12/24, 50%) was the predominant bacteria, and the isolates were cefoxitin and oxacillin resistant which established MRSA identity. While coagulase negative *staphylococci* (CoNS) were found in (6/24, 33.3%) of bacterial growth. 5 positive cultures had poly-bacterial infection, all of them showed MRSA. 15/39 specimens showed no growth.

Rendering the disc diffusion test results, MRSA isolates were 100% resistant to amoxicillin, augmentin, cefotaxime, ceftriaxone, vancomycin, clindamycin, amikacin, followed by chloramphenicol (66.7%), linezolid (64.7%), and imipenem (14.3%) as shown in **figure (1)**. High frequency of MDR was observed in the isolates (70.8%).

According to PCR results, 37/39 (94.9%) of samples harbored 16S rRNA for staphylococci and 35 of them (89.7%) were methicillin resistant. **Figure (2)** shows representative agarose gel electrophoresis of PCR products of amplified 16S rRNA (a), *mecA* (b), *femA* (c), *lukS* (d), and *nuc* (e) genes. A comparison between culture results and PCR results is demonstrated in **table (4)**. PCR for 16S rRNA and *mecA* genes yielded positive results in 14 negative blood culture samples. A discordant result was noticed in one case with a culture-positive and PCR-negative result. Unfortunately, we were unable to obtain a second specimen. The mortality percentage was high among cases infected with *mecA* positive isolates as demonstrated in **figure (3)**.

**Table 1.** Sequence of primers used for PCR

Primer	Primer sequence (5'-3')	Target gene	Annealing temp.	Product size
16S rRNA-F 16S rRNA-R	GCA AGC GTT ATC CGG AAT T CTT AAT GAT GGC AAC TAA GC	16S rRNA	55°C	597 bp
<i>mecA</i> -F <i>mecA</i> -R	ACG AGT AGA TGC TCA ATA TAA CTT AGT TCT TTA GCG ATT GC	<i>mecA</i>	55°C	293 bp
<i>lukS</i> -F <i>lukS</i> -R	CAG GAG GTA ATG GTT CAT TT ATG TCC AGA CAT TTT ACC TAA	<i>lukS</i>	58.5°C	151 bp
<i>femA</i> -F <i>femA</i> -R	CGA TCC ATA TTT ACC ATA TCA ATC ACG CTC TTC GTT TAG TT	<i>femA</i>	55°C	450 bp
<i>nuc</i> -F <i>nuc</i> -R	GCG ATT GAT GGT GAT ACG GTT AGC CAA GCC TTG ACG AAC TAA AGC	<i>nuc</i>	66°C	279 bp

**Table 2.** Characteristics of the study sample

Variable	Study Sample (n = 39)
<b>Age (years)</b>	38.05 ± 11.78 35 (30 – 45)
<b>Age Group:</b> Under 20 years 20 – 30 years 30 – 40 years 40 – 50 years 50 – 60 years Over 60 years	1 (2.6%) 11 (28.2%) 12 (30.8%) 10 (25.6%) 3 (7.7%) 2 (5.1%)
<b>Sex:</b> Male Female	23 (59%) 16 (41%)
<b>Diagnosis:</b> Acute myeloid leukemia Acute lymphoblastic leukemia Aplastic anemia Hairy cell leukemia	25 (64.1%) 10 (25.6%) 3 (7.7%) 1 (2.6%)
<b>Mortality:</b> Survival Death	17 (43.6%) 22 (56.4%)
<b>White Blood Cells (WBC) Data</b>	
<b>WBC Count (x10<sup>3</sup>)</b>	16.04 ± 42.11 1.95 (0.82 – 4.36)
<b>WBC Count Classification:</b> Leukopenia Normal Leukocytosis	29 (74.4%) 4 (10.3%) 6 (15.4%)
<b>Neutrophil Count (x10<sup>3</sup>)</b>	3.85 ± 9.71 0.625 (0.291 – 2.94)
<b>Neutrophil Count Classification:</b> Mild neutropenia Moderate neutropenia Severe neutropenia	16 (41%) 5 (12.8%) 18 (46.2%)
<b>Clinical Data</b>	
<b>Body Temperature Classification:</b> Low grade fever High grade fever	26 (66.7%) 13 (33.3%)

<b>Prophylactic Antibiotic Regimen:</b>	
Amikacin – Tazocin – Diflucan	18 (46.2%)
Amikacin – Tazocin – Mecamine	1 (2.6%)
Augmentin	1 (2.6%)
Ceftriaxone – Maxipime	1 (2.6%)
Diflucan	1 (2.6%)
Maxipime – Tavanic	2 (5.1%)
Mecamine	3 (7.7%)
Meropenem	6 (15.4%)
None	6 (15.4%)
<b>Empirical Antibiotic Treatment Regimen:</b>	
Amikacin – Tazocin – Diflucan	6 (15.4%)
Augmentin	6 (15.4%)
Diflucan – Meropenem	1 (2.6%)
Maxipime – Tavanic	1 (2.6%)
Meropenem	3 (7.7%)
Samples taken before empirical treatment	22 (56.4%)
<b>Risk Factors</b>	
<b>Patients on Chemotherapy</b>	31 (79.5%)
<b>Clinical Risk Factors:</b>	
Anal Fissure, palate ulcer	2 (5.1%)
Epistaxis, abdominal pain	1 (2.6%)
Hepatomegaly with right lower limb edema	1 (2.6%)
High bleeding tendency	1 (2.6%)
Jaundice	1 (2.6%)
Severe abdominal pain	3 (7.7%)
Pleural effusion	1 (2.6%)
Pulmonary embolism	1 (2.6%)
Maxillary fungal infection	1 (2.6%)
Swelling in mandibular gland	1 (2.6%)
Throat and ear pain	4 (10.3%)
UTI	3 (7.7%)
Continuous data are presented as mean ( $\pm$ SD) and median (IQR). Categorical data are presented as count (%).	

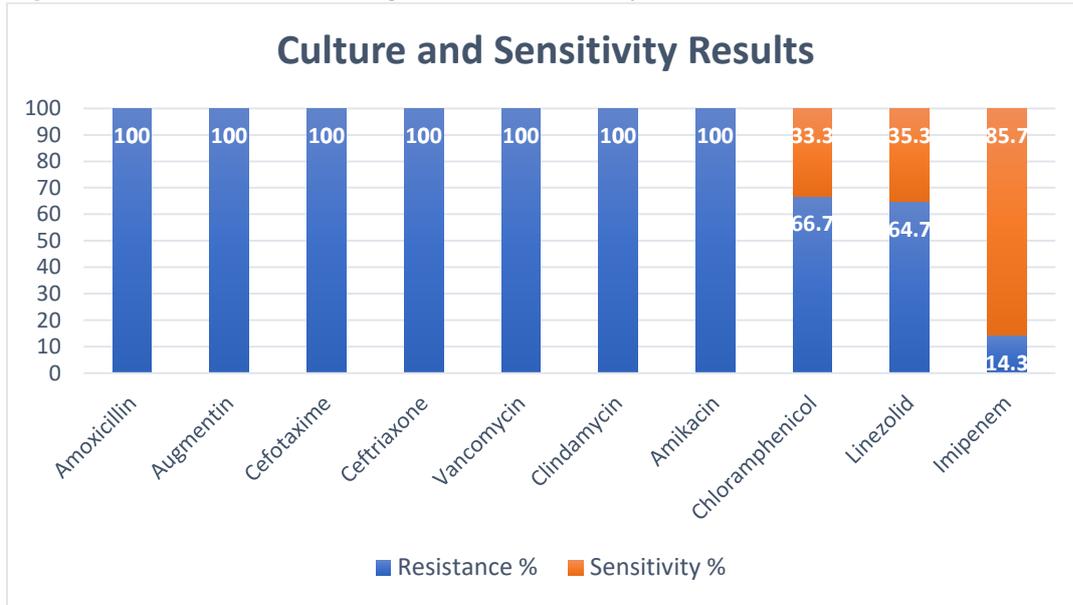
**Table 3.** Antibiotics sensitivity profile for MRSA isolates based on minimal inhibitory concentration (MIC)

Antibiotic	MIC		
	S	I	R
Vancomycin	( $\leq 2$ $\mu$ g/ml) 2 (16.6%)	(4-8 $\mu$ g/ml) 0 (0%)	( $\geq 16$ $\mu$ g/ml) 10 (83.3%)
Linezolid	( $\leq 4$ $\mu$ g/ml) 12 (100%)	- 0 (0%)	( $\geq 8$ $\mu$ g/ml) 0 (0%)
Cefoxitin	( $\leq 4$ $\mu$ g/ml) 0 (0%)	- 0 (0%)	( $\geq 8$ $\mu$ g/ml) 12 (100%)
Oxacillin	( $\leq 2$ $\mu$ g/ml) 0 (0%)	- 0 (0%)	( $\geq 4$ $\mu$ g/ml) 12 (100%)

**Table 4.** Culture results versus PCR results

Culture result	16S rRNA	<i>mecA</i>	<i>femA</i>	<i>lukS</i>	<i>nuc</i>
MRSA (no=12)	100%	100%	58.3%	50%	50%
CNS (no=6)	100%	83.3%	0%	0%	50%
Others (no=13)	92%	84.6%	76.9%	30.7%	53.8%
No growth (no=8)	87.5%	87.5%	37.5%	50%	50%

**Figure 1.** Stacked bar chart showing culture and sensitivity results for MRSA isolates



**Figure 2.** Representative agarose gel electrophoresis of PCR products of amplified genes

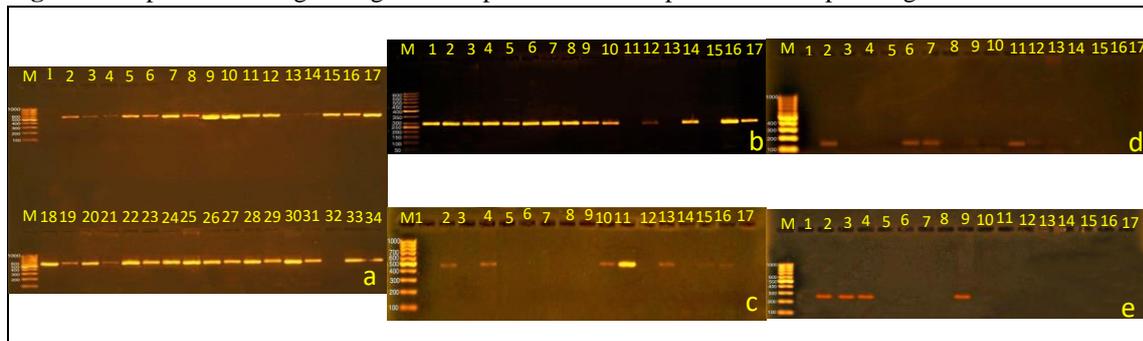
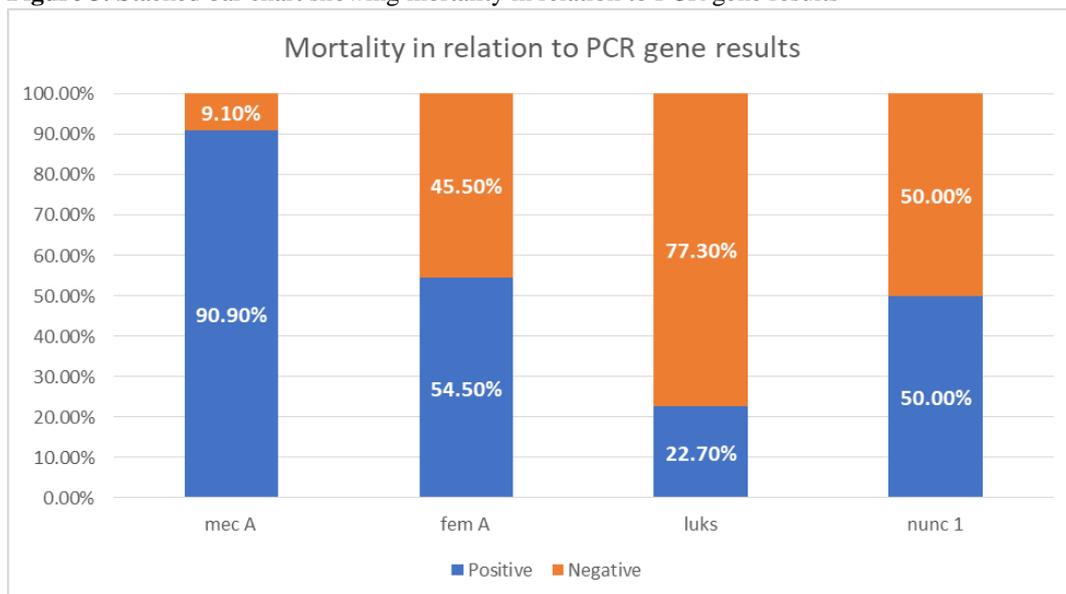


Figure 2: Representative agarose gel electrophoresis of PCR products of amplified 16S rRNA (a), *mecA* (b), *femA* (c), *lukS* (d), and *nuc* (e) genes. Lanes: 2-17,18-31,33,34 (a) represents 16S rRNA directed PCR positive results giving the expected PCR products of 597 bp. Lanes 1-10,12,14,16,17 (b) represents *mecA* directed PCR positive results giving the expected PCR products of 295 bp. Lanes 2,4,10,11,13,15,16 (c) represents *femA* directed PCR positive results giving the expected PCR products of 450 bp. Lanes 2,6,7,11 (d) represents *lukS* directed PCR positive results giving the expected PCR products of 151 bp. Lanes 2,3,4,9 (e) represents *nuc* directed PCR positive results giving the expected PCR products of 279 bp.

**Figure 3.** Stacked bar chart showing mortality in relation to PCR gene results



## Discussion

A medical crisis must be regarded when treating cancer patients who have fever and neutropenia as any delay in providing the proper empirical antibiotic medication can lead to a high rate of mortality and morbidity. Although there are verified guidelines for diagnosing and treating febrile neutropenic patients, the evolving patterns of antibiotic resistance limit the implementation of these guidelines. Current information on prevalent resident pathogens' epidemiology and their profiles of resistance needs to be considered for optimal empirical treatment policies in neutropenic patients since prompt empirical antibiotics are crucial at the beginning of fever.

Blood culture is a fundamental diagnostic tool in managing febrile neutropenia because it makes it easier to identify the pathogen with the appropriate antimicrobial susceptibility profile. Though, our culture positivity was 24/39 (61.5%) which was higher than other studies conducted in Ethiopia 27% [18], India 39.7% [19], France 40.7% [16], and Ireland 20.4% [20]. These differences are attributed to variations in the worldwide incidence of BSI because of various factors including the sample size, detection methods, infection control measures in hospitals, and geographical regions.

In this study the culture positivity was made up by Gram-positive more than Gram-negative bacteria which is consistent with other similar studies [3,16,18,20]. However, a study conducted in India, confirmed that Gram-negative organisms were predominant, and the overall Gram-positive organisms were only 12.3%. They presumed that was due to the decreased application of longstanding intravenous catheterization, as well as the absence of prophylactic antibiotic practice among their neutropenic patients [19].

In this study *staphylococci* were the most common Gram-positive isolated bacteria 18/19 (94.7%), which was in accordance with other studies [3,20,21]. 66.6% of *staphylococci* were *S. aureus* and that was higher than the reported results in previous studies 30.1% [20], 26.3% [21].

Crucially in our study all *S. aureus* isolates were methicillin-resistant (MRSA). This was consistent with another study conducted in Egypt [22]. However, our results differ to some extent from those of **Morris et al.** who observed that amongst their isolated *S. aureus*, 89.3% were methicillin-resistant (MRSA) [20]. On the other

hand, much lower results were reported by other studies. **Li et al.** detected that MRSA was 44% among *S. aureus* bacteremia in cancer patients [23], **Nam et al.** reported that 53.5 % of 43 *S. aureus* isolates were MRSA [24], **Montazeri et al.** confirmed that 29 out of 38 *S. aureus* isolates were MRSA [25], and **Kara et al.** demonstrated MRSA in 19.2% of *S. aureus* strains [3]. Moreover, none of *S. aureus* isolates in the study of **Raad et al.** was methicillin resistant [16]. These findings highlight the frightening high levels of MRSA isolates in our study.

Blood culture results revealed that *S. aureus* was the most common isolated Gram-positive bacteria (30.8%), followed by CoNS (15.4%). Even though these results differ from some earlier studies [2,3,20,26] that reported CoNS were the most isolated Gram-positive bacteria, they are consistent with those of **Worku et al.** who declared that *S. aureus* (31.5%, 17/54) was the commonly isolated bacteria followed by CoNS (29.6%, 16/54) [18].

The universal increase in resistant bacteria among febrile neutropenic patients has important consequences on the choice of an effective empirical or prophylactic therapy. We observed a prominent antimicrobial resistance among Gram-positive bacteria, which encompasses methicillin resistance in *staphylococci*. Crucially, it was found that MRSA isolates were 100% resistant to amoxicillin, augmentin, cefotaxime, ceftriaxone, vancomycin, clindamycin, amikacin by the disc diffusion method. Also, they showed a high level of resistance to chloramphenicol (66.7%) and linezolid (64.7%). Remarkably, imipenem showed the least level of resistance (14.3%). The MIC of vancomycin for MRSA has been steadily rising globally. It has been reported that the high-level of vancomycin MIC (>2 µg/mL) is related to failure of treatment and higher mortality rates in patients having infections caused by MRSA [2]. The MIC of vancomycin for 10/12 (83.3%) MRSA isolates was  $\geq 16$  µg/mL (VRSA). Significantly, MRSA isolates revealed considerable susceptibility to linezolid (MIC  $\leq 4$  µg/ml). Despite this, linezolid is a bacteriostatic drug, it may be inadequate for patients with neutropenia. Furthermore, it has been reported that linezolid's effectiveness was remarkably reduced in cases of hematological malignancy [2].

Vancomycin is the most often used medication for treating MRSA infections, but there is much controversy regarding the best strategy for

treatment. The quest for other anti-MRSA drugs has been spurred forward by MRSA treatment failure. Teicoplanin, tigecycline, linezolid, daptomycin, dalbavancin, and quinupristin are some of the novel MRSA-fighting medications that have been created over the last years [21].

Due to the widespread usage of venous catheters, the prevalence of FN attributed to MRSA and MRCNS has been rising lately. This is why, in accordance with the recommendations of the Infectious Diseases Society of America (IDSA), the prompt administration of anti-MRSA medicines such as vancomycin, linezolid, or daptomycin is more usually taken into consideration [2]. A properly dosed vancomycin regimen is advised as the first choice for treatment of MRSA BSI [23]. Nevertheless, the National Comprehensive Cancer Network (NCCN) instructions advise against employing vancomycin as the sole element of empirical treatment for cancer patients, since this might worsen the outcome for those who have MRSA bacteremia [27]. Therefore, knowledge regarding the incidence and contributing factors for MRSA BSI and associated mortality in cancer patients is urgently needed for improved handling of MRSA in those individuals.

A significant healthcare issue that requires attention is the expanding increase in multidrug resistance. Hence, it is essential to guarantee that antibiotics are used appropriately, and the prevalent local pathogens' antimicrobial susceptibility profile must be used to guide patients' therapy. The frequency of MDR bacteremia among our patients was 70.8%. On the other hand, **Raad et al.** stated that only 2.9% of BSI were caused by MDR bacteria in France [16]. Another study carried out in India concurred with our findings; they reported MDR bacteremia of 63.5% [21], and further supported the role of uncontrolled antibiotics' selling in the rising antimicrobial resistance issue among developing nations.

PCR results revealed the presence of *S. aureus* in 14 negative blood cultures and all of them were harboring *mecA* gene. This could be attributed to the application of antibiotic therapy on an empirical basis. A discordant result was noticed in one case with a culture-positive and PCR-negative result. This substantiates previous findings by **Rampini et al.** who reported 8 culture-positive PCR-negative staphylococcal spp. [28]. They clarified that their PCR-negative specimens had

extremely little amounts of bacteria as they took long incubation to show bacterial growth. Three of them showed the consistent isolates by PCR in the second sample. Likewise, **Mohamed et al.** couldn't detect 16S rRNA gene in one sample that grew MRCNS and two samples that grew Gram-negative bacilli, despite the yielded PCR results for both *nuc* and *mecA* genes [13]. Unfortunately, we were unable to obtain a second specimen because the considered patient passed away.

Despite the fact that *nuc* gene detection by PCR is contemplated for prompt recognition of *S. aureus* [8], it was not identified in all MRSA strains. This is barely supported by the findings of **Xu et al.** who reported that few *S. aureus* isolates were lacking the *nuc* gene [29]. Moreover, **Aksakal et al.** stated that 21 out of 23 *S. aureus* isolates yielded positive results for *nuc* gene by Real-Time PCR [30]. Furthermore, **Hoegh et al.** confirmed that *nuc* gene variations within *S. aureus* might cause methicillin-resistant and sensitive *S. aureus* misidentification [31], which supports our findings. The detection of *femA* gene was employed to differentiate MRSA from *mecA* positive CoNS. 23 (58.9%) isolates were proven to be MRSA by PCR detection of both *mecA* and *femA* genes. A higher frequency (85%) was reported by **Fri et al.** [5]. On the other hand, a lower frequency (47.8%) was described by **Nam et al.** [24]. The most striking observation in our study is the high frequency of *mecA* gene among both MRSA and CoNS isolates. This concurs well with **Bajpai et al.** who demonstrated a very high rate of MRCNS in their study [21], also **Aksakal et al.** stated that *mecA* gene was present in all MRCNS strains [30].

The *lukS* gene was detected in 41% of our specimens and its presence reflects the virulence of community acquired staphylococcal isolates. Corroborated with our findings, a study stated that out of 32 MRSA isolates, 13 (40.62%) were positive for presence of *luk-PVL* gene [32]. These findings are in contradiction with **Montazeri et al.** who noticed that the prevalence of *luk-PVL* toxin gene was (10.5%) [25].

Prophylactic antibiotic therapy was received by 84.6% of patients. The regimen including amikacin, tazocin and diflucan was associated with 46.2% of bacterial BSI. However, this was not statistically significant due to the use of several prophylactic antibiotic regimens in this study. These observations were much higher than

those described by **Worku et al.** (27.8%) [18], which emphasized that the extensive use of prophylactic antibiotics could be a risk factor for the development of MRSA BSI. It can be assumed that Gram-negative bacteria are less likely to recover being targeted by prophylactic and empirical antibiotic protocols, which favor Gram-positive bacteria. It was noticed that the incidence of culture-positive BSI in patients who received empirical antibiotic therapy was 43.6%, which doubts the effectiveness of the used regimens. However, in most cases intensive investigations don't identify the causal organism, and therapy is empirical relying on noticed infection trends. Moreover, physicians become compelled by antimicrobial resistance to pick the most effective empirical antibiotics for neutropenic patients with severe illnesses. Nevertheless, excessively employing broad-spectrum antibiotics raises resistance and mortality rates. Additionally, neutropenic patients on chemotherapy became immunosuppressed and vulnerable to MRSA BSI; being subjected to cytotoxic drugs, several invasive procedures, recurrent hospitalizations, and broad-spectrum antimicrobials. This explains the high frequency of patients on chemotherapy in our study (79.5%). High mortality rate was seen in our study (56.4%). The mortality rate in culture-positive cases was 62.5%. MDR was observed in 70.8% of our cases. These findings correlate fairly well with **Li et al.** [23] and further support the concept of the association between inappropriate empirical antibiotic treatment and increased mortality among patients with MRSA BSI. On the contrary, our findings are significantly higher than previously reported results [21,33].

Despite the small sample size and the lack of a clinical association in our study, the high mortality rate raises significant worries. It demonstrates the necessity of improving infection control policies and urges reevaluating our empirical antimicrobial strategy for FN.

### Conclusions

MRSA was the most frequent cause of BSI in patients with FN. Despite the fact that this study only focused on a small subset of patients with FN, our findings revealed that PCR-based blood bacterial analysis may be a useful tool for early diagnosis and proper management of FN. Localized microbiological and antibiotic-sensitivity profiles need to be taken into consideration while treating BSI in cancer patients, in adhering to the established

guidelines. This is possibly accomplished by regular bacterial monitoring and investigating their resistance profiles. Which also helps to direct effective antimicrobial empirical regimens and raise the standard of therapy. Crucially, the research zone should take rigorous standards of antibiotic stewardship along with infection control measures into concern.

### Conflicts of interest

The authors report no conflicts of interest.

### Author contributions

Conception and design: E.D., M.I., A.E. Acquisition of data: R.H., S.K., R.M. Analysis and interpretation of data: E.D., M.I., A.E., R.M. Drafting the article: R.H., S.K., R.M. Revising the article: E.D., M.I., A.E. All authors have approved the manuscript as submitted.

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