



Predominant Bacterial Pathogens and Resistance Profile of Blood Isolates: Blood Cultures' Surveillance from Tertiary Teaching Hospital

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Abstract

Background: Laboratory reporting of positive blood cultures (PBCs) is crucial and essential for infection control precautions and antibiotic stewardship programs at any healthcare setting, as it promotes the appropriate use of the empirical antibiotic prescription, and the implementation of infection control precautions. This study aimed to describe the epidemiological and microbiological data of patients with PBCs and to determine the resistance pattern of blood isolates.

Methods: The laboratory survey of all PBCs was conducted at Prince Sultan Military Medical City (PSMMC) in Riyadh, Kingdom of Saudi Arabia, from February to July 2019, where all PBCs were daily identified from microbiology reports and analyzed for demographic and microbiological data. However, contaminated and duplicated blood cultures were excluded.

Results: Total number of true PBCs was 632, of which 59.6% were gram-negative bacteria (GNB), and 56.8% were categorized as hospital-onset. The most frequently isolated bacteria were *Klebsiella* spp. (101 cases), *Staphylococcus aureus* (57 cases), *Pseudomonas* spp. (56 cases), *Enterococcus* spp. (50 cases), and *E. coli* (49 cases). Interestingly, *Klebsiella pneumoniae* and *Acinetobacter* spp were resistant to meropenem in 34% and 89% of cases, respectively. In addition, *S. aureus* was resistant to methicillin (MRSA) in 57%. In conclusion, our study showed a predominance of GNB in PBCs, with a high rate of MRSA and carbapenem-resistant organisms. The data could be useful for the update of the PSMMC antimicrobial guidelines.

Conclusion: Our study showed a high rate of PBCs with a great percentage of MRSA and Carbapenem resistant organisms.

Keywords: Blood Culture; Epidemiology; *Klebsiella pneumoniae*; MRSA; Carbapenem

Abbreviation

BC: Blood Culture; BSI: Bloodstream Infection; CO: Community Onset; CoNS: Coagulase-negative *Staphylococcus*; CPE: Carbapenemase-producing *Enterobacteriaceae*; DPBCs: Duplicated Positive Blood Cultures; EARS: European Antimicrobial Resistance Surveillance Network; GNB: Gram-negative Bacteria; GPC: Gram-positive Cocci; HAIs: Healthcare Associated Infection; HO: Hospital Onset; ICU: Intensive Care Unit; MDROs: Multidrug-resistant Organisms;

MRSA: Methicillin Resistant *Staphylococcus aureus*; NDM: New Delhi metallo-beta-lactamase; NHSN: National Healthcare Safety Network; OXA-48: Carbapenem-hydrolyzing oxacillinase-48; PBCs: Positive Blood Cultures; PMIs: Poly-Microbial Infections; PSMMC: Prince Sultan Military Medical City; PVC: Peripheral Venous Catheter; PVCBSI: Related Bloodstream Infections; REIs: Recurrent Infections; RIs: Relapsed Infections; SA: Saudi Arabia.

Introduction

Bloodstream infection (BSI) is a potentially life-threatening condition that can lead to organ dysfunction, septic shock, and even mortality. Blood cultures (BCs) are regarded as the “gold standard” for the diagnosis of bloodstream infections and the identification of the causative agents [1]. The detection of the pathogenic organisms will provide a better understanding of the pathogens encountered in routine clinical practice. Specifically, the susceptibility test will clearly illustrate the type of resistance and thus the corresponding antimicrobial therapy. In daily practice, physicians request a series of blood cultures in patients with suspected BSI and prescribe empirical antibiotics. The selection of this empiric treatment depends on several factors related to the patients' background and to the local ecology and epidemiology of BSI. Therefore, monitoring and surveillance of positive blood cultures are essential in every facility to guide the physicians for the prescription of the appropriate treatment [2].

Therefore, the study was conducted at PSMC, one of the largest tertiary and teaching medical center in the Middle East, with capacity of 1350 beds plus medical, surgical, intensive care, and organ transplantation units. This surveillance program was supervised by the infection control department, and the focus was on specific hospital-acquired infections (HAIs). Specifically, ventilator associated pneumonia, centerline associated blood stream infection, catheter-associated urinary tract infection, and multidrug-resistant organisms (MDROs). However, there was no routine surveillance or monitoring for positive blood cultures, either by hospital areas or by date of admission in the hospital. In general, laboratory monitoring of PBCs in any healthcare institution is crucial for infection control programs. In fact, it will accelerate and reinforce the implementation strategies of infection control precautions in areas with high rates of MDROs. This study aims to identify the common organisms responsible for BSI, along with their resistance patterns, and describe the epidemiological data of patients with PBCs.

Methods

This is an observational laboratory survey of identified PBCs, which was conducted at PSMC in Riyadh, Saudi Arabia, from February 2019 to July 2019. During the study period, all positive blood cultures were collected from Microbiology Laboratory electronic reports at PSMC. Demographical data was collected then illustrat-

ed as age, gender, location, date of admission, date of sample collection, and origin of infection. Microbiological data was categorized to determine the most common type of organism occur in different location. However, pediatric samples, contaminated blood cultures and duplicated positive blood cultures (DPBCs) were excluded and only true positive blood cultures (TPBCs) were included. All blood cultures were processed in the microbiological laboratory at PSMC, which is a certified laboratory by the College of American Pathologists. The microbial identification methods and susceptibility testing were applied in accordance to the clinical and laboratory standards institution guidelines. The prevalence of antibiotic resistance was calculated as the percentage of any given microorganism with intermediate or full resistance divided by the total number of tested isolates for a particular antimicrobial agent. All data was analyzed using descriptive statistics, and evaluated based on the normality of the data. Moreover, continuous variables such as age, date of admission and specimens collection was calculated as duration (days). Categorical variables such as gender, patient location, origin of infection, specimen type, site of the specimen, organism type along with the antimicrobial resistance test were presented as frequencies (N) and percentages (%).

Definitions

- **True positive blood culture incidence density rate:** Number of non-contaminated and non-duplicated blood cultures per patient per month identified >3 days after admission to the facility/Number of patient days for the facility x 1,000.
- **Duplicated positive blood cultures (DPBC):** Positive blood cultures from the same patient and for the same microorganism within 14 days from the first TPBC [3].
- **Contaminated blood culture:** A set of blood samples contained at least one of the following organisms in ≤50% of BCs obtained from one patient on the same day.
- (Coagulase-negative *Staphylococcus*, alpha-hemolytic *Streptococci*, *Micrococcus* spp., *Propionibacterium* spp., *Corynebacterium* spp., and *Bacillus* spp [4].
- **Community onset (CO):** Positive BC that obtained from outpatient and/or within 48 h from admission.
- **Hospital onset (HO):** Positive BC that obtained 48 h after admission.

- **Poly-microbial infections (PMIs):** Isolation of more than one organism from a single blood culture specimen and/or isolation of more than one organism(s) in different blood culture specimens during the same episodes of bloodstream infection (14 days) [5].
- **Relapsed infections (RIs):** PBC for the same patient with the same type of bacteria after 14 days from the first PBC [5].
- **Recurrent infections (REIs):** PBC for the same patient with different bacteria after at least 14 days from the last PBC [5].

Results

During the study period, 756 positive non-duplicated blood cultures were collected, 124 contaminated blood cultures were excluded, and thus 632 cases of TPBC were selected. In detail, TPBC were reported from 80 pediatric and 389 adult patients. Although gram-negative bacteria represented 59.6% of all microorganisms, fungemia was noted in 17 cases (2.7%), all of which were *Candida spp.* well, complete epidemiological data of TPBCs are summarized in (Table 1).

Table 1: Epidemiological data of true Positive Blood Cultures at PSMHC.

Data	Adult patients N = 389	Pediatric patients N = 80	Total N = 469
Gender			
Male	242	42	282
Female	147	38	187
Mean age (year)	59.4 (13-105)	1.9 (1W ¹ -12Y ²)	53.7
Number of TPBC ³	535	97	632
Types of micro-organism			
Bacterial	522/535 (97.5%)	93 (95.9%)	615 (97.3%)
Fungal	13/535 (2.5%)	4 (4.1%)	17 (2.7%)
Origin of TPBC ³			
Community onset	228/535 (42.6%)	45/97 (46.4%)	273 (43.2%)
Hospital onset	307/535 (57.4%)	52/97 (53.6%)	359 (56.8%)
Number of poly-microbial TPBC ³	49/535 (9.1%)	9 (9.27%)	58 (9.17%)
Number of patients with recurrent/relapse TPBC ³	68/389 (17.4%)	7/80 (8.75%)	75 (11.8%)
28 days-mortality rate	104/389 (26.7%)	9/80 (11.2%)	113 (24%)
TPBCs Incidence density rate	5.5 TPBC/1000 PD ⁴		

1W: Week, 2Y: Year, 3TPBC: true positive blood cultures/4PD: patient-days.

The most frequent isolated bacteria were *Klebsiella spp.* in 101 cases (16%), coagulase-negative *Staphylococcus* (CoNS) in 88 cases (14%), *Staphylococcus aureus* in 57 cases (9%), *Pseudomonas spp.* in 56 cases (8.8%), and *Enterococcus spp.* in 50 cases (8%), all isolated bacteria with their corresponding abundance are shown in (Figure 1). On the subject of the antimicrobial resistance profile,

the study found a high rate of resistance to Meropenem among *Klebsiella spp.* (34%), *Pseudomonas spp.* (28%) and *Acinetobacter spp.* (89%). Among *S. aureus* isolates, 57% were resistant to methicillin (MRSA); the antimicrobial resistance profiles of the isolated bacteria are summarized in (Table 2).

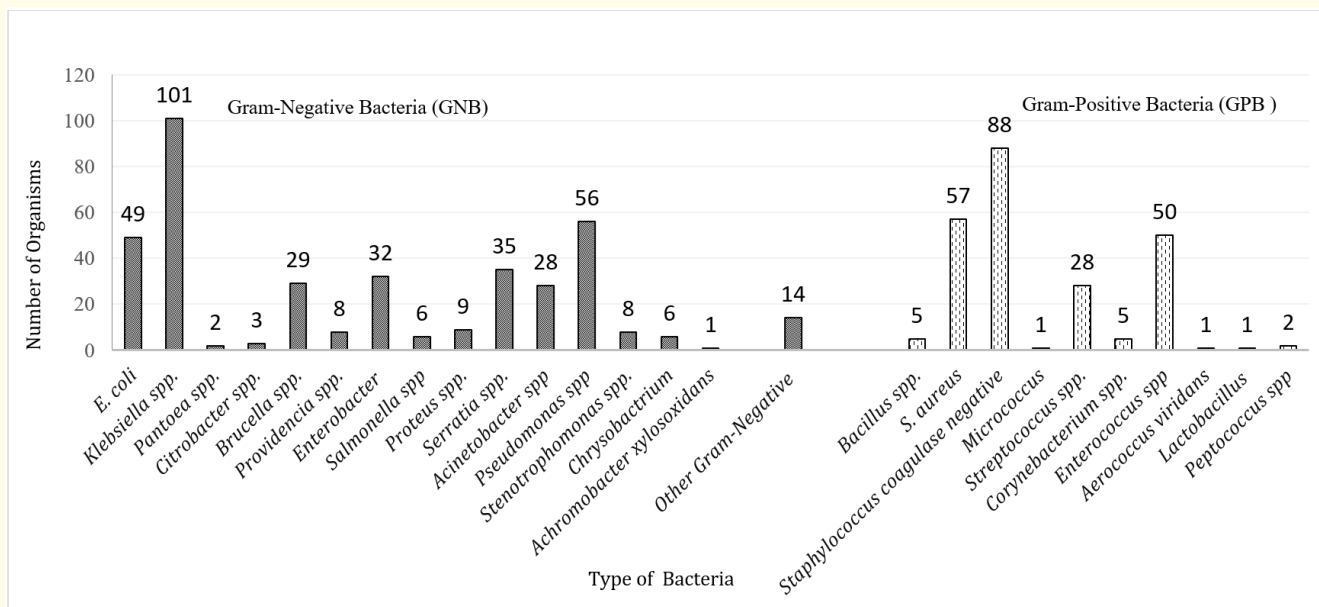


Figure 1: Isolated organisms from all true positive blood cultures at PSMHC.

Table 2: Antimicrobial resistant profile of most frequent isolates from true positive blood cultures (n = 373).

Antimicrobial drugs	<i>Klebsiella spp</i> n=(101)	<i>Pseudomonas spp</i> n=(56)	<i>E. coli</i> n=(49)	<i>Acinetobacter spp</i> n=(28)	<i>Enterobacter spp</i> n=(32)	<i>S. aureus</i> n=(57)	<i>Enterococcus spp</i> n=(50)
Ampicillin	(101)100%	0	(42)85%	(27)96%	(32)100%	NA	(3)6%
Augmentin	(51)50%	0	(15)30%	(27)96%	(30)93%	(27)47%	NA
Cefuroxime	(68)67%	0	(25)51%	(27)96%	(26)81%	NA	NA
Amikacin	(28)27%	(2)3.5%	(2) 22%	(19)67%	0	NA	NA
Ceftazidime	(61)60%	(12)21%	(22) 44%	(23)82%	(13)40%	NA	NA
Trimethoprim/ Sulfamethoxazole	(61)60%	NA	(29)59%	(14) 50%	(3)9%	(3)5%	NA
Ciprofloxacin	(49)48%	(5)8.9%	(27)55%	(25)89%	(3)9%	(19)33%	(28)56%
Piperacillin+ Tazobactam	(47)46%	(14)25%	(3)6%	(7)25%	(8)25%	NA	NA
Gentamicin	(39)38%	(7)12%	(10)20%	(22)78%	(2)6%	(5)8%	(6)12%
Imipenem	(33)32%	(21)37%	0	(23)79%	(1)3%	NA	NA
Meropenem	(35)34%	(16)28%	0	(25)89%	(1)3%	NA	NA
Tigecycline	(24)23%	NA	0	NA	(3)9%	NA	NA
Vancomycin	NA	NA	NA	NA	NA	0	(8)16%
Methicillin	NA	NA	NA	NA	NA	(33)57%	(25)50%
Rifampicin	NA	NA	NA	NA	NA	(2)3%	(23)46%
Tetracycline	NA	NA	NA	NA	NA	(8)14%	(22)44%
Clindamycin	NA	NA	NA	NA	NA	(9)15%	NA
Teicoplanin	NA	NA	NA	NA	NA	0	(8)16%

**Escherichia coli*.

Discussion

In the current study, gram-negative bacteria (GNB), mainly members of *Enterobacteriaceae*, were the predominant microorganisms isolated from TPBCs, hence, *Klebsiella spp.* were the most frequent bacteria responsible for TPBCs. This finding contradicts the findings from previous studies conducted in Saudi Arabia over the last decades, in their study, gram-positive bacteria (GPB) and in particular, *S. aureus*, were the main isolates of TPBCs. Indeed, several studies have indicated that *S. aureus* as the principal cause of BSI among adults and pediatric patients [6-9]. In a different study (2009), a clinical survey revealed that coagulase-negative *Staphylococcus* (CoNS) was the primary cause of bacteremia (n = 261, 23.7%), followed by *S. aureus* (n = 122, 11.1%) and *Escherichia coli* (n = 121, 11.1%) [8]. Moreover, same study stated the rate of BSI due to *K. pneumoniae* as 9% (n = 99), *P. aeruginosa* as 7.3% (n = 81), and *Acinetobacter spp.* as 5.3% (n = 59) [8]. Likewise, publication from the European Antimicrobial Resistance Surveillance Network (EARS-Net), which includes 198 laboratories in 22 European countries, indicated that *Escherichia coli* and *S. aureus* were the most common pathogens causing BSI [10]. Similarly, the surveillance network data from South Korea (2018) revealed that *E.coli* and *S. aureus* were the predominant bacteria isolated from BCs [11].

In contrast to this constant trend, single-center studies have recently reported an increase in the proportion of gram-negative pathogens among patients with BSI. Essentially, GNB were the source of approximately 25%–50% of all BSIs. In the other hand, published regional study has analyzed the culture reports of all blood samples collected for one year, the study disclosed that among 222 BSIs, 62.2% were due to GNB and 36.4% caused by GPB. Moreover, *K. pneumoniae* was the most frequent (28.4%) gram-negative pathogen, while *S. aureus* contributed to 11.3% of GPC [12]. A change in the trend and the predominance of GNB were observed also in the international healthcare institutions. Particularly, a study conducted in Switzerland has presented that CoNS, *S. aureus*, and fungi revealed a decreasing trends, while rates of *Enterococci* and GNB remained stable [13]. Furthermore, published analysis, 25-years study period, from Spain has described the etiology and outcomes of the short-term peripheral venous catheter (PVC)-related bloodstream infections (PVCBSI), they observed an increase in the prevalence of gram-negative PVCBSI over the last 25 years. The factors that were associated with GNB infection were being in the hospital for >7 days with a catheter *in situ* for >3 days, having undergone surgery, and having received antimicrobial

treatment with beta-lactams [14]. Similarly, in another study conducted among cancer patients, the cause of most PVCBSI was the presence of GNB instead of GPB [15].

Apparently, increasing of the extents gram-negative might be associated with PVCBSI, these studies claim to expand the prevention efforts of gram-positive central line infections, increase antimicrobial resistance, and/or changes in surveillance practices [16-18]. In addition, several factors are disturbing the local infection prevention practices and the prevalence of drug-resistance, which may explain the trend variability in a different hospital. Therefore, in our institution, each new patient admitted to the intensive care unit (ICU) and each high-risk patient for colonization or infection of MDROs, are systematically screened for MRSA and vancomycin-resistant *Enterococci*. Patients under the mentioned criteria will place under empirical contact precaution, while awaiting the results of their status. However, this routine screening is not performed for GNB; hence, a rapid molecular screening for MDROs and empirical contact precautions should be implemented to reduce the spread of such bacteria in or hospital.

The concerning finding from our study was the high-rate of GNB that resistant to Carbapenems antibiotics. In specific, *K. pneumoniae*, *Pseudomonas spp.* and *Acinetobacter spp.* were resistant to Meropenem and/or Imipenem in 34%, 39% and 89% respectively. This consequence can be attributed to the tertiary nature of our facility, as a significant number of patients were received with previous antibiotic prescriptions and multiple comorbidities. Also, other reasons might allied to that are the presence of long-term care patients colonized with MDROs in acute medical care units and the over prescription of Carbapenem prescription.

Over the latest decades (2010), a local study found that the rate of Carbapenem-resistant organisms in Saudi Arabia was less than 5%, the study recorded that there was 20 (1.17%) out of 1,706 of *K. pneumoniae* were Carbapenem-resistant [19]. Thus, during the last twenty years, Carbapenem resistant bacteria has been gradually increased universally and locally in SA. Consequently, the most important mechanism of resistance is the production of a carbapenemase enzyme that hydrolyzes Carbapenem especially among *Enterobacteriaceae* [20]. In fact, data from the National Healthcare Safety Network (NHSN; Centers for Disease Control and Prevention in Atlanta GA, USA) showed that 8.7% of *Klebsiella spp.* isolates that caused HAIs in 2006-2007 were Carbapenem-resistant

compared to less than 1% isolates in 2000 [21]. However, the rate of resistance to Carbapenem varies across regions and countries. A recent local study from southern region reported that *A. baumannii* isolates were extremely resistant to two Carbapenem drugs. Among tested strains, only 5 (0.05%) and 4 (0.04%) isolates were susceptible to Imipenem and Meropenem, respectively [22]. This process indicated that these isolates were more dangerous than previous identified MDR strains, which showed more than 90% susceptibility to both drugs [23]. Another recent survey conducted in a north region identified that among the 103 isolates of GNB isolated from BCs, 47.6% were multidrug resistant, 38.8% were extensive drug resistant, and 2.9% were pan-drug resistant, however, 46% of *K. pneumoniae* isolates were Carbapenemase producers [12]. Al-Otaibi conducted a retrospective study in 56 patients with malignancy that experienced 61 episodes of GNB bacteremia. The patients were admitted to the Oncology Unit and they demonstrated imipenem resistance (52.4%) among *P. aeruginosa* and *A. baumannii* [24]. Furthermore, in 38 European countries, over a period of two-years, the epidemiological status of Carbapenemase-producing *Enterobacteriaceae* (CPE) worsened with the rapid spread of Carbapenem-hydrolysing oxacillinase-48 (OXA-48) and New Delhi metallo-beta-lactamase (NDM)- producing *Enterobacteriaceae*. In 2015, 13 out of 38 countries reported interregional spread of or even an endemic situation for CPE, in contrast with 6 out of 38 countries in 2013, though, only three countries stated that they had not identified one single case of CPE [25]. Similar findings were reported in different countries worldwide, thus, Carbapenem-resistant *Enterobacteriaceae* (especially *K. pneumoniae*) were ranked among the recently published World Health Organization list of antibiotic-resistant "priority/critical" pathogens, for which research and development of new antibiotics is required [25,26].

Conclusion

To conclude, despite the fact that this is a single-center study and has limited duration of 6 months, it confirms the ongoing expansion and high-rate of CRE, which represents an increasing threat to patients' health safety at our institution and to other local tertiary hospitals. Therefore, reinforcement of infection control measures (hand hygiene, isolation precautions, environmental cleaning and disinfecting). The implementation of specific infection preventive measures and stewardship plans are crucial to limit the expansion of, mainly, CRE. These may include a routine screening of CRE for new admissions essentially in critical care units and

an identification of the types and mechanisms of CRE in colonized or infected patients by different cutting-edge molecular methods. Lastly, a stricter validation of Carbapenem prescription needs to be implemented, while a continuous CRE surveillance and monitoring are vital measures to control this situation.

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Conflict of Interest

The author(s) declared no potential conflicts of interest.

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