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Review

Epidemiology of bloodstream infection in Saudi Arabia

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Bloodstream infections are a challenge to clinicians due to high rates of morbidity and mortality. Moreover, rational antimicrobial therapy, if promptly started, can improve the prognosis of sepsis. The gold-standard blood culture has some limitations and it takes time to complete (about 2-5 days). This leaves the clinicians to start immediately broad-range antimicrobial therapy until microbiology culture's results available that may lead to complicate situation of patient further. Therefore, there is an urgent need for the development of more advanced diagnostic approaches that can overcome limitations of culture-based methods. This review gives an overview of the BSIs and its epidemiology over several cities and hospitals in Saudi Arabia. It also encompasses the diverse molecular diagnostic modalities developed for the rapid detection and identification of BSIs. Furthermore, the growing understanding of the host's inflammatory responses to infections has led to the discovery of immunological biomarkers as diagnostic tools for sepsis-related BSI. The rational use of immunological and molecular diagnostic markers in clinical settings may speed up diagnosis and prognosis of septic patients. This review shows that the percentage of BSIs in Saudi Arabia, from 1983 to 2015, ranges from 1% to 11% among patients with confirmed infection. It also indicated that *Staphylococcus aureus* and coagulase negative staphylococci (CoNS) were the most common Gram-positive pathogens; while *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the most Gram-negative ones from patients with BSI. The recommendation of this review is to initiate new surveillance systems of BSI in different regions and hospitals in Saudi Arabia and determining the antibiotic susceptibility profile to design antibiograms that may improve the antibiotic stewardship program for treating BSIs. A comparative study should be conducted for patients suspected to have sepsis, using the standard blood cultures, immunological markers and rapid molecular assays to set up a panel to help diagnose BSIs rapidly. This will allow early and optimal patient management and treatment in Saudi Arabia and therefore decrease overall healthcare costs.

Key words: Bloodstream infections, sepsis, molecular diagnosis, inflammatory markers, BSI in Saudi Arabia.

INTRODUCTION

Bloodstream infections (BSIs) remain a burgeoning public-health quandary worldwide and a leading factor in morbidity and fatality. They add substantially towards the healthcare burden and their management poses an ongoing challenging task to health-care providers and physicians (Anderson et al., 2007; Bassetti and Villa, 2012).

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Bacteriological culture to isolate the offending pathogen and knowledge about the sensitivity pattern of the isolates remain the gold standard in the definitive microbiological diagnosis and management of BSI. The emergence of antimicrobial resistance in BSI caused by bacterial pathogens can limit choice of treatment and complicate patient management.

Furthermore, the antimicrobial resistance rates are increasing now with varying patterns among different countries (Murphy et al., 2012). Additional treatment costs stem not only from extended hospital stays but the related medical costs incurred in hospitalized patients, all of which can be decreased with the proper prevention and control of nosocomial BSIs that may also reduce the mortality (Reier-Nilsen et al., 2009).

IMPACT OF BSIs

BSIs are the leading cause of morbidity and mortality worldwide. In spite of the methodological advantages, there is a scarcity of international studies investigating BSIs at the population level. However, none of these studies provides enough data from Saudi Arabia. The data provided here are from other parts of the world that may be similar to the situation in Saudi Arabia.

The limited reports on BSIs since the 1970s revealed rates ranging from 80 to 189 per 100,000 per year, considering all etiologies, with increased rates in the recent years. *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae* were the prevalent causative agents, occurring at 35, 25, and 10 per 100,000 persons, respectively. The BSI incidence has been reported to differ considerably amongst the different regions with varying rates of blood culturing, population demographics, and related health hazard determinants. Adequate insight into the prevalence of BSI is required in order to establish healthcare and research precedence and to evaluate the effectualness of preventive measures (Laupland, 2013).

There has been a remarkable change in the incidence of BSI among critically ill patients in Intensive Care Units (ICUs). This is due to the corresponding increase in numbers of elderly patients with comorbidities. This has been attributed to community-onset healthcare-associated (HCA) BSIs that are different from community-acquired and nosocomial BSIs. The incidence of HCA BSIs has a direct correlation with the resistance of bacteria to antimicrobials used in community-acquired (CA) infections. The recent upsurge in extensively-resistant bacteria worldwide has had major implications for the failure of antimicrobial therapies (Timsit and Laupland, 2012).

Severe BSI results in sepsis, a fatal inflammatory illness caused by the inappropriate response of the immune system. More than 750,000 people are afflicted annually in the United State with BSI, the prevalence being three cases per 1,000 individuals. Despite the advances in pharmacotherapy and supportive care, the survival rates have not improved much. The mortality varies from 25 to 30% for severe sepsis and 40 to 70% in cases of septic shock. Sepsis accounts for 20% of in-hospital deaths every year, which is almost the same as those occurring due to acute myocardial infarction (Timsit and Laupland, 2012). The continuum of inflammatory responses to severe microbial infections has been classified by the American College of Chest Physicians and Society of Critical Care Medicine as systemic inflammatory response syndrome (SIRS), sepsis, and severe sepsis, culminating in septic shock. SIRS is confirmed in the presence of two of the four following symptoms: fever, tachycardia, tachypnea, and leukocytosis or leucopenia. Some cases such as pancreatitis, autoimmune syndromes, trauma, burns, or surgery are usually predisposed to SIRS in the absence of an infection. The presence of an infection together with SIRS is defined as sepsis, though sepsis may not represent SIRS in some cases (Gauer, 2013).

EPIDEMIOLOGY OF BSI AND ITS PREVALENCE IN SAUDI ARABIA

The epidemiology of BSIs worldwide is continuously changing. Since 1980s, a progressive trend towards the predominance of Gram-positive pathogens as the causative agent of BSIs has been seen in developed countries, both in the community as well as in an HCA setting (Kollef et al., 2011). Conversely, Gram-negative microorganisms remained the most frequent BSI etiologic agents in developing countries. In 2011, the Gram-negative organisms re-emerged, notably in developed countries, and constituted 55% of the community-related BSIs (Shorr et al., 2006). Shorr and his team in 2006, reported the prevalence of bloodstream infections in patients from 59 hospitals in the United State within the period of 2002–2006. They found that 55.3% of all BSIs were healthcare-associated bloodstream infections. Furthermore, morbidity and mortality rates were higher with hospitalized patients with BSIs than those with community-acquired bloodstream infection (Shorr et al., 2006).

In Saudi Arabia, there have been only a few studies published on bloodstream infections in adults, pediatrics or neonates, wherein the majority of the cases were reported in ICUs (Al-Mously, 2013). Table 1 gives a brief overview of the studies that have been conducted on the BSIs in Saudi Arabia. A prospective study conducted at King Khalid University Hospital, Riyadh, Saudi Arabia, on patients who died from 1st January to 31st December 2004 due to BSIs, revealed that the mortality in these patients was attributable to the advancement in age and underlying health conditions. The bacterial isolates from these patients were identified and subjected to

Table 1. Studies that have been conducted on the BSIs in Saudi Arabia between 1983 and 2015.

Reference/Publishing Year	Year or period of study	Place of study/ unity	Population of interest	No of Patients	No and % of patients with BSIOR No of Isolates from Blood	Most common pathogens isolated
Haque et al. (1990)	1983-1988	King Khalid University Hospital, Riyadh (NICU)	Neonates	2117	190 BSI (9%)	*Staphylococcus epidermidis*
Elbashier et al. (1998)	1990-1995	Qatif Central Hospital, Qatif/ (ICU and PICU)	Adults and Pediatrics	84400	1324 BSI (1.6%)	*S. aureus,* CoNS, *E. coli,* K. pneumonia*; P. aeruginosa*
Asghar (2006)	2004-2005	4 main hospitals in Makkah City, Makkah	Adults and Pediatric	unknown	1626 BSI	*S. aureus,* CoNS, *E. coli,* K. pneumonia*; P. aeruginosa*
Babay (2007)	2004	King Khalid University Hospital, Riyadh	Adults		778 BSI (82 Died)	CoNS, *SA, P. aeruginosa*, *E. coli*
Al-Tawfiq and Mohandhas (2007)	1998-2004	Saudi Aramco Medical Services, Dhahran	Adults		476 isolates from patientswith BSI	A.*baumannii*
Al-Zamil (2008)	2003-2005	King Khalid University Hospital, Riyadh	Pediatrics	8244	259 BSI (3.1%)	*S. aureus, K. pneumoniae,E. coli*
Al-Tawfiq and Abed (2009)	2002-2006	Saudi Aramco Medical Services, Dhahran	Adults and Pediatrics	10499	1103 BSI (11%)	CoNS, *S. aureus*, *E. coli*, *Candida* spp.
Mahfouz et al. (2010)	2004-2005	Abha general hospital, Abha	Neonates	401	31 BSI (7.7%)	CoNS, *K. pneumoniae*, *Enterobacter cloacae*, *S. aureus*
Bahashwan et al. (2012)	2009-2010	Madinah Maternity and Children's Hospital, Madinah	Neonates	11968	728 BSI (6%)	S. *marcescens*, *K.pneumoniae*, CoNS
Al-Mously (2013)	2006-2010	King Khalid University Hospital, Riyadh	Critically ill adults		191 isolates	*A. baumannii*
Al-Tawfiq et al. (2013)	2004-2011	Saudi Aramco Medical Services, Dhahran	Adults	48161	215 BSI (0.5%)	-------
Al Thaqafi et al. (2014)	2002-2009	King Abdulaziz Medical City, Jeddah	Adult		258 patients with BSI with Candida Species	*C. albicans* Non-*Candida* spp
Al-Abdely et al. (2017a)	2013-2015	7 hospitals from 5 cities in Saudi Arabia	Adults and pediatrics	4551	187 BSI (4.1%)	*S. aureus, P. aeruginosa*
Al-Abdely et al. (2017b)	2013-2015	5 hospitals from five cities in Saudi Arabia	Adults and pediatrics	3769	106 BSI (2.8%)	*S. aureus, K. pneumoniae, Candida* spp.

antimicrobial susceptibility testing. Admission to ICU, intravascular catheterization, and respiratory tract infections were the risk factors that predisposed the patients to nosocomial BSIs. There was a preponderance of BSI episodes, which were monobacterial (86.5%) while more than 90% were HCA. The most prevalent were Coagulase-negative staphylococci (CoNS) representing 30.5% of all the isolates, whereas

Gram-positive bacteria together constituted 61%. Amongst the Gram-negative bacteria (38%), the most prevalent was the *Pseudomonas* species (7.3%) and *E. coli* (6%). *Candida glabrata* (Babay, 2007) was the most common fungal isolate (2.4%). Nevertheless, these old data are likely not reflective of the current pathogens causing BSIs in Saudi Arabia and recent studies on BSI should be conducted to reflect the real situation in Saudi Arabia.

A prospective survey conducted by Al-Tawfiq and Abed at Saudi Aramco Medical Services Organization from 2002 to 2006 determined the trend of HCA-BSI, as per the Centers for Disease Control and Prevention (CDC) guidelines. Whereas secondary BSIs persisted at a relatively constant rate, the primary BSIs and central line-associated BSIs (CLA-BSI) surged over time with the predominance of CoNS, *S. aureus*, and *E. coli* (Al-Tawfiq and Abed, 2009). Al-Tawfiq et al., in 2013, conducted another prospective study to evaluate the trends in device-associated infections (DAI) over time (2004–2011) at the adult intensive care units of the Saudi Aramco Medical Services Organization. They found that catheter-associated urinary tract infections (CAUTI) were the most common DAI with a prevalence of 42.2%, followed by a 38.5% prevalence of central line-associated bloodstream infections (CLABSI), and a 19.3% prevalence of ventilator-associated pneumonia (VAP). It was estimated that the total rate for each infection type per 1000 device-days was 8.18 for CAUTI, 10 for CLABSI, and 4.52 for VAP. They assessed the rates of DAI annually and indicated that there was a great decrease over time from when the study started until it was finished. The DAI rates were as follow: for CLABSI (16.3 versus 6.06), CAUTI (6.75 versus 3.41), and VAP (9.8 versus 1.3) ($p < 0.05$) (Al-Tawfiq and Abed, 2009). In 2017, a recent prospective multicenter study in five ICUs from five cities of Saudi Arabia revealed a substantial decline (56%) in the rate of CLA-BSI under the impact of the International Nosocomial Infection Control Consortium (INICC) (Al-Abdely et al., 2017b). The same group conducted another prospective surveillance study in 2017 at seven ICUs of five hospitals in five cities of Saudi Arabia, through the implementation of the INICC Multidimensional Approach (IMA), and found a slight drop in the device-associated HAI (Al-Abdely et al., 2017a).

The changing patterns of susceptibility to antibiotics with increasing resistance over the years and variations in the incidence and etiology of bacteremia in the hospitals was reported in 2007 by Al-Tawfiq and Mohandhas (Al-Tawfiq and Mohandhas, 2007). This emphasizes the need for conducting sentinel surveys of bacteremia in major hospitals so as to visualize the trend in bacteremia and antibiotic susceptibility profiles of the causative pathogens (Al-Tawfiq and Mohandhas, 2007). There is a scarcity of studies in Saudi Arabia on the nosocomial bacteremia caused by *Acinetobacter baumannii,* a major concern in critically ill patients. Therefore, the incidence, risk factors for infection, and antimicrobial sensitivity have been reported in a retrospective study carried out from April 2006 till April 2010 in a tertiary hospital in Saudi Arabia (Al-Zamil, 2008), wherein 191 isolates of *A. baumannii* were isolated from blood cultures of 191 patients. The majority of the *A. baumannii* isolates (about 40%) were recovered from blood obtained from patients in the surgical ICU. The highest resistance rates were observed for cefepime and piperacillin tazobactam (73.8 and 72.3%, respectively), followed by ciprofloxacin and gentamicin (68 and 66%, respectively). Imipenem (61.3%) and meropenem (3.9%) also revealed high resistance rates. Out of the total, 104 (78.8%) of these multi-drug resistant (MDR) strains were sensitive only to colistin and tigecycline (Al-Mously, 2013).

In another prospective study conducted in a 23-bed trauma ICU in Saudi Arabia, from January 2011 to December 2012, in accordance with the recommendations and guidelines of the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA), antimicrobial sensitivity of isolates of CLA-BSIs were measured in order to be benchmarked with the National Healthcare Safety Network (NHSN). In 2011 and 2012, the incidence rate of CLA-BSI was decreased by 58% from 3.87 to 1.5 per 1,000 central line days, respectively. The risk of CLA-BSI involved three factors. Namely, the proper training of health care, the use of a catheter cart, and the removal of unnecessary catheters. The etiologic agents identified were three *Klebsiella pneumonia* isolates with susceptibility to imipenem alone, one pandrug-resistant *A. baumannii*, and two *Enterococcus faecalis* isolates, of which one was resistant to vancomycin (Mazi, Begum, et al. 2014).

An eight-year (2002 to 2009) retrospective study on the epidemiological pattern of *Candida* BSIs in a tertiary care hospital of the King Abdulaziz Medical City, western Saudi Arabia, revealed an alarmingly increasing trend in candidemia episodes (Al Thaqafi et al., 2014). This could be attributed to the extensive and uncontrolled use of antifungal azoles in humans as well as agriculture.

Limited studies on the prevalence of neonatal BSI in Saudi Arabia have been reported. Distinct pediatric infection rates have been reported earlier from the different institutions in Saudi Arabia that varied with the age group and the hospital ward. For instance, while the incidences of BSIs were found to be as low as 9% in 1990 at the NICU of King Khalid University Hospital (Al Thaqafi et al., 2014), the Abha General Hospital reported 40.9% of such cases in 2010 [21(Prakash et al., 2011).

An analysis of the clinical and microbiological characteristics of BSI pediatric patients at the Maternity and Children Hospital in Al Madinah in Saudi Arabia, in a one year long study conducted from 2009 to 2010, showed that 728 out of 11,968 (6.1%, most were recovered from neonatal ICU) of the pediatric patients

were diagnosed with BSI after having at least one episode of positive blood culture. The reported overall mortality rate out of the 728 BSI patients was 11% (Al-Zamil, 2008). Gram-positive, Gram-negative, and yeast accounted for 63.8, 31.6, and 4.6% of the total pathogens, respectively. The most common Gram-positive pathogen was coagulase-negative staphylococci with 44%, while the most prevalent Gram-negatives organisms were *Serratia marcescens* and *Klebsiella pneumoniae*. The Gram-positive bacteria were mostly sensitive to cephalothin (82.3%) and vancomycin (72.2%), whereas the Gram-negative bacteria were mostly sensitive to ciprofloxacin (93%), piperacillin/tazobactam (92.9%), and meropenem (89.8%). On the sex-associated trends in the confirmed 728 BSI pediatric patients, 57% of 728 BSI patients were males, while the other 43% (313 patients) were females (Abo-Shadi, 2012). This is corroborated with the findings of Al-Zamil (2008), wherein 58.7% of the 259 pediatric patients with bacteremia were males and 41.3% females. The slightly higher rate of BSI in males (6.3%, 415 out of 658) over females (5.8%, 313 out of 5410), could be attributed to a higher admission rate of the former compared to the latter. The relatively higher rates (521 microbial isolates, 63%) were observed for the overall HA BSIs than the CA. The varying HA BSI rates (90% (Al-Tawfiq and Abed, 2009); 43% (Elbashier et al., 1998); 53% (Laupland et al., 2009) have been reported earlier. In a study by Al-Zamil (2008), 95% of *S. aureus* and 38% of *S. pneumonae* were resistant to penicillin G. An important observation noted was that the high resistance pattern of *Stenotrophomonas maltophila* and *Chryseobacterium meningosepticum* isolates to most of the antibiotics used. The treatment of *S. maltophila* is problematic owing to its high level of intrinsic resistance to multiple classes of antibiotics (Al-Zamil, 2008). Central venous catheterization has been found to be the most prevalent risk factor predisposing to BSI that extended the length of stay, increased the cost of treatment due to hospitalization; and was attributed to 4–37% of mortality (McLaughlin et al., 2012). Surprisingly, the mortality rate was higher in Pediatric ICU (PICU) than Neonatal ICU (NICU). This could be explained by the higher infection rate in PICU (186 out of 138, 1.34%) than NICU (280 out of 241, 1.16%) due to a problem in the infection control of this unit. The overall mortality among patients was 80 out of 728 (about 10%), of which 2.4% was directly correlated with infections (McLaughlin et al., 2012). Similar mortality rates were reported by other Saudi pediatric studies. In addition, the mortality rate in the NICU (for BSI) was 18.7%, which was comparable to a study performed previously in four main hospitals in Makkah in Saudi Arabia between 2004 and 2005 (13.4%) (Asghar, 2006).

DIAGNOSIS OF BSIs

BSI and its complications such as sepsis are the notable cause of fatality in critically ill patients, whether they are adults or neonates. Hence, the early diagnosis and treatment with rational antimicrobial therapy are of utmost priority in order to treat and manage acutely ill patients with BSI related-sepsis. The critically ill patient may remain asymptomatic with atypical signs, assessing their infectious status more difficult. The current culture-based approach for the detection of BSI is frequently too slow to allow start early and rational antimicrobial treatment and sometimes, may not identify the clinically important microorganisms. The delay in obtaining diagnostic results leaves the clinicians with no option but to initiate empiric antimicrobial therapy along with subsequent emergence of MDR strains, which complicates the treatment. There is an urgent need for rapid molecular and immunological diagnostic markers having attributes of high sensitivity and specificity, coupled with the feasibility of bedside monitoring and low cost (Jensen et al., 2008).

Laboratory and molecular diagnosis

The pathogenic microorganisms causing severe BSIs can be diagnosed by emerging detection technologies. The current gold standard for the identification of bacteremia or severe sepsis is blood culture and other culture-based techniques. All the same, these microbiological methods usually take time to complete and have a narrow sensitivity for fastidious (slow-growing) organisms. The pathogenic microorganisms can be rapidly identified within minutes to hours by nucleic acid-based techniques such as PCR amplification; using probes for hybridizing with specific pathogens, anti-bacterial, or anti-fungal activity; assays based on flow cytometer; and protein-based detection by mass spectrometry. Compared to detection and identification of live organisms in blood, interpretation results obtained from direct detection of pan-bacterial or pan-fungal nucleic acids in the blood is complicated, increasing the risk of contamination, ubiquitously present bacterial, fungal DNA, and the lack of a gold standard (Klouche and Schröder, 2008). Thus, it is necessary to develop rapid and improved high specific and sensitive phenotypic and molecular approaches to detect and identify BSI-related pathogens and their antibiotic resistance in order that suitable treatment could be initiated as early as possible (Huttunen et al., 2013). Table 2 gives a brief overview of the different diagnostic methods for BSIs.

The current gold standard for the diagnosis of BSIs is the automated machines, constant monitoring of blood cultures, Gram staining, sub-culture and phenotypic analysis, and antimicrobial susceptibility testing. The gold-standard culture is time-consuming and takes two to five days to complete, or even more in some cases such as endocarditis, which is inconsistent to make results available to clinicians to start rational therapy. Furthermore, culture-based methods may miss fastidious

Table 2. Diagnostic modalities for BSIs.

S/N	Diagnostic methods	Reference
A. Traditional methods - Gold standard but consumes time, have narrow sensitivity for fastidious microbes		
1	Blood culturing, Gram staining, sub-culture and phenotypic analysis and antimicrobial susceptibility testing	Datta et al. (2012), Klouche and Schröder (2008), Özenci, Tegmark-Wisell et al. (2008)
B. Molecular methods from positive blood cultures – Rapid but time lag in culturing, not for fastidious or non-culturable microbes, contamination risk		
1	RT-PCR	Hall et al. (2012)
2	PNA-FISH	Hall et al. (2012)
3	PCR/ESI-MS	Hall et al. (2012)
4	Multiplex PCR	Tang et al. (2007)
5	Pyrosequencing	Chan et al. (2012), Eigner et al. (2009), La Scola (2011), Motoshima et al. (2012)
6	MALDI-TOF MS	(Clerc et al. (2012), La Scola (2011), La Scola and Raoult (2009), Liesenfeld et al. (2014)
7	PCR coupled with microarray	Aittakorpi et al. (2012), Liesenfeld et al. (2014)
8	RT-PCR-based Film Array	(Altun et al. (2013)
9	Microarray-based multiplexed nucleic acid Verigene assay	Sullivan et al. (2013)
10	Time-lapsed microscopy coupled to image analysis	Sullivan (2017)
11	HRMA combined PCR	Ozbak et al. (2012), Ozbak (2018)
C. Molecular methods from whole blood – Rapid, suitable for fastidious or non-culturable microbes, no contamination risk		
1	Multiplex RT-PCR VYOO™	Bloos et al. (2012)
2	MagicPlex Sepsis Test	Opota et al. (2015)
3	Multiplex electrochemical Biosensor-based identification assay	Gao et al. (2017)
4	SepsiTest™	Wellinghausen et al. (2009)
5	LightCycler SeptiFast	Regueiro et al. (2010)
6	LOOXSTER® coupled with VYOO™	Liesenfeld et al. (2014)
7	BACSpectrum Assay	Ecker et al. (2010), Metzgar et al. (2016), Pfaller et al. (2016), Vincent et al. (2015)
8	T2 Magnetic Resonance-based assay	Pfaller et al. (2016)
9	PCR/semiconductor sequencing-based Genalysis and vivoDx system	Mwaigwisya et al. (2015)
D. Immunological methods		
1	PCT	Adib et al. (2012), Chiesa et al. (2000), Ghillani et al. (1989), Kim (2018), Köksal et al. (2007), Kopterides et al. (2010), Wacker, et al. (2013), Whicher et al. (2001)
2	CRP	Adib et al. (2012), Ghillani et al. (1989), Jaye and Waites (1997), Köksal et al. (2007), Wacker et al. (2013), Whicher et al.(2001)
3	IL-1, IL-6, IL-8, IL-10, IL-18, TNF-⍵, (s)CD164	Adib et al. (2012), Philip and Hewitt (1980), Russell (2012), Schulte et al. (2013)

microorganisms that are difficult or impossible to grow on culture. Confounding results may be obtained if antibiotics are given prior to blood sampling. In more than 50% of the cases, the blood cultures are reported to be negative while true bacterial or fungal sepsis are present (Özenci et al., 2008). This has directed the International Sepsis Committee to recommend that clinical judgment should prevail over the culture results in guiding the administration of antibiotics, including changes or discontinuation of the treatment. Hence, blood culture is not an ideal gold standard due to the delay in results that are incomplete and potentially misleading, as the recommendations are mostly to ignore delayed results in many cases. (Datta et al., 2012).

Most of the shortcomings of blood culture can be overcame by molecular techniques. An ideal molecular

approach would be to evaluate a patient's blood and provide all results needed to guide optimal antimicrobial therapy, and subsequently, provide data to assess its effectiveness over time. The current available molecular approaches are not sufficiently specific, rapid, or accurate enough to meet these objectives. These may represent the long-term targets for the diagnosis of BSIs (Datta et al., 2012).

A feasible target would be to analyze blood in conjunction with culture-based methods to identify the causative pathogens, inclusive of the fastidious microorganisms and determine some key determinants of drug resistance prior to the availability of the culture results. There is a scarcity of information on the molecular determinants of drug resistance but antibiotic-resistant genes have been identified in some species, such as those conferring resistance to methicillin, vancomycin, and carbapenem, which can be analyzed by molecular methods (Paolucci et al., 2010).

Quantitative measurements, being clinically relevant, are indispensable in microbiology laboratories. For instance, the bacterial load is prognostic of complexities and mortality but the inability to routinely monitor its load has obviated its value. Bacterial quantitation in blood by culture is difficult to accomplish and seldom applied in clinical laboratories, as it requires consequent plating on agar plates. The length of time to positive blood culture gives a weak indication of the bacterial load. However, it varies with the microbe(s) present. The quantitative molecular tests are precluded in BSIs due to various factors. Firstly, a wide spectrum of bacterial concentrations present in the infected blood challenges the lower limit of detection by current molecular techniques. Secondly, molecular approaches for pathogen DNA isolation from whole blood in adequate quantity and purity are inadequate. Thirdly, there are a plethora of microorganisms that may cause BSI and the quantitative molecular techniques encompassing all the potential causative microbes are lacking. Hence, the actual concentration of microbial DNA in BSI patients is rarely known. Nonetheless, a recent study suggests that quantitative analysis of bacterial DNA load could have implications in predicting the progression of BSI. Patients with pneumococcal pneumonia, having a high load of *Streptococcus pneumoniae* DNA, as analyzed by quantitative PCR, was a prime predisposing factor for shock and organ failure (Fazzeli et al., 2013).

An alternative approach is to analyze the blood specimens after growing in culture using molecular methods. The time lag and bias produced by culturing are the major drawbacks of direct molecular analysis of blood culture specimens. Further, this method is unsuitable for microorganisms that are difficult to culture (Ecker et al., 2010). Nonetheless, this method is more rapid compared to the standard method of phenotypic identification and antibiotic susceptibility testing that entails further a 24–72 h for post-culture positivity. PCR and other nucleic acid amplification-based techniques, as well as peptide and nucleic acid probes, have been reported that they can rapidly detect pathogens from positive blood cultures (Ecker et al., 2010).

The rapid detection of microbial DNA circulating in the blood has the potential to markedly speed up the time for diagnosis of BSIs and sepsis (Dark et al., 2009; Peters et al., 2004). Real-time PCR (RT-PCR) has been used for the diagnosis of BSIs rapidly in critically ill patients where rapid diagnosis and appropriate treatment are crucial (Dark et al., 2009). RT-PCR detects pathogen DNA and can therefore, reveal the presence of a pathogen even if the patient has been treated with antimicrobials. In addition, it can detect fastidious organisms and other non-cultural organisms (Dark et al., 2009). Since bacterial levels are often low in BSI, RT-PCR assays have to operate at high levels of sensitivity, capable of detecting a very low level of bacterial DNA in the sample (Dark et al., 2009). Molecular assays for detecting and identifying pathogen DNA in blood samples can be performed in a very short time to enable the rational selection of early antimicrobials in order to determine the optimum management plan of an infected patient.

Molecular methods for pathogen identification in positive blood cultures

Fluorescence in-situ hybridization (FISH) is a molecular technique utilizing peptide nucleic acid (PNA) that is used to identify several pathogenic species of bacteria and fungi (Hall et al., 2012). *S. aureus* was first identified from positive blood cultures by the U.S. Food and Drug Administration (FDA)-approved *S. aureus* PNA FISH kit (AdvanDx, MA, USA) using PNA probes to target *S. aureus* 16S rRNA and was able to differentiate *S. aureus* from non-*S. aureus* bacteria within hours.

A broad range amplification based on polymerase chain reaction coupled with Electron Spray Ionization Mass spectrometry (PCR/ESI-MS) has been developed wherein multiple primers pairs amplify the selected, broadly conserved regions of the microbial genomes. The target regions in the bacterial or fungal DNA encode ribosomal RNA while the essential proteins are encoded in the housekeeping genes. ESI-MS analysis of the amplified products is then carried out employing the automated PCR/ESI-MS equipment. The PCR amplicons are weighed with adequate mass accuracy by the mass spectrometer such that the nucleotide base composition (A, T, G, C) is inferred with respect to the amplicons present. The amplicon base-pair compositions are then correlated either with a pre-calculated database of surrogate markers of sequences of known micro-organisms or with the signatures from pre-determined (via PCR/ESI-MS) reference standards (Hall et al., 2012).

A new multiplex PCR assay has been developed that can concurrently identify a panel of bacteria and other

pathogenic microbes with precision and speed. The technique is based on the synchronized amplification of different segments of target DNA by employing two or more pair of primers in a single PCR-run, and is cost-effective and rapid (Hall et al., 2012). A StaphPlex system (Qiagen, CA, USA) has been designed to synchronously species-level identify and detect Panton–Valentine Leukocidin (PVL) in *Staphylococci* and several determinants of antibiotic resistance, directly from the blood cultures, wherein clusters of Gram-positive cocci are observed by Gram staining. This system employs a distinct target-enriched multiplex PCR to concurrently amplify, as well as identify, eighteen staphylococcus-specific genes. While the *tuf* and *nuc* gene targets are specific for CoNS and *S. aureus*, respectively, the *mecA* and *aacA* genes confer resistance to methicillin and aminoglycosides, respectively. The *ermA* and *ermC* genes contribute resistance to macrolides, lincosamides, and streptogramins, while the *tetM* and *tetK* genes afford tetracycline resistance. A Luminex suspension array characterizes the amplified products. The entire process, from the processing of blood cultures to procuring results, is accomplished in 5 h, which considerably curtails the time required for phenotypic characterization and antibiotic sensitivity testing (Tang et al., 2007).

A host of sequencing approaches has been used for the direct detection of microbial pathogens in positive blood cultures. The MicroSeq 500 kit (Perkin-Elmer Applied Biosystems, CA) has been successfully used by Woo and colleagues, in which the first 527 base pair fragments of the amplified 16S rRNA gene were sequenced (Chan et al., 2012). Pyrosequencing (Biotage, Sweden) has tremendous applications in identifying numerous bacteria, yeasts, and fungi, wherein fast, short-read sequencing of 30 bases can be performed in about thirty minutes. It can be carried out within a few hours in a 96-well microtiter plate from the bacterial colonies using a single PCR run for each pathogen. This technique is rapid and cost-effective as compared to the conventional sequencing and has been used for the classification, identification, and subtyping of different bacterial 16S rDNA regions (Chan et al., 2012). Using pyrosequencing, Maiko et al. (Motoshima et al., 2012) identified two distinct regions in the *Streptococcus* 23S rRNA gene in comparison to the formerly elucidated, universal 16S rRNA gene target. Pyrosequencing was found to be 97.8% concordant with the culture-based identification. When a single microorganism was isolated in blood cultures, the concordance may reach to higher than 98.8%. Although multiplex PCR or wide-range amplification methods with subsequent microbial sequencing subjected to conventional blood culture provides the benefit of obtaining results within a few hours, the costs incurred are higher compared to the conventional identification methods. The contamination of pathogen DNA in the blood sample during phlebotomy or processing further complicates this technique (Motoshima et al., 2012).

Bacterial colonies and genes conferring antimicrobial resistance have been identified in positive blood cultures using Matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (TOF-MS) as recently reviewed by La Scola et al (La Scola, 2011). This method provides rapid results with minimum labor as compared to the traditional methods. Presently, MALDI-TOF MS still entails microbial culturing and the determination of antibiotic resistance by traditional assays. Mixed pathogens cannot be identified due to dynamic-range issues in MALDI-TOF MS (La Scola and Raoult, 2009).

The gold standard, 16S-rRNA gene sequencing was recently compared on a large number of clinical isolates using the Bruker versus Shimadzu systems. Whereas the Bruker system identified 94.4% of the clinical isolates, the Shimadzu spectrometer detected 88.8%. A concordance of >99% was observed with the two systems using conventional as well as 16S identifications for the anaerobic bacteria while the streptococcal species remained unidentified (La Scola, 2011). Identification to the species level was reported by Eigner et al. (Eigner et al., 2009) in 80–100% of the clinical isolates. Additionally, MALDI-TOF MS has also been employed for the detection of yeast and yeast-like fungi. A recent report (Clerc et al., 2012) showed that the MALDI-TOF MS analysis on positive blood cultures helped to modify therapy in adults (13.4%) as well as pediatric patients (2.5%). Amalgamation of microorganism detection by MALDI-TOF and antimicrobial stewardship offering real-time review and intervention resulted in the reduced mortality, the shortened length of ICU stay, coupled with curtailing the time required for potent and optimum antimicrobial therapy, and the diminished recurrence of bacteremia. Though still dependent on positive blood cultures and Gram staining, it greatly improved the management of sepsis patients (Liesenfeld et al., 2014).

Prove-it sepsis (Mobidiag, Finland), a wide-spectrum novel PCR and microarray method, using positive blood cultures, is based on the amplification and identification of *gyrB*, *parE*, and *mecA* genes of about 50 bacterial species (Liesenfeld et al., 2014). A wide array of bacteria encompassing about 90% of the etiologic agents of sepsis can be identified by this assay that is reported to have 94.7% sensitivity and 98.8% specificity and is 18 hours more rapid than the conventional blood culture method. Recently, clinically important yeasts have also been identified with 99% sensitivity and 98% specificity (Aittakorpi et al., 2012). Unfortunately, the microbiological results have not been correlated with the clinical information. Spiking whole blood has correctly identified bacterial species with a detection limit of 11–600 CFU/mL (Laakso and Mäki, 2013).

The RT-PCR based-Film Array Blood Culture Identification Panel (Biofire, US) detects BSI-related six Gram-positive and ten Gram-negative bacteria besides the five *Candida* spp. and antibiotic resistance genes (*mecA, VanA/B*, and *KPC*) within 1 hour of positive blood cultures. The pathogens could be detected in 91.6% of samples having mono-microbial growth when compared

to the traditional blood culture-based identification method. While 7.8% of the pathogens undetected by blood culture were also not identified by the Film Array Panel, an additional pathogen was detected in 3.6% of the clinical isolates (Altun et al., 2013).

The Verigene Gram-positive and Gram-negative microarray-based multiplexed nucleic acid assays (Nanosphere, US) carried out on the Verigene system can detect and identify an array of Gram-positive (9 bacteria at species level and 4 at genus level) and Gram-negative pathogens (5 bacteria at species level and 4 at genus level) and their related antibiotic resistance markers in positive blood cultures. The assay can be performed in 2.5 hours and has shown to have a high sensitivity and specificity in comparison to the gold-standard of microbiological methods (Sullivan et al., 2013). In a multicenter study conducted in 2015, Siu and his group evaluated the specificity and sensitivity of Verigene Gram-positive blood culture and Gram-negative assay on 364 blood cultures. They indicated that this assay had a sensitivity of 98.9–100% and specificity of 80% (Altun et al., 2013). The recent FDA-approved Accelerate Pheno assay (Accelerate Diagnostics, Tucson, AZ) employs time-lapse microscopy coupled with image analysis for susceptibility assays to pre-define panels of drugs on blood cultures of several Gram-negative (*E. coli*, *Klebsiella* species, *Enterobacter* species, *Proteus* species, *Citrobacter* species, *S. marcescens, Pseudomonas aeruginosa, A. baumannii*), Gram-positive (*S. aureus, Staphylococcus lugdunensis,* CoNS species, *E. faecalis, E. faecium, Streptococcus* species), and yeast (*Candida albicans*, *Candida glabrata*) isolates in 6 hours (Sullivan, 2017).

Another new technology named high resolution melting analysis (HRMA) has been applied recently for direct detection of pathogens isolated from positive blood culture. HRMA, when combined with real time PCR, can be used for characterizing PCR amplicons based on differences in melting profile (curve shape) and therefore identifying a range of clinically important organisms from clinical isolates and positive culture (Cheng et al., 2006; Yang et al., 2009). Ozbak and co-authors in 2012 reported that the HRMA combined with PCR is a low-cost molecular approach that has ability for identification of the 21 organisms responsible for bloodstream infection from pathogens isolated from positive blood culture (Ozbak et al., 2012). In 2018, this PCR-HRMA approach was tested further for its ability for detection and identification of antibiotic resistant genes (Ozbak, 2018). It has been reported that 16S-PCR-HRMA assay has the potential for rapid detection and identification of vancomycin-resistant Enterococci (Ozbak, 2018).

Molecular diagnosis independent of blood culture

The emerging PCR-based techniques for direct detection of pathogen DNA from the whole blood have shown promise in early clinical diagnostic studies. The advantages and limitations of culture-based versus PCR techniques should be weighed prior to consideration in clinical practice (Deen et al., 2012). The major strengths of direct PCR from the blood include enhanced sensitivity, bypassing time-consuming culture, and leading to a significant decline in turnaround times in comparison to that entailed in PCRs from positive blood cultures. These amplification methods comprise of wide spectrum and multiplex PCR, most of which are CE (Conformité Européene, which literally means European Conformity) labeled, but the FDA has approved none.

The multiplex real-time PCR enables the rapid detection of pathogens directly from whole blood by concurrently amplifying multiple DNA targets with a mixture of primer. The assay is based on the amplification of non-coding internally-transcribed spacer (Jensen et al., 2008) regions of the ribosomal DNA that, being localized amongst the highly conserved genes, is remarkably heterogeneous among the bacterial and fungal genera and species, resulting in a notable identification with a narrow pool of slightly degenerate primers. Whereas PCR-based methods can detect the pathogens with higher sensitivity and more rapidity compared to conventional blood culture, presently, PCR can be a complementary to, but not replace, blood culture. A plethora of studies has reported a considerably elevated detection rate when combining both PCR and blood culture together than when one of them was used alone. Moreover, full detection and determining the antibiotic resistance profile cannot be currently done completely by PCR owing to the limitations in multiplexing potentialities (Mancini et al., 2010).

The Multiplex-PCR system VYOO (SIRSLab, Germany) was reported to have detected thirty-four bacterial and seven fungal species and five most prevalent antibiotic-resistance genes (*mecA, vanA, vanB, blaSHV, blaCTX-M*) (Bloos et al., 2012). In a study conducted by Bloos and his co-authors on 311 concomitant blood cultures and blood for VYOO obtained from 245 patients suspected to have sepsis; only 14.5% of blood cultures were positive, 30.1% of PCRs were found to be positive. Thus, the VYOO provides rapid and more frequently positive results that may help to early administering of antimicrobial therapy (Bloos et al., 2012).

The MagicPlex Sepsis Test (Seegene, Seoul, Korea) is a multiple-step automated system that couples conventional PCR with RT-PCR. The microbial DNA is amplified by PCR followed RT-PCR and allows identification of more than ninety pathogens at the genus level, 25 at the species level (nineteen bacteria and six fungi) as well as the antibiotic resistance genes (*mecA, vanA*, and *vanB*), with the time for results being 3 to 5 h (Opota et al., 2015). The sensitivity and specificity vary from 37 to 65% and 77 to 92%, respectively.

Recently, a multiplex electrochemical biosensor-based identification of species-specific sequences of 16S rRNA

of bacteria has been reported. The biosensor could identify bacterial clinical isolates spiked in whole blood with a 100% concordance to microbiological assays (Gao et al., 2017). Since the electrochemical sensing platform is portable, the assay can be easily carried out at the bedside (point-of-care, POC). Moreover, it diminishes the nonspecific amplification-related false positives, as it does not involve nucleic acid amplification.

Another commercial Real-time PCR (RT-PCR) approach is SepsiTest™ (SepsiTest; Molzym), which can be used for the identification of bacterial and fungal DNA that causes BSIs, but uses sequencing for species identification. Moreover, SepsiTest has recently gained European regulatory approval (CE Marked). SepsiTest™ involves subjecting white blood cells to gentle lysis followed by DNase treatment to scale down the human DNA content in the blood sample. Subsequently, bacteria and/or fungi analyzed under more stringent conditions, yielding pathogen DNA and negligible amounts of human DNA as a contaminant. PCR amplification of the 16S rDNA with subsequent sequencing helps to identify the microbes. In analytical studies, SepsiTest showed up to a 1000-fold higher sensitivity for spiked blood samples compared with the conventional method, with a detection limit as low as 50 Colony Forming Unit per milliliter (CFU/ml) of blood. Wellinghausen et al. (2009) investigated the SepsiTest™ for the identification of suspected bacteremia in critically ill patients and reported an 87% sensitivity and 85.5% specificity for the assay when compared with blood cultures. A potential shortcoming of this technique is that the freely circulating pathogen DNA released from the remote site microbial infections are digested together with the human leucocyte DNA. Similarly, the intracellular bacteria having membranes that lyse readily, may not be recovered (Wellinghausen et al., 2009). The requirement for an additional post-PCR sequencing step to achieve the identification of the pathogens detected is a significant disadvantage of SepsiTest™ since it substantially increases the time and cost of analysis.

In a prospective multicenter study conducted in 2016 on 236 samples from 166 ICU patients with clinically suspected sepsis collected from three different centers (Medical Center Cologne- Merheim, Germany; Heinrich Heine University Hospital, Germany and VU University Medical Center in Amsterdam, The Netherlands). The 16S/18S rDNA PCR SepsiTest™ assay (PCR-ST) was found to be far superior to conventional blood culture, with the detection of supplemental 71% BSI over blood culture alone (Nieman et al., 2016). The overall sensitivity and specificity of PCR-ST were reported to be 66.7% and 94.4%, respectively. The lower sensitivity was due to the detection of CoNS that were contaminants in the *S. aureus* and *Streptococcus* species. The results need to be supported by a clinical interpretation to rule out the contaminants from clinically relevant microorganisms, which is a major shortcoming of this approach.

The LightCycler® SeptiFast (Roche Molecular Systems, Germany) is a multiplex PCR-based assay that can identify twenty-five microbes directly from the blood. SeptiFast employs real-time PCR in a qualitative mode to detect 19 bacteria, five *Candida* species, and *Aspergillus fumagatus,* which are presumed to be responsible for more than 90% of all BSIs. This method entails nucleic acid extraction from whole blood by mechanically lysing with ceramic beads, followed by spin column-based nucleic acid purification resulting in the isolation of both human (from white blood cells) as well as pathogen DNA. The elutes are subjected to three real-time PCR amplifications for Gram-positive bacteria, Gram-negative bacteria, and fungi, which are identified using the respective hybridization probe-based fluorescence melting curve analysis (FMCA) software (Regueiro et al., 2010). The appropriate inhibitor controls for monitoring reaction efficiency are incorporated. The turnaround time is approximately 5 h. Recently, a semi-automated DNA extraction method was employed using MagNAPure (Roche Molecular Systems) that reduces the turnaround time to 3.5–4 hours without compromising the precision (Liesenfeld et al., 2014). The SeptiFast has been assessed in multicenter studies conducted primarily on adult ICU patients with suspected sepsis, severe sepsis, or septic shock, for CE registration, wherein the substantially greater positivity rate was reflected for SeptiFast than for blood cultures. In single-center studies conducted on ICU patients, the positivity rate for SeptiFast varied from 25%–35% while that of traditional blood cultures was 13 to 21% (Liesenfeld et al., 2014).

LOOXSTER® (SIRS-Lab) utilizes the methylation variations among bacterial/fungal and human DNA for the enrichment of the blood samples in pathogen DNA by affinity chromatography with subsequent 16S rDNA gene amplification utilizing a product, VYOO™ (Liesenfeld et al., 2014). This strategy was found to substantially alter the pathogen-human DNA ratio. Since nearly 90% of the human DNA was eliminated, the signal loss in subsequent amplification due to contaminating human DNA was remarkably reduced, coupled with at least a ten-fold enhancement in the sensitivity compared to the routine assay lacking in the pathogen DNA-enrichment step.

Another approach for the molecular identification of BSIs integrates wide spectrum PCR amplification with ESI-MS (PCR/ESI-MS). Earlier variants of this strategy, invented by Ibis Biosciences (a part of Abbott Molecular), were commercially called TIGER or Ibis T5000. Abbott PLEX-ID is the current version of the hardware platform conducting MS and the assay based on the Web App—PLEX-ID—for the direct evaluation of BSIs is known as BAC Spectrum Assay. This method utilizes primers targeting highly conserved genomic regions of bacterial or fungal DNA. The method was initially developed primarily for biodefense applications, for the detection of unknown microbes or those in samples with a multitude

of microorganisms, but difficult to culture. The technique is now being used for the diagnosis of BSIs (Ecker et al., 2010).

The BAC Spectrum assay for BSIs detects the bacterial DNA using eight amplification steps involving nine pairs of primer (including two multiplexed primer pairs), of which, three are specific for 16S rDNA, one for 23S rDNA, and the remaining four are designed for universally conserved housekeeping genes (*tufB*, *rplB,* *valS*, and *rpoB*). It is important to include primers specific for housekeeping genes. Firstly, in the event of difficulty in differentiating closely related bacterial species from the rDNA sequence, the information is integrated from the mass spectral signatures obtained from the housekeeping gene targets. Secondly, in case of mixed populations of microorganisms, there is a competitive amplification of the more abundant species as ribosomal primer pairs are used, resulting in the detection of the less abundant species at waning sensitivity. In contrast, the dynamic range of amplification is augmented when non-overlapping primer pairs are employed such as those targeting the housekeeping genes, as the competition among the mixed microbial population is thwarted (Anderson et al., 2007).

A major shortcoming of molecular diagnostic techniques lies in the fact that only three genes related to the phenotypic antibiotic resistance are presently analyzed by the BAC Spectrum assay. Namely, *mecA*, a molecular determinant of resistance to β-lactams; *vanA* and *vanB*, encoding resistance to vancomycin; and bla_{KPC}, conferring resistance to the carbapenems in *K. pneumoniae* and other Gram-negative bacteria. The molecular resistance determinants to other antibiotics would entail multiple region-genomic analyses of the microbes for inclusion in the present assay. Further advances in multiplex technology would facilitate the analysis of multiple drug-resistant markers by PCR/ESI-MS technology (Hall et al., 2012). Nonetheless, this strategy may help to target antibiotic therapy in patients who have initiated antimicrobials and those with negative culture results as evidenced in a recent multicenter study (Vincent et al., 2015).

Most infections, especially sepsis-related BSIs, are critical emergencies, requiring prompt therapeutic responses in order to mitigate the mortality and morbidity rates. The rapid culture and non-culture-based molecular techniques for the diagnosis of BSIs should be incorporated in our hospitals to facilitate timely treatment and minimize the morbidity and mortality, and hence, the economic burden. A recent study reported at a T2Candida panel presented a T2 Magnetic Resonance (T2MR)-based diagnostic approach for the detection of *Candida sp.* (Pfaller et al., 2016). This technique enables the detection of microorganisms at a threshold density of 1 CFU per ml of whole blood, in comparison to that needed for conventional PCR-based assays (100-1000 CFU/ml). New emerging technologies are being developed such as the PCR/semiconductor sequencing-based Genalysis for the detection of pathogens as well as their antimicrobial resistance genes directly in whole blood within 2–3 h. The vivoDx system for the identification and antimicrobial susceptibility testing of microorganisms, based on the coupling of DNA-delivery bio-particles with DNA, causing bacteria to express luciferase, with a turnaround time of less than 4 h (Mwaigwisya et al., 2015).

Immunological diagnosis of sepsis-related BSIs in adults and neonates

The immune system responds to severe BSI, culminating in a fatal condition called septicemia or sepsis, in which inflammatory responses are triggered to ward off the infection. The traditional immunological markers of inflammation, such as the leukocyte count and C-reactive protein (CRP) may be affected by parameters apart from infection and may be gradually released during infection. This coupled with the absence of an effective antibiotic therapy in the early phase of infection may prove detrimental to the patient. In such patients, there is a need for development of specific, accurate, and rapid diagnostic biomarkers for detection and identification of BSI (Volante et al., 2004). Blood culture, though the gold standard diagnostic tool, may yield false-positive results due to contamination, and also may give false-negative results in case of bacterial infection caused by fastidious organisms (Folli et al., 1987). Even though it is time-consuming, culture-based methods, especially blood cultures, remain crucial for sepsis diagnosis and facilitate to design a targeted therapy (Volante et al., 2004). The major pitfall is that the cultures are prone to contamination risks.

Other laboratory diagnostic methods comprising of the evaluation of microbial antigens, leukocyte count, determination of interleukins, procalcitonin (PCT), and acute phase proteins like C-reactive protein (CRP), haptoglobin, fibrinogen, and α1-antitrypsin have been explored (Adib et al., 2012). Acute phase reactants are commonly used as early sepsis markers. While some studies showed CRP as a promising biomarker for neonatal sepsis, others reported PCT as an early sepsis marker in critically ill as well as young patients (Adib et al., 2012; Köksal et al., 2007; Wacker et al., 2013). PCT (14.5 kD), a precursor of calcitonin, has 116 amino acids that lack hormonal activity. PCT is detectable in the plasma of healthy volunteers two hours post-administration of bacterial endotoxins, with a rapid surge in 6–8 hours, after which it plateaus and drops to attain normal levels in 24 hours (Dandona et al., 1994; Whicher et al., 2001). Serum concentrations of PCT in severe sepsis correlate with the clinical status of the patient and the intensity of the immune response. Serum PCT levels correspond to the severity of microbial invasion and

decline rapidly following effective antimicrobial therapy. Patients with localized bacterial or viral infection have been reported to have slightly elevated PCT levels, the normal being <0.5 ng/ml. Concentrations higher than the baseline value reflect pathological conditions (Whicher et al., 2001). At this cut-off value, PCT surpasses CRP in sensitivity and can predict late-onset sepsis in low birth weight infants (Ghillani et al., 1989; Köksal et al., 2007). However, a recent study indicated an elevated optimal cutoff value of PCT in nosocomial BSI with impaired renal function (Kim, 2018).

Earlier studies have reported a rise in PCT post-intravenous administration of endotoxins, following an upsurge in levels of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 (a few hours later), but preceding the elevation of CRP, and it recedes following a drop in IL-6, but prior to CRP at the cessation of the inflammatory response in clinical sepsis. Though a classical acute phase protein and a sensitive inflammatory marker, CRP cannot distinguish between bacterial and other infections as it enhances the following surge in PCT, therefore, it is not used to monitor the progression of the infection (Jaye and Waites, 1997; Köksal et al., 2007; Whicher et al., 2001). On the other hand, PCT could be a marker for distinguishing the infectious from non-infectious events. It has been shown to be a marker for the severity of infections. PCT is thus, a more promising early inflammatory marker than CRP for sepsis in adults and neonates (Atlıhan et al., 2002), and helps to assess the degree of microbial invasion, the intensity of sepsis, and optimal response to antimicrobial therapy. In a 2011 study focusing on the period between 2008 and 2009, at a university-affiliated, tertiary care medical center (Johns Hopkins Bayview Medical Center, Baltimore, MD), the benefits of using procalcitonin as a diagnostic marker of BSI was evaluated. Twelve patients out of 16 patients with BSI have positive procalcitonin levels in their samples (Atlıhan et al., 2002). Since PCT has a higher (93%) negative predictive value (NPV), its estimation is of prime importance in neonates (Guibourdenche et al., 2002). The routine use of PCT for diagnosis and follow-up post-therapy of neonatal sepsis would help to curtail the futile usage of antibiotics and shorten the hospital stay, thereby mitigating the cost incurred. However, some studies indicated that PCT is not an ideal marker for detecting sepsis. For instance, in a one-year period prospective multicenter study in 2006 at teaching hospitals in Spain, López-Sastre and his group assessed the usefulness of PCT as a diagnostic marker of sepsis in neonates with clinical suspicion of neonatal sepsis. They concluded that PCT was not accurate enough to be a definite immunological marker of neonatal sepsis but that it could be used in conjunction with microbiological culture and other sepsis's identification methods (Atlıhan et al., 2002).

The immunological markers including PCT should thus be used to complement the clinical diagnosis (Köksal et al., 2007). A major pitfall in the usage of PCT lies in its expression in non-infectious disease scenarios (Folli et al., 1987). Nonetheless, the strategy of implementing PCT-based algorithms may help to scale down antibiotic administration in critically ill, sepsis patients without jeopardizing the clinical effectiveness (Chiesa et al., 2000; Köksal et al., 2007; Kopterides et al., 2010).

The absence of any reliable test for the early detection of neonatal sepsis calls for an ongoing quest for new biomarkers, including other cytokines. Pro-inflammatory as well as anti-inflammatory cytokines constitute a double-edged sword in sepsis; they are indispensable in the elimination of infection as well as a cause of tissue and organ damage when excessively produced (Folli et al., 1987). Cytokine levels in amniotic fluid and maternal blood serum are not normally assayed. Hence, while clinical signs and symptoms remain imperative to neonatologists, laboratory diagnostic tests would help to establish early sepsis. Currently, serum concentrations of inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, IL-10, IL-18, and TNF-◎and the inflammatory markers, soluble CD164 remain one of the most sensitive and specific sepsis indicators in the neonates (Adib et al., 2012; Philip and Hewitt, 1980). Both, pro- as well as anti-inflammatory cytokines mediate the pathophysiology of sepsis. Preceding the appearance of any clinical signs and positivity of regular laboratory tests, the cytokine levels are heightened in infants with sepsis. However, very few studies have evaluated the cytokine profiles in the sepsis diagnosis and prognosis. The analysis of cytokines and other inflammatory biomarkers may facilitate the diagnosis of early-onset (assaying umbilical cord blood) as well as late-onset infections (sequential assays performed while residing in neonatal ICU).

Gram-negative sepsis has been reported to be more associated with elevated levels of pro-inflammatory cytokines (IL-6 and TNF-*α*) than Gram-positive sepsis due to the interaction of lipopolysaccharides and toll-like receptor (TLR)-4. In yeast sepsis, IL-10 is induced. IL-6 induces the hepatic acute phase protein synthesis and downregulates the TNF- *α* and IL-6 production that limits the inflammatory reaction. IL-8, a neutrophil chemoattractant, leads to inflammation-mediated tissue damage and multiple organ dysfunctions.

The cytokines are thus more reliable early diagnostic markers of neonatal sepsis than CRP. The inflammatory cytokines ascertain the degree of microbial invasion, as well as sepsis intensity, together with the response of post-antimicrobial therapy. Though both pro- (IL-6 and TNF-*α*) and anti-inflammatory cytokines (IL-4 and IL-10) increase during sepsis, IL-4 persists at low levels owing to its short half-life. IL-6 and IL-10 are significantly elevated in most septic patients, while TNF-*α* and IFN-*γ* are mostly associated with the disease severity. The benefit of employing cytokines in the routine diagnosis and post-therapeutic intervention follow-up of neonatal sepsis would be to scale down the unnecessary usage of

antimicrobials and expedite discharge, with accompanying cost-effectiveness (Russell, 2012; Schulte et al., 2013). The combination of cytokines with other inflammatory markers may expedite the diagnosis and the intervention of sepsis, optimizing clinical management.

CONCLUSION

In summary, the diagnosis of BSIs and sepsis-related BSIs continues to challenge the clinicians as it may result in a high mortality and morbidity rate without early and optimal therapeutic intervention. Antibiotic therapy, if promptly started, can improve the prognosis of sepsis. A 7.6% decline in the survival rate of sepsis-related BSIs patients has been reported for every hour of delay in the administration of effective therapy. The survival rate varies from approximately 80% with adequate therapy within the first hour of onset of symptoms, to less than 10% when the treatment is not given within 24 h. As per the recommendations of the International Committee on Surviving Sepsis(Rhodes et al., 2017), a patient with signs and symptoms of sepsis should be offered empirical treatment with broad-spectrum antibiotics, covering all likely pathogens, within the first hour, although a more targeted therapy would be appropriate. The Committee further recommended the drawing of blood for culture prior to antimicrobial therapy to effectively target the therapy after the availability of the culture results. Another recommendation is that once the pathogen has been identified and the antimicrobial susceptibility tests are done, empiric antimicrobial therapy should be narrowed to focus therapy. The current gold-standard blood culture, for detection and identification of BSIs and anti-microbial susceptibility profile, takes time to complete (2-5 days). This delay in results leaves the clinicians with no option but to immediately start empiric antimicrobial therapy, with subsequent possible emergence of MDR strains that complicates the treatment. Moreover, in case of the absence of infection from the blood culture, the administration of broad-spectrum antimicrobial therapy would increase the risk of developing antimicrobial resistance and increase healthcare costs. It is also important to take into consideration the potential for drug intolerances and toxicity. Therefore, there is a need for rapid, accurate, specific, sensitive and cost-effective molecular and immunological diagnostic approaches for rapid diagnosis of BSIs in order to ensure effective antimicrobial therapy for treating patients with sepsis-related BSIs. Tests of maternal inflammatory markers are low specific; culture tests are not immune from contamination risk and they have high turnaround times. Several molecular and immunological approaches have been developed to be applied directly on whole blood or positive blood cultures; this may lead to rapid and accurate diagnosis of severe BSIs with more effective therapeutic intervention and favorable clinical outcomes. Nonetheless, the shortcomings of the current diagnostic methods are substantial and may need to be complemented with traditional blood culture results. The reduction of the BSI burden needs to be weighed against the potential costs that might be incurred.

This review indicates that BSIs account for 1-11% of all patients confirmed to have infection in Saudi Arabia from 1983 up to 2015. This review has also showed that *Staphylococcus aureus* and Coagulase negative *s*taphylococci (CoNS) were the most common Gram positive pathogens while *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were the most Gram negative ones over patients with BSI. The major recommendations that stem from this review include initiating surveillance systems of BSI in different regions of Saudi Arabia with routine antibiotic susceptibility testing to design antibiograms that may improve the antibiotic stewardship program for treating BSIs. A comparative study should be initiated for patients suspected of having sepsis, using the standard blood cultures, immunological markers, rapid CE-approved molecular techniques, and microbiological investigations in order to set up a well-established panel to help diagnose BSIs rapidly and to guide and optimize patient management and treatment. The preventive strategies under INICC that have been proven effective in the ICUs of Saudi Arabia should be extended to all the hospitals to mitigate sepsis-associated BSIs rates.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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