

Antimicrobial Resistance in Pathogens Causing Pediatrics Bloodstream Infections in Madinah Maternity and Children's Hospital, Saudi Arabia

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ABSTRACT

Background: Bloodstream infection is one of the most common life-threatening condition in hospitalized pediatrics especially if associated with resistant microbes.

Aims: Determination the incidence, predisposing factors, microbiological and antimicrobial resistance patterns in suspected bloodstream infection (BSI) pediatric patients.

Place and Duration of Study: Different wards of Madinah Maternity and Children's Hospital, Saudi Arabia, during one year period from July 1, 2009 to June 30, 2010.

Methodology: Blood cultures were performed to all cases (n= 11968) using Bactec 9240 instrument Blood Culture Systems. Microorganisms were identified by colony morphology, Gram stain and biochemical profiles. BD Phoenix™ was used in confirmation of identification of all BSI Gram-negative isolates. Antibiotic susceptibility pattern of isolates was further done by using disk diffusion method.

Results: 728 cases (6.1%) were diagnosed of-with bloodstream infection after having a one positive blood culture according to positive blood cultures. The overall mortality rate was 11%. Gram-positive, Gram-negative and yeast accounted for 63.8%, 31.6% and 4.6% of the total isolates, respectively. Coagulase-negative staphylococci were the most prevalent Gram-positive isolates (44%); while *Serratia marcescens* and *Klebsiella pneumoniae* were the most common Gram-negatives. Gram-positive bacteria were mostly sensitive to cephalothin (82.3%) and vancomycin (72.2%), while Gram-negative bacteria were mostly sensitive to ciprofloxacin (93%), piperacillin/tazobactam (92.9%), and meropenem (89.8%).

Conclusion: The incidence rate of BSI is highest in ICU neonates. Therefore, special attention should be given to the quality of care provided for them to improve safety. There was appreciable resistance to commonly used antibiotics; and continued monitoring of antibiotic resistance is of great importance to ensure the proper use of antibiotics and to detect any increasing trends in resistance.

Keywords: Bloodstream infections; pediatrics; antimicrobial resistance; Saudi Arabia.

1. INTRODUCTION

Bloodstream infections (BSIs) are among the most common hospital-acquired infections in pediatric patients, and are responsible for approximately 10% to 30% of the cases (Gray, 2004). Bacteremias result in are related to up to 50% of a hospital mortality rate and to excessive healthcare costs (DiGiovine et al., 1999, Orsi et al., 2002).

Risk factors for hospital acquired BSI include: prematurity, prolonged hospitalization, intensive care unit (ICU) admission, indwelling catheterization and mechanical ventilation (Babay et al., 2005).

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27 Most of the literature concerning BSI is generated from adult patients that cannot be directly
28 extrapolated to children because of significant differences in age, underlying medical
29 conditions, or-and type, and distribution of pathogens (Yogaraj et al., 2002).

30 Bacteriological culture to isolate the offending pathogen and knowledge about sensitivity
31 pattern of the isolates remain the gold standard in the core-of definitive- microbiological
32 diagnosis and management of BSI. Furthermore, rates of antimicrobial resistance are
33 increasing rapidly nowadays with patterns of large variations between countries. Thus,
34 knowledge of local antimicrobial resistance patterns is essential to guide empirical and
35 pathogen-specific therapy within any hospital.

36 Antimicrobial resistance in nosocomial BSIs is a major health care-associated (HA) problem
37 that has a negative impact on patient morbidity and mortality (Foglia et al., 2007). Factors
38 that result in increased resistance in nosocomial pathogens include the inappropriate and
39 high use of antibiotics, limited availability of infection prevention and control programs,
40 limited surveillance systems to measure HA infections; and increased use of invasive
41 medical devices and invasive procedures at ICUs (Hsueh et al., 2002, Paskovaty et al.,
42 2005). An important consequence of antimicrobial resistance is treatment failure for serious
43 infections in hospitalized patients, that is often associated with higher rates of morbidity,
44 mortality, longer hospital stays, and increased costs of medical care (Carmeli et al., 2002,
45 Engemann et al., 2003).

46 Although some studies on the incidence of BSI in pediatric patients have been conducted in
47 Saudi Arabia, information on its incidence is lacking in Madinah Region. Thus, the aim of the
48 present study was to determine the incidence, predisposing factors, microbiological and
49 antimicrobial resistance patterns in pediatric patients suffering from BSI in different wards of
50 Madinah Maternity and Children's Hospital (MMCH); so that a guideline can be prepared for
51 empirical antibiotic therapy in the hospital.

52 53 **2. MATERIALS AND METHODS**

54 55 **2.1 Study location and patients**

56 The present retrospective study was conducted in MMCH, Madinah Munawarah city, located
57 in the western part of Saudi Arabia, during one year period from 1 July 2009 to 30 June
58 2010. MMCH, The hospital which has 500 beds, and provides primary, secondary and tertiary
59 health care; and is the main referral hospital for Madinah region.

60 Clinical standards of practice in hospitals entail collecting blood for culture from patients
61 exhibiting clinical signs of infection (e.g., fever $>38^{\circ}\text{C}$). During the study period, 11968
62 suspected BSI pediatric patients (new born to 14 years) were involved from different wards
63 at MMCH [Pediatric Intensive Care Unit (PICU), Neonatal Intensive Care Unit (NICU),
64 Minimal Care Unit (MCU), In-Patient Wards (IPWs) and Out-Patient Department (OPD)]
65 were included in the study. Their mean age was 803.83 ± 1234.4 days with min. age of 2 days
66 and max. of 14 years.

67 The clinical data collected from each patient medical record included: age, gender, location
68 (hospital ward), clinical diagnosis, length of hospitalization and risk factor including using
69 intravenous devices, urinary catheters, hemodialysis shunts, endotracheal tubes, ventilator
70 support, and previous antibiotic treatment given within the last month. Gestational age and
71 birth body weight was additional data collected for NICU deaths patients. The date of death
72 of patients was compiled from the patient administrative system.

Criteria used for Laboratory-confirmed BSI diagnosis should meet at least one of the following criteria: (1) ~~the patient had a recognized pathogen cultured from a~~ at least one set of positive blood cultures for a known pathogen, which was not related to an infection at another site; (2) ~~The patient experienced~~ had at least one of the following signs or symptoms: fever (> 38°C), chills, hypotension (systolic blood pressure > 90 mmHg), and a common skin contaminant e.g., diphtheroids (*Corynebacterium* spp.), *Bacillus* spp., *Propionibacterium* spp., coagulase-negative staphylococci (including *Staph. epidermidis*), viridans group streptococci, *Aerococcus* spp., or *Micrococcus* spp. that was isolated cultured from two or more blood cultures drawn on separate occasions (Liu et al., 2010). In neonates, in addition to the above criteria, the criteria included; toxic appearance, lethargy, hypotension, hypoventilation, hyperventilation or cyanosis.

An episode of BSI was defined as isolation of one or more recognised bacterial or fungal pathogens from one or more blood cultures. Only the first isolate per patient is counted, unless at least 14 days has passed without a positive blood culture with the same organism, after which an additional episode is recorded. ~~Where an episode was polymicrobial, a~~ All significant organisms were reported in a polymicrobial episode.

If a potential skin contaminant was isolated (e.g. coagulase negative staphylococcus, diphtheroid, etc), and the organism was isolated from two or more blood cultures drawn on separate occasions within 48 hours, the patient must have at least one of the following signs and symptoms within 24 hours of the blood culture being collected: ~~(For patients aged >1 year: fever (>38°C), chills, rigors, hypotension~~ (For patients aged >1 year):; and ~~for patients aged ≤1 year: fever (>38°C core), hypothermia (<36°C core), apnoea or bradycardia~~ (For patients aged ≤1 year) and the organism was isolated from two or more blood cultures drawn on separate occasions within 48 hours.

BSI was considered as community acquired which if developed before ~~(outpatient)~~ or within 48 hours after admission ~~(except for unless an organisms~~ with a long incubation period as *Salmonella typhi* is isolated) and was not maternally-acquired, ~~was designated as community acquired, and it was considered to be hospital acquired~~ ;- BSI and that if it occurred more than 48 hours after hospital admission or within 48 hours of discharge and were or in direct relation to medical procedures undertaken in the hospital ~~was considered to be hospital acquired~~.

2.2 Isolation, identification and antibiotic susceptibility testing of microbial isolates from BSI cases

Blood samples for culture were obtained from pediatric patients by venipuncture after ~~preparation-sterilization~~ of the skin with 70% isopropyl alcohol or from umbilical catheters using aseptic techniques. Pediatric blood culture bottle (Bactec Peds plus/F 40-ml culture vial, aerobic resin) was inoculated aseptically with 1-3 ml of blood from each case in accordance with the manufacturer's instructions. The bottles were then incubated in the Bactec 9240 instrument (Becton Dickinson, USA) for at least 7 days.

Specimens from the positive bottles, were gram stained, sub-cultured on the following media: blood agar, chocolate agar, MacConkey's agar and Sabouraud dextrose agar. Chocolate agar and blood agar plates were incubated in a CO₂ incubator with 5% to 10% CO₂ for up to 48 hours while other culture plates were incubated aerobically overnight at 37°C.

Identification of blood culture isolates was based on colony morphology, test results of catalase, coagulase, oxidase, urease, germ tube, optochin, bacitracin and biochemical profiles using API tests (BioMérieux, France) specific for gram-negative organisms. BD Phoenix™ NMIC/ID panels were also used in to confirmation of the identification of all BSI Gram-negative isolates.

Furthermore, antibiotic susceptibility testing was done according to National Committee for Clinical Laboratory Standards (NCCLS, 2005).

3. RESULTS

According to Ppositive blood cultures were reported for 728 out of 11968, 6.1% (6.1% 728/11968) and were diagnosed as BSI at MMCH during the one year study period.

Table 1 shows some demographic characteristics of the 728 confirmed BSI pediatric patients. The highest mean duration of hospital stay and mortality rate was reported from MCU (9 months). The highest number of BSI microbial isolates was reported from NICU (280 isolate), and OPD (278 isolate), which also had the highest number of patients (241 and 275 patient respectively) than other departments.

Table 1. Some demographic characteristics of the 728 confirmed BSI pediatric patients

Characteristics	NICU	PICU	MCU	IPWs	OPD	Total
No. of patients	241	138	6	68	275	728
Male	139	70	5	41	160	415
Female	102	68	1	27	115	313
Mean duration of hospital stay	37 days	28 days	9 months	15 days	0	
Total No. of microbial isolates	280	186	9	74	278	827
Patient deaths	45	30	4	1	0	80
Mortality rate	18.7% (45/241)	21.7% (30/138)	66.7% (4/6)	1.5% (1/68)	0 (0/275)	11% (80/728)

NICU= Neonatal Intensive Care Unit, PICU= Pediatric Intensive Care Unit, MCU= Minimal Care Unit, IPWs= In-Patient Wards, OPD= Out-Patient Department.

With regard to the place of acquisition of infection, 63% of microbial isolates (521/827) were acquired in the hospital, as compared to 37% (No/total) acquired in the community.

The different microbial isolates from BSI pediatrics were illustrated in table 2. A total of 827 microbial isolates were detected from 728 BSI pediatric patients. The number of microbial isolates was more than number of the total cases because some patients had multiple episodes of BSI with different organisms.

Most BSI cases 720 (98.9%) were monomicrobial, while 8 cases only (1.1%) cases only were polymicrobial (i.e., two different organisms were isolated from each case from the same blood culture at the same time).

Gram-positive bacteria were the most common BSI causative agents causing BSI in those pediatric patients. They were 528 isolates accounting for (63.8%) of the total isolates, whilst 261 isolates (31.6%) were Gram-negative bacteria. Furthermore, candidemia by Candida

spp. (*C. albicans* and other *Candida* spp.) were the only fungal species as isolated from found in only 38 cases (4.6%) of the total BSI cases. and no other fungi was detected.

Coagulase-negative staphylococci (CoNS) were the most common pathogens accounting for 44% of the total isolates, followed by *Streptococcus* spp. (10.6%). Among the Gram-negative bacteria, *Serratia marcescens* (7.5%), *Klebsiella* spp. (5.8%) and *P. aeruginosa* (3.9%) were the most common Gram-negative isolated pathogens (Table 2). Add the (%) to the table next or below the total numbers in Table 2 since authors used the percent in the above paragraph).

Table 2. Distribution of the BSI microbial isolates in the different wards of MMCH.

Microbial isolates	No. of isolates					Total (%)
	NICU	PICU	MCU	IPWs	OPD	
Gram-positive organisms (total)	150	74	4	58	242	528 (63.8)
CoNS	106	53	2	37	166	364
MRSA	22	3	1	5	0	31
MSSA	5	4	0	4	14	27
<i>Streptococcus</i> spp.	14	10	1	9	54	88
<i>Micrococcus</i> spp.	1	1	0	0	1	3
<i>Enterococcus</i> spp.	2	2	0	3	1	8
<i>Corynebacterium diphtheria</i>	0	0	0	0	4	4
<i>Bacillus</i> spp.	0	1	0	0	2	3
Gram-negative organisms (total)	112	95	5	14	35	261 (31.6)
<i>Klebsiella</i> spp.	26	11	1	2	8	48
<i>Pseudomonas aeruginosa</i>	25	3	1	0	3	32
<i>Enterobacter</i> spp.	9	18	0	3	4	34
<i>Acinetobacter</i> spp.	6	12	1	2	3	24
<i>E. coli</i>	11	4	0	2	3	20
<i>Salmonella</i> spp.	5	0	0	1	5	11
<i>Stenotrophomonas maltophilia</i>	4	4	1	0	0	9
<i>Chryseobacterium meningosepticum</i>	4	0	0	1	0	5
<i>Citrobacter</i> spp.	1	1	0	1	1	4
<i>Haemophilus influenzae</i>	1	2	0	0	2	5
<i>Moraxella</i> spp.	0	0	0	0	2	2
<i>Brucella</i> spp.	0	0	0	1	3	4
<i>Achromobacter</i> spp.	1	0	0	0	0	1
<i>Serratia marcescens</i>	19	40	1	1	1	62 (23.7)
Fungi	18	17	0	2	1	38 (4.6)
<i>Candida albicans</i>	4	1	0	0	0	5
Non <i>Candida albicans</i>	14	16	0	2	1	33
Total no. of isolates	280	186	9	74	278	827

CoNS = Coagulase-negative staphylococci, MRSA = Methicillin-resistant *S. aureus*, MSSA = Methicillin-sensitive *S. aureus* NICU = Neonatal Intensive Care Unit, PICU = Pediatric Intensive Care Unit, MCU = Minimal Care Unit, IPWs = In-Patient Wards, OPD = Out-Patient Department.

Gram-positive bacteria showed a high level of sensitivity to cephalothin and vancomycin. Methicillin resistant *Staphylococcus aureus* (MRSA) was resistant to all the tested antibiotics except vancomycin (Table 3). Add the concentration of each of the antibiotics tested to Table 3 and 4)

Antibacterial resistance rate of BSI Gram-negative isolates is represented in Table 4. All *Candida albicans* isolated were susceptible to amphotericin B and fluconazole, while, 21%

176 of non *C. albicans* were resistant to fluconazole and 18.4% were resistant to amphotericin B
 177 [\(not shown in a table\).](#)

178 **Table 3. Antibacterial resistance rate of BSI Gram-positive isolates understudy (n=**
 179 **528)**

Antibiotic	Antibacterial resistance rates No. (%)							
	CoNS n=364	MRSA n=31	MSSA n=27	<i>Streptococcus</i> n=88	<i>Enterococcus</i> n=8	<i>Micrococcus</i> n=3	<i>Bacillus</i> n=3	<i>Corynebacterium.</i> n=4
PG (---µg)	337	31	27	65 (73.4)	8 (100)	3 (100)	NT	NT
E	313	31	3 (10)	23 (26.1)	8 (100)	3 (100)	NT	NT
AP	332	31	27	58 (65.9)	8 (100)	3 (100)	NT	NT
KF	36	31	0 (0)	17 (19.3)	8 (100)	0 (0)	NT	NT
CD	154	30	3	29 (33)	6 (75)	0 (0)	NT	NT
TS	186	31	3	67 (76.1)	4 (50)	0 (0)	NT	NT
VA	96	25	5	19 (21.6)	0 (0)	0 (0)	NT	NT

180
 181 *MRSA* = methicillin-resistant *S.aureus*; *MSSA* = methicillin-sensitive *S.aureus*;; *PG* = Penicillins G,
 182 *E*= Erythromycin, *AP* = Ampicillin, *KF* = Cephalothin, *CD* = Clindamycin, *TS* = Cotrimoxazole,
 183 *VA* = Vancomycin, *NT* = Not tested.

Table 4. Antibacterial resistance rate of BSI Gram-negative isolates under study (n= 261)

Antibacterial resistance rates No. (%)													
	<i>Klebsiella</i> n=48	<i>E. coli</i> n=20	<i>S. marcescens</i> n=62	<i>Enterobacter</i> n=34	<i>P. aeruginosa</i> n=32	<i>Salmonella</i> n=11	<i>Citrobacter</i> n=4	<i>Acinetobacter</i> n=24	<i>C. meningosepticum</i> n=5	<i>S. maltophilia</i> n=9	<i>Brucella</i> n=4	<i>Achromobacter</i> n=1	<i>Moraxella</i> n=2
Beta-lactams													
CTX	17 (35.4)	2 (10)	0 (0)	0 (0)	32 (100)	0 (0)	2 (50)	6 (25)	5 (100)	9 (100)	NT	NT	0 (0)
CAZ	17 (35.4)	2 (10)	3 (4.8)	0 (0)	2 (6.3)	7 (63.6)	2 (50)	6 (25)	5 (100)	6 (66.7)	NT	NT	0 (0)
CRO	20 (43.3)	2 (10)	22 (35.5)	0 (0)	20 (62.5)	11 (100)	2 (50)	12 (50)	3 (6)	9 (100)	NT	NT	2 (100)
CPD	18 (36.7)	2 (10)	13 (21)	0 (0)	32 (100)	7 (63.6)	2 (50)	18 (75)	5 (100)	9 (100)	NT	NT	0 (0)
KF	27 (56.3)	14 (70)	62 (100)	31 (91.1)	32 (100)	11 (100)	2 (50)	24 (100)	5 (100)	9 (100)	NT	NT	2 (100)
CXM	16 (33.3)	2 (10)	62 (100)	34 (100)	32 (100)	7 (63.6)	2 (50)	18 (75)	5 (100)	9 (100)	NT	NT	0 (0)
FOX	11 (22.9)	0 (0)	62 (100)	31 (91.1)	32 (100)	7 (63.6)	2 (50)	24 (100)	5 (100)	9 (100)	NT	NT	2 (100)
FEP	17 (35.4)	2 (10)	0 (0)	0 (0)	2 (6.3)	4 (36.4)	2 (50)	6 (25)	5 (100)	9 (100)	NT	NT	0 (0)
AMP	48 (100)	20 (100)	62 (100)	31 (91.1)	18 (100)	7 (63.6)	4 (100)	24 (100)	5 (100)	9 (100)	NT	NT	0 (0)
PIP	27 (56.3)	16 (80)	3 (4.8)	0 (0)	2 (6.3)	7 (63.6)	2 (50)	18 (75)	5 (100)	9 (100)	NT	NT	0 (0)
ATM	17 (35.4)	2 (10)	13 (21)	0 (0)	7 (21.9)	7 (63.6)	2 (50)	24 (100)	5 (100)	9 (100)	NT	NT	0 (0)
IMI	0 (0)	0 (0)	0 (0)	0 (0)	18 (56.3)	0 (0)	0 (0)	6 (25)	5 (100)	9 (100)	NT	NT	0 (0)
MEM	0 (0)	0 (0)	2 (3.2)	0 (0)	4 (12.5)	0 (0)	0 (0)	6 (25)	5 (100)	9 (100)	NT	NT	0 (0)
β-lactams/β-lactamase inhibitors													
AUG	27 (56.3)	4 (20)	62 (100)	28 (82.4)	32 (100)	0 (0)	2 (50)	24 (100)	5 (100)	9 (100)	NT	NT	0 (0)
TZP	0 (0)	0 (0)	3 (4.8)	0 (0)	2 (6.3)	0 (0)	0 (0)	4 (25)	5 (100)	6 (66.7)	NT	NT	0 (0)
non β-lactams													
GM	12 (25)	0 (0)	3 (4.8)	0 (0)	5 (15.6)	11 (100)	0 (0)	4 (25)	5 (100)	9 (100)	NT	NT	0 (0)
AK	2 (4.2)	0 (0)	3 (4.8)	0 (0)	5 (15.6)	11 (100)	0 (0)	4 (25)	5 (100)	9 (100)	NT	NT	0 (0)
CIP	3 (6.3)	0 (0)	0 (0)	0 (0)	5 (15.6)	0 (0)	0 (0)	4 (25)	0 (0)	6 (66.7)	NT	NT	0 (0)
TS	21 (43.8)	12 (60)	5 (8.1)	3 (8.8)	32 (100)	7 (63.6)	2 (50)	24 (100)	5 (100)	3 (33.3)	NT	NT	2 (100)

185 CTX = Cefotaxime, CAZ = Ceftazidime, CRO = Ceftriaxone, CPD = Cefpodoxime, KF = Cephalothin, CXM = Cefuroxime, FOX = Cefoxitin, FEP =
 186 Cefepime, AMP = Ampicillin, PIP = Piperacillin, ATM = Aztreonam, IMI = Imipenem, MEM = Meropenem, AUG = amoxicillin-clavulanic acid, TZP =
 187 Piperacillin/tazobactam, GM = Gentamicin, AK = Amikacin, CIP = Ciprofloxacin, TS = cotrimoxazole, NT = Not tested.
 188 .

DISCUSSION

BSI still carries high morbidity and mortality in hospitalized patients despite the availability of current sophisticated therapeutic modalities (Blomberg et al., 2007).

In the present investigation, we analyzed clinical and microbiological characteristics of BSI pediatric patients in a Saudi hospital (MMCH); and results showed that 6.1% (728/11968) of pediatric patients were had at least one episode of ~~ving~~ BSI. Previous studies from Saudi Arabia have documented widely varying pediatric infection rates between individual institutions depending on the age group and the hospital ward. The observed incidence of BSI in NICU was 9% at King Khalid University Hospital, Riyadh (Haque et al., 1990), and 40.9% at Abha general hospital (Mahfouz et al., 2010). In contrast to our finding, some-a studies conducted in India reported show higher positivity rate (between 36-55%) in pediatric patients, e.g. in India, it was reported to be between 36-55 percent (Jain et al., 2003, Mahapatra et al., 2002).

In the current study, 415/728 (57%) of the confirmed 728 BSI pediatric patients were as males and 313 (43%) were females. Similar finding have been reported by Al-Zamil, (2008) in Riyadh, where 58.7% of the total 259 pediatric patients with bacteremia were males and 41.3% were females.

Out of the total 11968 admitted pediatric patients, 6558 (54.8%) (6558/11968) were males and the rest were females. Thus, the rate of BSI in males (6.3%, 415/6558) was higher than females (5.8%, 313/5410). This is in accordance to data of Meyer et al. (2007) in their investigation of a total of 1699 patients in 20 hospitals, where male patients had a significantly higher risk of acquiring BSI than female patients (P=0.002). This slight difference could be attributed to a higher admission rate of males compared to females.

The overall hospital-acquired BSI (521 microbial isolates, 63%) were relatively 521 microbial isolates (63%) of the total BSI isolates were acquired in the hospital higher than the community acquired. Different hospital-acquired blood stream infection rates (90%, Babay et al. (2007); and 43% (Elbashier et al., 1998) were reported by earlier in comparison with other Saudi studies, Babay et al. (2007) reported over 90% of isolates from 778 BSI patients were hospital acquired, while, Elbashier et al. (1998) found 43% only of BSI were acquired in the hospital.

~~We tried also to assess~~ The assessment of the risk factors associated with BSI revealed that ~~Of which,~~ the duration of hospitalization prior to the occurrence of BSI is an important factor reported in the literatures especially for cases of multidrug resistance nosocomial BSIs (Biedenbach et al., 2004). In the present study, the highest duration of hospital stay in MMCH (9 months) and mortality rate (4/6 patients, 66.7%) (Table 2) were as reported from the MCU (Table 1), whereas some patients had polymicrobial infections. In MMCH, MCU is used for hospice-type patients who are terminally ill or incurably ill. Most of those patients were mechanically ventilated or under continuous use of intravascular and central venous catheterization. Central venous catheterization was reported to be the most common risk factor for BSI that extends the length of stay, increases the cost of hospitalization; and attributed with to 4-37% of mortality (Rosenthal et al., 2003, McLaughlin et al., 2012).

The overall mortality among our patients was (80/728, 11%) (Table 1). The same Similar mortality rates was were reorted by corded by other Saudi pediatric studies (Abou Elella et al., (2010); in the pediatric cardiac ICU, King Abdulaziz Medical City. In addition, Elbashier et al., (1998); and Babay et al., (2007), reported an overall mortality among BSI patients of

236 | ~~10.5%. Our recorded~~The 11% mortality rate in the current study was higher than that
237 | reported by Gray (2004) where, the overall mortality rate directly attributable to infection was
238 | 2.4%. In addition, the mortality rate in the NICU was 18.7% in our investigation, which is
239 | comparable to a study performed previously in four main hospitals in Makkah (13.4%) by-
240 | Asghar, (2006). Recently, Ballot et al. (2012), reported a mortality rate of 6.3% in the early-
241 | onset sepsis and 19.6% in the late-onset sepsis groups.
242 |

243 | Due to the diversity of clinical conditions and wards, it was not possible to attribute the cause
244 | of death to BSI as a cause of mortality in all our patients. As, mortality could be attributed to
245 | different factors such as the causative organisms, age, underlying diseases and ICU
246 | admission (Babay, 2007). With respect to the organisms, the most commonly isolated
247 | organisms in our patients who died of BSI in this work were CoNS, *Klebsiella* spp. and
248 | *Candida* spp. In a study by Babay (2007), CoNS, *S. aureus* and *Enterococcus* spp. were the
249 | most leading to death reported in another study (Babay (2007)).

250 | In this investigation, 33 cases (73%) were preterm among neonates deaths. Premature
251 | neonates are at especially high risk of infection because of their lack of protective maternal
252 | antibodies, underdeveloped innate immunity and their fragile, easily damaged skin.
253 | However, while the newborn intrinsically faces an increased risk of infection, failures at
254 | critical points in the system of care can greatly increase this risk (Mahfouz et al., 2010).

255 | In this study, the most common reasons for NICU admission were respiratory distress
256 | syndrome, intrauterine growth retardation, premature rupture of membranes, congenital
257 | heart defect, hyaline membrane disease, very low birth weight, sepsis, septic shock,
258 | hydrocephalus and prematurity. Different primary reasons for admission in a study carried
259 | out in to the NICU in Abha general hospital, KSA by Mahfouz et al. (2010), included the five
260 | primary reasons for admission to the NICU were preterm & hyaline membrane disease,
261 | transient tachypnoea of newborn, meconium asphyxia syndrome, birth asphyxia and
262 | congenital anomaly (Mahfouz et al. (2010)).

263 | Most BSI cases in this work were monomicrobial (98.9%), and the polymicrobial BSI was
264 | caused by *S. viridians*, CoNS, *Klebsiella* spp., *Acinetobacter*, *Candida* spp., *Enterobacter*
265 | spp. and *S. marcescens*. Polymicrobial bacteremia was reported found in 5.2% in a previous
266 | local study by Al-Zamil (2008). The causative agents involved in the polymicrobial
267 | bacteremia was previously reported to be caused by included *E. coli*, *P. aeruginosa*, *K.*
268 | *pneumoniae*, *K. oxytoca*, *S. marcescens*, *E. cloacae* and *A. baumannii* (Emori and Gaynes,
269 | 1993).

270 | The Gram-positive bacteria (63.8%) were more prevalent in our study than Gram-
271 | negative bacteria (31.6%, which is in agreement with previous studies. In earlier studies,
272 | Gram-positive, Gram-negative bacteria and yeast accounted for 66.2%, 31.3% and 2.2% of
273 | the isolates, respectively (Gray, 2004), and 78.6%, 20% 1.3% were *C. glabrata* (Babay et
274 | al., 2005). Babay (2007) found Gram-positive bacteria were the most common isolates
275 | accounting for 61% of the total isolates in King Khalid University Hospital, Riyadh. Babay et
276 | al. (2005) reported 78.6% of the total isolates were Gram-positive bacteria, 20% Gram-
277 | negative bacteria while 1.3% were *C. glabrata*. Asghar (2006) detected Gram-positive
278 | organisms, Gram-negative organisms bacteria and *Candida* spp. in 56.6%, 37.5% and 5.9%
279 | of cases. All the above local studies were in accordance to our results as Gram-positive
280 | bacteria were more prevalent than Gram-negative. Also, this finding is in agreement with the
281 | general concept reported in some international studies that noting the increase in septicemic
282 | cases among Gram-positive bacteria compared with Gram-negative (Karchmer, 2000,
283 | Hadziyannis et al., 2004, NCCLS, 2005). On the other hand, some studies performed in

284 Saudi Arabia (Al-Orainey et al., 1989, Elbashier et al., 1998) revealed a high occurrence of
 285 Gram-negative compared with Gram-positive ~~organisms~~ bacteria.

286 Gram-positive cocci ~~have begun to started~~ reemerging as predominant pathogens in BSI in
 287 the early 1980s and it has been suggested that this change is due to evolution of medical
 288 care (Warren et al., 2001, Siefert and Wisplinghoff, 2005).

289 CoNS was the most frequent isolate in this study, ~~which~~ This could be due to the increasing
 290 use of intravascular devices in the medical care of MMCH.

291 CoNS are one of the most common causes of nosocomial BSI and also the most common
 292 blood culture contaminant (Haddon et al., 1999). These contaminants have been noticed to
 293 be common in pediatric patients since it is difficult to take blood samples from very young
 294 infants due to much skin manipulation leading to contamination. In addition, only single
 295 samples are usually taken from these patients and this adds to the difficulty of interpretation
 296 (Babay et al., 2005). However, it needs to be pointed out that *S. epidermidis* and other
 297 CoNS can cause sepsis particularly in preterm infants, immunosuppressed patients and
 298 patients with intravascular devices (Haimi-Cohen et al., 2002). Furthermore, the increased
 299 isolation of CoNS in blood cultures may reflect a change from regarding these organisms as
 300 normal skin flora to viewing them as clinically significant (Ahmed et al., 2009).

301 Recently, Ballot et al. (2012) reported the most common isolate overall was CoNS
 302 accounting for 19.1% of the total isolates, ~~while~~ Saied et al (2011) reported CoNS as the
 303 second most frequent isolate accounting for 25.5% of the total isolates recovered from
 304 nosocomial BSI, only exceeded by *K. pneumoniae* (26.7%).

305 The second most causative agents after CoNS in the cases under study were *Streptococcus*
 306 spp., of whom (1.7%) were *S. pneumoniae*, which was also previously reported (Haddon et
 307 al., 1999). ~~*S. pneumoniae* was reported previously as the most common pathogen in children~~
 308 ~~with bacteremia~~ Al-Zamil (2008) reported that the most common Gram-positive isolates from
 309 pediatric BSI were *S. aureus* (18.7%) and *Streptococcus* spp. (16.3%).

310 The proportion of blood culture isolates of methicillin-resistant *S. aureus* from children ~~that~~
 311 ~~are methicillin-resistant~~ is increasing (Khairulddin et al., 2004). Bacteraemia due to MRSA is
 312 associated with high mortality compared with methicillin sensitive *S. aureus* bacteraemia
 313 (Ahmed et al., 2009).

314 Worldwide, MRSA rates vary widely, from <1% in Denmark, Sweden, and The Netherlands
 315 to >40% in the United Kingdom, Greece, and Italy (Tiemersma et al., 2004). ~~MRSA in the~~
 316 ~~study of Gray (2004) reached 15.5%.~~ In our study, we encountered 31/58 (53.45%) isolates
 317 of MRSA of the total 58 *S. aureus* isolates ~~(53.45%)~~, with the highest proportion in NICU
 318 (22/31, 71%) suggesting a high dissemination in the hospital. The MRSA ratio infections in
 319 MMCH was higher than reported by Gray, 2004) which was 15.5%, and that reported by
 320 Khairulddin et al. (2004) in children < 15 years (13.1%); but lower than a study from Egypt
 321 by Saied et al. (2011) from Egypt (64%).

322 Increasing MRSA infection rates have been reported in tertiary care centers in Saudi Arabia,
 323 from 0.8 to 2.3 per 1,000 admissions between 2000 and 2004 (Balkhy et al., 2007).
 324 Promoting hand hygiene and isolation precautions are important measures to prevent the
 325 spread of MRSA that should be implemented and enforced to control the spread of these
 326 highly resistant isolates.

327 The most frequently isolated Gram-negative bacteria in our study were *S. marcescens* (62
328 (23.7) which is *S. marcescens* is a well-recognised cause of outbreaks of hospital-acquired
329 nosocomial infections, particularly in NICUs (Madani et al., 2011).

330 Several studies showed that *E. coli* and *Pseudomonas* spp. were the most common isolates
331 among Gram-negative organisms from blood cultures (Anthony et al., 2000, Diekema et al.,
332 1999, Lark et al., 2000, Gray, 2004, Asghar, 2006, Babay, 2007).

333 *P. aeruginosa* BSI in immunocompromised patients can lead to death within the first 72
334 hours (Munson et al., 2003). Wendt et al. (1998) and Weinstein et al. (1995) found that *P.*
335 *aeruginosa* was the commonest Gram-negative pathogen. On contrary, *P. aeruginosa* was
336 isolated from 3.9% of all BSI cases in this study, which is in agreement the 3.5% reported by
337 . This rate concurs with Al-Zamil, (2008), who found that 3.5% of cases was *P. aeruginosa*.
338 *P. aeruginosa* BSI in immunocompromised patients can lead to death within the first 72
339 hours (Munson et al., 2003). On contrary, to our results, Wendt et al. (1998) and Weinstein et
340 al. (1995) reported *P. aeruginosa* as the commonest Gram-negative pathogen isolated
341 from BSI cases.

342

343 The incidence of BSI caused by *K. pneumoniae* in this investigation was 5.6%, which. The
344 rate is comparable to that reported previously by Haddon et al. (1999) (7.4%); and Al-
345 Zamil, (2008) (6.6%).

346 *Candida* BSI contributes to increased length of hospital stay, increased hospital costs, and
347 most importantly, increased morbidity and mortality (Garey et al., 2006). *Candida* spp. was
348 implicated in 4.6% of all BSI episodes representing the fifth most common etiologic agent in
349 this study. Previous studies indicated that *Candida* spp. represent the fourth to seventh most
350 common etiologic agent causing septicemia (Lark et al., 2000, Hadziyannis et al., 2004,
351 Rangel-Frausto et al., 1999). However, the rate infections with *Candida* spp. isolates in our
352 among the study cases was more than what has been reported by Babay et al. (2005)
353 who detected *C. glabrata* 1.3%, and Saied et al. (2011) reporting budding yeast in 3.8%, but
354 less than Gomaa et al. (2001) report of detected candidemia in 14% of the neonates.
355 Recently, Saied et al. (2011) reported a proportion of 3.8% nosocomial BSIs due to budding
356 yeast.

357
358 The three departments generating the greatest number of microbial isolates in MMCH were
359 NICU, OPD and PICU. In agreement with other international studies, Our results confirmed
360 that ICU is the most important department generating the greatest number of positive
361 cultures, which is quietly normal, since most of the patients in the ICU are those who are the
362 critically ill or immunocompromised patients requiring mechanical support and indwelling
363 devices (Unal et al., 2004).

364 Increases in neonatal septicemia have been linked to increased use of prosthetic devices in
365 hospitals in recent years (Brodie et al., 2000); or to better methods of detection of organisms
366 in blood with more sensitive equipment such as the Bactec 9240 instrument (Gomaa et al.,
367 2001), that was used in the present study. The Bactec 9240 instrument facilitates the early
368 detection of microorganisms from blood cultures of newborn infants when compared with
369 other blood culture methods (Garcia-Prats et al., 2000).
370 Periodic testing and analysis of antibiotic resistance would enable physicians to detect
371 trends in the resistance pattern to commonly prescribed antibiotics in a given organism.

Gram-positive bacteria isolated in this study, showed a very low level of resistance to cephalothin and vancomycin (Table 3). Babay et al. (2005) reported none of the Gram-positive bacteria were resistant to vancomycin. Our results also showed resistance of a large proportion of isolates to penicillin G (90.4%) and ampicillin (73.1%). In a study of Al-Zamil (2008), 95% of *S. aureus* and 38% of *S. pneumoniae* was resistant to penicillin G.

An important observation was noted which is the susceptibility pattern of *S. maltophilia* & *C. meningosepticum* isolates, showing a high resistance pattern to most antibiotics used. Treatment of *S. maltophilia* is problematic owing to its high level of intrinsic resistance to multiple classes of antibiotics (Vartivarian et al., 1994). A number of factors, including multidrug efflux pumps and outer membrane impermeability, likely contribute to the intrinsic antibiotic resistance of *S. maltophilia* (Cullmann, 1991; Alonso and Martinez, 2000).

C. meningosepticum, exists in water systems and wet surfaces of the hospital environment. Colonization of water taps with *C. meningosepticum* has been associated with outbreaks in intensive care units (Hoque et al., 2001). It is highly pathogenic for premature infants and may also cause infections in immunosuppressed patients with usual sites being lungs, bloodstream, central nervous system and biliary tree (Bloch et al., 1997). *C. meningosepticum* is resistant to multiple antibiotics (Arvanitidou et al., 2003).

All our *C. albicans* isolated were susceptible to amphotericin B and fluconazole- as in our study. Babay et al. (2005), reported the sensitivity of all fungal isolates to all antifungal agents tested.

The high resistance rates for the commonly used antibiotics in this study could be explained by the fact that resistance develops wherever antibiotics are abused, overused, misused and dispensed at levels lower than the suggested treatment guidelines. This means that instead of wiping out the infection altogether, antibiotics kill only the non-resistant organisms, while leaving behind their tougher counterparts to replicate and spread resistance genes. Resistance to β -lactams, especially third-generation cephalosporins and non β -lactams, among clinical isolates of Gram-negative bacteria is increasing worldwide (Andrews, 2001). Previous use of more than two different families of antimicrobials in patients with BSI increased the risk for ESBL-producing strains (Quirante et al., 2011). The Gram-negative isolates among the studied patients demonstrated a high level of resistance towards cefpodoxime, cefotaxime, ceftazidime, ceftriaxone and aztreonam. This type of resistance is a marker for the presence of extended-spectrum β -lactamases (ESBL) (Nathisuwan et al., 2001). Thus, phenotypic screening of beta-lactamases should be performed; and *bla* genes could be identified.

4. CONCLUSION

BSIs are a major cause of morbidity and mortality in pediatrics. The pathogen distribution was different from those in the developed countries with a predominance of CoNS, *Streptococcus* spp. and *Serratia marcescens*. The incidence rate of BSI is high in ICU infants. So, special attention should be given to the quality of care for these neonates to improve safety. This could be done through education, training and staffing, proper selection of catheters and sites, use prophylactic antimicrobial lock solution in patients with long term catheters who have a history of multiple catheter-related BSI, and maximal sterile barrier precautions by following 2011 CDC guidelines for the prevention of intravascular catheter-related infections (<http://www.cdc.gov/hicpac/BSI/02-bsi-summary-of-recommendations-2011.html>).

418 There was appreciable resistance to commonly used antibiotics. Physicians should receive
419 regularly updated antibiograms to be familiar with trends for the antimicrobial agents used
420 frequently in their hospitals. This information is crucial for clinical care management and for
421 improving antimicrobial prescribing habits and performance-based infection prevention and
422 control practices. It is also very important to define the potential of resistant organisms
423 colonization surveillance in all ICU patients to predict etiology of subsequent infection and
424 improve adequacy of empiric antimicrobial treatment.

425 **Competing interests**

426 AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST.

427 **ETHICAL APPROVAL**

428 Ethical Committee of the Madinah Maternity and Children's Hospital & the Scientific
429 Committee of Taibah University approved the study.

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