Neonatal Sepsis 1991–2001: Prevalent Bacterial Agents and Antimicrobial Susceptibilities in Bahrain

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Key Words

Neonatal sepsis, Bahrain · Group B streptococci · Yeasts · Antibiotic resistance

Abstract

Objectives: To investigate the organisms causing neonatal sepsis and their modifications over an extended period, to assess their changing sensitivities to antibiotics and to verify whether the policy for screening pregnant women for group B streptococci (GBS) carriage is desirable in our settings. **Subjects and Methods:** Medical records of all infants with positive blood culture from the Neonatal Intensive Care Unit at Salmaniya Medical Complex between 1991 and 2001 and Bahrain Defense Force Hospital between 1999 and 2001 were reviewed. **Results:** Of the 7,978 neonates in both hospitals 335 (4.19%) had culture-proven bacteremia. Gram-positive bacteria were isolated at constant rate over the 11-year period. The main agents isolated were coagulase-negative *Staphylococcus* (CoNS) in 138 cases (41%), *Staphylococcus aureus* in 28 newborns (8%) and GBS in 26 patients (7.8%, 0.2/1,000 live births). All of them were sensitive to penicillin G, erythromycin and clindamycin. Gram-negative bacteria were declining but *Escherichia coli* was isolated in 35 cases (10%). Of special concern is the increasing percentage (5.7%) of *Candida* isolation. No clear trend

toward increasing resistance was observed, although a major difference among the two institutions was evident. *Klebsiella* and *Enterobacter* spp. showed resistance to many of the antibiotics tested, thereby posing difficult therapeutic choices. **Conclusion:** Good quality specimens are essential to evaluate the role of CoNS. The increasing threat of fungal infection must be carefully tackled. Specifically tailored policies for GBS prevention must be defined according to the local epidemiology.

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Introduction

Neonatal sepsis continues to be an important cause of morbidity and mortality both in term and in low-birth-weight infants despite advances in healthcare [1–3]. Neonatal infection is of either early or late onset, defined by development of symptoms before or after the 1st week of life. Group B streptococci (GBS, *Streptococcus agalactiae*) infection remains a common cause, although significantly declining [3], of early onset of neonatal infection at least in the USA [2–4], and is an increasing problem in developing countries [5]. However, in both the Arabian Gulf and the Indian subcontinent region the etiological picture of neonatal sepsis seems to differ substantially

[6–8]. In Saudi Arabia [6, 7] *Staphylococci* were the predominant organisms with the majority being *Staphylococcus epidermidis*. In India, *Staphylococcus aureus* and gram-negative bacteria were the most predominant pathogens causing neonatal sepsis [8]. Several studies have documented an increase in neonatal sepsis due to gram-negative organisms [9–11]. There are no data on neonatal sepsis in the Kingdom of Bahrain.

The main aim of this study is to determine the prevalent bacterial agents of neonatal sepsis and its modification over the study period, to assess their changing sensitivities to antibiotics. Furthermore, our goal was to assess the role played by GBS in this population in order to appraise the need of introducing the universal prenatal screening to detect vaginal and rectal GBS colonization as recently recommended by Centers of Disease Control (CDC) [4].

Subjects and Methods

This retrospective study covered a period of 11 years (January 1991–December 2001) for Salmaniya Medical Complex (SMC) and 3 years for Bahrain Defense Force Hospital (BDF). The SMC has 1,000 beds while BDF has 420 beds; both constitute the two major hospitals in the Kingdom of Bahrain. The vast majority of the positive blood cultures were from Bahraini children. The blood culture registers of the Microbiology Laboratory in each hospital were reviewed to identify the positive blood cultures from the Neonatal Intensive Care Unit and Nursery. The data extracted include the patient's hospital number, culture date, organism isolated and their antimicrobial susceptibilities. We report here the total number of patients with at least one positive blood culture. Rarely two or three sets of blood samples are collected in these patients. When two blood cultures were sent they were counted as 1 patient if they yielded the same organism. If different organisms were cultured they were considered contaminated and not included in the study. The numbers presented here refer to one blood culture per patient. Intrapartum prophylaxis for GBS was not administered to any of the pregnant women during the 11-year review period at both hospitals.

Based on the units' policy, every neonate was investigated according to the sepsis screening protocol (which included complete blood count, differential platelet count and tests for acute phase reactants) and this was usually repeated whenever there was a suspicion of sepsis while in the unit. Screening was either partial (blood and urine cultures) for infants who did not appear ill on admission or complete (blood, urine and cerebrospinal fluid culture) if the infant had definite symptoms of infection such as fever, lethargy or appeared septic at any time. Blood samples (3–5 ml) were obtained after thorough preparation of the skin with iodine solution and cultured by using conventional blood culture procedures [12].

All samples were cultured using an automated culture system (BacT-Alert, bioMérieux, Durham, N.C.; Bectec 9240, Beckton Dickinson, Franklin Lakes, N.J., USA). Bacterial isolates were identified by conventional procedures described in the manual by

Lenette et al. [12]. Antibiotic susceptibility testing was performed using the disk diffusion method described by Bauer et al. [13]. The disks were obtained from Mast Group Ltd., Merseyside, UK. Only antibiotics tested over the entire period were considered for the analysis of multiresistance. Drugs tested for gram-positive bacteria include: penicillin G, fusidic acid, clindamycin, tetracycline, cephalothin, erythromycin, chloramphenicol, vancomycin, and amoxicillin-clavulanate. For gram-negative bacteria ampicillin, ciprofloxacin, chloramphenicol, gentamicin, sulfamethoxazole-trimethoprim, and ceftriaxone were used and for Pseudomonadineae amikacin, ampicillin, aztreonam, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, meropenem, sulfamethoxazole-trimethoprim were used. The standard quality control strains, *S. aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, were tested every day, starting in the year 2000. The medium used was Mueller-Hinton agar (Difco; Becton Dickinson, Cockeysville, Md., USA). External quality control was run for the period 1994–2000 with the support of the WHO Collaborative Center for external quality assessment, Belgium. Statistical analysis was done by chi-square test; *p* value of <0.05 was considered as significant.

Results

Trend of Isolates at SMC 1991–2001

During the 11-year period, a total of 7,160 newborn infants were admitted to the Neonatal Intensive Care Unit and Nursery in SMC. Of these 288 (4.0%) had culture-proven bacteremia. The vast majority of them were of Bahraini nationality. No difference was observed between male and female children in susceptibility to infection.

Since 1991 the number of gram-negative bacteria isolated in blood cultures from neonates decreased in a highly significant manner ($p < 0.001$); while the isolation rate of gram-positive organisms plateaued in the period 1995–2001 at a very high isolation rate (table 1). At SMC isolation rate of gram-positive organisms started to increase sharply in 1995 (77.7%), followed by a minor decline in subsequent years to 69.4% (table 1). The difference in the positivity between the 1995–1998 and the 1999–2001 intervals was not statistically significant.

Of special concern is the trend in isolation of yeasts, mainly *Candida albicans*. The organism was present in 2.4% of patients at SMC 10 years ago to increase threefold in the period 1999–2001, up to 6.5% (NS) (table 1).

Comparison between the Two Major National Hospitals in the Period 1999–2001

In BDF Hospital during a 3-year period a total of 818 blood cultures from neonates were processed and 47 (5.7%) had culture-proven bacteremia, while for SMC

Table 1. Total positive blood cultures and microorganisms isolated in neonates up to 6 weeks at SMC and BDF

	SMC			BDF	Total
	1991–1994	1995–1998	1999–2001	1999–2001	
Gram-positive blood cultures	127	99	62	47	335
Gram-positive bacteria					
CoNS	19	63	35	21	138
<i>S. aureus</i>	23	4	1	–	28
GBS	10	7	4	5	26
<i>S. pneumoniae</i>	3	2	1	–	6
Viridans streptococci	3	–	2	–	5
Streptococci non-A-B	–	1	–	–	1
Enterococcus spp.	6	3	1	–	10
Diphtheroids	–	1	1	–	2
	64 (50.3%)	81 (81.3%)	45 (72.5%)	26 (55.3%)	216 (64.4%)
Gram-negative bacteria					
<i>E. coli</i>	25	3	3	4	35
<i>Klebsiella</i> spp.	14	6	4	–	24
<i>Pseudomonas</i> spp.	1	2	2	1	6
Enterobacter spp.	8	–	2	7	17
Acinetobacter spp.	4	1	1	1	7
Proteus spp.	3	–	–	–	3
Eikenella spp.	–	1	–	–	1
Citrobacter spp.	–	–	1	–	1
Flavobacterium spp.	4	–	–	–	4
Aeromonas spp.	1	–	–	–	1
<i>H. influenzae</i>	–	1	–	–	1
	60 (47.2%)	14 (14.1%)	13 (20.9%)	13 (27.6%)	100 (29.8%)
Yeast (<i>C. albicans</i>)	3 (2.4%)	4 (4.0%)	4 (6.5%)	8 (17.0%)	19 (5.6%)

2,260 blood cultures were examined and 62 (2.7%) showed some growth. The difference is statistically significant ($p < 0.001$).

In BDF Hospital gram-positive microorganisms were more frequently isolated than gram-negative microorganisms (55.3, 27.6%, respectively) (table 1), but at SMC isolation rates were 69.4 and 24.1%, respectively (NS). It is evident that in both hospitals, gram-positive organisms were more frequently isolated.

Regarding fungemia, in SMC four cultures (6.5%) yielded *Candida* spp. compared to eight (17%) at BDF (NS). It seems that both institutions have shared characteristics regarding the organisms isolated, indicating a common epidemiology without any statistically significant difference.

Bacterial Isolates at the Two Institutions

For the sake of clarity the whole data from SMC are included. There were no significant differences between the two major hospitals with regard to the common bac-

terial isolates. The main agents isolated (table 1) were coagulase-negative *Staphylococcus* (CoNS) in 138 cases (41%) followed by *E. coli* in 35 cases (10%), *S. aureus* in 28 cases (8%), GBS in 26 cases (7.8%), and *Klebsiella pneumoniae* in 24 cases (7.2%). Among gram-positive pathogens CoNS were consistently the most common agents isolated, followed by *S. aureus*, and GBS (table 1).

Regarding the role of gram-negative bacteria in neonatal sepsis we decided to concentrate our analysis on the last 3 years in consideration of the sharp decline already mentioned above. *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Pseudomonas* spp. are the most common isolates at both institutions (table 1). Although interesting, yet difficult to explain, variations can be observed, probably the most important single factor being the different antibiotic policies adopted at the two institutions.

For the more recent period, the isolation in decreasing order was CoNS, *C. albicans* and *S. agalactiae*; that affected 9 babies during 1999–2001 (4 at SMC and 5 at

Table 2. Multiresistant strains^a isolated from blood culture at SMC and BDF

Microorganisms	SMC			BDF
	1991–1994 ^b	1995–1998 ^b	1999–2001 ^b	1999–2001 ^b
Gram-positive bacteria				
CoNS	8 (19)	1 (63)	10 (35)	19 (21)
Methicillin (R)	0	0	0	0
<i>S. aureus</i>	5 (23)	0 (4)	1 (1)	0
Methicillin (R)	0	0	1 (1)	0
GBS	2 (10)	0 (7)	0 (4)	0 (5)
<i>Enterococcus</i> spp.	1 (6)	1 (3)	0 (1)	0
<i>S. pneumoniae</i>	0 (3)	0 (2)	0 (1)	0
<i>Viridans</i> streptococci	0 (3)	0	0 (2)	0
Subtotal	16 (64; 9.3%)	2 (79; 2.5%)	12 (45; 6.6%)	19 (26; 73.1%)
Gram-negative bacteria				
<i>Citrobacter</i> spp.	0	0	1 (1)	0
<i>E. coli</i>	1 (25)	0 (3)	3 (3)	3 (4)
<i>H. influenzae</i>	0	1 (1)	0	0
<i>Klebsiella</i> spp.	3 (14)	0 (6)	0 (4)	0
<i>Enterobacter</i> spp.	1 (8)	0	1 (2)	7 (7)
<i>Acinetobacter</i> spp.	1 (4)	0 (1)	1 (1)	1 (1)
<i>Pseudomonas</i> spp.	1 (1)	0 (2)	0 (2)	0
Subtotal	7 (52; 13.4%)	1 (13; 7.7%)	6 (13; 46.1%)	11 (12; 91.6%)
Grand total	23 (116; 19.8%)	3 (92; 3.2%)	18 (58; 31.3%)	30 (38; 78.9%)

^a Defined as resistance to 3 or more of the antibiotics tested.^b Number of resistant strains (total isolates).

BDF) out of 38,987 deliveries reported (National Health Statistics Annual Reports) (table 1). Time of onset (early vs. late) of the clinical symptoms for GBS was in the late group (>1 week) for the majority of the patients (7 out of 9).

Evolution of Antibiotic Resistance Patterns

The comparison of the multiresistant strains between the two hospitals is given in table 2. For SMC, data related to the whole study period are reported. Gram-positive resistance showed an apparent increase from 9.3% in 1991–1994 to 26.6% in 1999–2001 with identical testing techniques being used over the period (NS). For the most part *S. epidermidis* isolates were sensitive to the antibiotics tested, as was *S. aureus* to methicillin. All GBS strains were sensitive to penicillin G, clindamycin and erythromycin, one was resistant to tetracycline and cephalothin, and one showed resistance to gentamicin.

For gram-negative strains there was a statistically significant increase from 13.4% in 1991–1994 to 46.1% in 1999–2001 ($p < 0.01$). *Klebsiella* isolates showed multiple

resistances due to the presence of a hospital strain characterized by production of extended spectrum β -lactamase. High resistance values were detected in isolates from BDF in both gram-positive (73.1%) and gram-negative organisms (91%). The rate of isolation of multiresistant gram-positive as well as gram-negative bacteria was significantly higher in BDF than SMC ($p < 0.001$ and < 0.025 , respectively).

Discussion

In Bahrain in the recent years CoNS, *C. albicans*, *S. agalactiae*, *Enterobacter* spp. and to a lesser extent *E. coli* constitute the most frequent isolates of neonatal sepsis in the two major hospitals. The overall prevalence of the main pathogens shifted significantly during the study period, the two major findings being a marked decrease in gram-negative isolates contrasted by a sharp increase in isolation rate of yeasts while the isolation of gram-positive pathogens seems to be relatively constant.

The role of gram-negative pathogens seems conflicting and depending upon each particular institution with some reports of increasing isolation [14] probably linked to specific prophylactic strategies such as widespread use of antepartum antibiotics.

The trend we observed for fungi is similar to the results of other reports [15–18] showing that yeasts are emerging as important nosocomial bloodstream pathogens with possible spread within neonatal intensive care units and thereby posing a potential risk to neonates, including low birth weights. This is the most striking feature of our results, which calls for specific diagnostic and therapeutic policies and guidelines.

Gram-positive organisms are consistently present with CoNS being the most frequent isolates similar to several other studies [4, 6, 7] including Saudi Arabia [6, 7], a neighboring country to Bahrain. As this organism is present on normal skin it is always difficult to ascertain the clinical significance of its occurrence in blood culture, especially if one can, as in our case, rely only on one blood culture bottle. Although the accuracy of the staff in collecting blood was certainly consistent with the recommendations in the manual of clinical microbiology [12] it may not be possible to completely exclude a breach in the technique that could lead to blood contamination. Whereas the general rule of collecting two (or three) sets during 24 h can be easily applied to adults, it is not always easy to apply it to premature, underweight and severely ill children. Thus, the real clinical relevance of *S. epidermidis* remains in our opinion difficult to assess.

GBS ranked third (when considering the last period) among the isolates with a total of 9 strains (11.7%, 1/4,331 live births) and a total of 26 isolates over an 11-year period (7.8%) similar to previous studies [6, 7], which is much lower than the reported prevalence in UK or US [14]. We should stress here that intrapartum prophylaxis for GBS neonatal sepsis is not currently applied in Bahrain.

Based on our findings the recommendation of CDC for prevention of GBS disease including universal prenatal screening for pregnant women seems not to be appropriate for our epidemiological setting. The burden and the cost of effecting the policy will require a careful discussion before being implemented if one estimates 30,000 specimens/year – including vaginal and rectal swabs for 15,000 pregnant women – this will add up to 75,000 Euro to prevent 3 infections in a year. Therefore we recommend prenatal screening in the presence of additional risk factors for GBS disease [19, 20] such as: longer duration of membrane rupture, maternal age, presence of GBS at

previous testing or previous delivery of a baby with GBS disease. Indeed some of the above can be considered indications for starting intrapartum prophylaxis without waiting for the microbiological report.

A related issue is the need to test the GBS strains for antibiotic sensitivity. According to our sensitivity profile, penicillin G seems to maintain its full effectiveness and no clindamycin or erythromycin resistance has been detected. Again we feel that CDC guidelines for GBS testing are not applicable in our situation as a routine approach although it might be of interest to run Gulf collaborative studies to monitor the evolution of resistance in these important pathogens.

Of greater concern is that CoNS showing resistance to more than three antibiotics rose from 1.5% in 1995–1998 to 28% in 1999–2001 in SMC and to 90% in BDF. The difference between the two hospitals for the same time interval was statistically highly significant ($p \leq 0.001$). *Enterobacter* and *Acinetobacter* species also showed worrying high rates of multiple resistances. This trend among gram negatives is disquieting and requires careful observation, stressing the constant need for rigorous policies and incessant education for proper antibiotic use in hospitals. A supplementary major finding of this paper relevant for establishing national policies is the significantly higher rate of isolation of multipliresistant strains at BDF. Although this can partially be explained with the dissimilar patient population, nevertheless there is an urgent need for unified national guidelines in support of appropriate antibiotic use.

Conclusion

Good quality specimens are essential to evaluate the role of CoNS. The increasing threat of fungal infection must be carefully tackled. Specifically tailored policies for GBS prevention must be defined according to local epidemiology.

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