Original Article

High mortality among patients with positive blood cultures at a Children's Hospital in Tbilisi, Georgia

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Abstract

Background: The etiology and outcomes of blood-stream infections (BSI) among paediatric patients is not well described in resource-limited countries including Georgia.

Methodology: Patients with positive blood cultures at the largest paediatric hospital in the country of Georgia were identified by review of the medical and laboratory records of patients who had blood for cultures drawn between January 2004 and June 2006.

Results: Of 1,693 blood cultures obtained during the study period, 338 (20%) were positive; of these, 299 were included in our analysis. The median age was 14 days from a range of 2 days to 14 years of which 178 (60%) were male; 53% of the patients with a positive culture were admitted to the Neonatal Intensive Care Unit (NICU). Gram-negative bacilli (GNB) represented 165 (55%) of 299 cultures. Further speciation of 135 (82%) of 165 Gram-negative rod (GNR) was not possible because of lack of laboratory capacity. Overall, mortality was 30% (90 of 299). Among the 90 children who died, 80 (89%) were neonates and 68 (76%) had BSI caused by Gram-negative organisms. In multivariate analysis, independent risk factors for in-hospital mortality included an age of less than 30 days (OR=4.00, 95% CI 1.89-8.46) and having a positive blood culture for a Gram-negative BSI (OR = 2.38, 95% CI 1.32-4.29).

Conclusions: A high mortality rate was seen among children, particularly neonates, with positive blood cultures at the largest paediatric hospital in Georgia. Because of limited laboratory capacity, microbiological identification of common organisms known to cause BSI in children was not possible and susceptibility testing was not performed. Improving the infrastructure of diagnostic microbiology laboratories in countries with limited resources is critical in order to improve patient care and clinical outcomes, and from a public health standpoint, to improve surveillance activities.

Key words: blood infections, mortality, children, Georgia

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Introduction

Georgia gained independence from the Soviet Union in 1991. Following independence, there was a collapse of the public health and medical infrastructure. During the early 1990s, an increase in infant mortality and decreased immunization rates in Georgia were observed, both of which are markers of a health care system in crisis [1,2]. Georgia is currently a lower middle income country and despite much advancement, it remains a country in economic transition. The health care system in Georgia is currently undergoing many changes, moving from a centralized system based on the former Soviet model to a decentralized, market driven system [3].

Generally, there is a lack of information regarding rates of blood-stream infections among paediatric and adult patients in resource-limited areas. This is in part due to lack of laboratory infrastructure and surveillance capacity. There are limited data in Georgia regarding paediatric blood-stream infections and outcomes among patients with positive blood cultures. We therefore conducted a study designed to determine the etiology and outcomes of paediatric patients with positive blood cultures in the country of Georgia. The study was initiated to obtain baseline data in an effort to establish a surveillance system for invasive bacterial infections in children and to aid in developing

guidelines for empiric antibiotic use in suspected bacterial infections.

Materials and Methods

The study was conducted at Central Children's Hospital (CCH) in Tbilisi, Georgia. CCH is a 363-bed referral hospital for the entire country of Georgia and the largest paediatric hospital in Georgia. The population of Georgia is 4.7 million with 17.3% below the age of 14 [4]. The CCH microbiology laboratory processes an average of 30 specimens per day and 9,100 specimens per year. The study was approved by the Emory University Institutional Review Board (IRB) and the CCH Ethics Committee.

Data collection and definitions

A retrospective study was performed. All patients with at least one positive blood culture between January 2004 and June 2006 were identified by a review of medical and laboratory records. Data were collected using a standard data collection form. For each patient, the following demographic and clinical information was collected: date of birth, gender, internal displacement status (*i.e.*, persons who were internally displaced as a result of a civil war in 1992-1993), admitting hospital department, date of blood culture collection, date of hospital admission, number of sets obtained for culture (*e.g.*, one or two), blood culture results, white blood cell count, temperature on admission, in-hospital mortality. Neonatal blood-stream infections (BSI) were defined as positive blood culture(s) in an infant less than 30 days old. Nosocomial blood-stream infections were defined as neonatal BSIs and all cultures in children over 30 days old who tested positive more than 48 hours after admission to the hospital. Information on previous admissions to the hospital and treatment was not available.

Laboratory methods

Blood cultures were collected by the nursing staff after cleaning the skin with chlorhexidine or alcohol. A total of 2-3 ml of blood was drawn and placed in two aerobic blood culture bottles using a sterile needle. The microbiology laboratory did not have the capacity to perform anaerobic cultures. Blood culture bottles were incubated at 37ºC. Subcultures were performed from all blood culture bottles regardless of positive (turbid) or negative appearance. These "blind" subcultures were performed at 24 hours, 48 hours and seven days after collection. All the subcultures were plated onto blood agar, chocolate agar, and MacConkey agar plates. Agar was made in the CCH microbiology laboratory because no commercially prepared media was available in Georgia during the study period. Human blood (5%) rather than sheep's blood was used for preparation of culture media. Incubators were available for growing cultures at the proper temperatures; therefore, plates were incubated for 18 to 24 hours as follows: blood and chocolate agar plates were kept in a candle jar at 35ºC, MacConkey agar plates aerobically at 35ºC. Gram stains were performed routinely on all identified positive subcultures. All blood culture bottles were discarded after the seventh day of incubation. Some of the identifications were not performed beyond Gram staining the positive culture due to lack of available equipment.

Statistical analysis

Data were entered in Microsoft Excel 2000 (Microsoft Corp., Redmond, WA) and analyzed using SAS software, version 9.1 (SAS Institute, Cary NC). The Mantel-Haenszel odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated for dichotomous variables. Multivariate analysis was done using an unconditional logistic regression model. A P value of $\leq .05$ was defined as statistically significant.

Results

During the study period, 1,693 blood cultures were obtained from paediatric patients (a mean of 94 blood cultures per month). Of these, 338 (20%) children had a least one positive culture, and 39 (12%) of the 338 were excluded from further analysis because thirty had incomplete or missing medical records and nine had fungemia.

The median age of the 299 children included in analysis was 14 days and the mean age was 186 days from a range of 2 days to 14 years of which 178 (60%) were male. A total of 203 (68%) of 299 patients were neonates; 62 (21%) were internally displaced persons (IDP). The majority of patients were admitted to the CCH Neonatal Intensive Care Unit (NICU) (156 [53%] of 297 children); 75 (25%) were admitted to general neonatal departments; seven (2%) to the neurology neonatal department; 31 (10%) to the Paediatric Intensive Care Units (ICU); 17 (6%) to general paediatric departments; eight (3%) to the infectious diseases unit; and two (0.7%) who were seen in the Emergency Department were not admitted

Table 1. Pathogens recovered from blood cultures of 299 infants and children

Organisms	**Newborn babies (age 0-30 days) N = 203***	**Infants (age 31 days – < 12 months) N = 73**	**Children 1-5 years N = 13**	**Children > 5 years N = 10**	**Total N = 299***
Gram-negative aerobic bacteria					
Gram-negative rods (GNR)	118 (58.1)	13 (17.8)	3 (23.1)	1 (10.0)	135 (45.2)
Klebsiella spp.	15 (7.4)	2 (2.7)	1 (7.7)	0	18 (6.0)
Pseudomonas spp.	8 (3.9)	1 (1.4)	1 (7.7)	0	10 (3.3)
Salmonella spp.	0 (0)	1 (1.4)	0	1 (10.0)	2 (0.7)
Gram-positive aerobic bacteria					
Coagulase negative *Staphylococcus* (CNS)	50 (24.6)	44 (60.3)	8 (7.3)	7 (70.0)	109 (36.5)
Staphylococcus spp.	6 (3.0)	6 (8.2)	0	1 (10.0)	13 (4.3)
Streptococcus spp.	2 (0.99)	3 (4.1)	0	0	5 (1.7)
Enterococcus	2 (0.99)	2 (2.7)	0	0	4 (1.3)
Listeria monocytogenes	2 (0.99)	0	0	0	2 (0.7)
Gram-positive rods (GPR)	1 (0.5)	1 (1.4)	0	0	2 (0.7)

*One infant had mixed blood culture with *S. aureus* and *Klebsiella*, so there was a total of 204 isolates from 203 infants; the total was 300 isolates from 299 children

Table 2. Univariate analysis of risk factors for in-hospital death for children with BSI admitted to CCH

Risk factor	**Died in hospital N = 90 n (%)**	**Alive at discharge N = 209 n (%)**	**OR (95% CI)**	***P* value**
Age group				
0-30 days	80 (89)	123 (59)	5.59 (2.74-1.41)*	**< .001***
31 days - < 12 months	7 (8)	66 (31)		
1-5 years	3 (3)	10 (5)	1.00	
> 5 years	0 (0)	10 (5)		
Gender				
Male	53 (59)	125 (60)	0.96 (0.58-1.59)	0.88
Female	37 (41)	84 (40)	1.00	
Internally displaced person				
Yes	10 (11)	52 (25)	0.38 (0.18-0.78)	**0.007**
No	80 (89)	157 (75)	1.00	
Temperature at admission				
< 37^{o}C	86 (96)	124 (59)	14.74 (5.21-41.69)	**< 0.001**
≥ 37^{o}C	4 (4)	85 (41)	1.00	
White blood count, per mm^{3}				
≥ 10	52 (58)	128 (61)	0.87 (0.52-1.43)	0.58
< 10	38 (42)	81 (39)	1.00	
Organisms isolated				
Gram-negative	68 (76)	96 (46)	3.61 (2.08-6.27)	**<0.001**
Gram-positive	22 (24)	112 (54)	1.00	
Mixed	0 (0)	1 (0.5)	undefined	

*OR and *P* value for comparison of newborn (age 0-30 days) to all other age groups combined (age >30 days).

to a hospital ward. At the time of the patients' admission or presentation, the median body temperature was 36.6ºC from a range of 34.0-39.8ºC); thirteen (4%) of 299 children had hypothermia (a temperature of less than 36ºC. Median white blood count was 11.0/mm^3 from a range of 1.8-57.5/mm^3. Two sets of blood for cultures were obtained from 14 of 299 (5%) children and 285 (95%) had only one blood sample obtained for culture. Among 96 children aged older than 30 days, 30 (31.3%) had cultures performed in > 48 hours of admission to the hospital. A total of 233 (78%) of 299 children had nosocomial infection.

Pathogens recovered from blood cultures are shown in Table 1. Gram-negative rod (GNR) bacteria (165 [55%] of 299) and coagulase-negative *Staphylococcus* (CNS) (109 [36%] of 299) accounted for the majority of recovered pathogens. Further identification of the majority (135 of 165 [82%]) of GNR bacteria was not possible due to lack of laboratory capacity. The significance of a positive culture for CNS was difficult to assess because only a single blood culture was obtained in nearly all cases. No *H. influenzae* or *S. pneumoniae* were identified in blood cultures. Neonates were more likely to have a positive culture for a Gram negative bacteria compared to children older than 30 days (69% versus 25%, respectively, OR = 6.77, 95% CI 3.91-11.74).

Mortality among those with a positive blood culture was 30% (90 of 299 children died). Among 90 children who died, 68 (76%) had a positive blood culture from which Gram-negative organisms were recovered (59 had GNR not identified, five had *Pseudomonas* spp, and four had *Klebsiella* spp), and 22 (24%) had positive blood culture from which Gram-positive organisms were recovered (17 CNS, two *Enterococcus* spp, one *Listeria monocytogenes*, one *S. aureus*, and one had an unidentified Gram-positive rod organism). Mortality was significantly higher in neonates compared to infants and children over 30 days old (OR = 5.59, 95% CI 2.74-1.41), children with body temperature at admission less than 37^{o}C (OR = 14.74, 95% CI 5.21-41.69), and those with a positive blood culture for a Gram-negative organism (OR = 3.61, 95% CI 2.08-6.27). Internally displaced persons had significantly lower mortality (OR = 0.38, 95% CI 0.18-0.78) than other patients with a positive blood culture; among IDPs, 45% were newborn, and among non-IDPs, 74% were newborn infants. In multivariate analysis, independent risk factors for mortality were a positive blood culture in a neonate (< 30 days of age) (OR = 4.00, 95% CI 1.89-8.46), and having a positive culture for a Gram-negative organism (OR = 2.38, 95% CI 1.32-4.29).

Discussion

This study is one of the first studies to assess the etiology and mortality among paediatric patients with positive blood cultures in Georgia. Most (68%) positive blood cultures occurred among neonates (age < 30 days), and Gram-negative bacteria and CNS were most commonly recovered from blood cultures (82%). Unspecified GNR accounted for 45% of all positive blood cultures; further identification of these GNR to the genus and species level was limited due to lack of laboratory capacity. We suspect that *E. coli* may have accounted for many of these Gram-negative infections given that neonates were the most common paediatric patients to have these organisms recovered. Neonatal infections due to Gram-negative pathogens have also been reported from other resource-limited countries in neonatal surveillance [5-8].

The second most commonly recovered organism was CNS, which is not only one of the most common causes of nosocomial blood-stream infections, but also the most common blood culture contaminant [5,8,9,10]. Because almost all the patients had only a single blood culture obtained, it was not possible to assess whether patients who had CNS recovered had a true bacteremia or if recovery was due to skin contamination. However, it is important to note that a substantial proportion of children with positive blood cultures for CNS (17 [16%] of 109) died. It is also possible that recovery of CNS could be a marker for other factors such as a central venous catheter, prolonged hospital stay, underlying co-morbidities, or intracranial shunts. Guidelines are needed in Georgian hospitals to help ensure first that two sets of blood cultures are obtained to help distinguish true CNS bacteremia from contamination, and secondly that staff obtaining blood cultures receive ongoing training on aseptic technique when drawing blood cultures [11,12]. Surprisingly, there was no *Staphylococcus aureus* identified that could point to poor quality control for coagulation.

The mortality rate in patients with blood-stream infections was very high and most of those who died were neonates. Utilizing antimicrobial agents with *in vitro* activity against the pathogen recovered could improve patient outcomes and survival [8]; however, the lack of laboratory capacity at this institution and

many hospitals in resource-limited areas hampers effective care of patients with bacteremia. The hospital's clinical laboratory did not have the capacity to fully identify many of the pathogens recovered to the genus and species level and did not have the capacity to perform susceptibility testing. Surveillance data (*e.g.*, antibiogram) is also important in assisting in appropriate empiric antibiotic choices. On an individual level, a positive blood culture should directly impact patient care if the physician incorporates the result into decision making, such as improving the link between the microbiology laboratory and clinical practice in order to improve patient care. The lack of laboratory capacity appears to also impact suboptimal clinical practices. Anecdotal evidence suggested that antibiotics are often given for several days prior to blood being obtained for culture and because of the lack of laboratory capacity, we suspect that sometimes cultures are not obtained by clinicians when bacteremia or sepsis is suspected.

Like health care facilities in other former Soviet republics, the laboratory at this children's hospital is currently facing many problems relating to aging infrastructure, depreciated and obsolete equipment, and severe financial constraints that all lead to limited laboratory capacity. Staff training and acceptance of modern medical practices are also barriers in this transitioning health care system. In the microbiology laboratory, routine supplies are sometimes difficult to obtain, such as glass culture plates that are reused after sterilization. Funds are not sufficient to obtain commercially manufactured media; all media are made in the hospital without the use of a vacuum hood. A system for automated blood cultures is currently cost-prohibitive. The state of these laboratories is a public health emergency and it is necessary to address this issue as Georgia and other countries reform and modernize their health care systems [13].

In our study, there was no recovery of either *S. pneumoniae* or *H. influenzae* in blood cultures. These pathogens are common causes of invasive bacterial infections in an unvaccinated paediatric population [14-17]. The World Health Organization (WHO) estimates that *H. influenzae* is the leading cause of bacterial meningitis in children under five years of age and the second leading cause of deaths due to bacterial pneumonia in resource-limited areas where vaccination is not performed, which is the current situation in Georgia [18-20]. *S. pneumoniae* is possibly the most important pathogen of infancy, especially in developing countries [15]; however, it is possible that many cases of invasive pneumococcal or *H. influenzae* infections in Georgia go undiagnosed due to the laboratory's limited resources, especially considering the estimated prevalence of these two pathogens in other developing countries. There is a possibility that lab-prepared culture media made with human blood might inhibit or not support the growth of strains of *S. pneumoniae* or *H. influenzae*. It is unclear whether such fastidious organisms can be recovered by current practice without appropriate control materials such as ATCC strains of *S. pneumoniae* or *H. influenzae*. Good quality control and quality assurance programs for laboratory practice are necessary in laboratories that commercially prepare culture media, yet these programs are not available [18,19]. Another possible explanation for the lack of recovery of these two organisms is that the patients received antibiotics prior to the collection of blood cultures, either as inpatients or as outpatients prior to admission. Antibiotics are readily available at pharmacies in Georgia without the need of a prescription. It is clear that a laboratory surveillance system for *S. pneumoniae* and *H. influenzae* will be an important first step for surveillance activities on the prevalence and incidence of invasive pneumococcal and *H. influenzae* infections, and to measure the impact of the introduction of vaccination for both pathogens. As noted, measures to build laboratory capacity will ultimately improve diagnostic and surveillance capabilities, and implementation of vaccines against major bacterial infections in children is urgently needed.

This study was subject to several serious limitations. The lack of laboratory capacity affected the ability to identify many Gram-negative bacteria to the genus and species level. Also due to lack of laboratory capacity, susceptibility testing was not performed. Because only a single blood culture was obtained in the large majority of cases, we could not evaluate whether CNS was a contaminant or a true pathogen. Some patients had fungemia; although these were likely true pathogens, we limited our study to patients with bacterial BSI. No data on previous admissions to the hospital and treatment during current hospitalization were available. In addition, we were not able to readily assess whether mortality was due to the bacteremia because of the lack of ability to distinguish between true bacteremias and contaminants. However, a high

mortality was seen among those patients who had a positive blood culture.

In summary, a high mortality rate (30%) was seen among patients with positive blood-stream infections at the largest children's hospital in Georgia. Microbiological identification of common organisms known to cause blood-stream infections in children is difficult in the Republic of Georgia due to limited resources and laboratory capacity in the clinical microbiology laboratory. Improving the infrastructure and capacity of diagnostic microbiology laboratories in resource-limited countries is a major public health need and it is critical to improve patient care and clinical outcomes as well as establishing and conducting appropriate microbiological surveillance including that for vaccine preventable diseases.

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References

1. Skarbinski J, Walker HK, Baker LC, Kobaladze A, Kirtava Z, Raffin TA (2002) The burden of out-of-pocket payments for health care in Tbilisi, Republic of Georgia. JAMA 287: 1043-1049.
2. Khetsuriani N, Imnadze P, Dekanosidze N. Diptheria epidemic in the Republic of Georgia, 1993-1997 (2000) J Infect Dis Suppl 1 181: S80-S85.
3. Collins T (2003) The aftermath of health sector reform in the Republic of Georgia: effects on people's health. J Community Health 28: 99-113.
4. The World Factbook (2006) The Central Intelligence Agency. Available: https://www.cia.gov/cia/publications/factbook/geos/gg.html# People. Accessed 10 October 2006.
5. Newton O, English M (2007) Young infant sepsis: aetiology, antibiotic susceptibility and clinical signs. Trans R Soc Trop Med Hyg 101: 959-66.
6. Stoll B.J (1997) The global impact of neonatal infection. Infections in Perinatology 24: 1-21.
7. Abucejo-Ladesma E, Simoes EA, Lupisan SP, Sombrero LT, Quiambao BP, Gozum LS, Herva E, Ruutu P (2008) Arivac Health Organization priority for developing countries. J Hosp Infect 68: 285-292.
8. Shears P (2007) Poverty and infection in the developing world: healthcare-related infections and infection control in the tropics. J Hosp Infect 67: 217-224.
9. Obonyo CO, Lau J (2006) Efficacy of Haemophilus influenzae type b vaccination of children: a meta-analysis. Eur J Clin Microbiol Infect Dis 25: 90-97.
10. Whitney CG, Farley MM, Hadler J, Harrison LH, Bennett NM, Lynfield R, Reingold A, Cieslak PR, Pilishvili T, Jackson D, Facklam RR, Jorgensen JH, Schuchat A (2003) Active Bacterial Core Surveillance of the Emerging Infections Program Network. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N Engl J Med 348: 1737-1746.
11. Wenger JD, DiFabio J, Landaverde JM, Levine OS, Gaafar T (1999) Introduction of Hib conjugate vaccines in the non-industrialized world: experience in four 'newly adopting' countries. Vaccine 18: 736-742.
12. Levine OS, O'Brien KL, Knoll M, Adegbola RA, Black S, Cherian T, *et al*. (2006) Pneumococcal vaccination in developing countries. Lancet 367: 1880-1882.
13. World Health Organization (1998) Global program for vaccines and immunization. The WHO position paper on Heamophilus influenzae type b conjugate vaccines. Wkly Epidemiol Rec 73: 64-68.
14. World Health Organization (1999) Global program for vaccines and immunization. The WHO position paper on pneumococcal vaccines. Wkly Epidemiol Rec 74: 177-184.
15. Kobaladze NK (2005) [Improvement of the vaccine prevention quality as a basis of the social welfare]. Georgian Med News 123: 68-71. [Article in Russian]
16. Consortium (2007) Serious community-acquired paediatric infections in rural Asia (Bohol Island, Philippines): bacterial meningitis in children less than 5 years of age. Scand J Infect Dis 39: 983-989.
17. Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA (2005) Hospital-acquired neonatal infections in developing countries. Lancet 365: 1175-1188.
18. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in pediatric intensive care units in the United States (1999) National Nosocomial Infections Surveillance System. Pediatrics 103: e39.
19. Weinstein MP (2003) Blood culture contamination: persisting problems and partial progress. J Clin Microbiol 41: 2275-2278.
20. Allegranzi B, Pittet D (2007) Healthcare-associated infection in developing countries: simple solutions to meet complex challenges. Infect Control Hosp Epidemiol 28: 1323-1327.
21. Pittet D, Allegranzi B, Storr J, Bagheri Nejad S, Dziekan G, Leotsakos A, Donaldson L (2008) Infection control as a major World Health Organization priority for developing countries. Hosp Infect 68: 285-292.

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