Organisms and antibiotic sensitivity patterns in septicemic children of Lahore, Pakistan

Sundus Ameen^a, Zahid Qureshi^a, Hassan Ejaz^b, Aizza Zafar^b, Sana Riaz^a

^aDivision of Biochemistry, Department of Chemistry, Government College University, ^bDepartment of Microbiology, Children Hospital and Institute of Child Health, Lahore 54000, Pakistan

Background: The right understanding of antibiotic susceptibility patterns is important for cost effectiveness and outcome of care of septicemic patients.

Objectives: To determine antibacterial resistance patterns against commonly used therapeutic antibiotics in septicemic children of Lahore by isolating and analyzing the most frequently occurring bacterial pathogen from blood samples.

Methods: Twelve hundred blood samples from children with septicemia were processed at the Lahore Identification of Bacteria Laboratory following the Clinical Laboratory Standard Institute reference 2006. Antibiotic sensitivity was analyzed using the Kirby–Bauer disc diffusion method.

Results: The most effective antibiotics against the frequently isolated *Klebsiella* spp. were piperacillin–tazobactam (87.74%). High antimicrobial resistance was observed with cefuroxime (95.58%) and cefixime (93.38%).

Conclusion: Klebsiella are the most frequently occurring bacterial pathogens from blood samples of children with septicemia. Moreover, the antibiotics to which there was most resistance belong to the cephalosporins, which are among the foremost used antibiotics in Pakistan.

Keywords: Antibiotic, Klebsiella species, septicemic children, Pakistan

Despite ongoing major advances in the development of potent antimicrobial agents, humans remain subject to bacterial and fungal infection through mechanisms of virulence [1]. Bacterial sepsis affects any organ partly by releasing toxins [2]. Neonatal sepsis occurs often in first 90 days of life. Clinical manifestations include temperature, diarrhea, abdominal pain, vomiting, respiratory tract symptoms, jaundice, and anxiety [3]. The rate of septicemia in children is 0.5/1000 per live births and the risk is increased in infants with respiratory distress at the time of birth, in males (2:1), and in neonates with innate compromise [4].

Declining age of mother, low socioeconomic status, poverty, and unhygienic community environments play a significant role and increase risks [5]. Onset can appear in the first 6 to 72 hours of life and are commonly caused by group B streptococci

and Gram-negative pathogens; mainly Escherichia coli [6]. Mothers may show rectal and vaginal cultures with group B Streptococcus and thus 30% of their infants also become colonized with this organism [7]. The risk of persistent disease increases with the colonization. Within the first 6 h after birth 1/100 of the colonized infants will develop persistent disease due to group B streptococci [8]. There is also an increasing risk of non typeable Haemophilus influenza sepsis in children with septicemia [9]. Other Gram-negative enteric bacilli (e.g., Klebsilla spp.) and Gram-negative bacteria enterococci (e.g., Enterococcus faecalis, Enterococcus faecium), group D streptococci (e.g., Streptococcus bovis), and hemolytic streptococci account for most of the cases of children with septicemia. The rate of mortality due to K. pneumonia is high [10] because it affects lungs and is often responsible for lung suppuration, gastrointestinal, and urinary tract infections [11-13]. In most children with septicemia Klebsiella sepsis presents with joint tenderness, respiratory distress, coma, bloody diarrhea, and meningitis oral mouth ulcers [14, 15].

Correspondence to: Sana Riaz, Division of Biochemistry, Department of Chemistry, Government College University, Lahore 54000, Pakistan. E-mail: sanariazpk@gmail.com

Klebsiella spp. have different patterns of antimicrobial susceptibility. They are usually resistant to ampicillin and carbenicillin, but susceptible to aminoglycosides in Pakistan. Multidrug-resistant strains of *Klebsiella* spp. are not uncommon [16].

Materials and methods

Sample collection and processing

A total of 1200 blood samples from children with septicemia were collected and processed in the Microbiology Department of the Children Hospital and Institute of Child Health, Lahore, from February to November 2010.

Identification of bacteria

Preliminary identification of bacteria from 1070 positive neonatal blood samples was performed using Gram Staining, a catalase test, and oxidase test approved by the Clinical Laboratory Standard Institute (CLSI) [17], and criteria API 20 E (Biomereux) were employed. Results of preliminary identification tests were followed by biochemical tests. Gram-negative rods were subjected to the following routine biochemical tests; urease test, citrate utilization test, and triple sugar iron (TSI).

Antibiotic sensitivity test

All organisms isolated were tested using the Kirby–Bauer disc diffusion method with Muller Hinton agar plates. Antibiotic disks used in this study included Augmentin, cephalexin, cefixime, ciprofloxacin, ceftazidime, nalidixic acid, nitrofurantoin, pipemidic acid, ceftizoxime, imipenem, oxacillin, and vancomycin (Merck). The study was approved by the Ethics Committee of The Children Hospital and Institute of Child Health, Lahore. Informed consent was signed.

Results

A total of 1200 blood samples were collected and processed and 1070 samples were positive. There were 57.8% males (618 samples) and 42.2% females (451 samples) as shown in **Table 1**. The most common pathogens isolated from blood samples were *Klebsiella* spp. (38.13%). The next most common organisms found were staphylococci (16.8%) and *Escherichia coli* (9.15%). Other species included *Pseudomonas* (8.31%), *Burkholderia* (6.07%), and *Salmonella* (2.99%). The details are shown in **Table 2**.

Age	No. of isolates	Percentages	Frequency distribution by sex	
			Male (%)	Female (%)
<1 month	718	67.10	392 (36.6)	326(30.46)
1 11 months	224	20.93	151 (14.11)	73 (6.822)
1 5 years	96	8.97	57 (5.32)	39 (3.64)
6 10 years	21	1.96	16(1.49)	5 (0.46)
>10 years	11	1.02	2(0.18)	9 (0.84)
Total	1070	100	618.46 (57.8)	451.54 (42.2)

 Table 1. Age group and sex distribution of cases with bacterial growth

Table 2. Frequency of different bacterial species isolated from blood samples

Bacterial pathogens	Frequency	%	
Escherichia coli	98	9.15	
Klebsiella spp.	408	38.13	
Streptococcus spp.	38	3.55	
Pseudomonas spp.	89	8.31	
Staphylococcus aureus	78	7.2	
Other Staphylococcus spp.	180	16.8	
Other Enterobacter spp.	39	3.64	
Acinetobacter spp.	7	0.65	
Ralstonia spp.	5	0.46	
Serratia spp.	14	1.30	
Burkholderia spp.	65	6.07	
Salmonella spp.	32	2.99	
Chrysomonas spp.	17	1.58	
Total	1070	100	

Antibiotic susceptibility patterns for *Klebsiella* spp. were highly sensitive to piperacillin-tazobactam (87.74%), and ciprofloxacin (81.37%). Good sensitivity patterns were also observed with amoxicillin + clavulanic acid (Augmentin) (62.25%). Low sensitivity was observed with cefuroxime (4.41%), cefixime (6.61%), gentamycin (10.53), and ceftazidime

(16.17%).

Discussion

The results of present study are in accordance with the one carried out [18] at the Neonatal Unit of Ghurki Trust Teaching Hospital Lahore, where Gramnegative organisms were isolated from 80% of the patients. *E. coli* was the most common isolate, followed by *Klebsiella* spp. (76%) and *Pseudomonas* (13%).

Similar findings were attained [19], where the *E. coli, Klebsiella*, and *Pseudomonas species* constituted 77% of the total organism isolated. According to this analysis, more than 60% of the cases of sepsis are due to Gram-negative isolates and the most commonly isolated organism is *Klebsiella* spp. (41%).

A very low sensitivity and prevalence of *Klebsiella* spp. were noticed [20] in the Department of Pediatrics, Khyber Teaching Hospital, Peshawar. *Escherichia coli* was the most common organism found (36.6%), followed by *Staphylococcus aureus* (29.5%), *Pseudomonas* spp. (22.4%), *Klebsiella* spp. (7.6%), and *Proteus* (3.8%). Antibacterial sensitivity pattern for *Klebsiella* spp. revealed highest sensitivity to piperacillin–tazobactam (87.74%), and ciprofloxacin (81.37%), and amoxicillin + clavulanic acid (62.25%).

The case records of all admitted to the septicemic children unit of Al Wasl Hospital (Dubai) in a period of 60 months was analyzed. One hundred and six neonates had confirmed sepsis. *Klebsiella pneumoniae* were found to be the causative organism in 24% of cases [21].

The authors have no conflicts of interest to declare.

References

 Linkin DR, Fishman NO, Patel JB, Merrill JD, Lautenbach E. Risk factors for extended-spectrum beta-lactamase-producing Enterobacteriaceae in a neonatal Intensive care unit. Infect Control Hosp Epidemiol. 2004; 25:781-3.

- 2. Marrie TJ, Durrant. Review of pulmonary and extrapulmonary manifestation of infection due to *Coxiella burnetii*. Eur Respire J. 1989; 21:713-9.
- 3. Calderwood DA. Survey of septicemia in intensive care unit. Pediatr Infect Dis J. 1993; 41:56-64.
- Liberman RP, Wallace CJ, Blackwell CA. Innovations in skills training for the seriously mentally ill: the UCLA social and independent living skills module. Innovation Research. 1993; 2:43-59.
- Sturenburg E, Mack D. Extended-spectrum βlactamases: implications for the clinical microbiology laboratory, therapy, and infection control. J Infect. 2003;47:273-95.
- Sundsfjord A, Simonsen GS, Haldorsen BC, Haaheim H, Hjelmevoll SO, Littauer P. Genetic methods for detection of antimicrobial resistance. A PM I S. 2004; 112:815-37.
- Spanu T, Luzzaro F, Perilli M, Amicosante G, Toniolo A, Fadda G. Occurrence of extended spectrum β-lactamases in members of the family *Enterobacteriaceae* in Italy: implications for resistance to β-lactams and other antimicrobial drugs. Antimicrob agents Chemother. 2002; 46:196-202.
- Wu TL, Chia JH, Su LH, Kuo AJ, Chu C, Chiu CH. Dissemination of extended-spectrum â-lactamaseproducing *Enterobacteriaceae* in pediatric intensive care units. J Clin Microbiol. 2003; 41:4836-8.
- Kollef MH, Fraser VJ. Antibiotic resistance in the intensive care unit. Ann Intern Med. 2001; 134: 298-314.
- Higgins C. Microbiological examination of blood for septicemia. Nurs Times. 1995; 91:34-5.
- Khan IK, Akram DS. Neonatal sepsis etiological study. JAMA. 1987; 37:327-30.
- 12. <u>Chacko B, Sohi I. Early onset neonatal sepsis. Ind J</u> Pediatr. 2005; 72:23-6.
- Mondal GP, Raghavan M, Bhat BV, Srinavasan S. Neonatal septicemia among inborn and outborn babies in a referral hospital. Ind J Pediatr. 1991; 58: 529-33.
- Narla VR. Bacteraemia in neonates. J Infect. 1985; 10: 126-42.
- 15. <u>Parrilio JE. Septicemia present and future.</u> Ann Intern Med. 1991; 115:491-2.
- Waddell WW, Balsley RE, Grossman W, Charlottesville VA. The significance of positive blood culture in new born infants. J Pediatr. 1984; 68:321-5.
- 17. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility

testing; 16th informational supplement. M100-S16. Clinical and Laboratory Standards Institute, Wayne, Pa., 2006.

- 18. Rizwan W, Tahira S, Muhammad K, Abdul W. Epidemiology of sepsis. Prof Med J. 2005; 12:451-6.
- 19. Bhutta ZA. Epidemiology of neonatal Sepsis in Pakistan. An analysis of available evidence and

implication for care. JSPSP. 1996; 6:12-7.

- 20. Rehman S, Hameed A, Roghani MT, Ullah Z. Multidrug resistant neonatal sepsis in Peshawar, Pakistan. Arch Dis Child Fetal Neonatal Ed. 2002; 87:F52-4.
- 21. Koutouby A, Habibullah J. Neonatal sepsis in Dubai, United Arab Emirates. J Trop Pediatr. 1995; 41:177-80.