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Bacteriological Analysis of Blood Culture Isolates from Neonates in a Tertiary Care Hospital in India

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ABSTRACT

This study was undertaken to determine the profile and antibiotic sensitivity patterns of aerobic isolates from blood cultures of neonates in a tertiary care hospital in New Delhi, India. All blood culture reports (n=1,828), obtained during August 1995-September 1996 from newborns admitted to the Department of Pediatrics and the Neonatal Intensive Care Unit at the University College of Medical Sciences and GTB Hospital, Delhi, were analyzed, and the sensitivity patterns were recorded. The positivity of blood culture was 42% (770/1,828). Most (93.2%) bactaeremic episodes were caused by a single organism, while polymicrobial aetiology was observed in 52 (6.8%) cases. Gram-negative organisms were isolated in 493 (60%) of 823 cases, with *Klebsiella* (33.8%), *Enterobacter* (7.5%), *Alcaligenes faecalis* (4.9%), and *Escherichia coli* (4.6%) being the common microbes. *Staphylococcus aureus* (24.4%), followed by coagulase-negative staphylococci (7.9%), were the major Gram-positive isolates. Most (80%) Gram-positive isolates were sensitive to vancomycin, and 50-75% of the Gram-negative isolates were sensitive to ciprofloxacin and amikacin. It is concluded that *Klebsiella* and *Staphylococcus aureus* remain the principal organisms responsible for neonatal sepsis in a tertiary care setting.

Key words: Septicaemia; Drug resistance, Microbial; Microbial sensitivity tests; Antibiotics; Neonate; Retrospective studies; India

INTRODUCTION

Neonatal septicaemia is an important cause of morbidity and mortality among neonates in India, with an estimated incidence of approximately 4% in intramural livebirths (1). An early and accurate aetiological diagnosis is not always easy, especially since the disease may start with minimal or non-specific symptoms. Delayed treatment until clinical recognition of signs and symptoms of sepsis entails risk of preventable mortality, notwithstanding the fact that presumptive antibiotic therapy may result in over-treatment. Of necessity, many more babies are evaluated and treated for sepsis than the number who actually have the condition. Aetiological causes also do not remain the same. A wide variety of bacteria—both aerobic and anaerobic—can cause neonatal septicaemia. To compound the problem, both regional and temporal differences in aetiologic agents exist (1-7).

The uncertainty surrounding the clinical approach to treatment of neonatal septicaemia can be minimized by periodic epidemiological surveys of aetiological agents and their antibiotic sensitivity patterns leading to recognition of the most frequently-encountered pathogens in a particular neonatal setting.

This study was undertaken to determine the profile and antibiotic sensitivity patterns of aerobic isolates from blood cultures of neonates in a tertiary care hospital in New Delhi, India.

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MATERIALS AND METHODS

An analysis was conducted on all blood culture reports obtained during August 1995-September 1996 from newborns admitted to the Department of Pediatrics and the Neonatal Intensive Care Unit (NICU) at the University College of Medical Sciences (UCMS) and GTB Hospital, Delhi. Most of these newborns were delivered at the UCMS and GTB Hospital. The neonatal intensive care unit is a 32-bedded level II^+ unit and caters only to babies born in this hospital. The number of livebirths at this hospital ranges from 10,000 to 12,000 per annum, and approximately 12-18% of these are admitted to the NICU. Neonates born in other hospitals or at home are admitted to the Pediatrics Ward. On average, 60-80 extramural newborns are admitted to the Pediatrics Ward per month.

Blood culture was done for all neonates suspected to have septicaemia. Septicaemia was suspected in the following settings:

At birth: All neonates (i) born to mothers with maternal fever, prolonged rupture of membranes for more than 24 hours, foul-smelling or meconium-stained liquor, or frequent (>3) unclean vaginal examinations, and/or (ii) having severe prematurity, or birth asphyxia necessitating active resuscitation.

After birth: All newborns with lethargy, refusal of feeds, abdominal distension, respiratory distress, instability in temperature, pathological jaundice, convulsions, autonomic disturbances, and bleeding manifestations with constitutional symptoms.

Following procedures: All newborns undergoing an exchange transfusion.

All blood cultures were collected from a peripheral vein with proper aseptic precautions before starting any antibiotic therapy. Approximately, 3 mL of blood was inoculated into brain-heart infusion broth and incubated at 37 ˚C. Sub-cultures were made on both blood agar and McConkey agar after 24 and 48 hours. Growth, if any, was identified by the standard bacteriological techniques (8), including Gram staining, colony characteristics, biochemical properties, and slide agglutination where appropriate. Antibiotic sensitivity was performed by Stoke's disc-diffusion method (9). *Staphylococcus aureus* NCTC 6571 was used as the known standard strain.

Aerobic spore bearers, wherever grown, were regarded as contaminants. The remaining isolates were included in analysis. Further categorization of the isolates as true pathogen, trivial pathogen, and contaminant was not considered appropriate in absence of repeated sampling and clinical correlation.

RESULTS

In total, 1,828 blood cultures from neonates were evaluated. These included 1,358 (74.3%) intramural and 470 (25.7%) neonates born outside the hospital. An aetiology could be established in 770 (42.1%) neonates. Of all culture-positive isolates, 616 (80%) belonged to the intramural cohort. In total, 830 (45.4%) samples were bacteriologically sterile, and 228 (12.5%) grew contaminants.

In culture-positive neonates, the cause of septicaemia was monomicrobial in 718 (93.2%) cases and polymicrobial in 52 (6.8%) cultures. Detailed aetiology

Table 1. Distribution (frequency) of bacterial isolates (n=823) from newborns with clinically-suspected septicaemia

Isolate	Distribution (frequency)
Gram-negative bacilli (n=492)	
Klebsiella species	206
Klebsiella rhinoscleromatis	49
Klebsiella pneumoniae	17
Klebsiella aerogens	3
Klebsiella oxytoca	3
Enterobacter species	62
Alcaligenes faecalis	40
Escherichia coli	38
Acinetobacter calcoaceticus	22
Acinetobacter species	11
Citrobacter species	9
Pseudomonas aeruginosa	10
Pseudomonas maltophila	1
Proteus sp.	4
Providencia sturtii	1
Salmonella typhimurium	5
Salmonella barielly	1
Corynebacterium aquaticum	3
Unidentified Gram-negative	7
Gram-positive cocci (n=309)	
Staphylococcus aureus	201
Coagulase-negative staphylococci	65
Enterococcus faecalis	35
Enterococcus species	5
Streptococcus viridans	2
Streptococcus species	1
Gram-negative cocci (n=1)	
Moraxella catarrhalis	1
Fungi (n=21)	
Candida	20
Mycelial fungi	1

of the 823 isolates is provided in Table 1. These included Gram-positive cocci (309/823, 37.5%), Gram-negative bacilli (492/823, 59.8%), and *Candida* species (20/823, 2.43%). Staphylococci and *Klebsiellae* were the most common Gram-positive and Gram-negative organisms together accounting for 32.3% (266/823) and 33.8% (278/823) of the isolates respectively. Other common Gram-negative isolates were *Enterobacter* (62/823, 7.5%) and *Escherichia coli* (38/823, 4.6%).

An association of two organisms was observed in 51 cases. In only one case, *S. aureus, Klebsiella* sp., and *Enterococcus* sp. were isolated from a single culture specimen. Thus, the total number of specimens containing polymicrobial strains was 52. Gram-positive organisms were present in 20 (39.2%) of these cultures. These were either associated with another Gram-positive bacterium (n=5) or a Gram-negative organism (n=15). A combination of two Gram-negative bacteria was observed in 30 (58.9%) cases. In 3 (5.9%) neonates, Gram-negative organisms were grown along with *Candida* species.

Table 2 and 3 show the antibiotic sensitivity patterns of the common organisms isolated. Seventy to eighty percent of *S. aureus* were resistant to the commonly-used antibiotics, including penicillin, cloxacillin, cefelexin, and gentamicin. More than 80% of the Gram-positive organisms, including *S. aureus,* were sensitive to vancomycin. Of the two aminoglycosides studied, amikacin scored over gentamicin in terms of sensitivity for staphylococci. Most Gram-negative isolates (50-75%) were sensitive to ciprofloxacin. Fifty percent of the *Klebsiella* and *E. coli* isolates were also sensitive to cefotaxime and amikacin.

Table 2. Antibiotic sensitivity patterns of Gram-positive isolates

Antimicrobial	*Staphylococcus aureus* (n=201)		Coagulase-negative staphylococci (n=65)		*Enterococcus faecalis* (n=35)	
	No.	%	No.	%	No.	%
Penicillin	40/191	20.9	20/62	32.2	3/26	11.5
Erythromycin	60/174	34.5	35/60	58.3	14/32	43.7
Cloxacillin	49/161	30.4	20/45	44.4	0/21	0
Vancomycin	56/68	82.3	9/9	100	9/11	81.8
Amikacin	72/130	55.4	29/37	78.4	7/21	33.3
Gentamicin	51/183	27.9	36/64	56.2	1035	28.6
Cefelexin	55/188	29.2	34/59	57.6	6/32	18.8
Cefotaxime	43/117	36.7	21/37	56.8	8/24	33.3
Co-trimoxazole	16/55	29.1	10/27	37	0/10	0
Ciprofloxacin	17/35	48.6	16/24	66.7	-	-

DISCUSSION

The findings of our study are similar to those of the National Neonatal Perinatal Database (1) wherein *Klebsiella* was the predominant pathogen in 29% of cases. *S. aureus* has been predominantly isolated in several studies (3,10). Recent data from Pakistan reveal that *S. aureus*, *Klebsiella,* and *E. coli* are the common organisms isolated in neonatal units at Karachi and Peshawar, and most of these strains are multidrug-resistant (11,12). Group B *Streptococcus*, a common cause of neonatal sepsis in the West, is infrequent in India and in other tropical countries (13).

Culture-positivity for aerobic organisms in neonates vary from 25% to 60% (1,2,13,14). It is possible that many anaerobes were being missed in these studies, including the present one. In 1974, Chow *et al*. demonstrated the significance of anaerobes in neonatal sepsis (15). In a recent Indian study, anaerobes were isolated in 6.6% cases of neonatal blood cultures (10). However, the feasibility, logistics, and cost-effectiveness of routine anaerobic cultures for neonatal sepsis need to be explored further. Zaidi *et al.* reported that anaerobic blood cultures are rarely helpful in the majority of paediatric patients and usually show positive results only in clinical settings associated with anaerobic infections (16).

The clinical significance of relatively low-virulence isolates, such as coagulase-negative staphylococci, *Enterococcus faecalis*, non-fermentative Gram-negative bacilli other than *Pseudomonas*, *Cornyebacteria aquaticuam*, *Moraxella catarrhalis*, *Streptococcus viridans,* and *Candida* species, is difficult to ascertain. These organisms can cause true bacteraemia, or their

Table 3: Antibiotic sensitivity patterns of Gram-negative isolates

Organism	No.	Cefelexin	Cefotaxime	Amikacin	Gentamicin	Ciprofloxacin	Chloramphenicol
Klebsiella sp.	206	28/144 (19.4)	70/167 (41.9)	89/176 (50.6)	36/203 (17.7)	66/98 (67.3)	75/205 (36.6)
Klebsiella rhinoscleromatis	49	10/49 (20.4)	8/47 (17.0)	2/45 (4.4)	0/47 (0.0)	0/39 (0.0)	1/49 (2.0)
Klebsiella pneumoniae	17	5/15 (33.3)	7/12 (58.3)	11/14 (78.6)	5/17 (29.4)	3/4 (75.0)	3/14 (21.4)
Enterobacter sp.	62	2/42 (4.8)	4/35 (11.4)	14/38 (36.8)	12/45 (26.7)	20/33 (60.6)	21/44 (47.7)
Alcaligenes faecalis	40	1/35 (2.9)	21/29 (72.4)	8/31 (25.8)	8/38 (21.1)	16/33 (48.5)	4/40 (10.0)
Escherichia coli	38	15/36 (41.7)	17/30 (56.7)	15/30 (50.0)	16/36 (44.4)	11/16 (68.8)	25/36 (69.4)
Acinetobacter calcoaceticus	22	2/22 (9.1)	6/12 (50.0)	5/15 (33.3)	5/22 (22.7)	7/12 (58.3)	4/22 (18.2)
Acinetobacter sp.	11	1/10 (10.0)	6/6 (100)	5/5 (100)	4/11 (36.4)	2/4 (50.0)	4/11 (36.4)
Pseudomonas aeuroginosa	10	0/3 (0.0)	3/5 (60.0)	4/10 (40.0)	2/9 (22.2)	6/8 (75.0)	0/3 (0.0))
Citrobacter sp.	9	2/9 (22.2)	2/5 (40.0)	3/6 (50.0)	4/9 (44.4)	2/4 (50.0)	6/9 (66.7)

Figures in parentheses indicate percentages

isolation may represent simple contamination. It would be injudicious to dismiss such isolates as contaminants.

In polymicrobial sepsis, the association of two different organisms did not exhibit any particular pattern or trend. A neonate already infected with one microbe may have acquired the second one from the hospital environment, or both the bacteria could be nosocomial in origin. Most previous studies failed to document polymicrobial sepsis, either because of unawareness of its significance or disregard for the second organism in an already positive culture (1-7,14,17). In a recent Indian report, polymicrobial aetiology was documented in 8% of cases (10), which is very similar to the incidence of 6.8% in our study. A western study reported an incidence of 3.9% of polymicrobial sepsis in infants in an intensive care nursery (18). There is a need to correlate the occurrence of polymicrobial sepsis with clinical outcome in neonatal septicaemia.

Vancomycin is still the drug of choice for *S. aureus*, but resistance to this drug has also been reported (19). A similar trend may also be expected in the developing world due to its lower cost and increased availability. A combination of ciprofloxacin and amikacin appears to be the best choice for infections due to *Klebsiella*. These findings are in tandem with the National Neonatal Perinatal Database (1). Treatment with ciprofloxacin is also indicated in multidrug-resistant *S. aureus* in the paediatric age group, but its use in neonates is still experimental due to lack of safety data (3,19).

We did not distinguish between community- and hospital-acquired infections for analyzing the results. Being a retrospective study of microbiological records, correlation with neonatal morbidity and mortality and other markers of sepsis was also not possible. Inclusion of these data would have definitely enhanced the utility of this study.

Clinical recognition of neonatal sepsis is not always straight-forward. Appropriate intervention requires an early aetiological diagnosis. Microbial aetiology of neonatal septicaemia is diverse. Several studies on neonatal sepsis have documented the diversity of bacteria and their temporal variability. The present study reiterates the earlier findings and emphasizes the importance of periodic surveys of microbial flora encountered in particular neonatal settings to recognize the trend.

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