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Clinical significance of polymicrobial bacteremia in newborns

Piyush Gupta,[1] Ghanshyam D Kumhar,[1] Geetinder Kaur[1] and VG Ramachandran[2]

[1]*Department of Pediatrics and* [2]*Department of Microbiology, University College of Medical Sciences and GTB Hospital, Delhi, India*

Objective: To identify whether polymicrobial bacteremia in newborns is associated with any predisposing factors, distinguishing clinical features, or higher mortality.
Methods: Results of blood cultures obtained over a period of 1 year from neonates admitted to the paediatric ward and Neonatal Intensive Care Unit of a tertiary care hospital were retrospectively analysed. The study group included all cases with polymicrobial bacteremia (isolation of two or more organisms). Controls (double the number of study cases) were randomly selected from the monomicrobial group. Case records of included cases were retrieved and scrutinized.
Results: Of 770 positive cultures during the study period, 52 (6.8%) cultures were positive for more than one organism. Complete case records were retrieved for 40 polymicrobial and 78 monomicrobial cases. The two groups were comparable for maternal and neonatal parameters including: maternal and obstetric complications; period of gestation; mode of delivery; birthweight and perinatal asphyxia; clinical symptomatology; invasive therapeutic interventions; and mortality.
Conclusions: Isolation of more than one organism from the blood culture of a suspected septic newborn is not rare. It does not always represent a true invasion by multiple organisms. Polymicrobial isolation per se should not be the criterion for hastily changing the therapeutic decisions.

Key words: bacteremia; newborn; polymicrobial.

Polymicrobial bacteremia refers to the isolation of more than one microorganism from a single blood culture specimen at a given time. Invasion of the bloodstream by more than one organism is facilitated by a susceptible host; immunosuppression induced by malnutrition, disease or drugs; and exposure to invasive monitoring or intensive care.[1,2] True polymicrobial bacteremia is a well-defined clinical entity in adults.[2,3] Incidence of polymicrobial sepsis in the paediatric population is reported to range from 3.2 to 23%.[4,5] However, incidence of polymicrobial bacteremia and its relevance in newborns is not well understood. A sick neonate admitted to a Neonatal Intensive Care Unit (NICU) presents with an ideal scenario for simultaneous or sequential invasion of the bloodstream by multiple organisms.

Jarvis *et al*.[6] reported the incidence of polymicrobial bacteremia to be 9.8% that increased to 25% during an outbreak of *Klebsiella pneumoniae* and *Enterobacter cloacae* sepsis in a NICU. In another study,[7] newborn infants exposed to umbilical venous catheters and total parenteral nutrition given simultaneously with a dextrose/electrolyte solution were more likely to have polymicrobial bloodstream infections. Faix and Kovarik[8] reported the incidence of polymicrobial bacteremia in neonates to be 3.9% in all cases of culture-proven sepsis in a non-outbreak setting, with a higher mortality (70%) as compared to monomicrobial sepsis (23%). No other systematic studies are available to shed light on the risk factors or clinical significance of polymicrobial bacteremia in newborn infants.

The present study was conducted to find out the incidence of polymicrobial bacteremia in the NICU of a tertiary care hospital in India and identify whether it is associated with any predisposing factors, distinguishing clinical features, or higher mortality.

METHODS

Blood cultures obtained from neonates admitted to the NICU and paediatric wards of a tertiary care referral hospital over a period of 1 year were retrospectively analysed. The NICU is a 32-bedded level II + unit and caters only to babies born in this hospital. The number of live births at our 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 paediatrics ward. On an average, 60–80% extramural newborns are admitted to the paediatrics ward every month. Of the admitted newborns, 60–70% are preterms. Postoperative or cardiac newborns contribute little to our neonatal admissions.

These blood cultures were obtained from newborns admitted with suspected septicemia. Septicemia was suspected on the basis of the presence of one or more of the following criteria: (i) history of antenatal risk factors like maternal fever, prolonged rupture of membranes (>24 h), foul-smelling vaginal discharge, frequent (>3) unclean vaginal examinations, severe prematurity and birth asphyxia necessitating active resuscitation; (ii) neonate presenting with symptoms suggestive of sepsis such as lethargy, refusal to feed, respiratory distress, thermal instability, bleeding manifestations etc.; and (iii) all neonates undergoing invasive procedures such as exchange transfusion.

Blood cultures were collected from a peripheral vein with proper aseptic precautions before the initiation of antibiotic therapy. Skin was disinfected with 70% alcohol beginning in the centre and encircling an area of approximately 5 cm. When the alcohol was dry, the area was swabbed with tincture iodine in the same manner. When the iodine was dry, blood was collected from the chosen vein by a dry sterile needle and syringe. Blood was never collected through an existing intravenous catheter. Approximately 3 mL of blood was immediately inoculated into brain–heart infusion broth and incubated at 37°C. Subcultures were made on Blood agar and McConkey's agar at 24 and 48 h. Growth, if any, was identified by standard bacteriological techniques[9] including Gram stain, biochemical reactions and slide agglutination, where appropriate. The antibiotic sensitivity was performed by Stoke's disc diffusion method.[10] Aerobic spore bearers, wherever grown, were regarded as contaminants. The remaining isolates were included in the analysis.

Correspondence: Dr Piyush Gupta, Block R-6-A, Dilshad Garden, Near Telephone Exchange, Delhi 110095, India. Fax: +91 11 22590495; email: drpiyush@satyam.net.in
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The positive blood cultures were then classified into monomicrobial and polymicrobial bacteremia. Polymicrobial bacteremia was defined as the growth of two or more pathogenic organisms in a single blood culture and monomicrobial bacteremia as the growth of a single pathogen. Polymicrobial and monomicrobial cases were listed separately in a dated sequence. Case records of all polymicrobial cases were retrieved from the Medical Records Department. Using a computer-generated random number table, double the number of polymicrobial cases were randomly chosen from the monomicrobial group. The clinical record sheets of these newborns were also retrieved. Subjects with untraced or incomplete records were excluded from the study.

Records of those who were finally included in the two groups were scrutinized for the following information.

1. *Maternal complications*: fever, premature rupture of membranes, foul-smelling vaginal discharge, increased maternal white cell count, diarrhoea or urinary tract infection within 2 weeks before delivery and meconium-stained liquor.
2. *Neonatal characteristics*: sex, gestational age, birthweight, weight to gestation, mode of delivery, birth asphyxia and need for active resuscitation.
3. *Clinical symptoms*: presence of any of the following symptoms along with the date of onset was recorded: (i) general symptoms such as lethargy, refusal to feed, prefeed aspirates, fever and hypothermia; (ii) alimentary symptoms like abdominal distension, vomiting, diarrhoea and hepatomegaly; (iii) respiratory symptoms including respiratory distress, apnea and cyanosis; (iv) CNS symptoms such as full anterior fontanel, convulsions, staring (vacant) look and irritability; (v) haematological symptoms such as pallor, splenomegaly or conjugated hyperbilirubinemia; and (vi) complications such as intracranial, pulmonary or gastrointestinal bleed because of disseminated intravascular coagulation, osteomyelitis, arthritis, umbilical sepsis, necrotizing enterocolitis and shock.
4. *Invasive intervention received*: parenteral nutrition (blood, plasma or lipid transfusion), exchange transfusion, indwelling i.v. catheters and artificial ventilation.
5. *Outcome*: death or discharge, day of amelioration of symptoms and duration of hospital stay.

Initial empiric therapy consisted of a combination of ampicillin and gentamicin. In case of non-response or clinical worsening, antibiotics were changed to cefotaxime and amikacin in most instances, or as dictated by sensitivity reports.

The data were entered in Microsoft Excel and analysed by SPSS. Chi-square test or Fisher's exact test was used for comparison of categorical variables and unpaired '*t*'-test for continuous variables. Maternal and neonatal factors were studied as predisposing factors for polymicrobial bacteremia. The presence and day of onset of clinical symptoms and complications and mortality were compared between the two groups to ascertain the clinical significance of polymicrobial bacteremia.

RESULTS

Of 1828 blood cultures obtained during the study period, 770 (42.1%) cultures grew an organism, 830 (45.4%) were bacteriologically sterile and 228 (12.5%) grew contaminants. Of the 770 positive cultures, 718 (93.2%) were monomicrobial and 52 (6.8%) were polymicrobial. A detailed analysis of these monomicrobial cultures has been presented earlier.[11]

Two organisms were grown simultaneously in 51 cultures. Gram-positive organisms were present in 20 (39.2%) of these cultures. These were associated with another Gram-positive bacteria ($n = 5$) or a Gram-negative bacteria ($n = 15$). A combination of two Gram-negative bacteria was observed in 28/51 (54.7%) cases. In three (5.9%) neonates, Gram-negative organisms were grown along with *Candida* species. The pattern of association of two organisms is shown in Table 1. In only one case, three organisms (i.e. *Staphylococcus aureus*, *Klebsiella* sp. and *Enterococcus* sp.) were isolated from a single culture specimen.

Predisposing factors

As shown in the study flow chart (Fig. 1), 40 neonates with polymicrobial bacteremia and 78 neonates with monomicrobial bacteremia were finally included in the study. The two groups were comparable with regard to neonatal and maternal characteristics. The mean (SD) birthweight in the polymicrobial group was 1946.2 (611.1) g (median: 1725 g; range: 1000–3500 g) and 1957.1 (617.1) g in the monomicrobial group (median: 1800 g; range: 900–3750 g). The gestational age in the polymicrobial group ranged between 30 and 40 weeks with a mean of 35.9 ± 2.4 weeks and 35.4 ± 2.8 weeks in the polymicrobial and monomicrobial groups, respectively. The two groups were comparable in sex ratio ($P = 0.85$), number of inborn and

Table 1 Polymicrobial bacteremia in neonates: combinations of two organisms

Organisms	Associated with organism (number of cases)
Gram-positive	Gram-positive/negative
1. *Staphylococcus aureus*	*Klebsiella* sp. (3), *Streptococcus fecalis* (3), *Alcaligens fecalis* (2), *Enterococcus fecalis* (2), *Acinetobacter*, *Enterobacter*, *Proteus* sp. (1 each)
2. Coagulase negative staphylococci	*Acinetobacter* sp., *Alcaligens fecalis*, *Pseudomonas aeuroginosa* (1 each)
3. *Streptococcus viridans*	*Citrobacter* sp. (1)
4. *Enterococcus* sp.	*Klebsiella* sp. (1)
5. *Enterococcus fecalis*	*Cirtobacter* sp. (1), *E. coli* (1)
Gram-negative	
1. *Klebsiella* sp.	*E. coli* (6), *Enterobacter* sp. (6), *Alkaligans fecalis* (3), *Klebsiella rhinoscleromatis* (1), *Citrobacter* sp. (1)
2. *Enterobacter* sp.	*Pseudomonas aeuroginosa* (2), *Proteus* sp. (1), Gram-negative bacilli (1), *Klebsiella rhinoscleromatis* (1)
3. *E. coli*	*Klebsiella rhinoscleromatis* (1)
4. *Alkaligens fecalis*	*Enterobacter* species (1), Gram-negative bacilli (1) *Klebsiella rhinoscleromatis* (1)
5. *Providencia stuartii*	*Acinetobacter* sp. (1)
6. *Klebsiella aerogens*	*Klebsiella rhinoscleromatis* (1)
Fungi	
1. *Candida* sp.	*Klebsiella* sp. (2), *Enterococcus fecalis* (1)

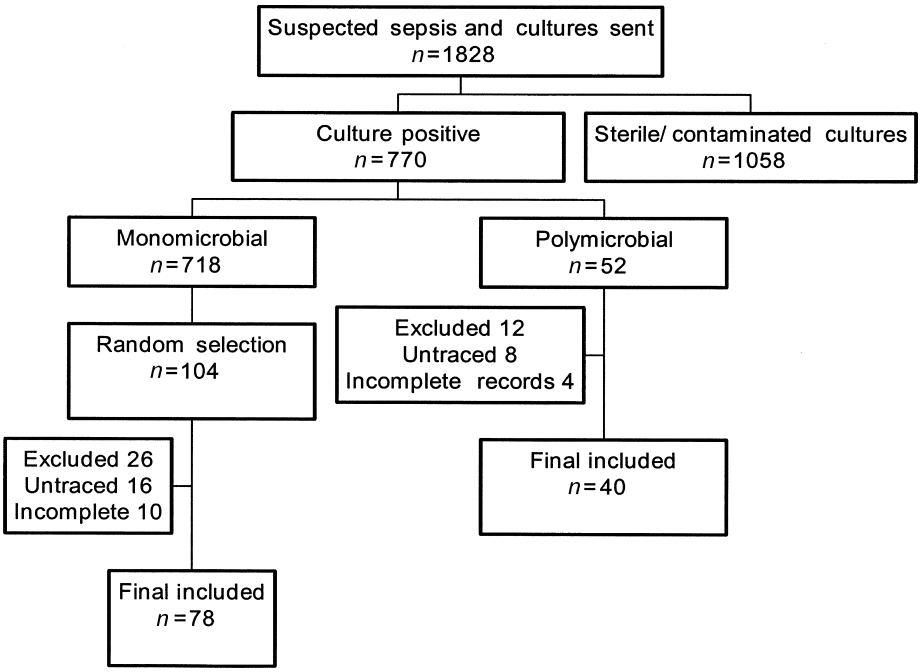

Fig. 1 Study flow chart.

outborn babies ($P = 1.0$), appropriate for gestational age and intrauterine growth-retarded babies ($P = 0.24$) and normal versus assisted deliveries ($P = 0.84$). The number of preterm babies in the polymicrobial group was 23/40 (57.5%) as compared to 46/78 (58.9%) ($P = 0.45$). The incidence of birth asphyxia was also comparable between the two groups ($P = 0.49$). Maternal and obstetric complications were present in 18 (45%) of polymicrobial cases and 45 (57.7%) of monomicrobial cases ($P = 0.24$). Presence of maternal complications like fever, premature rupture of membranes, foul-smelling vaginal discharge or meconium-stained liquor was also not different between the two groups.

Clinical presentation and outcome

Four (10%) of the newborns were asymptomatic in the polymicrobial group as compared to 14 (17.9%) in the monomicrobial group ($P = 0.26$). Table 2 provides a comparative analysis of clinical presentation in symptomatic babies between the two groups. Mean (SD) day of onset of symptoms was 3.4 (6.1) days in the polymicrobial group as compared to 3.6 (6.3) days in the monomicrobial group (95% CI; −2.9, 2.3). Symptoms occurred within 48 h (early-onset sepsis) in 27/36 (75%) in the polymicrobial as compared to 49/64 (76.6%) cases in the monomicrobial group ($P = 1.0$). With regards to treatment received by newborns in both groups, no statistically significant difference was detected in the number of newborns subjected to parenteral nutrition (blood, dextrose, plasma or lipids), exchange transfusion, indwelling i.v. catheters or ventilation.

Of 40 newborns in the polymicrobial group, 18 (45%) died as compared to 28 of 78 (35.9%) deaths in the monomicrobial group ($P = 0.34$). The number of deaths directly attributable to sepsis was also comparable in the two groups (14/18; 77.8% in the polymicrobial group and 21/28; 75% in the monomicrobial group). In the neonates who survived, the mean (SD) duration of symptomatic improvement (polymicrobial: 5.1 (6.6); monomicrobial: 6.4 (6.8); 95% CI of mean difference: −4.7, 2.2) and hospital stay (polymicrobial: 10.1 (5.3); monomicrobial: 12.3 (8.9); 95% CI of mean difference: 2.0, −6.2) was comparable between the two groups. The age at death in the two groups was also comparable (polymicrobial: 7.3 (6.3); monomicrobial: 8.6 (10.7); 95% CI of mean difference: −7.3, 4.7).

DISCUSSION

The incidence of polymicrobial bacteremia in newborns at our centre (6.8%) was similar to that reported by Paerregaard and Brown (6%) and Thomas *et al.* (8%).[12,13] Faix and Kovarik[8] attempted to identify true pathogens among multiple isolations and thus found a lower incidence of 3.9%. Results of Jarvis *et al.*,[6] who reported 25% incidence of polymicrobial episodes, cannot be generalized as they had a very small sample size (20 newborns) studied over a 48-h period. We reported an association of maximum three aerobic organisms. Previous studies have documented growth of up to five organisms simultaneously, but that included anerobic cultures as well.[3] Different species of

Table 2 Clinical presentation of symptomatic polymicrobial and monomicrobial bacteremia: a comparative evaluation

	Polymicrobial ($n = 36$)	Monomicrobial ($n = 64$)	*P*-value
Clinical manifestations			
General	32 (88.9)	59 (92.2)	0.72
Respiratory	27 (75)	53 (82.8)	0.35
Gastrointestinal	29 (80.6)	45 (70.3)	0.34
Haematological	17 (47.2)	37 (57.8)	0.40
Neurological	8 (22.2)	15 (23.4)	1.0
Complications	15 (41.7)	21 (32.8)	0.39
Invasive intervention			
Exchange transfusions	9 (25)	14 (22.9)	0.72
Parenteral nutrition	9 (25)	13 (20.3)	0.59
Indwelling i.v. catheters	15 (41.7)	21 (32.8)	0.39
Ventilation	4 (11.1)	1 (1.6)	0.06

Klebsiella or *S. aureus* were one of the two isolates in 37 out of five isolates, in keeping with the fact that these two are the most commonly isolated organisms in the NICU.

We could not single out any predisposing factor for polymicrobial bacteremia in our series. Also, there was no difference in the clinical presentation, complication and mortality between subjects growing one or more organisms. 'Isolation of more than one organism from blood cultures is in itself intrinsically dangerous' is too simplistic an explanation and does not take into account other factors. Our results indicate that in our newborn unit, contamination rather than true invasion was responsible for the second organism in most polymicrobial instances. In the absence of well-laid-down laboratory criteria, the bacteriologist reports all the isolates with their antibiogram and the treating physician unhesitatingly approves an aggressive intervention, partly as a precautionary measure and partly because of the inability to truly ascribe significance to the occurrence of multiple isolates. As a consequence, a bacteriologist's report, instead of serving as a guide to provide direction to appropriate therapy, wrongly alerts the physician to indulge in overzealous intervention.

The decision of true invasion by multiple organisms can be made only retrospectively, based on the clinical course of the disease. This too, is by and large subjective in nature and cannot be fully relied upon. Except for four, all subjects in the polymicrobial group were clinically septicemic; and even in these four, the blood tests and C-reactive peptide/protein (CRP) values were not entirely normal. However, the fact that these children did not, in any way, behave differently from the monomicrobial group, indicates that probably the second organism was a contaminant.

Several workers have attempted to distinguish the contaminating bacteria and etiologically significant bacteria through such methods as (i) repeated sampling and demonstration of same isolate with comparable characteristics; and (ii) PFGE, plasmid profile, OMP profile and other typing methods with serial isolates.[14] Such procedures are neither pragmatic nor routinely available in hospital diagnostic laboratories. Even repeated cultures to attempt isolation of the same bacterial agents may not succeed because of the intermittent nature of the bacteremia. Moreover, results of these procedures are not available at a time when they can be useful to the physician. Thus objective clinical and microbiological attributes, needed to differentiate true from contaminated polymicrobial isolates, are not routinely available at the time of instituting therapy. Simple bacteriological criteria are required to confidently ascribe etiological significance to each of the isolates in polymicrobial bacteremia, especially in asymptomatic patients in a hospital setting. Till that time, one has to resort to the clinical course and not the culture report; this in essence is the crux of our findings.

Our study had its share of limitations. We did not attempt the isolation of anaerobes. This would have resulted in a higher incidence of multiple isolations. We did not use special medias to facilitate more bacterial growths. A study has indicated that in approximately 25% cases, the second organism would escape detection if multiple or complex medias are not used.[1] Also, we did not attempt to discriminate between a contaminant and a true pathogen, based on the clinical features. This could be the reason for failing to document a higher mortality in newborns with polymicrobial isolation. We also did not attempt a detailed analysis of cause of death in the polymicrobial group, as to whether the death occurred because of the underlying disease(s) or could be attributed to multiple invading organisms per se. There was a trend to increased mortality in the polymicrobial group. This failed to reach a statistically significant difference, probably because the study was insufficiently powered.

Notwithstanding these limitations, we conclude that isolation of more than one organism from the blood culture of a suspected septic newborn is not uncommon. It does not always represent a true invasion by multiple organisms. Polymicrobial isolation per se should not be the criterion for hastily changing the therapeutic decisions. All such results need to be viewed with caution, with a close eye on the clinical course of the disease.

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Queries

Q1 Au: Please confirm the expansion of CRP is OK

Q2 Au: Please define PFGE and OMP

Q3 Au: Please confirm the spelling of second author ‘Brunn’ has been changed to ‘Brown’