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Pathogens Identified by Minimally Invasive Tissue Sampling in India and Pakistan From Preterm Neonatal Deaths: The PURPOSE Study

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Background. We identified pathogens found in internal organs and placentas of deceased preterm infants cared for in hospitals in India and Pakistan.

Methods. Prospective, observational study conducted in delivery units and neonatal intensive care units. Tissue samples from deceased neonates obtained by minimally invasive tissue sampling and placentas were examined for 73 different pathogens using multiplex polymerase chain reaction (PCR).

Results. Tissue for pathogen PCR was obtained from liver, lung, brain, blood, cerebrospinal fluid, and placentas from 377 deceased preterm infants. Between 17.6% and 34.1% of each type of tissue had at least 1 organism identified. Organism detection was highest in blood (34.1%), followed by lung (31.1%), liver (23.3%), cerebrospinal fluid (22.3%), and brain (17.6%). A total of 49.7% of the deceased infants had at least 1 organism. *Acinetobacter baumannii* was in 28.4% of the neonates compared with 14.6% for *Klebsiella pneumoniae*, 11.9% for *Escherichia coli/Shigella*, and 11.1% for *Haemophilus influenzae*. Group B streptococcus was identified in only 1.3% of the neonatal deaths. *A. baumannii* was rarely found in the placenta and was found more commonly in the internal organs of neonates who died later in the neonatal period. The most common organism found in placentas was *Ureaplasma urealyticum* in 34% of the samples, with no other organism found in >4% of samples.

Conclusions. In organ samples from deceased infants in India and Pakistan, evaluated with multiplex pathogen PCR, *A. baumannii* was the most commonly identified organism. Group B streptococcus was rarely found. *A. baumannii* was rarely found in the placentas of these deceased neonates.

Keywords. neonatal death; organ pathogen PCR; *Acinetobacter baumannii*; *Klebsiella pneumoniae*; Group B Streptococcus.

In 2020, 2.4 million neonatal deaths occurred globally [1]. Worldwide neonatal mortality rate was 17 deaths per 1000 live births, with 20 deaths per 1000 in India and 40 deaths per 1000 in Pakistan [2]. Neonatal deaths are attributed to infections, birth asphyxia, and congenital anomalies [3]. Infection is considered the primary cause for more than one-third with bacterial infection accounting for most of the infection-related neonatal deaths [4]. The common bacterial pathogens identified are Group B streptococcus (GBS), *Ureaplasma* species, *Escherichia coli* and *Staphylococcus aureus,* whereas respiratory syncytial virus, cytomegalovirus, and parvovirus are common viral pathogens [5, 6]. *Klebsiella* and *S. aureus* have been identified as a cause of hospital-acquired neonatal sepsis in low and middle-income countries (LMICs) [7]. A recent study from South Africa attributed 70% of childhood deaths to hospital-acquired infections from *Acinetobacter baumannii*, *Klebsiella pneumoniae,* and *S. aureus* [8]. The burden of infection was higher among preterm compared with term neonatal deaths [9].

Because the sensitivity of methods identifying pathogens varies (ie, blood culture, antigen detection, and targeted polymerase chain reactions [PCRs]), comparison of results across studies is difficult, especially for LMICs [10]. Identifying the pathogens contributing to neonatal deaths is needed to implement interventions to reduce the burden of infection-related mortality.

To address gaps in knowledge about infection-related mortality, we undertook a prospective study of causes of death in

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preterm neonates in 2 south Asian sites [11]. Minimally invasive tissue sampling (MITS) [12, 13] was performed on deceased infants to obtain lung, liver, and brain organ tissue and cerebral spinal fluid (CSF) and blood for detection of pathogens using real-time PCR [14, 15]. The placenta was also evaluated.

MATERIALS AND METHODS

Study Settings

The Project to Understand and Research Preterm Pregnancy Outcomes and Stillbirths in South Asia (PURPOSe), was conducted in sites in India and Pakistan from July 2018 to February 2020 [11]. Briefly, all preterm live-born infants were recruited in India at 3 hospitals associated with the Jagadguru Jayadeva Murugarajendra Medical College, Davangere, and in Pakistan at Jinnah Postgraduate Medical Center, and the National Institute of Child Health, Karachi. Infants were admitted to a study neonatal intensive care unit (NICU) or discharged home and then followed until death or 28 days postpartum. Among neonatal deaths, permission was requested from the family to perform MITS. This report focuses on PCR-based detection for pathogens from the MITS samples, including blood, CSF, brain, lung, and liver as well as the placental samples. Although infants who died in the obstetric unit after birth were eligible and included, nearly all participants received care in a study NICU before death.

MITS Sampling

MITS uses needle biopsies to obtain organ and fluid samples from deceased individuals [12]. Less invasive than autopsy, these tissues were evaluated to determine the presence of pathogens by PCR. Tissue samples for PCR analysis were collected aseptically by trained technologists usually within 1 hour of death, placed into tubes with RNAlater (Thermofisher; Waltham, Massachusetts), transported to the laboratory on dry ice, and kept at −80°C until processing.

Molecular Detection

All sample preparation and molecular procedures were conducted by the Indian and Pakistani teams onsite. The blood, CSF, and tissue samples were initially processed using proteinase K and homogenized using beads and the Precellys 24 homogenizer (Bertin Technologies; Rockville, Maryland). Total nucleic acid (TNA) was extracted from the homogenized samples using the EZ1 Virus Mini kit (Qiagen; Germantown, Maryland) on the EZ1 Advanced XL automated extractor (Qiagen). PCR was performed for molecular detection using customized assays developed by the Centers for Disease Control and Prevention (CDC) (Atlanta, Georgia) and loaded onto TaqMan Array Cards (TACs; ThermoFisher). Two array cards were designed, 1 to specifically detect pathogens from the lung tissue and the other to detect pathogens from all other biological samples. The respiratory TACs contained assays to detect 43 pathogens (19 bacteria, 21 viruses, 1 fungus, and 2 bacterial toxins). The blood/CSF/tissue TAC detected 54 pathogens (Supplementary Table 1). For several pathogens, the target was combined for more than 1 organism. Where noted, a positive result may indicate that at least 1 of the indicated pathogens was present (eg, *Ureaplasma* spp. indicates that either *Ureaplasma urealyticum* or *Ureaplasma parvum* was present).

Both TACs contained an assay control to identify the presence of PCR inhibitors and a sample extraction control (human RNA polymerase) to confirm sufficient TNA. Additionally, 2 loading lanes were designated for a no template control to identify any exogenous contaminants and a positive template control to validate the proper amplification for each custom assay. All newly prepared TAC lots were validated by the US CDC.

The no template control, the positive template control, and the sample TNAs were loaded on the TAC and the card was run on the QuantStudio 7 Flex Real-time PCR instrument (ThermoFisher). All pathogen PCR data were analyzed using customized QuantStudio Real-Time PCR software, version 1.2. Amplification at any cycle threshold was considered positive if the amplification curve and fluorescence level were deemed appropriate with some exceptions in which specific cycle threshold value cutoffs were designated for select target pathogen assays (eg, *E. coli*, Chikungunya virus, Dengue virus, and *K. pneumoniae*) to indicate a positive or indeterminate result. Additionally, for pathogens with multiple target assays (eg, toxigenic vs nontoxigenic *Corynebacterium diphtheriae*), an algorithm determined final results based on the combined results of each assay [15].

Statistical Analyses

The sample size was 350 preterm neonatal deaths per site to provide sufficient precision to detect causes of death contributing to >20% of the deaths with 95% power. We estimated that 50% of the parents would consent for additional MITS analyses, including the PCR analyses. Descriptive statistics were used to calculate frequencies of the variables of interest. All data were entered and reviewed at each site. Descriptive analyses were performed using SAS (SAS v.9.4, Cary NC).

Ethical Approvals

The study was approved by the respective ethical review committees from India (JJM Medical College, JN Medical College), Pakistan (Jagadguru Jayadeva Murugarajendra Medical College, the National Institute of Child Health, and Aga Khan University), and the data coordinating center, RTI International. All women provided informed written consent before participation and the study procedures conducted as per hospital protocol following receipt of written informed consent from the parents of the neonates.

Role of Funding Source

The funder provided input on the original study design but did not have a role in the collection, analysis, and interpretation of data, in the writing of the report, nor in the decision to submit the paper for publication. K. J., J. K., and E. M. M. had access to the full data set for analyses.

RESULTS

Of the 3470 live born preterm infants, 2032 were from India and 1438 were from Pakistan (Figure 1). By 28 days of age, 329/2032 (16.2%) died in India and 475/1438 (33.0%) died in Pakistan, a total of 804 neonatal deaths. Of these, 377/804 (46.9%) consented for MITS, 222 neonates in India, and 155 from Pakistan. Because of multiple pregnancies, the analytical sample included 348 mothers and 377 neonatal deaths.

The sociodemographic characteristics of the 348 study mothers are presented (Table 1). Most women (79.0%) were 20 to 30 years old. Indian women had higher levels of education compared with Pakistani women (ie, 76.0% vs 41.0% with 5–12 years of schooling, respectively). In both sites, about 95% of the women were homemakers and few (2.9%) had outside employment. More women from India were primagravidas, and about half (51.4%) from both sites were gravida 1 to 3. In Pakistan, 18.7% of the women were gravida >3 compared with 3.3% of the Indian women. Maternal morbidities such as anemia (71.0%) and antepartum hemorrhage (15.1%) were higher in Pakistan compared with India, whereas hypertensive disorders were diagnosed more frequently in India (40.2% vs 24.5%, respectively).

Table 2 presents the characteristics of the 377 babies who died by day 28. About one-quarter (24.8%) were products of multiple births and 55.3% were male. Most (238/376 or 63.2%) had a birth weight of 1000 to 2499 g and 130/377 (34.5%) weighed <1000 g. Most deaths were 28 to 36 weeks' gestation, more frequently at the Indian site (72% vs 57%, respectively). Fetal growth restriction was observed in a higher proportion of neonates (35.6%) in India compared with Pakistan (18.7%). About half (50.4%) the deceased infants had no pathogen identified in any internal organ: 18.6% were positive for 1 pathogen, 17.0% for 2 to 3 pathogens, and 14.1% for >3 pathogens.

Table 3 presents the PCR testing for the internal tissues. Of the 377 infants with at least 1 specimen, the number of each specimen available for testing ranged from 331 for lung to 370 for blood. We restricted the presentation of the analyses

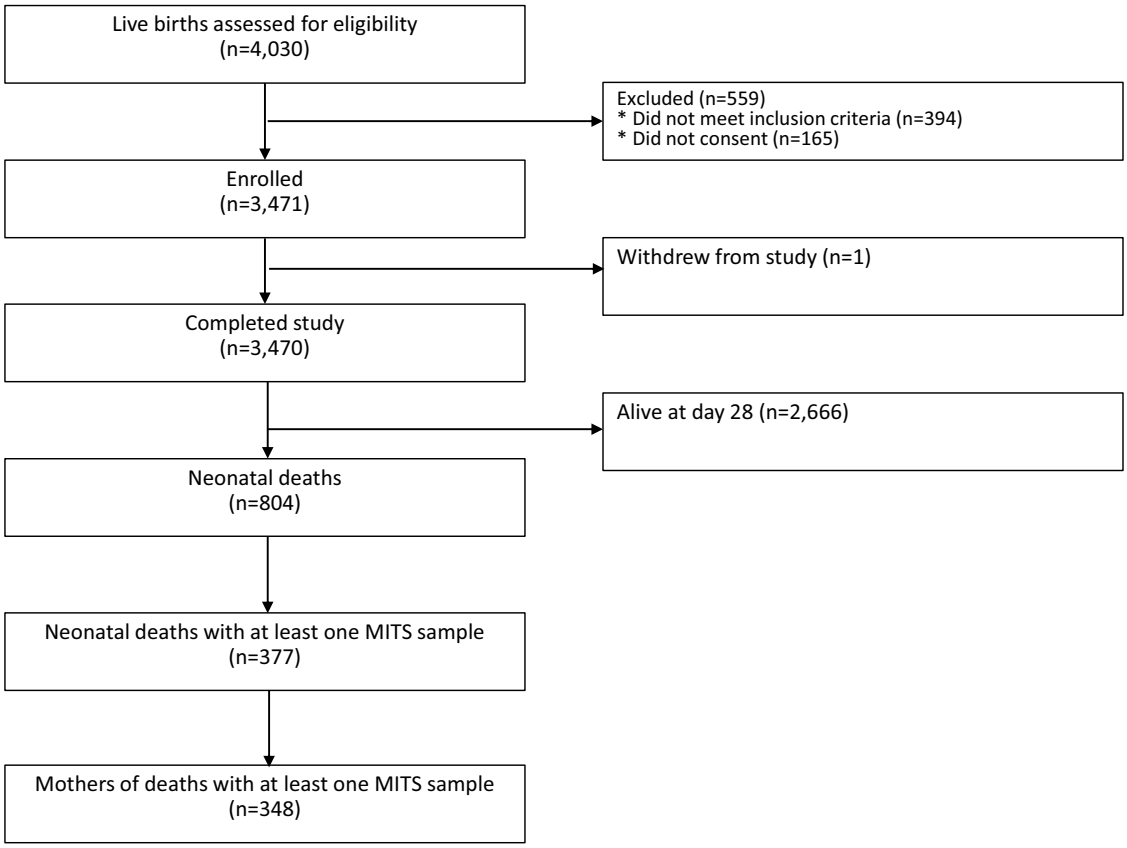

Figure 1. Data results. Abbreviation: MITS, minimally invasive tissue sampling.

Table 1. Maternal Sociodemographic Characteristics

	Overall	India	Pakistan
Mothers enrolled with preterm death and MITS done	348	209	139
Maternal age, n (%)	348	209	139
<20 y	31 (8.9)	19 (9.1)	12 (8.6)
20–30 y	275 (79.0)	170 (81.3)	105 (75.5)
>30 y	42 (12.1)	20 (9.6)	22 (15.8)
Maternal education, n (%)	347	208	139
No formal schooling, illiterate	56 (16.1)	19 (9.1)	37 (26.6)
No formal schooling, literate	32 (9.2)	2 (1.0)	30 (21.6)
1–4 y	14 (4.0)	7 (3.4)	7 (5.0)
5–12 y	215 (62.0)	158 (76.0)	57 (41.0)
>12 y	30 (8.6)	22 (10.6)	8 (5.8)
Maternal occupation, n (%)	348	209	139
Homemaker	332 (95.4)	200 (95.7)	132 (95.0)
Employed	10 (2.9)	8 (3.8)	2 (1.4)
Other or don't know	6 (1.7)	1 (0.5)	5 (3.6)
Gravida, n (%)	348	209	139
0	136 (39.1)	91 (43.5)	45 (32.4)
1–3	179 (51.4)	111 (53.1)	68 (48.9)
≥3	33 (9.5)	7 (3.3)	26 (18.7)
Antenatal care received, n (%)	348	209	139
Yes	334 (96.0)	209 (100.0)	125 (89.9)
No	14 (4.0)	0 (0.0)	14 (10.1)
Any hypertensive disorders, n (%)	118 (33.9)	84 (40.2)	34 (24.5)
Anemia, n (%)	178 (51.1)	79 (37.8)	99 (71.2)
Antepartum hemorrhage, n (%)	40 (11.5)	19 (9.1)	21 (15.1)

Abbreviation: MITS, minimally invasive tissue sampling.

Table 2. Characteristics of PURPOSE Study Neonatal Deaths

	Overall	India	Pakistan
Variable			
Enrolled, n	377	222	155
Multiple birth, n (%)	94 (24.8%)	49(22.1%)	45(29.0%)
Male, n (%)	208 (55.3)	131 (59.0)	77 (50.0)
Birth weight (g), n (%)	376	221	155
<1000	130 (34.6)	76 (34.4)	54 (34.8)
1000–2499	238 (63.2)	141 (63.8)	97 (62.6)
≥2500	8 (2.1)	4 (1.8)	4 (2.6)
Gestational age (w), n (%)	377	222	155
20.0–27.6	128 (34.0)	62 (27.9)	66 (42.6)
28.0–36.6	249 (66.0)	160 (72.1)	89 (57.4)
Fetal growth restriction (intrauterine growth restriction), n (%)[a]	108 (28.6)	79 (35.6)	29 (18.7)
Positive pathogens, n (%)	377	222	155
0	190 (50.4)	103 (46.4)	87 (56.1)
1	70 (18.6)	34 (15.3)	36 (23.2)
2–3	64 (17.0)	42 (18.9)	22 (14.2)
>3	53 (14.1)	43 (19.4)	10 (6.5)

Abbreviation: PURPOSE, The Project to Understand and Research Preterm Pregnancy Outcomes and Stillbirths in South Asia.

[a]Fetal growth restriction is defined as birthweight less than the INTERGROWTH-21ST 10th percentile weight, which are not available for gestational age <24 weeks or ≥43 weeks and fetuses missing sex or birthweight.

to organisms present in at least 1% of the infants evaluated. Next, we present the percent of the internal organs positive for specific organisms. Across both sites, there were 1749 tissues with PCR tests performed: 25.7% of these tests identified at least 1 pathogen. The frequency of organism detection was highest in blood (34.1%), followed by lung (31.1%), liver (23.3%), CSF (22.3%), and brain (17.6%). The pathogen with the highest frequency of detection was *A. baumannii,* found in 14.1% of all tissue samples, followed by *K. pneumonia* (4.9%) and *E. coli/Shigella* (4.5%). *A. baumannii* was also found twice as often in at least 1 individual's tissue samples: 28.4% compared with 14.6% for *K. pneumoniae*, 11.9% for *E. coli/Shigella*, and 11.1% for *Haemophilus influenzae. A. baumannii* was found more often in 2 and 3 and >3 tissues of a deceased neonate than any other organism. Several other organisms were found in <10% of the cases. Of note, GBS was identified in only 1.3% of the cases. Parvovirus B19 (2.8%) and cytomegalovirus (0.6%) were the most common viruses detected. Other viruses identified included Dengue, Parechovirus, Chikungunya, and influenza, but in <1%. *Candida albicans* was found in 1.6% of the cases. In a pattern different from the other pathogens, *H. influenzae* was found only in lungs, whereas every other organism was detected in more than 1 type of tissue.

The results were generally similar for both sites, *A. baumannii* was the most common organism followed by *K. pneumonia,* and both were found in all tissue types tested (Supplementary Table 2). *A. baumannii* was also found in the most tissues and in relatively similar proportions of *A. baumannii* (31.1% and 24.5%) at both sites. *K. pneumoniae* was found in 20.7% of the deaths in the Indian site and 5.8% in the Pakistani site. Overall, the number of pathogens detected in the 5 tissues was greater in India than Pakistan.

Table 4 presents the PCR analysis of 719 placental samples; 45.2% had >1 pathogen detected of which *U. urealyticum/U. parvum* was most commonly found at 34.1%. No other organism was found in >4% of the samples. *A. baumannii* was found in 1.1% of the placental samples with a similar low frequency in both sites.

We compared the organisms by the age at which the baby died. For *A. baumannii, K. pneumonia, E coli/Shigella, and H. influenza,* the proportion of the neonatal deaths positive for that organism increased by age at death (Table 5). As an example, the frequency of *A. baumannii* increased from 19.3% in babies who died in the first 3 days to 51.7% at 4 to 7 days, to 62.5% from 8 to 14 days and 68.8% from 15 to 28 days. A similar trend was observed for *K. pneumonia* and *H. influenza*. In contrast, there was no apparent increase in the proportion of

Table 3. Pathogens Identified by Polymerase Chain Reaction in the Internal Organ Tissues of 377 Preterm Neonatal Deaths in India and Pakistan Combined

	Babies With at Least 1 Positive Sample	Babies With 2–3 Positive Samples	Babies With >3 Positive Samples	All Tissues Tested	Liver	Lung	Brain	Cerebrospinal Fluid	Whole Blood
Number	377	377	377	1749	352	331	346	350	370
At least 1 positive pathogen, n (%)	…	…	…	450 (25.7)	82 (23.3)	103 (31.1)	61 (17.6)	78 (22.3)	126 (34.1)
Acinetobacter baumannii	107 (28.4)	33 (8.8)	28 (7.4)	247 (14.1)	39 (11.1)	69 (20.8)	25 (7.2)	38 (10.9)	76 (20.5)
Klebsiella pneumoniae	55 (14.6)	17 (4.5)	3 (0.8)	86 (4.9)	17 (4.8)	7 (2.1)	7 (2.0)	12 (3.4)	43 (11.6)
Escherichia coli and/or *Shigella* species	45 (11.9)	19 (5.0)	2 (0.5)	78 (4.5)	18 (5.1)	0 (0.0)	6 (1.7)	16 (4.6)	38 (10.3)
One or more serotypes of *Haemophilus influenzae*	42 (11.1)	0 (0.0)	0 (0.0)	42 (2.4)	0 (0.0)	42 (12.7)	0 (0.0)	0 (0.0)	0 (0.0)
Ureaplasma urealyticum and/or *parvum*	29 (7.7)	3 (0.8)	0 (0.0)	34 (1.9)	6 (1.7)	0 (0.0)	4 (1.2)	5 (1.4)	19 (5.1)
Enterococcus faecium	28 (7.4)	4 (1.1)	0 (0.0)	33 (1.9)	5 (1.4)	0 (0.0)	0 (0.0)	9 (2.6)	19 (5.1)
Candida albicans	23 (6.1)	5 (1.3)	0 (0.0)	28 (1.6)	6 (1.7)	0 (0.0)	1 (0.3)	2 (0.6)	19 (5.1)
Parvovirus B19 (human parvovirus)	21 (5.6)	6 (1.6)	6 (1.6)	49 (2.8)	13 (3.7)	0 (0.0)	9 (2.6)	14 (4.0)	13 (3.5)
Staphylococcus aureus	9 (2.4)	3 (0.8)	0 (0.0)	13 (0.7)	2 (0.6)	2 (0.6)	4 (1.2)	3 (0.9)	2 (0.5)
Pseudomonas aeruginosa	6 (1.6)	1 (0.3)	0 (0.0)	7 (0.4)	1 (0.3)	1 (0.3)	1 (0.3)	0 (0.0)	4 (1.1)
One or more species within the *Rickettsia* genus	6 (1.6)	0 (0.0)	0 (0.0)	6 (0.3)	1 (0.3)	0 (0.0)	2 (0.6)	1 (0.3)	2 (0.5)
Human cytomegalovirus	5 (1.3)	1 (0.3)	1 (0.3)	10 (0.6)	3 (0.9)	4 (1.2)	1 (0.3)	1 (0.3)	1 (0.3)
Streptococcus agalactiae (Group B Streptococcus)	5 (1.3)	1 (0.3)	0 (0.0)	6 (0.3)	0 (0.0)	0 (0.0)	3 (0.9)	0 (0.0)	3 (0.8)
One or more genotypes of human parechovirus	5 (1.3)	0 (0.0)	0 (0.0)	5 (0.3)	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	4 (1.1)
Salmonella enterica subspecies Paratyphi A	5 (1.3)	0 (0.0)	0 (0.0)	5 (0.3)	3 (0.9)	0 (0.0)	0 (0.0)	1 (0.3)	1 (0.3)
Plasmodium vivax	4 (1.1)	0 (0.0)	0 (0.0)	4 (0.2)	1 (0.3)	0 (0.0)	1 (0.3)	1 (0.3)	1 (0.3)
Rubella virus	4 (1.1)	0 (0.0)	0 (0.0)	4 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (1.1)
Streptococcus pneumoniae	4 (1.1)	0 (0.0)	0 (0.0)	4 (0.2)	1 (0.3)	2 (0.6)	0 (0.0)	0 (0.0)	1 (0.3)

Restricted to pathogens identified in >1% of the babies tested.

Table 4. Polymerase Chain Reaction Pathogen Results From Placental Tissue and Membranes for India and Pakistan Combined

	All	Placenta Tissue	Placenta Membranes
Tested	719	360	359
Tissues with at least 1 positive pathogen	325 (45.2)	138 (38.3)	187 (52.1)
Ureaplasma urealyticum and/or *parvum*	245 (34.1)	104 (28.9)	141 (39.3)
Staphylococcus aureus	28 (3.9)	9 (2.5)	19 (5.3)
Streptococcus agalactiae (Group B streptococcus)	24 (3.3)	13 (3.6)	11 (3.1)
Escherichia coli and/or *Shigella* species	23 (3.2)	10 (2.8)	13 (3.6)
Enterococcus faecium	15 (2.1)	5 (1.4)	10 (2.8)
Human cytomegalovirus	13 (1.8)	4 (1.1)	9 (2.5)
Candida albicans	13 (1.8)	2 (0.6)	11 (3.1)
Parvovirus B19 (human parvovirus)	10 (1.4)	4 (1.1)	6 (1.7)
Acinetobacter baumannii	8 (1.1)	4 (1.1)	4 (1.1)

positive tests for *U. urealyticum/U. parvum as* age of death increased.

We also investigated organism positivity related to various case characteristics. (Supplementary Table 3) Several observations stand out. The first is that no matter the birthweight, gestational age, associated condition or treatment, *A. baumannii* was the most common organism identified in the internal organs. The second important observation is that the lower birthweight and earlier gestational age infants were less likely to have any organism identified, as well as being less likely to have any specific organisms identified, including *A. baumannii*.

CONCLUSIONS

In this study, tissue samples collected via MITS from deceased preterm infants were evaluated for the detection of pathogens using multiplex PCR. Between 17.6% and 34.1% of the tissues

Table 5. Day of Neonatal Death by Positivity for the Most Commonly Detected Organisms

	Acinetobacter baumannii, n (%)	*Klebsiella pneumoniae*, n (%)	*Escherichia coli* and/or *Shigella* species, n (%)	*Haemophilus influenzae*, n (%)	*Ureaplasma urealyticum* and/or parvum, n (%)	*Enterococcus faecium*, n (%)	*Candida albicans*, n (%)
Total positive (days 0–28), n/N (%)	107/377 (28.4)	55/377 (14.6)	45/377 (11.9)	42/377 (11.1)	29/377 (7.7)	28/377 (7.4)	23/377 (6.1)
Time at death (d), n/N (%)	…	…	…	…	…	…	…
0–3	55/285 (19.3)	22/285 (7.7)	23/285 (8.1)	15/285 (5.3)	19/285 (6.7)	13/285 (4.6)	9/285 (3.2)
4–7	31/60 (51.7)	20/60 (33.3)	11/60 (18.3)	15/60 (25.0)	6/60 (10.0)	7/60 (11.7)	8/60 (13.3)
8–14	10/16 (62.5)	4/16 (25.0)	4/16 (25.0)	6/16 (37.5)	2/16 (12.5)	3/16 (18.8)	3/16 (18.8)
15–28	11/16 (68.8)	9/16 (56.3)	7/16 (43.8)	6/16 (37.5)	2/16 (12.5)	5/16 (31.3)	3/16 (18.8)

had at least 1 organism identified. The frequency of organism detection was highest in blood (34.1%), followed by lung (31.1%) and liver (23.3%). Overall, *A. baumannii* was the most prevalent organism detected (28.4%) compared with 14.6% for *K. pneumoniae*, 11.9% for *E. coli/Shigella*, and 11.1% for *H. influenzae*. GBS was rarely found in any of the tissues evaluated.

A. baumannii is commonly found in NICUs and is reported as the cause of neonatal sepsis and death [16–18]. *A. baumannii* is rarely reported as a cause of stillbirth and is not generally found in placentas or amniotic fluid [19]. *A. baumannii* was rarely found in placental tissues in our cohort. That the longer the infant was in the nursery before death, the greater proportion of deaths associated with the presence of this organism in fetal tissues, is further evidence of the nosocomial origin. Consistent with the age at death findings is the finding that the most preterm and lower birthweight infants were less likely to be positive for *A. baumannii* or any of the other organisms studied. We hypothesize that these infants were more likely to die soon after admission and have less time to acquire a nosocomial infection. For these reasons, it appears that *A. baumannii* infection in neonates is usually acquired after admission to the nursery. Other organisms, including *K. pneumoniae* and *E. coli/Shigella*, are often found in the maternal genital tract and may be transmitted to the fetus during labor or acquired as a nosocomial infection [20, 21]. GBS, although often transmitted to the fetus if present in the mother, was rarely found in any of the tissues evaluated. This finding is similar to results from previous studies from this region [22, 23].

A study from India investigating microbiological data from three NICUs reported two-thirds of the isolates to be gram-negative organisms, predominately *A. baumannii* (22%) followed by *K. pneumoniae* (17%) and *E. coli* (14%) [24]. Therefore, the high frequency of *A. baumannii* in the tissues of neonatal deaths in India and Pakistan as well as *K. pneumoniae* and *E. coli,* was not unexpected. Because many infants in NICUs receive intravenous fluids and are ventilated either with continuous positive airway pressure or through intubation, and are subjected to many other invasive procedures, preterm newborns cared for in NICUs are especially vulnerable to nosocomial infections [25]. Although we did not study prevention of infection in preterm neonates, given the high likelihood of nosocomial infection associated with *A. baumannii* and other organisms, limiting unnecessary invasive interventions, and paying strict attention to sterile technique overall and especially when invasive interventions are used would seem to be imperative [25].

That GBS was rarely found in the preterm neonatal deaths in this study might be considered surprising based on reports from North America, Europe, and Africa [26]. However, there are a number of reports from south Asia documenting very low levels of GBS colonization in pregnant women or as a cause of infection or death in neonates [22, 23]. That our findings were not due to problems with the PCR assay was verified by the low level of GBS found by rectovaginal culture in this same population (data not shown). One possible explanation for the rarity of GBS in the internal organs is that more than half of the deceased neonates received antibiotics before death. GBS was likely more sensitive to these antibiotics than *A. baumannii* or some of the other organisms that have a high degree of antimicrobial resistance. However, in mothers admitted in Pakistan, before any antibiotics were given, the prevalence of GBS on rectovaginal culture was quite low, consistent with other reports from south Asia.

This study had a number of strengths and weaknesses. Among the strengths are the large sample size, data collected independently at 2 sites, central quality oversight, internal organ samples collected by MITS for PCR testing, and PCR testing using a methodology validated by the CDC. The relatively similar results obtained independently from 2 sites support the validity of the results. One weakness was that only about half the families consented to MITS and PCR pathogen detection. Additionally, we acknowledge that the participating facilities are not necessarily representative of either India and Pakistan or other LMICs. We also are aware that detection of an organism, even in an internal organ, does not prove that organism caused the death. However, given that *A. baumannii, K. pneumoniae,* and *E. coli* were often found in more than

1 internal organ and have been reported to cause neonatal deaths in NICUs around the world, lends credence to the likelihood that these organisms played an important role in the preterm neonatal deaths in this study.

A key finding from this study is the higher proportion of probable nosocomial-acquired *A. baumannii*–associated death in neonates >4 days of age. *K. pneumoniae* and *E. coli* are also considered to be an important contributing pathogen identified in tissues of neonatal deaths and their prevalence in internal organs also increased as the age at death increased [21].

We have considered why *A. baumannii* might play a prominent role in preterm neonatal deaths. Assuming we are correct that the organism is most often acquired as a nosocomial infection in the nursery, the infant first needs to be in a nursery. Home births and those not admitted to a nursery are likely to be spared infection from this pathogen. Second, there are much data to suggest that preterm infants are more susceptible to infection than term infants [27–30]. Furthermore, we have documented, especially in the Pakistan nursery, the large patient load and staffing shortages, conditions that are often associated with increased risk of nosocomial infections. Therefore, it is likely that our findings related to *A. baumannii* and the other infections are of more than academic interest and could be a starting point to initiating interventions that lead to a reduction in mortality from these infections, especially in south Asia.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. N. K. G., I. A., and R. L. G. conceived of the analyses and drafted the first manuscript with input from J. K., E. M. M., and R. S. N. K. G., I. A., S. S., S. S. G., S. M. D., G. G., R. L. G., A. A., E. M., and R. M. S. developed the study protocols. N. K. G., I. A., S. H., M. S. S., A. Z., S. S. T., S. S., S. S. G., S. M. D., G. G., and S. Y. oversaw the study implementation. K. H., J K., A. A., and E. M. M. reviewed the data and K. H. performed study analyses. All authors reviewed and approved the final manuscript.

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