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Original Article

The changing profile of neonatal pathogens and susceptibility pattern: prospective observational study from a tertiary center in South India

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Abstract

Introduction: Changing the pattern of neonatal pathogens poses challenges to the management of neonatal sepsis. Profile and antimicrobial susceptibility of neonatal pathogens were evaluated in this prospective observational study in a tertiary center.

Methods: Neonates with risk factors and clinical features of sepsis were screened. Blood culture performed in positive-screen babies. Antimicrobial susceptibility was also evaluated. Statistical significance was tested by the chi-square test and *t* test accordingly. Univariate analysis was performed to study possible correlations in this regard.

Results: Out of 431 suspected cases, 89 neonates (20.65%) had sepsis. The rate of early and late-onset sepsis (LOS) was 48.3% and 51.7%, respectively. The clinical spectrum included septicemia 68 (76.5%), congenital pneumonia 13 (14.7%), meningitis 5 (5.7%), and septic arthritis 3 (3.4%), respectively. Gram-positive bacteria constituted 61 (68.5%), while gram-negative was 28 (31.5%) ($P<0.05$). *Staphylococcus aureus* (23.6%) and methicillin-resistant *S. aureus* (MRSA) (22.5%) were the most common isolates. *Acinetobacter* (15.8%), coagulase-negative staphylococcus areus (CoNS) (11%), *Klebsiella* (7.9%), enterococci (8%), *E. coli* (4.5%), and ß hemolytic streptococci (1 case) were other detected pathogens. MRSA, *Acinetobacter*, and coagulase-negative *S. aureus* as a single entity involved in sepsis pathogenesis (50.6%) showed a positive correlation with inborn babies, pre-term, low birth weight, and early-onset sepsis (OR; 95% CI: 2.20; 0.94–5.20, 1.82; 0.79–4.22, 1.25; 0.55–2.89 and 1.05; 0.46–2.50 respectively). Susceptibility pattern was penicillin (12.3%), ampicillin (6.7%), cloxacillin (42.9%), cefotaxime (8%), cefazolin (37.9%), cefoperazone sulbactam (81.5%), piperacillin-tazobactam (68.9%), gentamicin (63.5%), amikacin (47.9%), vancomycin (88.9%), linezolid (88.6%), co-trimoxazole (55.4%), and clindamycin (50%).

Conclusion: Gram-positive pathogens and opportunistic pathogens like *Acinetobacter* predominate over the conventional gram-negative pathogens in neonates. Of note, penicillin, ampicillin, and cefotaxime are not suitable for the empiric treatment of neonatal sepsis.

Introduction

Sepsis is one of the common causes of neonatal deaths worldwide.[1] Among the neonatal deaths, 24% occurred by sepsis according to an epidemiologic analysis performed in India.[2] The incidence of culture-proven sepsis in India was also reported as 8.5 per 1000 live births.[3] It has been well-established that the Gram-negative bacteria were the well-known pathogens of neonatal sepsis in India and other developing countries.[4-6] While, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Acinetobacter* are emerging as the predominant pathogens.[2,7-16] Moreover, multi-drug resistant (MDR) and extended-spectrum ß lactamase (ESBL) pathogens are also increasingly reported.[2,4] In low resource settings, due to the delay in obtaining the results of conventional bacteriological studies, empiric antibiotics remains the mainstay of management of neonatal sepsis. Therefore, the choice of empiric antibiotics plays a crucial role to blunt the early and late outcome of neonates with sepsis. Conventionally, the first-line empiric antibiotics of choice are ampicillin and aminoglycosides; however, a multitude medical centers have been using third-generation cephalosporin instead. In the context of the changing pattern of neonatal pathogens, the emergence of MDR pathogens, and extended spectrum ß lactamase pathogens, planning the antibiotic policy in neonatal

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intensive care units (NICUs), and choosing empiric antibiotics by physicians are considered new challenges. To address this issue periodic surveillance of bacterial profiles in the locality and analyzing their susceptibility pattern remain to be determined. Here, an observational prospective study on the profile and the antibacterial susceptibility of neonatal pathogens was conducted.

Methods

This study was conducted from March to September 2020 in the in-born and out-born NICUs of Government Medical College Kozhikode, which is a referral center for 6 districts of Kerala state in India. Newborn babies of 0 to 28 days of age admitted in the in-born and out-born NICUs showing any of the clinical features consistent with sepsis or having any of the risk factors of early-onset sepsis (EOS) were subjected to sepsis screening. The clinical features considered were fever ≥ 38 °C, poor feeding, poor activity, lethargy, drowsiness, persistent irritability, paradoxical cry, abdominal distension, loose stools, hypothermia, exaggerated physiological neonatal jaundice, neonatal cholestasis, bulging anterior fontanels, seizures, persistent vomiting, apnea, respiratory distress, grunting, unexplained tachycardia, and low volume peripheral pulses or mottling of skin. The risk factors of EOS were low birth weight, pre-term delivery, diagnosed fever ≥ 38 °C in the mother during the one week prior to the delivery, foul smelling or meconium stained liquor, rupture of amniotic membranes > 24 hours before delivery, single unclean or ≥ 3 sterile vaginal examinations, prolonged labor or perinatal asphyxia. Sepsis workup was carried out by performing (i) complete blood count, band cell count, and C-reactive protein in all the babies, (ii) chest X-ray, urine analysis, urine culture, and pus culture test when indicated, and (iii) lumbar puncture (LP) in babies suspected of with late-onset sepsis (LOS). The specimens of 1 mL cerebrospinal fluid (CSF) and urine were collected for cultures. Babies with two or more positive sepsis screening tests, abnormal CSF findings, chest X-ray showing pneumonia, positive urine culture, or pus cultures were subjected to blood culture sampling by adding 16 drops of blood to 10 mL of culture medium (brain heart infusion broth, HiMedia, Mumbai, India) adhering to the standard protocol. Inoculated culture mediums were incubated at room temperature for 7 days. Bacterial colonies were identified macroscopically by the presence of opalescence, gas bubbles, or clot formation and microscopically by the presence of hemolysis of erythrocytes. Broths were subjected to subculture anaerobically on blood agar (HiMedia, Mumbai) and aerobically on MacConkey agar (HiMedia, Mumbai) at 37 °C. The organisms were identified based on the appearance, chemical and microscopic examination obtained in these media.[17] Bacterial isolates were categorized as pathogenic, when both the agars were showing the growth and contaminant if (*i*) only one of the agars was showing the growth, (*ii*) the time to appear the growth was ≥ 3 days after inoculation, or (*iii*) multiple bacterial colonies were observed. When a *Staphylococcus aureus* growth was detected, it was tested for susceptibility to cefoxitin by modifying the Kirby-Bauer disc diffusion method. Strains showing ≤ 21 mm inhibition zone in 30 µg cefoxitin disc were screened as MRSA. Further, the minimum inhibitory concentration (MIC) of oxacillin was determined on these strains by broth microdilution method using 0.0125 to 128 µg/mL solution of oxacillin. The strains with a MIC > 0.4 µg/mL of oxacillin were also confirmed as MRSA. Antimicrobial susceptibility testing was performed for penicillin, ampicillin, cloxacillin, cefazolin, piperacillin-tazobactam, cefotaxime, cefoperazone sulbactam, gentamicin, amikacin, co-trimoxazole, clindamycin, and linezolid, using Mueller Hinton agar plates (HiMedia, Mumbai) using the Kirby-Bauer disc diffusion method.[18] The susceptibility to vancomycin was also tested by agar dilution method according to the CLSI guidelines in which a MIC of ≤ 2 µg/mL was the cut-off for susceptibility. Culture-proven sepsis was diagnosed when growth of pathogenic bacteria was identified in the blood, CSF, pus, or urine. Babies in whom the pathogens were isolated from CSF, urine, or pus were also diagnosed as culture-proven sepsis. Coagulase-negative *S. aureus* was considered a pathogen, only if the baby was demonstrating clinical features of sepsis. Babies presenting ≤ 72 hours of birth were defined as EOS and > 72 hours as LOS. All the newborns included in the study were closely monitored during their NICU stay. Prolonged hospital stay was defined as hospitalization lasting ≥ 14 days.

Statistical analysis

Statistical analysis was performed using SPSS version 18.0 (https://www.ibm.com/analytics/spss-statistics-software). The demographic data and clinical spectrum of neonatal sepsis were compared between the neonates with EOS and LOS and the distribution of pathogens in babies with EOS, LOS, in-born, and out-born babies were also studied. The susceptibility of individual pathogens to different antimicrobial agents was detected, which was further analyzed by taking the entire pathogens, total gram-positive, and gram-negative bacteria as discrete variables. Statistical significance was evaluated by the chi-square test for categorical variables and *t* test for continuous variables and P value < 0.05 was considered significant. We also analyzed MRSA, CoNS, and *Acinetobacter* infections together as a single variable, and odds ratio (OR) and 95% confidence interval (CI) were calculated to study its correlation with different variables.

Results

A total of 1812 babies were admitted in in-born and out-born NICUs during the study period, out of which 431 babies were screened for neonatal sepsis, including 201

in-born and 230 out-born babies. A flow chart depicting the layout of newborn babies for the study is shown in Figure 1. 89 (20.65%) culture-proven sepsis were detected in various culture media, including blood (84), pus (3), and CSF (2), respectively. Among the 89 babies with sepsis, 43 (48.3%) were EOS, 46 (51.7%) were LOS. Also, 51 (57.3%) of cases were inborn, and 38 (42.7%) were out-born babies. The mean birth weight was 2.36 kg ± 0.78 ranging from 0.7-4.49 kg and the mean gestational age was 36.4 ± 3.12 weeks. The mean hospital stay was 12.18 ± 8.15 days. The mortality of newborn babies with sepsis was 11 (12.4%) with 6 (14.0%) in in-born babies and 5 (10.9%) in out-born babies (*P* value = 0.6). The demographic pattern and the clinical profile of newborns with sepsis are shown in Table 1. The details of pathogens isolated and their distribution among EOS, LOS, in-born, and out-born babies are described in Table 2. The antimicrobial effectiveness of commonly used agents against the total NICU pathogens (as a group), gram-positive pathogens (as a group) and Gram-negative pathogens(as a group) is detailed in Table 3. The susceptibility patterns of the isolates to commonly used antimicrobial agents are detailed in Table 4. In Table 5 we analyzed the association of various risk factors for infection with MRSA, CoNS, and *Acinetobacter*. Here univariate analysis was performed by taking all the above mentioned infections together as a single variable.

Table 1. Demographic pattern and clinical profile of newborns with sepsis

Parameters	Total (n = 89)	EOS (n = 43)	LOS (n = 46)	*P* value
Male	45(50.6%)	22(51.2%)	23(50%)	0.91
Female	44(49.4%)	21(48.9%)	23(50%)	0.91
Term	48(54.0%)	24(55.8%)	24(52.2%)	0.71
Preterm	41(46.0%)	19(44.2%)	22(47.8%)	0.73
AGA	39(43.8%)	18(41.9%)	21(45.7%)	0.72
LBW	49(55.1%)	24((55.9%)	25(54.4%)	0.89
LFD	1(1.1%)	1(2.4%)	0	0.29
Normal Delivery	51(57.3%)	24(55.9%)	27(58.7%)	0.79
LSCS	38(42.7%)	19(44.2%)	19(41.3%)	0.79
Septicemia	68(76.5%)	30(69.8%)	38(82.7%)	0.15
Congenital pneumonia	13(14.7%)	13(30.3%)	0	0.00
Meningitis	5(5.7%)	0	5(10.9%)	0.03
Septic arthritis	3(3.4%)	0	3(6.6%)	0.08

AGA, Appropriate gestational age, LBW, Low birth weight, LFD, Large for date; LSCS, Lower segment cesarean section.

Discussion

Sepsis remains significant factor affecting the morbidity and mortality of neonates. Apart from its effect on early outcome like mortality and prolonged hospital stay, it significantly contributes to the adverse neurologic outcome also. Being a government institution, our NICUs manage more babies than their capacity, posing a challenge to the delivery of optimum management and prevention of neonatal infections. The prevalence of neonatal sepsis in our study was 20.7% which is low compared to the 41.6% and 42% reported in previous studies in India.[4,7] Of this, 16.6% belonged to the out-born babies whose constituted a large portion of our study population, and demographic difference mainly led to this overall low prevalence of sepsis. Moreover, our findings showed that the EOS occurred more commonly in in-born babies, whereas LOS was mainly observed in the out-born babies ($P < 0.05$). Congenital pneumonia represented as a 14.7% participation in sepsis pathogenesis in our study compared to the 8-9.7% reported from other developing countries.[7,19] The mortality rate in our study was 12.4% which was similar to an Indian study conducted by Thakur et al, which reported similar data in both EOS and LOS babies.[7]

It is worth noting that available evidence has revealed the predominance of gram-positive pathogens involvement over conventional gram-negative pathogens among neonates.[7,20-22] In our study, 68.5% of isolates were related to gram-positive. Thakur et al have also demonstrated the prevalence of 60% and 40% of gram-positive and gram-negative pathogens in neonatal septicemia, respectively.[7]

According to our results, *S. aureus* (23.6%) was the most common pathogen in this era, which was in accordance with previous reports in India.[7-10] It has been reported that 30% to 70% of humans are colonized with *S. aureus,* imparting them potential carriers of this pathogen.[23] So neonates nursed by adult caregivers can be easily colonized and infected with *S. aureus* after birth.[23,24] Monthly 1200 to 1400 deliveries take place in our institution, resulting in the post-natal wards being crowded and customarily

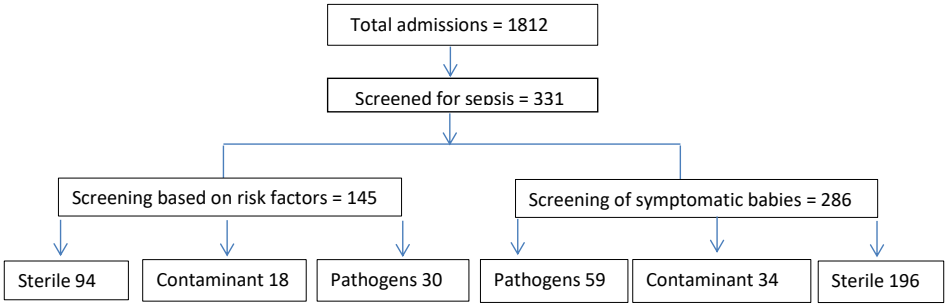

Figure 1. Flow chart depicting NICU admissions, number of babies screened based on risk factors, and clinical features of sepsis

Table 2. Pathogens isolated in early-onset sepsis, late-onset sepsis, inborn, and out-born babies

Pathogens	Total	EOS	LOS	*P* value	Inborn	Out born	*P* value
Gram positive	61(68.5%)	31(50.8%)	30(49.2%)	0.32	36(59.1%)	25(40.9%)	0.39
S. aureus	21(23.6%)	13(30.3%)	8(17.4%)	0.16	13(25.5%)	8(21.0%)	0.63
MRSA	20(22.5%)	9(21%)	11(24%)	0.74	11(21.6%)	9(23.7%)	0.82
CoNS	11(12.4%)	7(16.3%)	4(8.7%)	0.28	8(15.7%)	3(7.9%)	0.83
Enterococci	8(9.0%)	2(4.7%)	6(13%)	0.17	4(7.9%)	4(10.6%)	0.67
ß Streptococci	1(1.2%)	1(2.3%)	0	0.34	0	1(2.7%)	0.25
Gram negative	28(31.5%)	12(42.8%)	16(57.2%)	0.32	15(53.6%)	13(46.4%)	0.39
Klebsiella	7(7.9%)	2(3.8%)	5(10.9%)	0.28	1(2%)	6(15.8%)	0.02
Acinetobacter	14(15.8%)	6(14%)	8(17.4%)	0.66	11(21.6%)	3(7.9%)	0.08
Enterobacter	3(3.4%)	2(4.7%)	1(2.1%)	0.52	1(2%)	2(5.3%)	0.40
E. coli	4(4.5%)	2(4.7%)	2(4.4%)	0.95	2(4%)	2(5.3%)	0.77
MC&A	45(50.6%)	22(48.9%)	23(51.1%)	0.55	30(66.7%)	15(33.4%)	0.05
MDR[a]	5(5.7%)	1(2.3%)	4(8.7%)	0.19	2(4%)	2(5.3%)	0.42

MC&A, MRSA, CoNS & *Acinetobacter*.
[a]3 pathogens were MDR *Acinetobacter* and 2 pathogens MDR *Klebsiella*.

Table 3. Antimicrobial effect of commonly used agents against total NICU pathogens, gram-positive pathogens, and gram-negative pathogens

Antibiotic	Total pathogens (n = 89) effective/tested in/(%)	Gram positive (n = 61) effective/ tested in/(%)	Gram negative (n = 28) effective/ tested in/(%)	*P* value
Penicillin	8/65/(12.3)	8/52/(15.4)	0/13/(0.0)	0.00
Ampicillin	5/75/(6.7)	5/56/(8.9)	0/19/(0.00)	0.007
Cloxacillin	24/56/(42.9)	24/52/(46.2)	0/4/(0.00)	0.00
Cefotaxim	2/25/(8)	0/3/(0.00)	2/22/(9.1)	0.00
Cefazolin	25/66/(37.9)	23/47/(48.9)	2/19/(10.6)	0.01
Cefoperazone sulbactam	22/27/(81.5)	1/1/(100))	21/26/(80.8)	0.00
PTZ	20/29/(68.9)	0/3/(0.00)	20/26/(76.9)	0.00
Gentamicin	52/82/(63.5)	39/57/(68.5)	13/25/(52)	0.29
Amikacin	11/23/(47.9)	0/2/(0.00)	11/21/(52.4)	0.00
Co-trimoxazole	31/56/(55.4)	28/48/(58.4)	3/8/(37.5)	0.00
Vancomycin	32/36/(88.9)	32/35(100%)	0/1(81%)	0.00
Linezolid	31/35/(88.6)	31/34/(91.5)	0/1/(0.00)	0.00
Clindamycin	5/10/(50)	5/9/(55.6)	0/1/(0.00)	0.23

PTZ, piperacillin tazobactam.

2 to 3 caregivers will be involved in the care of each of these babies apart from the mother. Conclusively, above-mentioned factors will contribute to the colonization and subsequent infection with *S. aureus* in these babies.

In our study, MRSA was the second most common pathogen (22.5 %), after methicillin-sensitive *Staphylococcus aureus* (MSSA) which constituted 23.6% of babies with sepsis. Similar to this observation, MRSA contributed to 50% of *S. aureus*-induced infections according to a previous study performed in India.[11] For the first time in 1981, it has been reported that the MRSA-induced infections are progressively increasing in the NICUs in a new born nursery.[12-14] Among the known pathogens, *Acinetobacter* was considered as a low virulence pathogen caused predominantly opportunistic infections in immune-compromised patients. Later, it evolved into an important healthcare-associated infection globally, being the most common isolate according to a multicenter cohort study report from India.[2,15] Of note, pre-term delivery is one of the most important risk factors for the incidence of this infection.[25] In our study, *Acinetobacter* was isolated in 15.8% of cases of sepsis. Similar data was also shown by Kaistha et al, on neonatal septicemia.[16] Based on our report, six out of 14 *Acinetobacter* infections were presenting as EOS; however, further studies are warranted due to the increasing incidence of *Acinetobacter* infections as well as its occurrence in unusual settings like EOS.

We analyzed MRSA, *Acinetobacter*, and CoNS as a single entity and found that they constituted 50.6% of all isolate pathogens. These organisms have been also reported as the predominant healthcare-associated (HA) pathogens from other centers.[26] In neonates, the healthcare associated infections (HAI) are encountered commonly after 72 hours of life i.e., LOS.[26,27] However, we reported that nearly half (48.9%) of these infections were unusually caused by EOS. Conditions predisposing to longer hospitalization like pre-term delivery, low birth weight, and lower segment cesarean section deliveries showed a correlation with these infections in our study (OR; 95% CI, 1.82; 0.79-4.22, 1.25; 0.55-2.89 and 0.67; 0.29-1.55 respectively). Whether or not increased census in the labor rooms and NICUs together with the logistics of a government institution predisposes to the HA infections of newborn babies need further studies. Overcrowding and understaffing in the NICUs have been shown to increase the risk of healthcare-associated transmission, colonization, and epidemics of MRSA infections.[28] *Klebsiella* (7.9%) and *E. coli* (4.5%) contributed in a

Table 4. Susceptibility of isolates to commonly used antibiotics

Isolates	*S. aureus* (n = 21)	MRSA (n = 20)	*Acinetobacter* (n = 14)	CoNS (n = 11)	*E. coli* (n = 4)	*Klebsiella* (n = 7)	Enterococci (n = 8)	*Enterobacter* (n = 3)
Penicillin	15%	NT[a]	0%	0%	NT	0%	37.5%	0%
Ampicillin	0%	NT	0%	9%	0%	0%	50%	0%
Cloxacillin	100%	NT	0%	44.5%	NT	NT	NT	NT
Gentamicin	95%	44.5%	50%	50%	100%	40%	87.5%	100%
PTZ	NT	0%	28.6%	NT	100%	66.7%	NT	100%
Cefazolin	95.3%	0%	20%	33.4%	0%	0%	NT	0%
Vancomycin	NT	100%	0%	87.6%	NT	NT	100%	NT
Linezolid	NT	100%	0%	87.6%	NT	NT	100%	NT
Clindamycin	NT	66.7%	0%	50%	NT	NT	NT	NT
Cefotaxime	NT	0%	7.7%	NT	0%	0%	NT	50%
Cefoperazone	NT	100%b	23%	NT	100%	71.5%	NT	100%

PTZ, piperacillin tazobactam.
[a] Susceptibility not tested for; [b] Tested in one case only.

Table 5. Analysis of MRSA, CoNS, and *Acinetobacter* together as a single variable (n = 45)

Variables	Frequency/Total/(%)	Odds ratio	95% CI
Inborn	30/51(59%)	2.20	0.94-5.20
Out born	15/38(39.5%)	0.46	0.20-1.10
EOS	22/43(51%)	1.05	0.46-2.50
LOS	23/46(50%)	0.96	0.42-2.20
Prematurity	24/41(58.5%)	1.82	0.79-4.22
LBW	26/49(53%)	1.25	0.55-2.89
LSCS	17/38/(44.8%)	0.67	0.29-1.55

EOS, Early onset sepsis; LOS, Late onset sepsis; LBW, Low birth weight; LSCS, Lower segment cesarean section.

small proportion of isolates in our study, while 6 out of 7 *Klebsiella* sepsis and 2 out of 4 *E. coli* sepsis occurred in out-born babies.

When susceptibilities of all the isolates were analyzed against individual antibiotics, a very low susceptibility to ampicillin (6.7%), cefotaxime (8%), and penicillin (12.3%) was found, making these antibiotics unsuitable for the empiric treatment of neonatal sepsis. High resistance to third-generation cephalosporin was also reported previously.[4,5] The isolates showed good susceptibility to cefoperazone sulbactam (81.5%), and moderate susceptibility to piperacillin-tazobactam (68.9%), gentamicin (63.5%), and amikacin (47.9%) in our study. A similar pattern of susceptibilities was also shown in previous Indian study.[5]

Staphylococcus aureus showed a good susceptibility to cloxacillin, cefazolin, and gentamicin in our study, while, two similar studies showed 55.1% and 90% susceptibilities to gentamicin.[11,29] Given that MRSA was 100% susceptible to vancomycin and linezolid, it showed good susceptibility against aminoglycosides and clindamycin, making these agents an alternative antibiotic of choice in the treatment of MRSA infections.

Acinetobacter has also represented good susceptibility to cefoperazone-sulbactam and piperacillin-tazobactam, with moderate susceptibility to gentamicin. However, it has been proven that its susceptibility to commonly used antibiotics in the NICUs like cefotaxime (7.7%) and cefazolin (20%) was low.

Study limitations

Limitations of our study were (i) The proportion of contaminant growth in our study was high (12.1%), (ii) limited risk factors for *S. aureus*, MRSA, COoNS, and *Acinetobacter* infections were studied, (iii) isolates were identified by standard microbiological techniques only, in which molecular characterization of isolates was not performed due to the lack of facility, (iv) MRSA, CoNS, and *Acinetobacter* were not categorized into community-onset and hospital-acquired infections, and (v) CoNS sepsis may be overestimated as the diagnosis was based on growth from a single sample.

Conclusion

Gram-positive pathogens as well as opportunistic pathogens like *Acinetobacter* are overwhelming to the conventional gram-negative bacterial flora in neonates and current antibiotic therapy (e.g.., ampicillin, penicillin, and cefotaxime) are not suitable for the incidence of sepsis in neonatal subgroup.

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Authors' Contribution

Conceptualization: Krishnan Chakkiyar, Gireeshan Veluthedath Kuzhiyil, Mohandas Nair.
Data curation: Nabeel Valappil Faisal, Shameem Anathan Mohammed, Krishnan Chakkiyar.
Formal analysis: Krishnan Chakkiyar, Mohandas Nair, Gireeshan Veluthedath Kuzhiyil.

Study Highlights

What is current knowledge?

- Conventionally gram-negative bacterial flora predominates in neonates

What is new here?

- Currently Gram positive as well as opportunistic pathogens predominate in neonates and commonly used beta lactam antibiotics are found ineffective in the treatment of neonatal sepsis

Methodology: Krishnan Chakkiyar, Maya Sudhakaran, Gireeshan Veluthedath Kuzhiyil.
Project administration: Krishnan Chakkiyar, Gireeshan Veluthedath Kuzhiyil, Shameem Anathan Mohammed,.
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Validation: Krishnan Chakkiyar, Mohandas Nair, Maya Sudhakaran.
Writing–original draft: Krishnan Chakkiyar.
Writing–review & editing: Krishnan Chakkiyar.

Competing Interests
None.

Ethical Approval
Institutional Ethics Committee clearance (Ref. No. GMC KKD/RP/2020/IEC/398-27.2.2020) was issued and informed consent was obtained from the parents for participating in the study.

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