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Case Report

Sneathia species in a case of neonatal meningitis from Northeast India

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Here we report the detection of *Sneathia* species most closely related to *Sneathia sanguinegens*, an infrequently reported bacterium, in the cerebrospinal fluid of a neonate by a culture independent method. Even though on rare occasions, this bacterium was isolated previously from the blood of neonatal bacteraemia cases. To the best of our knowledge there exists no previous report of detection of *S. sanguinegens* in the cerebrospinal fluid even though recently there has been a report of isolation of closely related species, *Leptotrichia amnionii*. The neonate recovered following antimicrobial therapy for 21 days. We conclude that uncultivable or difficult- to-cultivate bacteria like *Sneathia* could be an emerging pathogen for neonatal infection.

INTRODUCTION

Sneathia is an emerging pathogen of the female genital tract having a significant role in obstetrics and gynaecological health [1]. *Sneathia sanguinegens* has been previously reported in post-partum as well as neonatal bacteremia [2]. Though initially assigned to genus *Leptotrichia* it was later shown that *Leptrochia sanguinegens* was distinct from *L. buccalis* and was assigned to the genus *Sneathia* as *S. sanguinegens* sp.nov [3]. Due to its fastidious nature, detection of this bacterium may not be possible by a culture-based method [4]. Reports available have implicated difficult to cultivate, uncultivated or previously unrecognized organisms in early onset neonatal infections [5]. To the best of our knowledge there exists no previous report of detection of *S. sanguinegens* in the cerebrospinal fluid (CSF), even though recently there has been a report of isolation of closely related species, *Leptotrichia amnionii* [6]. It has been suggested that *L. amnionii* is better assigned to genus *Sneathia* than *Leptotrichia* [7]. Here we report the detection of *Sneathia* species closely resembling *S. sanguinegens* in the CSF of a neonate by amplification and sequencing of the 16SrRNA gene. The bacteria could not be isolated by culture. The neonate recovered following antimicrobial treatment in combination with piperacillin and netilmicin for 21 days.

CASE REPORT

A female neonate was delivered at 36 weeks of gestational age by preterm vaginal delivery with maternal history of unexplained preterm labour of 16 h and rupture of membrane for 2 h in a tea garden hospital. The neonate was referred to a tertiary level care unit at 10 h of life for poor feeding and lethargy. On admission she was found to have poor muscle tone and was hypothermic. In view of maternal unexplained preterm labour and clinical symptoms in baby, sepsis was considered as a probability and antibiotics amikacin (15 mg/kg IV once daily) and ciprofloxacin (10 mg/kg IV 12 hourly) were started after taking blood culture sample which did not show any growth. Amikacin was started as the first line of treatment for sepsis and ciprofloxacin was combined in the backdrop of existing sensitivity pattern of the commonly encountered isolates in the setting. Sepsis screen was also positive (C-reactive protein value of 4.03 mg/dl with a laboratory cut-off value of 4 mg/dl and the total count of 15 600 cells/mm^3 of blood).

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At 34 h of life antibiotics were upgraded to second-line treatment, piperacillin (100 mg/kg IV once daily) and netilmicin (7.5 mg/kg IV once daily), based on the protocol of the unit (common culture sensitivity pattern of last 6 months) due to clinical deterioration of baby when CSF was also examined. The CSF was suggestive of meningitis. The CSF biochemical parameters had shown a protein value of 180 mg/dl and sugar of 10 mg/dl versus corresponding blood sugar of 70 mg/dl and total CSF cell of 920 cells/mm^3 with 80% polymorphs. The CSF sample was processed for isolation of bacteria. Briefly, the CSF was centrifuged at 1000× *g* for 10−15 min. One drop of sediment was used to prepare the Gram stain and one drop was used to streak the primary culture media (blood agar, chocolate agar and Mac Conkey agar). No bacteria could be seen in the gram stain. The inoculated plates were then incubated under aerobic condition at 37°C with 5% CO_2. A blood agar plate was also incubated under anaerobic condition. Cultures did not show any growth under aerobic as well as anaerobic conditions after 5 days. In the meantime 16SrRNA gene amplification was carried out directly on a CSF sample to exclude the presence of any organism. DNA was extracted as previously described [8]. The supernatant was used as DNA template. An ∼1400 bp amplified product of the 16SrRNA gene was amplified using universal primers (Forward primer: 5′-AGA GTT TGA TCC TGG CTC AG-3′ and Reverse primer: 5′-ACG GCT ACC TTG TTA CGA CTT-3′). The amplified product was purified using a commercial kit (High Pure PCR Product Purification Kit, Version 15, Roche, Germany) as per the manufacturer's instructions. An 884 consensus sequence was obtained after sequencing and editing of the amplified product. A search for similar sequence in the GenBank database had shown 100% homology to that of *Sneathia sanguinegens* strain NTS65407 with accession number HM567404 isolated from a case of arthritis [9]. The sequenced gene was deposited at Genbank with accession number KJ194587.

The neonate recovered with treatment with netilmicin for 7 days and piperacillin for 21 days and was discharged. On follow-up after 2 weeks the neonate was normal on neurological assessment.

DISCUSSION

Sneathia species are morphologically described as fastidious, gram negative, non-motile, anaerobic bacteria [2]. *Sneathia* species commonly inhabit the human vagina and has been accounted for preterm delivery as well as neonatal bacteraemia [1, 2, 4]. This could also explain the preterm delivery in the present case. A previous study on amniotic fluid samples from preterm births found *Sneathia sanguinegens* as the third most frequently encountered taxon, overall suggesting it to be an intra-amniotic pathogen. [4]. The first case of early onset meningitis due to *L. amnionii*, closely related to *S. sanguinegens*, was recently reported in a neonate with intrauterine growth retardation [5]. They could isolate this organism after prolonged incubation after 5 days. In our case we could not isolate this bacterium even after 5 days of incubation.

The antibiotics chosen for empirical treatment of a neonate is based on taking into account the probable pathogens and their susceptibility pattern in the setting [10]. The neonate showed clinical deterioration with initial treatment with amikacin and ciprofloxacin before the microbiological report was available. Ciprofloxacin also is not the treatment of choice for meningitis due to its poor penetration [10]. Previous authors have reported the ineffectiveness of aminoglycoside against *Sneathia* [9]. The neonate however responded to piperacillin and netilmicin combination which are shown to be effective against Gram-negative bacteria which are highly resistant [10, 11]. Since we could not isolate the *Sneathia* species by culture, its susceptibility pattern could not be determined.

We suspect that in our case the maternal genital tract could be a source for infection. Screening of the maternal genital tract for Group B Streptococci (GBS) and administration of prophylaxis against the same have shown the reduction in neonatal sepsis associated with this organism [12]. This provides evidence that the maternal genital tract is a source for neonatal infection. The programmes are targeted only to GBS. Maternal screening for other pathogenic bacteria likely to cause neonatal infections could also help in the reduction of fatal neonatal outcome in regions where they are the common aetiology. The problem could arise when such bacteria are uncultivable or difficult-to-cultivate.

We therefore conclude that uncultivable bacteria or difficult-to-cultivate bacteria like *Sneathia* could be an emerging pathogen for neonatal infection. This is of concern in developing countries with a high neonatal mortality rate and where most of the health facilities are not equipped with molecular diagnostics facilities; hence in most cases it will remain undiagnosed if dependent on conventional method alone.

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