Diagnosis of neonatal meningitis: Is it time to use polymerase chain reaction?

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SUMMARY
Group B Streptococcus (GBS) is a predominant causative pathogen of neonatal meningitis that is associated with a high rate of mortality and morbidity. The establishment of antenatal screening and intrapartum chemoprophylaxis has led to a significant reduction in the incidence rate of invasive GBS disease in developed countries. However, these strategies are not routinely practiced in most developing countries. To ensure good recovery of infants affected with GBS, a prompt diagnostic strategy and appropriate therapy are essential. We highlight here the case of a preterm male infant diagnosed with early-onset of GBS meningitis diagnosed by using polymerase chain reaction (PCR) method on the cerebrospinal fluid (CSF) of the infant. Initially the pathogen was not isolated in both blood and CSF cultures as sampling was performed after the administration of antibiotics. Hence, PCR was a crucial diagnostic test in facilitating the detection of the pathogen in CSF. We believe that PCR is a potentially fast and precise diagnostic method for infection in a newborn.

INTRODUCTION
Streptococcus agalactiae, or group B Streptococcus (GBS) is a common causative pathogen of neonatal meningitis. GBS meningitis has been associated with high rates of mortality and morbidity. A timely diagnosis and prompt treatment with the appropriate antibiotic is essential to ensure optimal outcome. Unfortunately, the commonly used diagnostic method to isolate GBS from the cerebrospinal fluid (CSF) sample lacks sensitivity despite high specificity.1 In this case report, we illustrate a case of GBS neonatal meningitis that failed to grow in the routine CSF culture but was instead detected via PCR.

CASE REPORT
A preterm male infant was admitted to the neonatal intensive care unit (NICU) of Hospital Serdang at 6 hours of life for recurrent episodes of symptomatic hypoglycaemia. He was born at 36 weeks of gestation via an emergency caesarean section for unprovoked foetal heart rate deceleration. His birth weight was 3100gm and the Apgar score was normal. The mother presented with late-onset pregnancy-induced hypertension on the day of delivery with no history of fever or premature rupture of chorionamniotic membrane. No GBS screening was performed throughout the course of her pregnancy. On examination, the baby had jitteriness and a weak cry. His body temperature was 36.3°C, pulse rate was 144 beats/minute with immediate capillary refilling time and blood pressure was 62/49mmHg. His anterior fontanelle was normotensive on palpation and he was actively moving all four limbs with normal tone and reflexes.

On further examination, blood sugar profile ranged between 1.6-2.4mmol/L, thus requiring multiple doses of Dextrose 10% bolus and intravenous glucose infusion at the rate of 15mg/kg/min. His condition deteriorated a day later despite the normalisation of blood glucose level. He developed respiratory distress requiring non-invasive respiratory support. Haematological indices were within the normal limits (haemoglobin 14.7g/dL, total white cell count 19,100/μL with 60% neutrophils, 22% lymphocytes and platelet 150,000/mm3). Blood culture was sterile for bacterial growth, even though the C-reactive protein was elevated (19mg/dL).

In view of that, he was treated as presumed sepsis and commenced on intravenous c. penicillin and gentamycin. However, no clinical improvement was observed after 48 hours. A repeated haematological test revealed a reduced platelet count (haemoglobin 12.9g/dL, total white cell counts 15,200/μL and platelet 92,000/mm3). In addition, CSF microscopy showed pleocytosis (60 polymorphonuclear cells/mm3) with a ratio of CSF glucose to serum glucose of 0.79 and CSF protein of 862mg/dL. However, the microbiological study of both blood and CSF did not yield any microorganism. CSF sample was also sent for polymerase chain reaction (PCR) test to amplify the 16S rRNA gene and it turned out to be positive. The primer sequences used were: primer U1 (F): 5′-CCAGCAGCCCGTAATACG-3′ and primer U2(R): 5′ATCGGYTACCTTGTTACGACTTC-3′. Following that, the PCR product was sent for sequencing and the species was identified as S. agalactiae. A high vaginal swab sample was taken from the mother subsequently and a similar pathogen was isolated in the culture.

Following the diagnosis, the antibiotic treatment was escalated to a high dose intravenous c. penicillin and cefotaxime for 21 days. Upon completion of the antibiotics, his symptoms resolved and he was discharged home on day 25 of life. He continued to do well on follow-up.
DISCUSSION
GBS meningitis is a devastating infection associated with significant mortality and morbidity in the neonatal population. It is associated with a 30% mortality rate and the survivors are predisposed to a high risk of neurological sequelae. Even though the overall incidence and mortality of GBS meningitis have declined in recent decades, its morbidity remains virtually unchanged. Therefore, prompt diagnostic strategies with appropriate therapies is crucial to ensure better outcome for the affected infants.

Nevertheless, accurate diagnosis of early-onset GBS meningitis is often difficult due to the non-specific clinical symptoms manifested by infants. Thus, the definitive diagnosis is highly dependent on the positive identification of infecting microorganisms isolated in the CSF culture. Unfortunately, the conventional microbiological diagnostic method is associated with low sensitivity and high false-negative culture results, especially if the CSF sampling is done after intrapartum or postnatal exposure to antibiotics. In our experience, the diagnosis of neonatal meningitis is often presumptive based on a combination of clinical suspicion and clues from the CSF microscopy. However, the CSF indexes vary according to age and normal values of CSF in infancy are poorly defined. A study evaluating the use of >20/mm³ CSF leucocyte count as a cut-off value for the diagnosis of neonatal meningitis subsequently showed that 13% of confirmed meningitis cases had normal CSF parameters. In addition, CSF protein and glucose are also considered as poor predictors of meningitis due to possible overlapping of values between infants with or without meningitis. This is further complicated by the rapid sterilisation once antibiotics have been initiated. In other words, it is difficult to predict the diagnosis of neonatal meningitis solely based on CSF parameters. Thus, the identification of the offending microorganism is still paramount in the optimisation of antibiotic therapy and in order to minimise the risk of severe neurological sequelae.

To date, several PCR-based methods have been explored as a diagnostic tool for bacterial meningitis. Apart from having a significantly higher sensitivity and specificity for direct detection of bacteria from CSF, the PCR method has also been proven to be able to produce the result within a day, thus representing a shorter turn-around time than the conventional methods. Moreover, PCR yields higher sensitivity and specificity in detecting the pathogen in CSF among individuals who have been on antibiotic treatment because it is not affected by the viability of microorganisms. Therefore, even though the cost of PCR is higher, it can lead to faster and more accurate detection of pathogens in the CSF. As a result, it enables the appropriate chemotherapy to be initiated early prior to CSF collection and subsequently contributes to a better prognosis in patients with GBS meningitis.

CONCLUSION
Microbiological studies remain the gold standard for the diagnosis of neonatal meningitis. However, its low sensitivity often results in false-negative results. Therefore, PCR is more promising and helpful method in investigating infants who are clinically suspected of meningitis, especially those who have been treated with antibiotics prior to CSF sampling.

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REFERENCES