Microbiology diagnostic approach in identifying *Streptococcus pneumoniae*: Case report of *Streptococcal meningitis*

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**ABSTRACT**

**Introduction:** Bacterial meningitis remains a serious global health problem. *Streptococcus pneumoniae* as the causative organism, is fastidious. Microbiology diagnostic approach is needed to identify *S. pneumoniae* and antimicrobial susceptibility tests (AST). This study aims to report *S. pneumoniae* meningitis has been successfully cured strongly related to diagnostic stewardship.

**Case:** A 1 year old child who fell, suffering from vomiting, fever and seizures was brought to the Emergency Department. Multi-slice Computerized Tomography shows cerebral edema and intracerebral hemorrhage. Laboratory blood tests and cerebrospinal fluid analysis strongly indicate bacterial meningitis. Presumptive Gram microscopy of Cerebrospinal Fluid (CSF) and initial identification of CSF cultures on lysed blood agar under microaerophilic conditions, consistently showed lancet-shaped and encapsulated Gram-positive diplococci. Our Clinical Microbiology Laboratory finally identified the growth of *S. pneumoniae* from blood and CSF cultures. Patients were treated with ceftiraxone 50 mg/kg body weight/8 hours in the first 10 days as empirical and switched later definitively to ciprofloxacin 10 mg/kg body weight/12 hours according to the AST result on day 11 to day 26. On the 31st day, the patient recovered and was discharged.

**Conclusion:** The case of a 1 year-old child with *S. pneumoniae* meningitis has been successfully cured strongly related to diagnostic stewardship by the Clinical Microbiology Laboratory. Supplementation media with sodium bicarbonate can improve *S. pneumoniae* recovery as a basis for identification and AST. Proper inoculation and AST need to be accelerated because *S. pneumoniae* easily undergoes autolysis.

**Keywords:** bacterial meningitis, diagnostic stewardship, fastidious, *Streptococcus pneumoniae*,

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**INTRODUCTION**

Bacterial meningitis is a serious central nervous system (CNS) infection and life-threatening condition that requires a rapid diagnosis and treatment. The incidence of bacterial meningitis has decreased but the mortality rate is still high as well as long-term neurological sequelae and decreasing quality of life, especially in developing countries. The major causes of bacterial meningitis are *N. meningitidis*, *S. pneumoniae* and *H. influenza*, those are fastidious. Meningitis caused by *S. pneumoniae* occurs most often in children and the elderly, with an incidence of 17 cases in 100,000 children under 5 years of age. The case fatality rate can increase to 70% without appropriate treatment.

*S. pneumoniae* is fastidious and fragile bacteria, that grows well at 35-37°C with 5% CO₂ in the laboratory. Rapid identification of bacterial pathogen meningitis is very important for treatment guiding and reducing neurological sequelae as well as mortality. Clinical Microbiology Laboratory plays an important role for causative pathogen identification of meningitis and providing antimicrobial susceptibility patterns for definitive treatment. The discovery of fastidious bacteria is a challenge for routine clinical microbiology laboratories in many health facilities.

**CASE PRESENTATION**

A one-year-old girl has a seizure, the latter approximately 15 minutes, with a history of falling down from 0.5-1m stairs five days before, entering the hospital emergency room. With the seizure therapy, her consciousness did not improve and she was transferred to the Pediatric Intensive Care Unit then. The patient is still unconscious, has a fever, no more convulsions, diarrhea or vomiting, and neither cough nor shortness of breath. The patient was referred to Dr. Sardjito Hospital, a reference and Academic Hospital for further treatment.

On physical and neurological examination, there was a patent airway with RR 35/min without any chest retractions, a strong pulse 140/min, good response to pain, spastic bi-hemiparesis, physiological reflex +3 in all extremities, positive pathologcal reflex, neck stiffness without Brudzinski neck sign or contralateral neck sign and Kernig sign neither. The result of the laboratory examination are presented in Table 1 and 2.

Figure 1 - 3 shows the microscopic examination of CSF or isolate growth, as a presumptive result as well as early...
Table 1. Haematology and Chemistry Laboratory results.

<table>
<thead>
<tr>
<th>Hematology</th>
<th>Reference range</th>
<th>Unit</th>
<th>2022/03/16</th>
<th>2022/03/17</th>
<th>2022/03/18</th>
<th>2022/03/31</th>
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<tbody>
<tr>
<td>Hemoglobin</td>
<td>9.6 - 15.6</td>
<td>g/dL</td>
<td>11.0</td>
<td>8.1</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>5.50 - 17.50</td>
<td>10^3/µL</td>
<td>25.0</td>
<td>14.6</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>22.0 - 46.0</td>
<td>%</td>
<td>83.9</td>
<td>74.5</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td>37.0 - 73.0</td>
<td>%</td>
<td>12.1</td>
<td>19.8</td>
<td>30.8</td>
<td></td>
</tr>
<tr>
<td>Monocytes %</td>
<td>2.0 - 11.0</td>
<td>%</td>
<td>3.9</td>
<td>5.1</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>150 - 450</td>
<td>10^3/µL</td>
<td>574</td>
<td>362</td>
<td>715</td>
<td></td>
</tr>
<tr>
<td>Chemistry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN</td>
<td>5.00 - 18.00</td>
<td>mg/dL</td>
<td>5.57</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Creatinine</td>
<td>0.18 - 0.35</td>
<td>mg/dL</td>
<td>0.201</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood sugar</td>
<td>74 - 106</td>
<td>mg/dL</td>
<td>108</td>
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<tr>
<td>Sodium</td>
<td>136 - 145</td>
<td>mmol/L</td>
<td>128.2</td>
<td>133.1</td>
<td>133.8</td>
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</tr>
<tr>
<td>Potassium</td>
<td>3.5 - 5.1</td>
<td>mmol/L</td>
<td>3.81</td>
<td>4.02</td>
<td>4.37</td>
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<tr>
<td>Chloride</td>
<td>98 - 107</td>
<td>mmol/L</td>
<td>90.4</td>
<td>92.0</td>
<td>99.3</td>
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<tr>
<td>Magnesium</td>
<td>1.7 - 2.3</td>
<td>mg/dL</td>
<td>2.54</td>
<td>2.32</td>
<td>2.20</td>
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<tr>
<td>Albumin</td>
<td>3.80 - 5.40</td>
<td>g/dL</td>
<td></td>
<td></td>
<td>3.19</td>
<td></td>
</tr>
<tr>
<td>Immunology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procalcitonin</td>
<td>&lt; 0.50</td>
<td>ng/mL</td>
<td>18.7</td>
<td>1.46</td>
<td>0.19</td>
<td></td>
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</tbody>
</table>

Table 2. Cerebrospinal fluid (CSF) laboratory result.

<table>
<thead>
<tr>
<th>Cytology</th>
<th>Reference range</th>
<th>Unit</th>
<th>17/03/2022</th>
<th>18/03/2022</th>
<th>22/03/2022</th>
<th>28/03/2022</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarity</td>
<td>Cloudy</td>
<td>Clear</td>
<td>Clear</td>
<td>Clear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell Count</td>
<td>0 - 5</td>
<td>cell/µL</td>
<td>250</td>
<td>153</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>PMN</td>
<td>0 - 6</td>
<td>%</td>
<td>95</td>
<td>72</td>
<td>69</td>
<td>11</td>
</tr>
<tr>
<td>MN</td>
<td>54 - 100</td>
<td>%</td>
<td>5</td>
<td>28</td>
<td>31</td>
<td>89</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>-</td>
<td>cell/µL</td>
<td>100</td>
<td>300</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Chemistry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>0.02 - 0.05</td>
<td>g/dL</td>
<td>0.56</td>
<td>0.11</td>
<td>0.12</td>
<td>0.38</td>
</tr>
<tr>
<td>Glucose</td>
<td>50 - 80</td>
<td>mg/dL</td>
<td>0.14</td>
<td>3</td>
<td>14</td>
<td>38</td>
</tr>
<tr>
<td>Nonne</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.10 - 2.40</td>
<td>mmol/L</td>
<td>11.50</td>
<td>7.29</td>
<td>6.94</td>
<td>3.27</td>
</tr>
<tr>
<td>Pandy</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>LDH</td>
<td>-</td>
<td>U/L</td>
<td>762</td>
<td>538</td>
<td>650</td>
<td>97</td>
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<tr>
<td>Presump</td>
<td>Diplococcus Gram positive</td>
<td>Diplococcus Gram positive</td>
<td>S.pneumoniae</td>
<td>S.pneumoniae</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Organism</td>
<td>AZM, CXM, FEP, CIP, DO, TE, SXT, C, CTX.</td>
<td>AZM, CRO, CIP, SXT, C, CTX.</td>
<td>S.pneumoniae</td>
<td>S.pneumoniae</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Antibiotic susceptible</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic resistant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: PMN=polymorphonuclear cell, MN=mononuclear cell, AZM=Azithromycin, CXM=Cefuroxime, FEP=Cefepime, CIP=Ciprofloxacin, DO=Doxycycline, TE=Tetracycline, SXT=Trimethoprim/Sulfamethoxazole, C=Chloramphenicol, CTX=Cefotaxime, CRO=Ceftriaxone, P=Penicillin, CAZ=Cefazidime.

detection and biochemical test of identification in the cultivation process. Optochin test resulted good susceptibility of *S.pneumoniae* to 5 ug Optochin disc, negative catalase and oxidase test. The result of radiodiagnostic MSCT showed cerebral edema and intracranial hemorrhage. The cytological examination from Anatomical Pathology Laboratory did not reveal any malignant cells. The patient was treated with Cefepime 50 mg/kg body weight for 8 hours during...
the first 10 days of stay in the PICU, then
was switched to Ciprofloxacin 10 mg/kg of
body weight per 12 hours for the following
14 days after the AST presented. The
patient's condition was improved, without
more fever nor seizures. She had improved
of consciousness, normal response on
any stimulation and got spontaneous
movements. Finally, the patient was
discharged after 24 days of treatment and
continuing evaluation in an outpatient
clinic.

**DISCUSSION**

A culture of cerebrospinal fluid is the
definitive diagnosis of bacterial
meningitis. S.pneumoniae is a Gram-
positive bacteria, lanceolate and
encapsulated diplococcus. Identification of
S.pneumoniae in culture medium is
accompanied by careful observation of
morphological characteristics and four main phenotypic characteristics, namely α-hemolysis, negative catalase test, susceptible Optochin test and bile
solubility test. S.pneumoniae is a fastidious
and brittle bacteria, easy to autolyze due to
its autolysin enzyme.

Based on our country epidemiology,
there are only a few reports of
S.pneumoniae as a pathogen due to the
difficulties of recovering it in vitro. S.pneumoniae is better grown by direct
inoculation of the clinical specimen
(in this case cerebrospinal fluid) into
lysed blood agar that has been added
sodium bicarbonate without through the
enrichment broth. Inoculated medium is
incubated in a CO₂ atmosphere at 37°C
overnight. Macroscopically, colonies are
round, small and smooth, flat, gray in
color, well-defined edges with greenish
areas around them indicate their property
of alpha-hemolytic. Bicarbonate is in
equilibrium with CO₂ through the
formula CO₂ + H₂O → H₂CO₃ → HCO₃⁻ + H⁺. Based on this theory, lysed blood agar
(BA) medium was developed with the
addition of sodium bicarbonate powder,
poured when it has begun to cool before
it is solidified. Ersoy et al in 2017 reported
that the addition of sodium bicarbonate
into the culture medium could change the
bacterial structure and gene expression.
In this case, S.pneumoniae was successfully
grown rapidly on lysed BA with sodium
bicarbonate supplementation. In this
-growing media, the characteristic colonies
of S.pneumoniae was fit to its true nature.
Gram staining showed the morphology of
the encapsulated Gram-positive
diplococcal lancet (slightly oval). Capsule
test showed an enlarged capsule indicated
to S.pneumoniae more clear. The catalase
test resulted negative and optochin test
showed susceptibility. The biochemical test
using the Analytical Profile Index (API) 20
STREP V8.0 showed S. pneumoniae in a
significant ID of 97.9%.

Antimicrobial susceptibility test (AST)
showed that there was no critical value of
multidrug resistance. Firstly, the patient
was treated Cefepime as an empirical
treatment of bacterial meningitis and
switched to Ciprofloxacin on day 11 as a definitive antibiotic based on
AST result. Microbiology based evaluation of Cerebrospinal fluid on the day 6th
and 14th after definitive treatment resulted
sterile culture. This was in line with the improvement of its comprehensive laboratory result (WBC, procalcitonin, CSF analysis).

The prevalence of antibiotic resistance is increasing in the world. Bacteria have a fairly high adaptability, causing antibiotic resistance with the target of mutation of the antibiotic component and their efflux.⁸ Antimicrobial stewardship is the most important activity which is needed microbiology data accurately and timely. Susceptibility testing is essential for tracking changes in phenotype as well as geography distribution to address the success on antimicrobial resistance control.

The patient’s consciousness was improved, got clinically better without any fever nor seizures and discharged on the day 31 admission to be continued evaluation in a pediatric outpatient clinic. Diagnostic and antibiotic stewardship practices in this patient have met a good patient outcome.

**CONCLUSION**

We report the case of a one-year-old girl with bacterial meningitis, the cerebrospinal fluid culture showed *S. pneumoniae*, which is fastidious bacteria. The specific technical approach in clinical microbiology laboratory can improve the recovery of this bacteria as a basis for its identification and AST. Diagnostic and antibiotic stewardship practices as part of holistic effective management in this patient have met the good patient outcome.

**ETHICAL CONSIDERATION**

Patient’s parents had signed written informed consent regarding the publication of their medical data in the journal article, IC number 02004600.

**CONFLICT OF INTEREST**

The author reports no conflicts of interest in this work.

**AUTHOR CONTRIBUTIONS**

AD led and supervised the laboratory examination of the patient, suggested laboratory-based clinical decisions and

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**Table 3. Empiric and definitive antibiotic time-scheme related to AST result.**

<table>
<thead>
<tr>
<th>Culture specimen</th>
<th>Gram and bacterial identification</th>
<th>Antibiotic susceptibility</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Gram-positive <em>S. pneumoniae</em></td>
<td>AZM, CRO, CIP, SXT, C, CTX</td>
<td></td>
</tr>
<tr>
<td>LCS</td>
<td>Gram-negative</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Notes: AZM=Azithromycin, CXM=Cefuroxime, FEP=Cefepime, CIP=Ciprofloxacin, DO=Doxycycline, TE=Tetracycline, SXT=磺胺甲恶唑, C=Chloramphenicol, CTX=Cefotaxime. The same box color represents the same sequence specimen procedure.
CASE REPORT
management, supervised the development of the manuscript, and agreed to the final version for publication. TAMA held the laboratory examination and its follow-up, wrote the manuscript preparation and as correspondent submitting process.

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REFERENCES