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

Andaru Dahesihdewi[1], Tama Sari Girsang[1*]

[1]Department of Clinical Pathology and Medical Laboratory of Sardjito Hospital/ Faculty of Public Health Medicine and Nursing Universitas Gadjah Mada Yogyakarta;

*Corresponding author:
Tama Sari Girsang;
Department of Clinical Pathology and Medical Laboratory of Sardjito Hospital/ Faculty of Public Health Medicine and Nursing Universitas Gadjah Mada Yogyakarta;
tamagirsang19@gmail.com

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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 cefepime 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.[1] 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.[2]

S.pneumoniae is fastidious and fragile bacteria, that grows well at 35-37°C with 5% CO_2 in the laboratory.[2] 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.[3] 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 pathological 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.**

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

Table 2. **Cerebrospinal fluid (CSF) laboratory result.**

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

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=Ceftazidime.

detection and biochemical test of identification in the cultivication 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

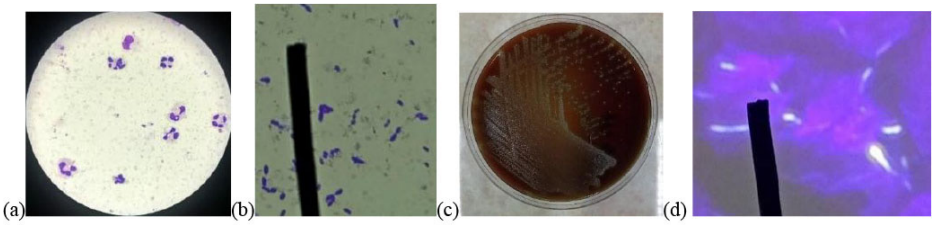
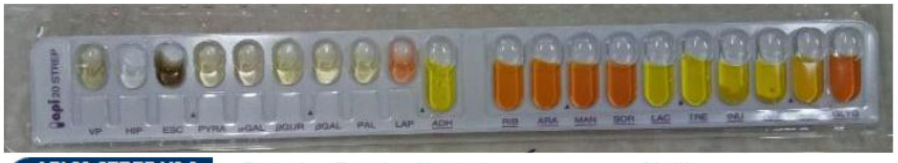


Figure 1. **(a)** Patient's CSF with many pmn cells ; (b) CSF with Gram positive diplococcus ovoid (Lancet-shaped); (c) *S. pneumoniae* grows on blood agar with the addition of sodium bicarbonate; (d) Capsule Test.

Figure 2. **(a)** Positive (susceptible) optochin test ; (b)Negative catalase test ; (c) Negative oxidase test.



API 20 STREP V8.0 Printout Export New test Modify

REFERENCE DATE 5/16/22

COMMENT

PRESUMPTIVE IDENTIFICATION	
Strip	API 20 STREP V8.0
Profile	4 1 4 0 4 7 1
Note	

Significant taxa	% ID	T	Tests against
Streptococcus pneumoniae	97.9	0.79	ßGAL 79%

Next taxon	% ID	T	Tests against		
Streptococcus salivarius ssp salivarius	1.8	0.44	VP 85%	PYRA 1%	

Figure 3. Biochemical test using Analytical Profile Index (API) 20 STREP V8.0.

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.[1] *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.[3] *S.pneumoniae* is a fastidious and brittle bacteria, easy to autolyze due to its autolysin enzyme.[4]

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.[5] *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 CO_2 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 CO_2 through the formula $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$.[6] 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.[7] 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 in children[8] 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

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

	Empiric Treatment: *Cefepime* 50 mg/kg body weight/8 hours intravenous														Definitive Treatment: *Ciprofloxacin* 10 mg/kg body weight/12 hours intravenous						Treatment: Phenobarbital 17 mg/12 hours po, Acetazolamide 175 mg/8 hours po	
	16/3	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1/4	2	8	14	
Culture specimen		LCS	LCS				Blood						LCS								Patient was discharged
Presumptive Gram			*Diplococcus* Gram positive	*Diplococcus* Gram positive				No microbe						No microbe							
Initial Identification				*Diplococcus* Gram positive	*Diplococcus* Gram positive																
Organism ID						*S.pneumoniae*	*S.pneumoniae*					Sterile					Sterile				
Antibiotic susceptible						AZM, CXM, FEP, CIP, DO, TE, SXT, C, CTX.	AZM, CRO, CIP, SXT, C, CTX.														

Notes: AZM=Azithromycin, CXM=Cefuroxime, FEP=Cefepime, CIP=Ciprofloxacin, DO=Doxycycline, TE=Tetracycline, SXT=Trimethoprim/Sulfamethoxazole, C=Chloramphenicol, CTX=Cefotaxime, CRO=Ceftriaxone. The same box's color represents the same sequence specimen procedure.

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

1. VanDemark M. Acute bacterial meningitis: Current review and treatment update. Crit Care Nurs Clin North Am [Internet]. 2013;25(3):351–61. Available from: http://dx.doi.org/10.1016/j.ccell.2013.04.004
2. Shadid J. Laboratory Methods for the Diagnosis of Meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae , and Haemophilus influenzae Laboratory Methods for the Diagnosis of Meningitis caused by Neisseria meningitidis , Streptococcus pneumoniae. [Internet]. 2011;2:14-323. Available from: https://apps.who.int/iris/bitstream/handle/10665/70765/WHO_IVB_11.09_eng.pdf?sequence=1&isAllowed=y
3. Bandettini R, Melioli G. Laboratory diagnosis of Streptococcus pneumoniae infections: Past and future. J Prev Med Hyg. 2012;53(2):85–8.
4. Burghout P, Cron LE, Gradstedt H, Quintero B, Simonetti E, Bijlsma JJE, et al. Carbonic anhydrase is essential for Streptococcus pneumoniae growth in environmental ambient air. J Bacteriol. 2010;192(15):4054–62.
5. Sanchez-Rosario Y, Johnson MDL. Media Matters, Examining Historical and Modern Streptococcus pneumoniae Growth Media and the Experiments They Affect. Front Cell Infect Microbiol. 2021;11(March):1–15.
6. Hinnu M, Kogermann K, Bumann D. Making Antimicrobial Susceptibility Testing More. Antimicrob agents Chemother. 2022;(10.1128/aac.02412-21):1–4.
7. Ersoy SC, Heithoff DM, Barnes L, Tripp GK, House JK, Marth JD, et al. Correcting a Fundamental Flaw in the Paradigm for Antimicrobial Susceptibility Testing. EBioMedicine [Internet]. 2017;20:173–81. Available from: http://dx.doi.org/10.1016/j.ebiom.2017.05.026
8. van de Beek D, Brouwer MC, Koedel U, Wall EC. Community-acquired bacterial meningitis. Lancet [Internet]. 2021;398(10306):1171–83. Available from: http://dx.doi.org/10.1016/S0140-6736(21)00883-7