〈Regular Article〉

A Study of Gram Stained Specimens Obtained from Pediatric Inpatients of Kawasaki Medical School Hospital - Achievements from an elementary research experiment by a 2nd year medical student

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ABSTRACT This study was designed as an elementary medical research experiment course for a 2nd year medical student. All Gram stained specimens deriving from upper respiratory tract specimens collected from pediatric inpatients admitted to the pediatric ward of our hospital during 2018 October 1st through 31st were examined; the result was assessed as to whether or not it was in concordance with past reports or not.

Gram-negative cocci (*Moraxella catarrhalis*-like), Gram-negative rods (*Haemophilus influenzae*-like), and Gram-positive diplococci (*Streptococcus pneumoniae*-like) were frequently encountered in this order; these three most encountered species were in concordance with previous reports, whereas their order was not. This difference in the order of frequency might be attributed to the legislation of vaccination against *Haemophilus influenzae* type b and *Streptococcus pneumoniae*.

doi：10.11482/KMJ-E201945083 *(Accepted on Sept 9, 2019)*

Key words：Gram Staining, Respiratory Tract Infection, Microscopic Examination

INTRODUCTION

Microscopic examination of Gram stained specimens is an important procedure in obtaining first-step information of the causative microorganism of bacterial infections. Past literature from our country reports that the major pathogens identified from pediatric upper respiratory tract specimens by gram staining are *H.influenzae*, *S.pneumoniae*, and *M.catarrhalis*[1－3]. However, whether these facts are applicable to current patients of our institute remain unproved. We hereby sought to evaluate the gram stained slides of pediatric inpatients at our institute.

The study was designed for a one-month elementary medical research experiment course for a 2nd year medical student.

MATERIALS AND METHODS

All Gram stained slides deriving from upper respiratory tract specimens collected from pediatric inpatients admitted to the pediatric ward of our hospital during 2018 October 1st thru 31st were subjected to this study. All specimens were collected for clinical diagnostic purposes. Appropriate written

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consents for secondary use of the stained slides were obtained from the guardian of the patients; opportunities for opting out of this consent were provided by announcement on our institutional Web page (URL: https://h.kawasaki-m.ac.jp/data/dept_016/ekigaku_s_dtl/). Only the Gram stained slides, anonymized and dissociated from clinical information, were subjected to this study.

The slides were initially evaluated at a magnification of 10×10 to estimate its quality by Geckler classification[4]. Following initial evaluation, precise observation at a magnification of 10×100 was carried out; Gram stainability, morphotype, existence of phagocytosis of microbes were carefully observed.

This study was approved by our institutional ethics board (No. 3261).

RESULTS

A total of 20 slides were eligible and subjected to microscopic examination. The distribution of the quality of slides by Geckler classification is summarized in Table 1. The frequency of microbes by their Gram stainability and morphotype as well as the frequency of phagocytosis are summarized in Table 2.

DISCUSSIONS

Initial evaluation proved 18 of 20 (90%) of the slides to be appropriate for examination (Geckler classification 4, 5 and 6). This result is concordant with a previous evaluation of 496 pediatric sputum specimens that report 87.1% of the specimens to be Geckler 4-6[5], suggesting that specimens of good quality are obtained in the pediatric ward of our institute.

Gram-negative cocci (*Moraxella catarrhalis*-like), Gram-negative rods (*Haemophilus influenzae*-like), and Gram-positive diplococci (*Streptococcus pneumoniae*-like) were frequently encountered in this order, sometimes phagocyted.(Table 2). *M. catarrhalis*, *H. influenzae*, *S. pneumoniae* are common bacteria found in the upper respiratory tract, pathogenic as well as colonized. Furthermore, causative bacterial agents are known to be limited in pediatric patients[6]. Another report also supports the estimation of species by Gram stain to be reliable with a specificity of 94.8~98.1% for the aforementioned three species[1]. Thus, even without final confirmation by bacterial culture and further tests, the authors presume that our estimation is likely to be reliable.

In past literature from Japan, the order of these three species differs from our results, which are reported in the following order – *H.influenzae*, *S.pneumoniae*, *M.catarrhalis*[1-3]. This difference might be attributable to the introduction of Hib vaccine (in 2008) and heptavalent pneumococcal

Table 1. Distribution of the quality of slides evaluated by Geckler classification

Geckler*	1	2	3	4	5	6
numbers	0	2	0	1	13	4

*Geckler 4-6 are considered appropriate for examination

Table 2. Obsereved frequency of microbes by Gram stainability and morphotype, existence of phagocytosis

	Mca -like	*Hin* -like	*Spn* -like	Gram(+)			Gram(−)		
				Cocci, chain	Cocci, cluster	Rods	Cocci, chain	Cocci, cluster	Rods
number	10	9	7	2	3	3	2	1	2
Phagocytosis observed	5	1	1	0	0	0	0	0	0

Mca, Moraxella catarrhalis; Hin, Haemophilus influenzae; Spn, Streptococcus pneumoniae

conjugated vaccine (in 2010) (replaced by tridecavalent from November 2011). Following public promoting of vaccination of these vaccines by wavering of the vaccination fee in 2011, which caused a rise in vaccination rates, these vaccines became mandatory in 2013. The aforementioned domestic reports are from the "pre-vaccination" era, whereas our result is from the "post-vaccination" era. Since the difference is the decline of *H.influenzae* and *S.pneumoniae*, the result might be well attributed to the vaccination against these two pathogens. Similar trends are reported in the literature. A study from the USA, describing the isolated organism of the nasopharynx of pediatric acute maxillary sinusitis, reports a statistically significant decline of *S.pneumoniae* isolation, whereas this was not the case with *H.influenzae*, *M.catarrhalis*, *Streptococcus pyogenes*, and *Staphylococcus aureus*[7]. Another study from Japan, describing the isolated microorganism from bacteriologically-confirmed pediatric, community-acquired pneumonia patients, reports a similar decline of *S.pneumoniae*[8]; however, no decline of *H.influenzae* was observed in this report, which is not in concordance with our result. Although the cause of this discrepancy is not clear, one possible explanation might be the differing study period. The post-vaccination specimens were collected during the year 2012 through 2013 in the aforementioned report, the period when Hib vaccine was not yet mandatory. Legislation might have promoted vaccination, resulting in reduced occasions of *H.influenzae* in the authors' result.

The authors are aware of the following limitations. The study period was one month, which might have been prone to potential seasonal effects. Fortunately, however, none of the three species are known to cause seasonal epidemics; thus seasonal bias is unlikely. Collected data was solely limited to Gram stained slide observations, omitting clinical/patient information; this was to facilitate the study design for a one-month course. The number of assessed slides was also not abundant; further accumulation of similar information is warranted for confirmation of our preliminary findings.

Finally, discrepancy in the order of the three major bacteria species encountered was found in our result from those of past reports; the decline of the two species is likely to be attributed to the legislation of mandatory vaccination against the two.

ACKNOWLEDGEMENTS

The authors thank the physicians of the Department of Pediatrics, who supported the study by achieving written informed consent for secondary use of the stained slides. The authors are grateful to the staff of the microbiology division of the clinical laboratory of Kawasaki Medical School Hospital for their assistance and instructive advice.

This study was designed and prepared for an elementary medical research experiment course for a 2nd year medical student. Part of this manuscript has been presented in Japanese at an intramural poster session, which was mandatory for the research experiment course.

This study was funded by the Department of Pediatrics, Kawasaki Medical School.

CONFLICT OF INTEREST

The authors have no conflicts of interest directly relevant to the content of this article.

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