Prevalence and predicting factors of Group A beta-hemolytic *Streptococcus* carrier state in primary schoolchildren

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ABSTRACT

Introduction : **Transmission of Group A *Streptococcus* from asymptomatic children to their surrounding carries a risk of acute rheumatic fever in susceptible people.**

Aim and Objectives : **We aimed to investigate the prevalence and predictors of GAS carrier state and evaluate the antibiotic sensitivity pattern of GAS in Jakarta, Indonesia.**

Material and Methods : **We enrolled 201 asymptomatic schoolchildren (6–12 years) using stratified random sampling from a primary school in Jakarta. None of the children had a history of rheumatic fever or rheumatic heart disease. All participants underwent physical examination, and laboratory tests include complete blood count, erythrocyte sedimentation rate, C-reactive protein, antistreptolysin O titer, and throat swab culture.**

Results : **The prevalence of GAS carrier was 13.9% (95% confidence interval: 9.2%–18.6%) in our study. On multivariate analysis, tonsillar enlargement was found to be the only predicting factor of GAS carrier (P = 0.03). GAS was sensitive to penicillin G, erythromycin, vancomycin, clindamycin, chloramphenicol, azithromycin, and tetracycline in 100%, 89%, 86%, 75%, 68%, 68%, and 32% of patients, respectively.**

Conclusion : **The GAS carrier state is common among school-age children affecting approximately 13.9% children. Tonsillar enlargement is a significant finding predictive of GAS carrier state. All isolates are still sensitive to penicillin and mostly sensitive to erythromycin but are increasingly resistant to tetracycline.**

Keywords : Acute rheumatic fever, antibiotic sensitivity testing, group A *Streptococcus*

INTRODUCTION

Group A beta-hemolytic *Streptococcus* (GAS) infection is common in school-age children (5–15 years), and the incidence of pharyngitis due to GAS in these children is approximately 37%.[1,2] About 0.3%–3% of children develop acute rheumatic fever following GAS pharyngitis.[3]

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GAS carrier is a condition wherein a throat swab from an asymptomatic person tests positive for GAS.[4] Its prevalence is estimated to be 6%–16% in school-age children and these patients are a source for transmission to the surrounding environment.[5-9] Studies have shown

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several factors predictive of GAS carrier state such as housing condition, gender, number of family members living in the same house, parent's education, economic status, nutritional status, tonsillar enlargement, and lymphadenopathy.[6,10,11]

In South East Asia, there is limited data regarding GAS carrier.[12] All countries within this region are categorized as tropical countries with 2 seasons (rainy and dry season). The possibilities of having GAS carrier during rainy season are high with the risk of transmission to their surroundings. Indonesia, as a lower-middle income country, has the fourth highest population in the world, estimated at over 270 million.[13] The sociodemographic condition facilitates GAS infection and the risk of acute rheumatic fever, however, there is scanty data about the incidence of acute rheumatic fever in Indonesia. There is an urgent need for contemporary data of this region. It would help to emphasize to the concerned authorities, the importance of its management due to the associated risk of development of acute rheumatic fever in the susceptible population.

Therefore, we performed this study to determine the prevalence and predictors of GAS carrier state and evaluate the antibiotic sensitivity pattern of GAS.

MATERIALS AND METHODS

Study population

We enrolled 201 school-age children using stratified random sampling from a public elementary school in South Jakarta, Indonesia, in November and December, 2019. We included children of 6–12 years of age with no history of acute respiratory infection or antibiotic use in the preceding 2 weeks. We excluded patients with a history of acute rheumatic fever or rheumatic heart disease. We also collected data on possible predictive factors including tonsillar enlargement, cervical lymphadenopathy, economic status, nutritional status, number of siblings, gender, number of people living in the same house, housing condition, and mother's education. The study was reviewed and approved by the Ethics Committee of Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia (Number: 1066/UN2.F1/ETIK/PPM.00.02/2019). We obtained an informed consent from all parents or guardians, before data and sample collection.

Specimen collection

Throat swab specimen was collected from every eligible child by trained medical staff. The swabs were placed in Amies Transport Media and sent on wet ice directly to the bacteriology laboratory at the Eijkman Institute for Molecular Biology, Jakarta, Indonesia. The swabs were transferred to skim milk tryptone glucose glycerol (STGG) media and stored at −80°C until analysis. We collected 6 ml of blood from every participant for complete blood count, erythrocyte sedimentation rate, antistreptolysin O (ASO) titer, and C-reactive protein at the Clinical Pathology Laboratory, Dr. Cipto Mangunkusumo Hospital, Jakata, Indonesia.

Isolation and identification of GAS

Throat swab in STGG media was thawed and vortexed. Two hundred microliter of swab inoculated STGG was inoculated into 5.0 ml THY broth (containing 2 μg/ml trimethoprim and 38 μg/ml sulfamethoxazole to inhibit the normal respiratory microbiota), and 1 mL rabbit serum was incubated at 37°C, for 5 h. 10 μl of cultured broth was inoculated and streaked onto blood agar plate at aerobic 37°C, for 24–48 h.[14] Cases with a presumptive GAS colony (white-grayish-colored colonies, having a diameter of >0.5 mm, and surrounded by a zone of β-hemolysis two to four times as large as the colony diameter) were subcultured on blood agar plate for bacitracin test, oxidase test, and Gram-staining.[14] To ensure that these beta-hemolytic isolates were not part of the staphylococcus group, culture tests on Mannitol Salt Agar (MSA) media were also carried out. The isolates confirmed as *Streptococcus* by Gram test was stored in STGG media at −80°C for further analysis.

Antibiotic sensitivity testing

Antibiotic sensitivity testing of GAS was performed by following Clinical and Laboratory Standards Institute (CLSI) guideline.[15] Pure and freshly grown culture was added into 5 mL of Mueller Hinton Broth in glass tube, and the turbidity was adjusted to 0.5 McFarland standard. Sterile cotton swab was dipped into suspension and pressed gently onto the wall of glass tube to remove excess fluid. The cotton swab was cultured onto Mueller Hinton Agar plate with 5% sheep blood. Antibiotic disks (Oxoid) of erythromycin, azithromycin, vancomycin, clindamycin, chloramphenicol, and tetracycline were placed onto the agar. Inoculated media were incubated in 37°C with 5% CO_2 for 20–24 h. Inhibition zone was measured and recorded. Sensitivity was determined according to breakpoints in CLSI guideline 2019. Antibiotic sensitivity testing by broth microdilution was performed for benzylpenicillin (Penicilin G) as per the recommendations of CLSI minimum inhibitory concentration breakpoint.[15]

Statistical analysis

Statistical analysis was performed using SPSS version 23 (IBM Corp., NY, USA). We considered $P < 0.05$ to be statistically significant. Data were expressed as median and range. We used Chi-square test or Fisher test for bivariate analysis. Independent variables with $P < 0.25$ were further evaluated with logistic regression analysis.

RESULTS

Two hundred and one throat swab samples were collected from school-age children in Jakarta. The baseline characteristics are described in Table 1. The age of the children was between 6 and 12 years with a median of 9 years. About 54.7% of participants were female (110/201). The laboratory tests revealed that the C-reactive protein (median), erythrocyte sedimentation rate (median), and leukocyte cell count (median) were 0.14 IU/ml, 12 mm, and 8260 cells/μL, respectively [Table 1]. We found GAS strains in 28 children. The prevalence of GAS carrier state was 13.9% (n = 28; confidence interval 95% 9.2%–18.6%) among school-age children. There were 50 children with elevated ASO titer out of whom, 9 children had a positive throat swab (9/50; 18%), as described in Table 2. Tonsillar enlargement was the only variable found to be predictive of GAS carriage on logistic regression analysis (P = 0.03). Other factors including cervical lymph node enlargement, economic status, nutritional status, number of siblings, gender, number of people in the house, house density, and mother's education were statistically insignificant, as reported in Table 3.

We found that all the GAS isolates were sensitive to penicillin G but they had varying degree of sensitivity to other antibiotics (erythromycin [89%], vancomycin [86%], clindamycin [75%], chloramphenicol [68%], azithromycin [68%], and tetracycline [32%]).

DISCUSSION

In this study, we found the proportion of GAS carrier was 13.9%, in concurrence with other studies, wherein it ranged from 10.9% to 16%.[6-9] The reason for the concurrence can be the similarity of the sociodemographic and economic background of our study to others. To our knowledge, our study is an important contribution to the literature from South East Asia region.[12]

The prevalence of carrier state has remained unchanged over the last two decades, especially in the developing countries. GAS infection commonly occurs during the fall and winter seasons,[16] but Indonesia is a tropical country with only two seasons (rainy and dry season). The study was conducted from November to December (rainy season) and therefore could have resulted in overestimation of the prevalence of GAS carrier state.

We attempted to determine the factors predictive of the GAS carrier state. Most of the children in our study belonged to low socioeconomic strata and lived in a crowded environment. Of all the factors studied, only tonsillar enlargement was a statistically significant predictor. In contrast to other studies which enrolled children between 15 and 18 years old, we recruited children between 6 and 12 years of age.[10]

Colonization of pharyngeal GAS is influenced by the crowded environment and frequency of GAS exposure. Gender and nutritional status were not predictive of the GAS carrier state as is reported in other studies.[7,9,10,17-19] Most of our participants had number of siblings <3, and the median number of siblings was one. In contrast to other studies, we did not find housing conditions and mother's education to be predictive of the carrier state.[6,8,10] Majority of our participants lived in house <9 msq but with good ventilation that can reduce GAS exposure.

We included only asymptomatic participants in the study. We also evaluated ASO level in all participants. There were nine children with elevated ASO level and positive GAS pharyngeal swab caused by prior GAS infection. The other 41 children also had elevated ASO level but negative GAS pharyngeal swab which was also caused by prior GAS infection. Elevated ASO level can be traced 1 week after GAS infection and reached a peak level in 3–5 weeks.[20]

To date, penicillin is the drug of choice for GAS infection and has excellent sensitivity.[21,22] We also tested sensitivity of other antibiotics toward GAS. As reported by other studies,[21-23] our study also demonstrated excellent sensitivity of penicillin toward GAS. This can be explained by inability of GAS to produce beta-lactamase, the absence of low-affinity penicillin-binding protein, and the absence of a genetic transfer mechanism that causes resistance.[24] Nevertheless, there is a growing number of antibiotic resistance to GAS, especially for erythromycin and clindamycin.[22,25] In this study, erythromycin still had good sensitivity (89%) but

Table 1: Baseline characteristics of patients

Characteristics	Total participants (n=201)
Gender, *n* (%)	
Male	91 (45.3)
Female	110 (54.7)
Age, median (range) years	9 (6-12)
Weight, median (range) kg	30 (15-79)
C-reactive protein, median (range) IU/mL	0.14 (0.1-25)
Erythrocyte sedimentation rate, median (range) mm in 1 hr	12 (2-65)
Leukocyte cells count, median (range)/uL	8260 (1120-16,980)

Table 2: Result of antistreptolysin O testing in the study population

	GAS carrier state		
	Yes (n=28)	No (n=173)	n=201
ASO (+)	9	41	50
ASO (–)	19	132	151

ASO: Antistreptolysin O, GAS: Group A β-hemolytic *Streptococcus*

Table 3: Bivariate and multivariate analysis of factors predictive of Group A β-hemolytic *Streptococcus* carrier state

Variable	GAS carrier		Unadjusted OR (95% CI)	*P*#	Adjusted OR (95% CI)	*P*##
	Yes (*n*=28)	No (*n*=173)				
Tonsillar enlargement, *n* (%)						
Enlarged	21 (75)	92 (53.2)	2.6 (1.1-6.5)	0.03	2.6 (1-6.5)	0.03
Normal	7 (25)	81 (46.8)				
Cervical lymphadenopathy, *n* (%)						
Yes	0	1 (0.5)		1*	-	-
No	28 (100)	172 (99.5)				
Gender, *n* (%)						
Female	14 (50)	96 (55.5)	0.8 (0.4-1.8)	0.58	-	-
Male	14 (50)	77 (44.5)				
Number of people living in the same house (people), *n* (%)						
>5	13 (46.4)	55 (31.8)	1.8 (0.8-4.2)	0.12	1.6 (0.7-3.8)	0.21
<5	15 (53.6)	118 (68.2)				
Nutritional status, *n* (%)						
Malnutrition	6 (21.4)	48 (27.2)	0.7 (0.2-1.8)	0.48	-	-
Normal-overweight	22 (78.6)	125 (72.8)				
Economic status, *n* (%)						
Low	16 (57.1)	114 (65.9)	0.6 (0.3-1.5)	0.37	-	-
Middle-high	12 (42.9)	59 (34.1)				
Housing condition (msq), *n* (%)						
<9	24 (85.7)	139 (80.3)	1.4 (0.4-4.5)	0.5	-	-
>9	4 (14.3)	34 (19.7)				
Mother's education, *n* (%)						
Low	7 (25)	43 (24.9)	1 (0.4-2.5)	0.98	-	-
Middle-high	21 (75)	130 (75.1)				
Number of sibling in the house (person), *n* (%)						
>3	5 (17.9)	27 (15.6)	1.1 (0.4-3.3)	0.76	-	-
<3	23 (82.1)	146 (84.4)				

#Bivariate analysis: Chi-square test, *Fisher's exact test, ##Logistic regression. GAS: Group A β-hemolytic *Streptococcus*, OR: Odds ratio, CI: Confidence interval

clindamycin had fair sensitivity (75%). We also found increased resistance of GAS to tetracycline (68%) as compared to other studies.[26-28] Antibiotic regulation in a country may determine its sensitivity pattern.

There were several limitations of this study. First, we performed a single measurement of the ASO blood level. We did not evaluate the change in the ASO titer subsequently. Second, there was no tracing of GAS infection in other family members, therefore, we could not estimate the transmission rate of GAS. Third, we did not do M-serotyping of GAS.

CONCLUSIONS

The GAS carrier state is common among school-age children affecting approximately 13.9%. Tonsillar enlargement is a significant finding predictive of GAS carrier state. Penicillin G is the drug of choice for GAS infection with excellent sensitivity. Erythromycin still shows low resistance toward GAS, but there is higher resistance to tetracycline.

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Conflicts of interest

There are no conflicts of interest.

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