

# Study of streptococcal antibody (anti-streptolysin O) among healthy children in Bangladesh

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Santosh Kumar Saha<sup>1</sup> , Kamrun Nahar Choudhury<sup>2</sup>,  
Salma Zareen<sup>2</sup>, Sabrina Mousum<sup>3</sup>, Mohammad Abdullah Al Mamun<sup>4</sup>  
and M Atiqul Haque<sup>3</sup>

## Abstract

**Objectives:** The standard reference value of anti-streptolysin O (ASO) titre for diagnosing acute rheumatic fever (RF) in children is yet to be determined in Bangladesh. We aimed to measure the upper limit of the normal (ULN) of ASO titre among healthy children to determine the cut-off value for the diagnosis of RF in Bangladesh.

**Methods:** A total of 400 healthy children aged 5–15 years with no history of fever, sore throat and impetigo during the last 8 weeks of the study were enrolled. The respondents were randomly selected from an urban non-slum area, an urban slum area and a rural area of Bangladesh. ASO titre was measured using a turbidimetric immunoassay based on the principle of an agglutination reaction. The 80th percentile value was considered as the ULN of ASO titre.

**Results:** Approximately 55% of the children were male. The mean (SD) age of children was 9.1 (2.7) years. The ULN of ASO titre for 5–15 years aged children was 217.4 IU/mL.

**Conclusion:** Our reference value of ASO titre at the 80th percentile will be an essential guide for clinicians to diagnose acute RF.

## Keywords

Anti-streptolysin O, upper limit of normal, children, rheumatic fever, Bangladesh

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## Introduction

Rheumatic fever (RF) is a common public health concern in Bangladesh, like other developing countries.<sup>1</sup> Diagnosis of RF is based on modified Jones criteria with recent evidence of group A beta-haemolytic streptococcal sore throat confirmed by a positive throat culture or a rapid streptococcal antigen test or an elevated or rising streptococcal antibody titre.<sup>2</sup> However, it is not always possible to identify the organism from the throat of suspected patients. Several antibodies like anti-streptolysin O (ASO) and anti-deoxyribonuclease B (ADNase B) are produced in response to group A beta-haemolytic streptococcal infection. Measurement of these antibodies against extracellular antigens of group A beta-haemolytic streptococci is necessary to confirm a recent infection where ASO titre is the most commonly used test.<sup>2</sup>

Empirical studies suggest that factors like age, geographical area, frequency of streptococcal infections and nutritional status of children might influence the ‘normal’ reference

level of the ASO titre in a country.<sup>3,4</sup> Several studies have suggested that the upper limit of normal (ULN) of ASO titres be calculated using the 80th percentile rather than 2 SD from the mean, as value beyond this ULN detect 80%–90% of patients with acute RF.<sup>2,5</sup> An increased level of ASO titres is related to the recent history of pharyngitis due to group A

<sup>1</sup>National Institute of Cardiovascular Diseases and Hospital, Dhaka, Bangladesh

<sup>2</sup>National Center for Control of Rheumatic Fever and Heart Disease, Dhaka, Bangladesh

<sup>3</sup>Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

<sup>4</sup>National Heart Foundation Hospital and Research Institute, Dhaka, Bangladesh

### Corresponding author:

Santosh Kumar Saha, Department of Pediatric Cardiology, National Institute of Cardiovascular Diseases and Hospital, Sher-E-Bangla Nagar, Dhaka 1207, Bangladesh.

Email: santu2002@gmail.com



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beta-haemolytic streptococcal infection. ASO titre remains low in healthy children during early life. As pharyngitis is common during childhood, an increasing ASO titre trend is observed between 5 and 15 years of age, which gradually declines with age and maintains a flat curve in adulthood.<sup>5</sup> According to child's age, the cut-off point of ASO titre has been specified by ULN in different studies from developed and developing countries.<sup>5-8</sup>

There is a lack of quantitative country representative data on the ULN of ASO titre for healthy children in Bangladesh. Rouf et al.<sup>9</sup> reported the normal ASO level of urban school children of Bangladesh using a semiquantitative laboratory method, whereas Zaman et al.<sup>10</sup> determined the ULN value of ASO titre of children quantitatively in a selected rural area in Bangladesh. Therefore, a mixed population (urban, rural and slum) based country representative data on ULN of ASO titre is yet to be determined in Bangladesh. The aim of this study was to estimate the ULN of ASO titre in children aged 5–15 years in Bangladesh.

## Methods

### Study area and sample

This cross-sectional study was conducted at purposively selected one urban non-slum, one urban slum and one rural area of Dhaka, the capital of Bangladesh. Sher-e-Bangla Nagar Thana (subdistrict) was selected as an urban non-slum area, Kalyanpur Porabari slum was selected as an urban slum area and Dhamsona Union (lowest administrative unit) of Ashulia Thana (subdistrict) was selected as a rural area. Four educational institutions for children, two from rural areas, one from urban slum areas and one from urban non-slum areas were randomly selected from a list of schools. Data were collected from March to May, 2012.

The sample size was calculated based on  $N = z^2 pq / d^2$ , where  $z = 1.96$ ,  $p = 0.5$ ,  $q = 0.5$ ,  $d(\text{precision}) = 0.045$  and non-response rate = 7%. Therefore, the sample size was  $507 \approx 510$ . This sample was equally allocated to both urban and rural areas. Thus, we approached 510 children in order to acquire primary data. However, parents/caregivers of 400 children finally consented to a blood test for their children for ASO titre, where 238 children were from rural, 92 children were from urban non-slum and 70 children were from urban slum areas. As a result, the dropout rate was 22%.

### Selection criteria

**Inclusion criteria:** Data were collected from 5- to 15-year-old school children from urban, urban slum and rural area as RF is common between 5 and 15 years of age.<sup>11,12</sup> A detailed examination of each child was done during school hours.

**Exclusion criteria:** Children with a history of acute RF, rheumatic heart disease (RHD), congenital heart disease,

fever  $>38^\circ\text{C}$ , recent history of throat infections or impetigo/skin sores (within 8 weeks), children who had received antibiotic treatment for any infection 4 weeks prior to enrolment, acute infections and inflammatory disorders, and so on were excluded. Children having tonsillopharyngitis were excluded as ASO titre rises within 1 week following the infection, peaks at 3–5 weeks and declines after 8 weeks.<sup>13</sup>

### Data collection procedure

Based on literature reviews,<sup>5,7-10</sup> a semi-structured questionnaire was developed and finalized after a consultative meeting with public health professionals, paediatric cardiologists and paediatricians. Prior to data collection, this questionnaire was pretested with 10 children (4% of final sample population) to determine its suitability for data collection.

Data were collected through face-to-face interviews with the parent or caregivers of the children. A detailed history, including sore throat beyond 8 weeks and results of blood tests, such as complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and ASO titre, were noted in the datasheet. A phlebotomist collected 5 mL of blood aseptically from each child's antecubital vein. Blood specimens were immediately stored in a cool box and transported to the laboratory of the National Center for Control of Rheumatic Fever and Heart Disease, Dhaka, on the same day. Blood samples were then centrifuged, and the serum was stored at  $2^\circ\text{C}$ – $8^\circ\text{C}$ . ASO titre was measured by the auto-analyzer (HUMALYZER-3000, Model no. 3500 of HUMAN GmbH D-65205, Wiesbaden, Germany) using a turbidimetric immunoassay based on the principle of an agglutination reaction. The test specimen was mixed with Quantia-ASO (Manufactured by Tulip Diagnostics Pvt. Ltd., Goa, India) latex reagent (R2) and activation buffer (R1) and was allowed to react. The presence of ASO in the test specimen results in the formation of an insoluble complex increasing turbidity, which is measured at wavelength 505–578 nm. The increase in turbidity corresponds to the concentration of ASO in the test specimen.

### Statistical methods

The 80th percentile values of ASO titre were considered to determine the cut-off value of ULN. This method has been adapted from previous studies.<sup>5,13</sup> The Statistical Package for Social Sciences (SPSS) version 16 for windows was used to analyze data.

### Ethical issues

Ethical approval was obtained from the Ethical Review Committee of the National Center for Control of Rheumatic Fever and Heart Diseases, Dhaka (memo no. 2011/2-1).

**Table 1.** Distribution of children according to laboratory findings.

Lab findings	Age group 5–9 years (n=226)	Age group 10–15 years (n=174)	p-Value
Total count of WBC (K/cu mm)	8358.85 ± 1891.93	8357.47 ± 1705.52	0.994*
Hb (g/dL)	12.8 ± 1.41	12.12 ± 1.72	0.089
ESR (mm/h)	18.23 ± 11.28	17.43 ± 9.41	0.448
Mean ASO (IU/dL)	150.37 ± 80.57	161.02 ± 82.92	0.196
CRP (mg/L)	4.53 ± 1.03	4.55 ± 1.01	0.854

WBC, white blood cells; Hb, haemoglobin; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

\*The t-test was done to measure the level of significance.

**Table 2.** ASO titre at 80th percentile.

Children	N	All age group	5–9 years	10–15 years
All children	400	217.4	215.5	219.9
Urban non-slum children	92	250.6	244.98	255.52
Urban slum children	70	280.1	288.10	268.22
Rural children	238	196.9	192.68	198.78

Formal approval was taken from the school authority for conducting this study in the premises of the institutions. We obtained written consent from the parents or caregivers for their children's participation in this study.

## Results

Approximately 55% of the children were male. The mean (SD) age of children was 9.1 (2.7) years. The total count, haemoglobin level, ESR and CRP levels of all children were within the normal range (Table 1).

The average ASO titre at the 80th percentile for all children was 217.4 IU/mL. The ASO titre at the 80th percentile was 215.5 IU/mL for 5–9 years and 219.9 IU/mL for 10–15 years aged children (Table 2).

Significant variations in ASO titre were found between all children from urban slum with rural area (mean, SD: 191.52, 87.77 and 133.26, 72.57,  $p$ -value=0.001) and urban with rural area (mean, SD: 183.80, 80.64 and 133.26, 72.57,  $p$ -value=0.001). Figure 1 also shows the mean ASO titre with a 95% confidence interval (CI) in different age groups of children from urban, rural and urban slum areas.

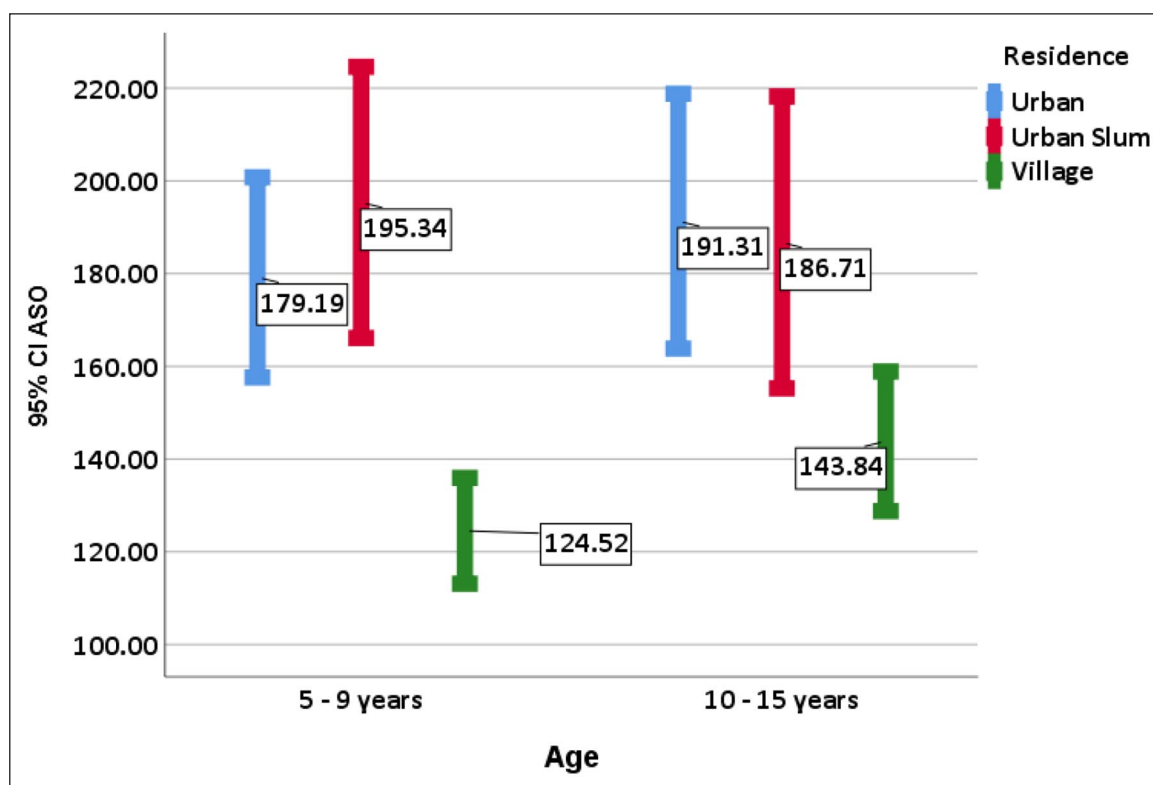
Children with a history of sore throat beyond 8 weeks had a significantly higher ASO titre than children without a history of sore throat (mean, SD: 176.05, 88.30 versus 139.26, 72.33,  $p$ -value=0.001). The 80th percentile of ASO titre was 253.36 IU/mL in those with a history of sore throat and 200.18 IU/mL in those who did not (Table 3).

## Discussion

The majority of the studies on the ULN of ASO titres recruited healthy volunteers.<sup>5,13–15</sup> In conjunction with the updated Jones criterion, the ASO titre aids in the final diagnosis of acute RF.<sup>2</sup> Therefore, the ASO titre is a routinely

used standardized test for detecting recent group A streptococcal (GAS) infection. More than 80% acute RF patients have elevated ASO titres.<sup>2,5</sup> To determine an increasing ASO titre, serum should be obtained and examined concurrently during the acute and convalescent phases. However, this is not always possible in a resource-scarce country like Bangladesh. Thus, a predetermined baseline value or ULN for ASO titre is needed to aid the diagnosis of acute RF.

The ASO titre can vary depending on the study population, geographic location, age group and seasonal variations.<sup>5,16</sup> In our study, ULN of ASO titre was found 217.4 IU/mL for children aged 5–15 years, comparable with an Indian study conducted by Sethi et al.<sup>8</sup> where ULN of ASO titre was found 239 IU/mL. Studies from developed countries reported a ULN of ASO titre between 170 and 276 IU/mL, which is similar to the findings of our study.<sup>5,13</sup> On the contrary, other studies from India, Bangladesh, Australia, Korea, USA and Egypt found the ULN of ASO titre with a range from 305 to 395 IU/mL, which is higher than that of our findings.<sup>7,10,17–19</sup> Most of these values exceeded the normal level of ASO titre set by laboratories ( $\leq 200$  IU/mL).<sup>14</sup> Okello et al. found a peak ASO titre in 5- to 14-year-old children (389 IU ULN) in their study, which is substantially higher than ours. However, they excluded anyone with a history of RF, RHD or invasive GAS illness, as well as anyone who had a sore throat or skin sore in the previous 14 days.<sup>20</sup> Our study excluded children with tonsillopharyngitis within 8 weeks because ASO titre rises within 1 week of infection, peaks at 3–5 weeks and then drops after 8 weeks. Our recommended ULN for ASO titre is less than 390 IU/mL reported in a prior Bangladeshi study by Zaman et al.<sup>10</sup> The decreased level of ASO titre ULN in the present study could be attributed to a substantial decline in streptococcal infection in our country.<sup>21</sup> Significant social and economic progress has occurred in Bangladesh during the last three decades, and indicators of human development,



**Figure 1.** Error diagram shows mean (95% CI) of ASO titre by children's age and residence.

**Table 3.** Association of ASO titre in children with a history of previous sore throat.

ASO titre	History of previous sore throat (beyond 8 weeks)		p-Value
	Yes (n = 172)	No (n = 228)	
80th percentile	253.36	200.18	>0.001
Mean (SD)	176.05 (88.30)	139.26 (72.33)	

The t-test was done to measure the level of significance.

such as poverty, hunger and inadequate sanitary conditions, all of which are risk factors for streptococci infection, have exhibited favourable developments.<sup>22–24</sup> Furthermore, frequent antibiotic usage for sore throat may be responsible for the decrease in ASO value.

Children of slum and urban areas showed higher ASO titres in our study than children of rural areas. Shet et al.<sup>16</sup> revealed that areas with endemic streptococcal infections have higher levels of typical streptococcal antibody titres. Mberu et al.<sup>25</sup> showed that the indicators for mortality and morbidity were worse in urban slums than in rural areas in Bangladesh. Therefore, we can assume that streptococcal infections rate is more in urban slum areas than in rural areas, which may explain why children in urban slums and urban non-slum areas had higher ASO titres than children in rural areas.

In our study, children with a history of sore throat had a considerably higher level of ASO titre. The 80th percentile of ASO titre was 253.36 IU/mL among those who had a

previous history of sore throat beyond 8 weeks, and it might be due to the frequent streptococcal throat infection. Kotby et al.<sup>21</sup> showed significantly higher ASO titre in children with a history of sore throat more than three times per year.

In our study, we did not find any significant differences of the ULN of ASO titre between the age group 5–9 years and 10–15 years ( $p$ -value > 0.05). This finding is comparable with studies conducted by Steer et al.<sup>13</sup> in Fiji Island and Saini et al.<sup>26</sup> in India. However, Danchin et al.<sup>17</sup> and Kotby et al.<sup>21</sup> showed an increasing trend of the ULN of ASO titre with age.

The limitation of this study was that we only used ULN of ASO titre because ADNase B was expensive and was not available in the study area. We did not investigate the presence of GAS by throat swab culture to detect healthy carriers in this study. Carrier asymptomatic (healthy) children may have a higher ASO than non-carrier healthy children.<sup>27–29</sup> However, it is not always possible to identify the organism



from the throat swab, and it is also expensive for resource-poor countries like Bangladesh. The data presented in this study are 10 years old, so the findings of this study should be compared to current data. These are also shortcomings of our research.

## Conclusion

The ASO titre 217.4 IU can be considered as the ULN among children aged 5–15 years in Bangladesh. Because there is no standard reference value for ASO titre in healthy Bangladeshi children, this 80th percentile cut-off value will be an essential guide for diagnosis of acute RF.

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## Author contributions

All authors contributed equally towards the development of this manuscript.

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Ethical approval

Ethical approval for this study was obtained from Ethical Review Committee of National Center for Control of Rheumatic Fever and Heart Diseases, Dhaka, Bangladesh (approval number, 2011/2-1\*).

## Informed consent

Written informed consent was obtained from the parents or caregivers for their children's participation in this study.

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## ORCID iD

Santosh Kumar Saha  <https://orcid.org/0000-0002-5402-3414>

## Supplemental material

Supplemental material for this article is available online.

## References

1. Bavdekar SB, Soloman R and Kamat JR. Rheumatic fever in children. *J Indian Med Assoc* 1999; 97(12): 489–492.

2. Guidelines for the diagnosis of rheumatic fever. Jones Criteria, 1992 update. Special Writing Group of the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young of the American Heart Association. *JAMA* 1992; 268(15): 2069–2073.
3. Johnson RD, Kaplan EL, Sramek J, et al. Laboratory diagnosis of group A streptococcal infections. Geneva: World Health Organization, 1996, <https://apps.who.int/iris/handle/10665/41879>
4. Zaman MM, Yoshiike N, Ahmed J, et al. Humoral immune response in a sample of Bangladeshi children with rheumatic fever. *Bangladesh Med Res Counc Bull* 1999; 25(2): 42–45.
5. Kaplan EL, Rothermel CD and Johnson DR. Antistreptolysin O and anti-deoxyribonuclease B titers: normal values for children ages 2 to 12 in the United States. *Pediatrics* 1998; 101(1 Pt 1): 86–88.
6. Blyth CC and Robertson PW. Anti-streptococcal antibodies in the diagnosis of acute and post-streptococcal disease: streptokinase versus streptolysin O and deoxyribonuclease B. *Pathology* 2006; 38(2): 152–156.
7. Karmarkar MG, Venugopal V, Joshi L, et al. Evaluation & reevaluation of upper limits of normal values of anti-streptolysin O & anti-deoxyribonuclease B in Mumbai. *Indian J Med Res* 2004; 119 Suppl: 26–28.
8. Sethi S, Kaushik K, Mohandas K, et al. Anti-streptolysin O titers in normal healthy children of 5–15 years. *Indian Pediatr* 2003; 40(11): 1068–1071.
9. Rouf MA, Ahmed J, Hassan MMM, et al. Study on streptococcal antibodies among school children of Dhaka city. *Chest Heart Bull*.1992; XVI: 55-58.
10. Zaman MM, Hassan MM, Ahmed J, Zareen S, Jalil MQ, Eshaque N, et al. Streptococcal antibodies among rural school children in Bangladesh. *Bangladesh Med Res Counc Bull* 2002; 28(1): 1–6.
11. Ahmed J, Mostafa Zaman M and Monzur Hassan MM. Prevalence of rheumatic fever and rheumatic heart disease in rural Bangladesh. *Trop Doct* 2005; 35(3): 160–161.
12. Haque KMHS, Hossain M and Mahmud RS. Epidemiology of rheumatic fever and rheumatic heart disease: observations in 15798 children of rural and urban areas. In: Faruq QO (ed.) *Proceedings of first national scientific conference on rheumatic fever and rheumatic heart disease*, Dhaka, Bangladesh, 4–5 May 1992, pp. 31–32. Dhaka: Ministry of Health and Family Welfare, Government of the People's Republic of Bangladesh and Japan International Cooperation Agency (JICA), Government of Japan, 1992.
13. Steer AC, Vidmar S, Ritika R, et al. Normal ranges of streptococcal antibody titers are similar whether streptococci are endemic to the setting or not. *Clin Vaccine Immunol* 2009; 16(2): 172–175.
14. Format Asfaw T, Shenkute D, Tilahun M, et al. Antistreptolysin O titres: normal values for children ages 5 to 15 at Debre Berhan Referral Hospital, Ethiopia. *J Family Med Health Care* 2018; 4(1): 1–4.
15. Sanjeevaiah CRP, Ahamed MM and Mariyappa BD. Prediction of upper limits of normal values of anti-streptolysin 'O' titres in normal healthy school going children of 6–16 years. *Am J Pediatr* 2019; 5: 74–77.

16. Shet A and Kaplan EL. Clinical use and interpretation of group A streptococcal antibody tests: a practical approach for the pediatrician or primary care physician. *Pediatr Infect Dis J* 2002; 21(5): 420–426.
17. Danchin MH, Carlin JB, Devenish W, et al. New normal ranges of antistreptolysin O and antideoxyribonuclease B titres for Australian children. *J Paediatr Child Health* 2005; 41(11): 583–586.
18. Kim S and Lee NY. Asymptomatic infection by *Streptococcus pyogenes* in schoolchildren and diagnostic usefulness of antideoxyribonuclease B. *J Korean Med Sci* 2005; 20(6): 938–940.
19. Khattab AA, Bahbah WA, Abou-Elela DH, et al. Determine upper limit of normal range of antistreptolysin-O titre in normal school children from 5 to 15 years old. *Menoufia Med J* 2021; 34: 2049.
20. Okello E, Murali M, Rwebembera J, et al. Cross-sectional study of population-specific streptococcal antibody titres in Uganda. *Arch Dis Child* 2020; 105(9): 825–829.
21. Kotby AA, Habeeb NM, Ezz El and Elarab S. Antistreptolysin O titer in health and disease: levels and significance. *Pediatr Rep* 2012; 4(1): e8.
22. Zaman MM, Yoshiike N, Chowdhury AH, et al. Nutritional factors associated with rheumatic fever. *J Trop Pediatr*. 1998; 44(3): 142–147.
23. Riaz BK, Selim S, Karim MN, et al. Risk factors of rheumatic heart disease in Bangladesh: a case-control study. *J Health Popul Nutr* 2013; 31(1): 70–77.
24. Human development report-Bangladesh: United Nations Development Programme. <https://hdr.undp.org/data-center/specific-country-data/#/countries/BGD> (2020, accessed 17 June 2022).
25. Mberu BU, Haregu TN, Kyobutungi C, et al. Health and health-related indicators in slum, rural, and urban communities: a comparative analysis. *Glob Health Action* 2016; 9: 33163.
26. Saini N, Kumar D, Swarnim S, et al. Comparison of anti-streptolysin O and anti-deoxyribonucleic B titers in healthy children to those with acute pharyngitis, acute rheumatic fever, and rheumatic heart disease aged 5–15 years. *Ann Pediatr Cardiol* 2019; 12(3): 195–200.
27. Abdissa A, Asrat D, Kronvall G, et al. Throat carriage rate and antimicrobial susceptibility pattern of group A streptococci (GAS) in healthy Ethiopian school children. *Ethiop Med J* 2011; 49(2): 125–130.
28. Bennett J, Moreland NJ, Williamson DA, et al. Comparison of group A streptococcal titres in healthy children and those with pharyngitis and skin infections. *J Infect* 2022; 84(1): 24–30.
29. Ozturk CE, Yavuz T, Kaya D, et al. The rate of asymptomatic throat carriage of group A streptococcus in schoolchildren and associated ASO titers in Duzce, Turkey. *Jpn J Infect Dis* 2004; 57(6): 271–272.