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Objectives: The study is aimed to estimate prevalence and factors associated with the same among school children aged 6–12years.

Materials and Methods: This cross-sectional survey was carried out from February to July 2010, in the diagnostic laboratory of Microbiology department. The study group was divided into four groups, namely, Group A; Group B; Group C; and Group D. A total of 1769 eligible children were enrolled for sampling of these schools. For each enrolled child in the study, a standard culture and antibiogram test with the Lancefield grouping technique was done in the assessment of the outcome.

Results: Among 1769 participants, 1029 (58.2%) were boys and 740 (41.8%) were girls. The overall prevalence of GABHS was estimated 27.9%. Group A 35.62%, Group B 37.5%, Group C 34.8%, and Group D 23.7%.

Keywords : *antibiogram, factors associated, GABHS, prevalence, school children.*

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The sensitivity was highest for vancomycin 100% and the resistance was highest for amoxicillin 79.1%.

Conclusions: The study confirmed that the prevalence of GABHS is 27.9%. Overall prevalence shows an endemic situation. Therefore, it is recommended that local health sectors should make provision for regular screening and prophylaxis.

Keywords : antibiogram, factors associated, GABHS, prevalence, school children.

I. Introduction

The prevalence of GABHS carriage in throat of normal asymptomatic school children varies from 13-15% depending upon the population studied, season and other factors.[1]

The GABHS has remained a significant human pathogen for centuries. This organism causes a wide variety of infections in humans, ranging from mild upper respiratory tract and skin infections to severe suppurative and invasive conditions like necrotising fasciitis and toxic shock syndrome. [2] of major concern is that post infectious sequelae like acute rheumatic fever (ARF) and post-streptococcal glomerulonephritis continue to occur worldwide despite efforts of clinicians, scientists and public health officials to comprehend their pathogenesis and devise ways of disease control. [2]

It is estimated that approximately 7 sore throat episodes per year, with 13.5% of these being caused by GABHS. [2]

Although ARF and rheumatic heart disease (RHD) have declined in many parts of the world, they continue to be a major cause of cardiovascular morbidity and mortality in India.[2] Sore throat caused by GABHS is one of the most common diseases during adolescence and early adulthood which makes a lot of problems and consumes a great budget for its treatment and complications, all over the world.[3, 4, 5, 6]

II. Materials And Methods

a) Study design and setting

This cross-sectional survey was carried out from February to July 2010, in the diagnostic laboratory of microbiology department.

b) Study population, sample size and sampling strategy

The study population consisted of school children from 6 to 12 years of age enrolled in various schools of Chitradurga. The sample size was calculated for the primary objective taking the prevalence to be 50% that gives the maximum sample size, with a 95% level of confidence and 5% bound on the error of estimation. The minimum sample size required was 400 children.

A list of all schools (the sampling frame) in the locality was prepared. They were divided into four groups, Namely, Group A (orphanage); Group B (residential schools); Group C (government schools); and Group D (private schools). The simple random sampling method was employed to select 1 school from each group of schools. A total of 1769 eligible children were enrolled for sampling of these schools. All of them were sampled, giving the response rate of 100%.

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c) Data Collection

The outcome variable was culture status of the received sample, whether positive or negative for GABHS parasite, which was determined from a throat swab. Data on the independent variable of socio-demographic characteristics were collected by trained research officers on a pretested and structured questionnaire addressed to the student.

d) Throat swab collection and laboratory testing

Apparently healthy children 6-12 years of age were studied. A team of doctors, technician and interns visited the school twice a week. A detailed history as per recommendation of the WHO for identification of streptococcal sore throat such as fever, soreness of throat, cough and watery nasal discharge were excluded.

For each enrolled child in the study, for the assessment of the outcome [7] the test was conducted at the diagnostic microbiology laboratory, by the principal investigator and co-investigator with the help of an experienced laboratory technician. The diagnosis of GABHS disease still relies on isolation of GABHS strains on sheep blood agar followed by presumptive identification based on bacitracin sensitivity on the results of more precise serogrouping methods such as the Lancefield grouping.

From the selected subjects the tonsillar and the pharyngeal mucosa were rubbed vigorously with sterile cotton swab applicator avoiding the surrounding tissues. Throat swabs were transported to the microbiology laboratory by using Todd Hewitt broth. Then swabs were plated onto crystal violet sheep blood agar, chocolate agar and MacConkey's agar. It was then incubated in a candle jar at 35®C for 18-24 hours, examined for the presence of GABHS and sub cultured onto blood agar for bacitracin sensitivity to differentiate GABHS from non GABHS. The isolates of GABHS were subjected for Lancefield grouping as per the manufacturer's instructions with the kit.

After diagnosis of GABHS was confirmed, antibiogram test was done for the isolates according to recommendations of the National Committee for Clinical Laboratory standards (NCCLS) using Cephalothin, erythromycin, tobramycin, tetracycline, amoxicillin, co-trimoxazole, vancomycin, Cloxacillin and penicillin antibiotic discs from HiMedia Laboratories Pvt. Ltd. Mumbai.

III. Ethical Clearance

The protocol for this study was approved by the Chairman, and the secretary, institutional ethical committee (IEC). The approval was in the agreement that patient anonymity must be maintained, good laboratory practice quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only, all work was performed according to the international guidelines for human experimentation in biomedical research.[8] Approval was obtained from the Head Master of each school studied and informed consent was obtained from each of the participating pupils. The participating students were given chocolates as an incentive. Culture positive subjects were referred to the primary health center in the area for immediate treatment.

a) Data management and statistical analysis

During data collection completed questionnaires were checked regularly to rectify any discrepancy, logical errors, or missing values. The data entry was carried out using Microsoft Office Excel worksheet and then the data were exported to statistical analysis software WINK SDA for further analysis. Variables were categorized in a biologically meaningful way where applicable. To the analyzed data, mean and standard deviation for continuous variables and proportion for categorical variables were computed. Crude associations of the binary outcome variable with each independent variable were assessed by the Chi - square test. The level of statistical significance was set as $P \leq 0.05$ and for each statistically significant factor.

Results: The results represent information collected from 1769 Throat swabs.

b) Descriptive Characteristics

Among 1769 participants, 1029 (58.2%) were boys and 740 (41.8%) were girls.

c) Prevalence of GABHS

The overall prevalence of GABHS was estimated as 493/1769 (27.9%) [Table 1]. Group A 35.62% (26/73), Group B 37.5% (51/136), Group C 34.8% (187/588), and Group D 23.7% (230/972). Higher prevalence was found in 6-10 years aged; the prevalence percentage declined with increase in age and showed lowest prevalence in subjects above 10 years of age. The prevalence was higher in boys as compared with girls. [Tables 2-5].

d) The resistance pattern of GABHS

The sensitivity was highest for vancomycin (100%) followed by tobramycin 77.1%, Cephalothin 74% and erythromycin 69.6%. The resistance was highest for amoxicillin 79.1% followed by penicillin 76.4%, Cloxacillin 74.6% and Gentamicin 70%. [Table-7]

The factors associated with the GABHS prevalence and carrier rate were estimated as in [Table 8].

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Table 1 : Aisolation of Gabhs from School Children

SEX	TOTAL NO.OF SPECIMENS PROCESSED	CULTURE POSITIVE	
		No.	%
Boys	1029	291	28.3
Girls	740	202	27.3
Total	1769	493	27.9

Table 2 : Group A (Basawamakkalu)

AGE	BOYS			GIRLS			TOTAL SAMPLED		
	TOTAL	POSITIVE		TOTAL	POSITIVE		TOTAL	POSITIVE	
		NO.	%		NO.	%		NO.	%
6-7	05	04	80	01	01	100	06	05	83.3
7-8	08	06	75	02	02	100	10	08	80
8-9	00	00	00	02	01	50	02	01	50
9-10	08	03	37.5	00	00	00	08	03	37.5
10-11	07	03	42.8	08	01	12.5	15	04	26.7
11-12	10	02	20	00	00	00	10	02	20
>12	16	02	12.5	06	01	16.7	22	03	13.6
TOTAL	54	20	37.03	19	06	31.6	73	26	35.6

Table 3 : Group- B (Orphanage)

AGE	BOYS			GIRLS			TOTAL SAMPLED		
	TOTAL	POSITIVE		TOTAL	POSITIVE		TOTAL	POSITIVE	
		NO.	%		NO.	%		NO.	%
6-7	15	12	80	07	05	71.4	22	17	77.3
7-8	10	06	60	08	04	50	18	10	55.5
8-9	12	06	50	10	03	30	22	09	40.9
9-10	12	04	33.3	07	03	42.8	19	07	36.8
10-11	14	04	28.6	05	01	20	19	05	26.3
11-12	11	01	9.1	03	00	00	14	01	07.1
>12	20	02	10	02	00	00	22	02	09.1
TOTAL	94	35	37.23	42	16	38.1	136	51	37.5

Table 4 : Group-C (Govt. School)

AGE	BOYS			GIRLS			TOTAL SAMPLED		
	TOTAL	POSITIVE		TOTAL	POSITIVE		TOTAL	POSITIVE	
		NO.	%		NO.	%		NO.	%
6-7	41	14	34.1	33	24	72.7	74	38	51.3
7-8	74	24	32.4	25	13	52	99	37	57.4
8-9	45	14	31.1	44	16	36.4	89	30	33.7
9-10	45	15	33.3	69	23	33.3	114	38	33.3
10-11	45	15	33.3	56	12	21.4	101	27	26.7
11-12	25	07	28	35	01	02.8	60	08	13.3
>12	29	09	31	22	00	00	51	09	17.6
TOTAL	304	98	32.2	284	89	31.3	588	187	31.8

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Table 5 : Group-C (Pvt. School)

AGE	BOYS			GIRLS			TOTAL SAMPLED		
	TOTAL	POSITIVE		TOTAL	POSITIVE		TOTAL	POSITIVE	
		NO.	%		NO.	%		NO.	%
6-7	88	24	27.3	59	14	23.7	147	38	25.8
7-8	85	24	28.2	58	16	27.6	143	40	27.9
8-9	84	23	27.4	58	16	27.6	142	39	27.5
9-10	86	21	24.4	60	14	23.3	146	35	24
10-11	82	19	23.2	56	13	23.2	138	32	23.2
11-12	77	17	22.1	53	11	20.7	130	29	22.3
>12	75	10	13.3	51	07	13.7	126	17	13.5
TOTAL	577	138	23.9	395	91	23.0	972	230	23.7

Table 6 : Table Showing the Total No.of Subjects Tested and Total No. of Gabhs Isolated

SEX	TOTAL NO.OF SPECIMENS PROCESSED	CULTURE POSITIVE	
		No.	%
GROUP-A	73	26	35.6
GROUP-B	136	51	37.5
GROUP-C	588	187	31.8
GROUP-D	972	230	23.7

Table 7 : Antibiotic Susceptibility of GABHS

ANTIBIOTICS	RESISTANCE	
	No.	%
Cephalothin (CF)	128	26
Erythromycin (E)	144	29.4
Tobramycin (T)	113	22.9
Tetracycline (Te)	241	48.9
Amoxicillin (Amx)	390	79.1
Co-Trimoxazole (S&T)	289	58.8
Vancomycin (Va)	0	0
Cloxacillin (Cx)	368	74.6
Gentamicin (Gm)	345	70
Penicillin (P)	376	76.4

Table 8 : Factors Associated with Prevalence of Gabhs

Variable	Determiner	Total no.	Positive	
			No.	%
Age	6-10	1061	355	33.5
	>10	708	139	19.6
Gender	Male	1029	291	28.3
	Female	740	202	27.3
Residence	Orphanage	73	26	35.6
	Minority	136	51	37.5
	Sub-urban	588	187	31.8
	Urban	972	230	23.7
Oral hygiene	Good	1263	216	17.1
	Bad	506	413	81.6
Care taker	Literate	1222	183	14.9
	Illiterate	547	246	44.9

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IV. Discussion

Healthy carriers of GABHS are sources for bacterial dissemination and are able to communicate the disease and even lead to severe epidemics.

There is evidence that asymptomatic throat infection caused by GABHS may lead to ARF.[9] Asymptomatic infections with subsequent rise in streptococcal antibody titers have also been reported in patients with previously diagnosed rheumatic fever (RF).[10]We observed that the prevalence of GABHS in the throat of asymptomatic school children was 27.9% which is in correlation with Koshi G et al [11], Prakash K et al. [11]

However, the prevalence of asymptomatic carriage of GABHS has been reported (11,12,13, 14,15,16,17,18) between 11-47% from various countries this could be attributed to various demographic factors.

In this study none of the school children showed the characteristic picture of streptococcal pharyngitis. This is not unusual, as in a 3 year study from Egypt. [19] none of the children had streptococcal exudative tonsillitis out of 1041 children examined.

The clinical picture alone is not reliable, as subclinical throat infection caused by GABHS is not uncommon. Also isolation of GABHS alone is not enough to suggest infection in asymptomatic children. Thus the demonstration of a rise in titer of antibodies against extracellular antigens of GABHS is essential for diagnosis of infection. [20]

a) Strength and Limitations

As per our knowledge this study is first of its kind in our locality focusing on school children of different living conditions. The throat swab testing by routine culture and Lancefield grouping method increased the validity of estimates.

Our study has certain limitations that need to be single throat swab examination for detection of GABHS, which could have underestimated the prevalence, as optimal laboratory diagnosis of GABHS requires the examination of at least two throat swabs collected over several days.

More recent studies have suggested throat culture is still considered as the 'gold standard' method for GABHS detection, giving results in about 90-95% of the cases and we have done the same.

However, Antibody studies are microbiologically important both for demonstration of GABHS pharyngitis as well as the clinical diagnosis of (RF/RHD).We were unable to perform antibody studies on subjects as we could not to get the written consent. This is one of the first few school survey studies carried out in this region and therefore, we wanted to develop a good rapport with the children before we could do intensive studies on them.

V. Conclusion

According to the present study, the streptococcal carrier rate in this area is comparable to the other studies in surrounding areas. Prevalence of GABHS from the throat of asymptomatic school children has been reported for the first time from this locality. Further epidemiological studies on this aspect are needed to substantiate the findings of our study.

In India, the streptococcal reference system has already been established and primary prevention method for RF and RHD, namely control of streptococcal infections is also going on. [21] To conclude, as methods of the streptococcal control program have now become cost effective, we strongly recommend such prevalence studies should be actively employed to prevent the high prevalence of GABHS pharyngitis and their sequelae, with special concern for children below 11 years of age.

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