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A one-year study of streptococcal infections and their complications among Ethiopian children

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SUMMARY

Post-streptococcal complications are known to be common among Ethiopian children. Little is known, however, about the epidemiology of beta-haemolytic streptococci in Ethiopia. A total of 816 children were studied during a one-year period: 24 cases of acute rheumatic fever (ARF), 44 chronic rheumatic heart disease (CRHD), 44 acute post streptococcal glomerulonephritis (APSGN), 143 tonsillitis, 55 impetigo, and 506 were apparently healthy children. Both ARF and APSGN occurred throughout the year with two peaks during the rainy and cold seasons. The female: male ratio among ARF patients was 1·4:1 and 1:1·9 among APSGN. The monthly carrier rate of beta-haemolytic streptococci group A varied from 7·5–39%, average being 17%. T type 2 was the most frequent serotype. Marked seasonal fluctuations were noted in the distribution of serogroups among apparently healthy children. Beta-haemolytic streptococci group A dominated during the hot and humid months of February–May. Strains were susceptible to commonly used antibiotics, except for tetracycline.

INTRODUCTION

Beta-haemolytic streptococci still remain a major public health problem despite their sensitivity to treatment with penicillin. Rheumatic fever was almost disappearing in the developed world and this was correlated with the improved standard of living and introduction of antibiotics. However, the resurgence of rheumatic fever in the USA since the mid 1980s, mostly among middle class people [1–4], and an increase of serious streptococcal infections in Europe observed in recent years [5–7] have proved that streptococcal infections are far from being controlled in the developed world.

The epidemiology and clinical patterns of streptococcal infections in the developed countries are well documented. In contrast, information from Africa is

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scanty [8–11]. The high prevalence rate of rheumatic heart disease reported from a few African countries, however, has indicated the importance of streptococci as pathogens in this part of the world [12–18]. In Ethiopia about 50–64% of all cases of heart morbidity among children are of rheumatic origin [13, 14, 18]. An average of about 100 cases of post-streptococcal glomerulonephritis per year are seen annually at the Ethio-Swedish Children's Hospital in Addis Ababa [19].

The purpose of the present study was to determine the throat carrier rate of beta-haemolytic streptococci among schoolchildren, to investigate streptococcal infections and their complications at a paediatric teaching hospital and to identify the prevalent serogroups and serotypes. Epidemiological and clinical features of the patients were also examined.

MATERIALS AND METHODS

Study populations

Sample collection was carried out during January to December 1990 at a paediatric teaching hospital, Ethio-Swedish Children's Hospital, located in the centre of Addis Ababa.

Schoolchildren. Forty to 48 schoolchildren below the age of 12 years and without any signs of upper respiratory tract or skin infection were randomly selected every month from three public elementary schools located in the nearby vicinity of the Ethio-Swedish Children's Hospital. A total of 506 children were screened for beta-haemolytic streptococci in their throats.

Tonsillitis. A request was made to all physicians who worked at the outpatient department of the hospital to send cases of tonsillitis who fulfilled the entry criteria to the bacteriology laboratory for culture. Clinical forms were distributed and the clinical symptoms of every patient were recorded by the physician responsible. A total of 143 clinically diagnosed tonsillitis patients, with sore throats, enlarged and reddened tonsils, cervical lymphadenopathy and/or exudates on the tonsils were studied.

Impetigo. From October 1990 to February 1991, 55 consecutive skin swabs from children with impetigo attending the outpatient department were cultured.

Acute post streptococcal glomerulonephritis. All 44 patients with acute post-streptococcal glomerulonephritis (APSGN) admitted to the hospital or referred to the renal clinic at the out-patient department during the study period were enrolled in the study. Criteria for APSGN included history of streptococcal infections, hypertension, oedema, red urine, proteinuria, and urine casts.

Acute rheumatic fever. Twenty-four cases of acute rheumatic fever (ARF) patients who fulfilled the revised Jones criteria of 1965 [19] were admitted to the hospital during January–December 1990. All were included in the study.

Chronic rheumatic heart disease. Chronic rheumatic heart disease (CRHD) patients with echocardiography proven lesions were selected from the out-patient cardiac clinic on weekly bases. Only patients on their fourth week after they had the last injection of benzathine penicillin were included. The first two patients who reported and fulfilled the entry criteria were selected every week. A total of 44 such cases, 22 males and 22 females, were studied.

Clinical information and demographic data for each patient were obtained. Clinical assessment of ARF and CRHD were performed. The demographic data included household size, number of rooms, number of siblings, parent's education and profession, availability of latrine, water supply, and cooking facilities.

Culture methods

Throat and skin swabs were collected using sterile cotton swabs and taken to the laboratory in an empty sterile test tube and inoculated within 2 h of collection onto sheep blood agar containing 1 mg/l of crystal violet. Plates were incubated anaerobically using BBL GasPak Plus anaerobic system (Becton Dickinson, USA) and examined after 18–24 h of incubation. Beta-haemolytic colonies were subcultured on sheep blood agar, sero-grouped and stored at −80 °C in Trypticase soy broth with 20% glycerol.

Identification

Beta-haemolytic streptococci were grouped by agglutination using Streptex kits (The Wellcome Diagnostics, UK) following the instructions from the manufacturer. Group A streptococci were T typed using commercial antisera (from the Institute of Sera and Vaccine, Prague, Czechoslovakia) according to the instructions given by the manufacturer. Briefly, bacterial cells were collected by centrifugation of an overnight culture in 2 ml Todd-Hewitt broth. Cells were then resuspended in 0·5 ml of 0·15 M Tris buffer, pH 7·5. Two drops of 5% trypsin were added to the suspension and incubated at 37 °C for 1 h. Agglutination tests were performed by mixing one drop of the trypsin digested bacterial suspension and one drop of the antisera on a glass slide. Results were recorded within 2 min. Suspensions which showed spontaneous reaction or which agglutinated more than one antiserum were subjected to further trypsinization. One more drop of 5% trypsin was added and incubation continued at 37 °C for another 1 h. If a strain still reacted with more than one serum another drop of trypsin was added and incubation was changed to 50 °C for 20 min.

Beta-haemolytic streptococci group C and G were further characterized using the biochemical tests provided by the API system version 3·0 (Bio Merieux, France). All strains positive for the Voges Proskauer test were classified as '*Streptococcus milleri*'. No further tests were done to speciate this group.

Opacity factor reaction

The microplate technique devised by Johnson and Kaplan [21] was used to detect opacity factor reaction. The supernatant from an overnight culture of Todd-Hewitt broth was used as a source of opacity factor.

Antibiotic susceptibility test

Strains were tested against 12 antibiotics by the disk diffusion method on iso-sensitest agar (Oxoid) enriched with 5% horse blood. The antibiotics were (Oxoid disks) ampicillin (10 μg), penicillin (10 μg), cefaclor (30 μg), cephuroxime (30 μg), cephatazidine (30 μg), chloramphenicol (30 μg), clindamycin (15 μg), erythromycin (15 μg), gentamicin (30 μg), tetracycline (30 μg), trimethoprim-sulphamethoxazole (25 μg) and vancomycin (30 μg). A suspension equivalent to 0·5 MacFarland turbidity was prepared from an over night culture on blood agar and was diluted to 1:100 for inoculation. The plates were incubated at 37 °C in 5% of CO_2 for 18–24 h and inhibition zones were measured using a pair of calipers. Histograms were plotted for each antibiotic and serogroup, and susceptibility interpretation was done according to the guidelines provided by the Swedish antibiotic reference group [22].

Statistical analysis of the data

The database and statistics system package of EPI Info version 5 (USD, Inc., Stone Mountain, Georgia, 1990) was used to analyse the data. The level of significance was determined by calculating the P value using the contingency tables.

RESULTS

Healthy schoolchildren

A total of 506, apparently healthy schoolchildren, 270 males and 236 females, were screened for beta-haemolytic streptococci by throat culture during the study period. Their age varied between 4 and 12 with a mean of 8·7 years. Monthly carrier rates of beta-haemolytic streptococci varied between 16·7–58·5%, average being 39·7%. Beta-haemolytic streptococci group A (GAS) accounted for 86 (16·9%), *Streptococcus equisimilis* for 61 (12·1%) and large colony group G (GGS) for 14 (2·7%) (Table 1). Only 47 (54·7%) of the GAS strains were T typable and the most frequent types were T2, 12, 4 and B3264 (Table 2). Only 30 (34·9%) of the GAS strains were OF negative. Figure 1 shows the monthly carrier rates of beta-haemolytic streptococci and the different serogroups. Carrier rates of GAS varied from 7·5% in October to 39% in March. As to the annual carrier rate, GAS was the predominant species. However, a marked seasonal fluctuation was noted among the serogroups. The central highlands of Ethiopia experiences three types of seasons in a year (Fig. 2). These are, the period during February–May, which is hot and humid accompanied by short rains, the period during June–September, the season of big rains, and the period during October–January which is dry and relatively cold. GAS strains were predominant during the short rains of February–May. The prevalence of *Streptococcus equisimilis* and GGS increased during the following two seasons as the prevalence of GAS strains decreased (Fig. 1). This difference in the prevalence of the groups in these three seasons was statistically significant ($P < 0·009$).

Tonsillitis

Throat swabs from 143 cases of tonsillitis were examined. Most of the patients (72·5%) were aged 3–8 years, the mean age was 6·9, 72 were males and 71 females. Beta-haemolytic streptococci were isolated from 86 (60·1%) patients, GAS accounted for 58 (40·6%), *Streptococcus equisimilis* for 6 (4·3%), and GGS for 2 (1·4%) (Table 1). Several T types were detected among the GAS (Table 2), 16

Table 1. *Distribution of beta-haemolytic streptococci among the different patient categories.*

Patient category	No. of cases	No. of beta-haemolytic streptococci isolated* Total	A	C†	G	Others‡
Schoolchildren	506	201 (39·7)	86 (16·9)	61 (12·1)	14 (2·7)	40 (7·9)
Tonsillitis	143	86 (60·1)	58 (40·6)	6 (4·3)	2 (1·4)	20 (13·9)
Impetigo	55	53 (96·3)	51 (92·7)	1 (1·8)	1 (1·8)	—
APSGN	44					
Throat		11 (25·0)	4 (9·1)	1 (2·3)	—	6 (13·6)
Skin		15 (34·1)	14 (31·8)	—	2 (4·5)§	—
ARF	24	8 (33·3)**	5 (20·8)**	—	—	3 (12·5)
CHRD	44	9 (20·4)	2 (4·5)	—	1 (2·3)	7 (15·9)
	816	383	220	69	20	76

* Numbers in parentheses indicate percentage of the total children examined in each group. †, All were *Streptococcus equisimilis*. ‡, Include *Streptococcus agalactiae*, *S. milleri* and non-typable. §, One case with double infection with group A and G. **, One strain was from skin lesion.

Table 2. *T typing pattern of beta-haemolytic streptococci group A*

T type	ARF	CRHD	APSGN	Tonsillitis	Carriers	Impetigo
2	3 (60·0)*	—	4 (22·2)	17 (29·3)	11 (12·9)	4 (7·7)
B3264	—	—	4 (22·2)	3 (5·2)	6 (7·0)	4 (7·7)
4	—	—	2 (11·1)	3 (5·2)	8 (9·3)	3 (5·8)
12	—	—	—	2 (3·4)	9 (10·5)	2 (3·8)
1	—	1(50)	—	4 (6·9)	2 (2·3)	—
25	—	—	—	2 (3·4)	1 (1·2)	2 (3·8)
9	—	—	—	3 (5·2)	—	1 (1·9)
6	—	—	—	1 (1·7)	2 (2·3)	—
27/44	—	—	—	—	2 (2·3)	2 (3·8)
11	—	—	—	—	3 (3·5)	—
44	—	—	1 (5·6)	1 (1·7)	—	—
8	—	—	—	1 (1·7)	1 (1·2)	—
3	—	—	—	—	1 (1·2)	1 (1·9)
5		—	—	1 (1·7)	—	—
23	—	—	—	—	1 (1·2)	—
13	—	—	1 (5·6)	—	—	—
8/Imp19	—	—	—	1 (1·7)	—	—
5/27/44	—	—	—	1 (1·7)	—	—
ND‡	—	—	—	2 (3·4)	—	1 (1·9)
NT	2 (40)	1 (50)	6 (33·3)	16 (27·6)	39 (45·3)	32 (61·5)
Total	5	2	18	58	86	52†

* Numbers in parentheses indicate percentage.
†, Two types were isolated from one patients skin swab.
‡, Not done.

(27·6%) were non-typable with the antiserum panel used. Thirty-three (56·9%) were OF negative.

The isolation rate of GAS was significantly higher ($P < 0{\cdot}001$) from tonsillitis cases than from asymptomatic school children (Table 1). *Streptococcus equisimilis* were more frequently isolated from schoolchildren than from tonsillitis cases ($P = 0{\cdot}006$). On the other hand, there was no difference in the isolation rate of GGS

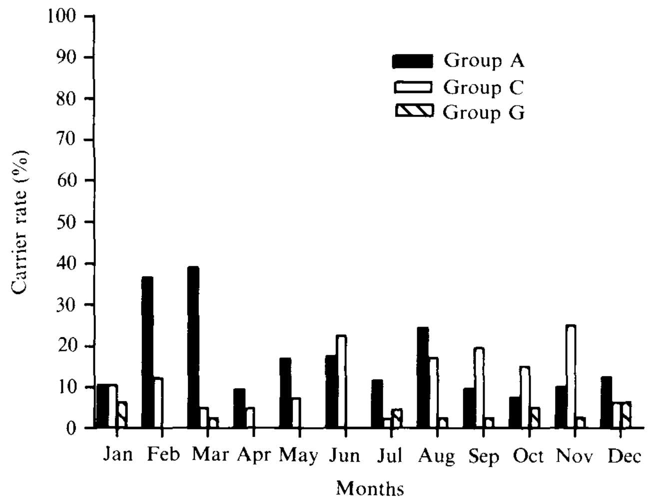

Fig. 1. Frequency of isolation of beta-haemolytic streptococci from throat swabs of schoolchildren as percentage of total children examined every month. All the streptococci group C isolates were *Streptococcus equisimilis*.

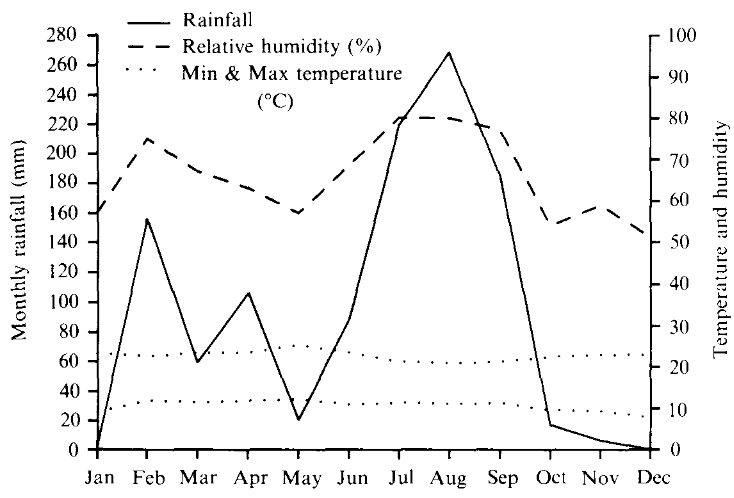

Fig. 2. Meteorological data of Addis Ababa, 1990.

among tonsillitis patients and healthy schoolchildren. The distribution of beta-haemolytic streptococcal serogroups in both symptomatic and asymptomatic cases was not different among the sexes.

Impetigo

Fifty-five consecutive skin swabs from impetigo cases, 30 females, 25 males, were cultured and 53 (96·3%) of them were positive for beta-haemolytic streptococci. GAS strains accounted for 51 (92·7%). *Streptococcus equisimilis* strains for 1 (1·8%) and GGS strains for 1 (1·8%) (Table 1). The T typing pattern of GAS is shown in Table 2. The majority of them (61·5%) were non-typable. Opacity factor production was detected in 30 (58·8%) of the GAS strains.

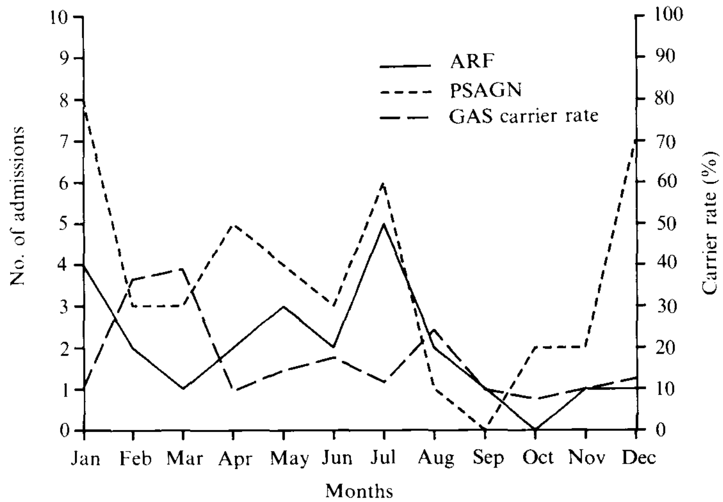

Fig. 3. Number of monthly admissions of ARF patients and APSGN patients in the Ethio-Swedish Children's Hospital during 1990 and GAS carrier rate among apparently healthy schoolchildren.

Acute post-streptococcal glomerulonephritis

From January to December 1990, 44 clinically confirmed cases of APSGN were studied. Figure 3 shows the monthly admissions. The highest incidence was seen during December and January. The female:male ratio was 1:1·9. Age varied from 3 to 11 years, with a mean age of 7. Fourteen (31·8%) had a history of sore throat. Only 11 (25%) were throat culture positive for beta-haemolytic streptococci, 4 (9·1%) were GAS, and 1 (2·3%) was *Streptococcus equisimilis* (Table 1). Twenty-seven (61·4%) had a history of recent skin infection but only 15 (34%) had the skin lesion at the time they reported to the hospital. All 15 were positive for beta-haemolytic streptococci, 13 cases were GAS, one was GGS and one GAS and GGS. Twelve (66·6%) of the GAS isolates were typable and five different T types were detected. The most common ones were type T2 and B3264 (Table 2). Most (61·1%) of the GAS isolates both from skin and throat of patients with APSGN were OF negative. If the 14 GAS skin isolates were taken separately, 10 (71·4%) were negative for opacity factor. This is high compared to the OF negativity of impetigo isolates 21/51 (41·2%) (see below), although not statistically significant (Yates corrected P value = 0·088).

Acute rheumatic fever

Twenty-four cases of acute rheumatic fever were admitted to the hospital during the study period. Fig. 3 shows the number of monthly admissions. The highest peaks were observed during the months of July and January. Age varied between 4–14 years, with a mean age of 9·5. The sex ratio was 1·4:1 (F/M). Fifteen (62·3%) had carditis, 5 (20·8%) had carditis and polyarthritis, 3 (12·5%) had polyarthritis and only 1 (4·2%) patient had chorea. Although 14 (58·3%) of the patients had a history of sore throat, only 7 (29·2%) of the throat cultures yielded beta-haemolytic streptococci (Table 1), only 4 (16·6%) of these were positive for

Table 3. *Demographic data of patients. Numbers (%) indicate children with the indicated feature*

Patient category	Total no.	Private latrine	No. Sibling (≤ 4)	No. rooms (≤ 2)	Family size (≥ 7)	Tap water*	'Mitad†'
ARF	24	6 (25)	18 (75)	17 (70·8)	15 (62·5)	8 (33·3)	9 (37·5)
CRHD	44	14 (31·8)	23 (52·3)	25 (56·8)	34 (77·3)	26 (59·1)	21 (47·7)
APSGN	44	17 (38·6)	23 (52·3)	26 (59·0)	29 (65·9)	28 (63·6)	17 (38·6)
Tonsillitis	140‡	74 (52·9)	99 (70·1)	55 (39·2)	96 (68·6)	123 (87·9)	108 (77·1)
	P value	0·01	0·02	0·005	0·5	< 0·0001	< 0·0001

* Availability of tap water either in the house or compound. †, Possession of 'mitad', an electric stove which has recently replaced the traditional biomass 'mitad'. ‡, Data were not available for three tonsillitis cases.

GAS. Three patients (12·5%) had a history of skin infection one of which was culture positive (Table 1). This patient had skin lesions all over the body which were almost healed when she came to the hospital. Throat culture from this patient was negative, but GAS were isolated from the skin lesions. Three of the five GAS isolated from acute rheumatic fever patients belonged to type T2 and the other two were non-typable. All strains from acute rheumatic fever patients were opacity factor (OF) negative.

Chronic rheumatic fever

Forty-four patients with chronic rheumatic heart disease were included in the study. Their age varied between 5 and 14 years with a mean age of 10·4. Beta-haemolytic streptococci were isolated from nine (20·4%) of the throat cultures (Table 1). GAS were isolated from two patients.

Social status

Demographic data of the four patient categories are given in Table 3. The patients did not differ in the number of siblings or family size ($P = 0·02$ and $P = 0·5$, respectively). Most of the patients (70·8%) with acute rheumatic fever lived in crowded conditions ($P = 0·005$). All the other social indicators studied showed a poorer standard of living of rheumatic fever patients. This is in agreement with the general observation [23], although, the recent outbreaks of ARF in the USA were mainly among middle class children [1–4].

Species identification and antibiotic susceptibility test

Table 4 shows categorization of beta-haemolytic streptococci group C and G based on the API classification. The majority (62·2%) of the beta-haemolytic streptococcal group G strains belonged to the 'milleri' group, of which 30/33 were identified as *S. milleri* II. A total of 84 group C isolates was treated and 69 (82·1%) of them were identified as *S. equisimilis*, 7 (8·3%) as *S. milleri* I, and 8 (9·5%) as *S. milleri* II.

All the 220 GAS isolates were susceptible to each of the antibiotics tested, except tetracycline where 150 (68·2%) were resistant with inhibition zone diameters of ≤ 20 mm. The remaining 70 (31·8%) isolates were susceptible, with

Table 4. *API classification of sero-group C and G beta-haemolytic streptococci from throat cultures of different patients.*

Source	Group C			Group G†		
	Total	*S. equisimilis*	*S. milleri*	Total	Group G‡	*S. milleri*
Carriers	72	61 (84·7)*	11 (15·3)	29	14 (48·3)	15 (51·7)
Tonsillitis	9	6 (66·6)	3 (33·3)	11	2 (18·2)	9 (81·8)
Impetigo	1	1 (100)	—	1	1 (100)	—
APSGN	1	1 (100)	—	6	2 (33·3)	4 (66·6)
ARF/CRHD	1	—	1 (100)	6	1 (16·7)	5 (83·3)
Total	84	69 (82·1)	15 (17·8)	53	20 (37·7)	33 (62·2)

* Numbers in parentheses indicate percentage.
† Lancefield grouping.
‡ According to API 20 classification.

an inhibition zone diameter of ⩾ 24 mm. Tetracycline resistance was not associated with any T type. On the other hand, resistance was more frequent among OF positive isolates, 97/117 (82·9%), compared to OF negative GAS, where resistance was detected among 55/103 (53·3%) of the isolates. This was statistically significant ($P < 0{\cdot}001$). There was no difference in the level of tetracycline resistance among isolates from sick children and healthy ones. *S. equisimilis*, GGS, and the milleri group were also susceptible to the 11 antibiotics with the exception to tetracycline, but one GGS strain was resistant to chloramphenicol with zone inhibition diameter of 15 mm. Resistance to tetracycline was detected in 4/32 of the milleri and 47/86 of *S. equisimilis* and GGS isolates.

DISCUSSION

The carrier rate of beta-haemolytic streptococci reported from tropical countries like Nigeria or Liberia was lower in comparison with temperate regions [24]. In the present study the carrier rate of GAS among apparently healthy schoolchildren, 16·9%, was markedly higher compared with the isolation rate of 1·9% from similar survey in Lagos [11], but comparable to the rate reported from Egypt, 13–24% [8]. A lower carrier rate, (4%), than in the present study (16·9%), has been reported earlier from a 6-week study among children in Addis Ababa [19]. Carrier rates of GAS have shown to be influenced by several factors such as age, place, season, and crowding. However, the prevalence rate of GAS among schoolchildren reported in the present investigation might reflect a true increase, because a similar trend has been observed in the frequency of chronic rheumatic heart disease among children in the Ethio-Swedish Children's Hospital [18]. This concomitant increase in carrier rate of GAS and the post streptococcal complications may implicate a change in the epidemiology of streptococci and streptococcal infections in Ethiopia. The repeated drought, famine, and civil war that has struck Ethiopia in the last years have obviously resulted in an influx of people to the big cities, causing crowding and lower standards of living.

Most studies have reported GAS as a predominant serogroup among throat isolates. However, surveys in Egypt [8], Liberia and Nigeria [9–11] and Thailand

[25] showed serogroups other than GAS, mainly C and G, to be more frequent. A 3-year study [11] over a decade after the first report from Nigeria [10] has shown a continued predominance of streptococci of group C and G. In the present study, the frequency of non-group A streptococci among isolates from tonsillitis cases was low. As to the carrier state, GAS was the predominant bacterial species on an annual basis. However, a considerable monthly variation was observed in the distribution of the serogroups. The preponderance of a certain serogroup is therefore seasonal as seen from the present investigation.

There is a growing recognition of the role of streptococci group C and G in human infectious diseases. A number of outbreaks of tonsillitis associated with beta-haemolytic streptococci group C and G have been reported [26–28]. Their role in sporadic community acquired pharyngitis has been questioned [29–31]. On the other hand, others [32] have emphasized their importance in tonsillitis among children. A community-wide outbreak of pharyngitis due to large colony group G streptococci has been reported among paediatric populations [33]. The importance of both group C and G streptococci increases with age [32, 33]. It has been suggested that streptococci group C and G might be important pathogens of the throat in the tropics, because of the predominance of both groups over GAS in the throat carrier state and tonsillitis in Nigerian and Liberian children [9–11]. Comparative studies of isolation rates in asymptomatic and symptomatic adult populations [34, 35] have demonstrated a strong correlation of streptococci group C with pharyngitis. In contrast, Hayden and co-workers [31] were unable to demonstrate any association of streptococci group C and G with tonsillitis among children. Our data do not show any difference in the distribution of GGS streptococci among tonsillitis and asymptomatic schoolchildren. *Streptococcus equisimilis* were in fact isolated more frequently from healthy carriers. The findings of the present study do not favour the non-group A streptococci as major aetiologic agents of tonsillitis among children.

Earlier reports from Ethiopia and other African countries have shown dominance of females among rheumatic heart disease patients. In Ethiopia a female to male ratio of 1·38:1 [14] and 1·3:1 [18], and in Uganda a ratio of 1·5:1 [15] have been reported. Ogunbi and colleagues [16] have shown a female to male ratio of 1·6:1 and 1·9:1 among rheumatic fever and rheumatic heart disease patients respectively in Nigeria. Our data also show a higher ratio of females to males, 1·4:1 among the acute rheumatic fever cases. This is in contrast to several reports from the USA [1, 2, 36]. On the other hand, our data and those of Maekawa and co-workers [37] from Japan and Hoffman [38] from Denmark showed that females and males are equally susceptible to throat streptococcal infection. In contrast APSGN was more frequent among boys than girls (1·9:1), which is in line with earlier reports [17]. The frequency of streptococcal skin infection was not different among the sexes as observed in the present investigation. Thus, the higher prevalence rate of rheumatic fever among girls and APSGN among boys did not seem to be due to an increased attack rate by GAS, but rather to differences in susceptibility among the sexes to the mechanisms underlying these complications.

The association of throat rather than skin streptococcal infection with rheumatic fever is well established [39]. However, a high prevalence of GAS impetigo [16, 40] and a predominance of group C and G rather than GAS among throat isolates lead some investigators to suspect that skin infection with GAS may be followed by rheumatic fever in Africa [16]. Moreover, increased anti-DNase levels but not of anti-streptolysin O (ASO titre), an indication of previous streptococcal skin rather than throat infection, have been observed among ARF patients in Trinidad [41, 42]. In Nigeria, streptococcal skin infections were more common than throat infections among rheumatic fever cases [16]. However, in the present study a history of skin infection was less frequent than a history of throat infection among the acute rheumatic fever patients. Beta-haemolytic group A streptococci were isolated from skin lesions, but not from the throat, of one of the three ARF patients with history of skin infections. It is not possible to determine whether the skin infection was responsible for the sequelae or not, because it may have followed a previous throat infection. Although our data suggest a major role of throat infections, they do not contradict a role of streptococcal skin infections in causing rheumatic fever.

Acute post-streptococcal glomerulonephritis may be associated with either skin or throat infection with GAS. In the tropics streptococcal skin infections are common and are often associated with APSGN [16, 40, 41]. In Egypt, however, throat rather than skin infections are more commonly found in APSGN patients [17]. Similar to our findings Axemo and co-workers [19] have reported a high frequency of skin infections among APSGN patients in Addis Ababa. In the present study 61·4% of the patients had a history of skin infection, while only 31·8% had a history of sore throat. Our data confirm the association of either skin or throat infections with APSGN among the study population.

A considerable number of the GAS isolates were non-typable using the antisera from Prague. Non-typable strains were more frequent among isolates from carriers compared to strains from patients, as also has been reported by Jelinkova and co-workers [43]. In southern Sweden, the non-typability rates among clinical isolates are considerably lower, from 4·6–16·8% from 1987–90 (C. Schalèn, unpublished data). There are two possible explanations for the high non-typability rates among the Ethiopian isolates. Non-typable GAS may dominate in this geographical area, or the isolates may possess T antigens unrecognizable by the antisera from Prague. Our findings emphasize a need for production of T antisera using local strains.

Beta-haemolytic streptococci isolated during our investigation were susceptible to most commonly used antibiotics, with the exception of tetracycline. Erythromycin resistance among GAS strains has been reported at different levels from several parts of the world [44–46]. Erythromycin resistance was not detected among the isolates of the present study. On the other hand, the level of tetracycline resistance among GAS, 68%, *Streptococcus equisimilis* and large colony group G, 53%, was high. There is no earlier information on the level of resistance to antibiotics of streptococci from Ethiopia. The frequency reported here is higher compared to frequencies from Denmark, 21% [38], from Sweden, 21% [47] and 4%, [48], but it is comparable to the level reported from Japan (70%) in 1979 [44]. T type 12 were associated with erythromycin resistance in Sweden [45] and Japan [44]. Tetracycline resistance among GAS in the present study was not type restricted. However, the level of resistance was higher among

OF positive isolates. The finding of the present study and a high level of tetracycline resistance among bacterial pathogens other than streptococci reported earlier from Ethiopia [49, 50] reflect the widespread use of tetracycline in the country.

Reports on the seasonal variation in the incidence of beta-haemolytic streptococci in a population are inconsistent. In the temperate regions the general observation is that the carrier rate of GAS is high during the winter season [24], while others have failed to demonstrate such periodicity [37, 51]. In a 3-year study of Egypt, a sharp rise in the isolation rate of GAS from schoolchildren was noted during late autumn and early winter with a minimum rate in the summer months [8]. Reports on seasonal variation of non-group A streptococci is limited [31, 32]. No specific pattern has been noted in the overall carrier rate throughout the year in the present study. However, the seasonal variation observed among the streptococcal serogroups is interesting. Group A streptococci predominate during the season of short rains and starts to decline during the following two seasons, heavy rains and the dry cold seasons. *Streptococcus equisimilis* and GGS showed an opposite trend. It is worthy to note here, that elimination of GAS by antibiotic treatment in symptomatic cases resulted in an invasion of the throat by either group C or G streptococci [29, 51]. It is known that bacterial antagonism exists among throat microflora. The viridans streptococci which constitute part of the normal flora produce bacteriocins which interfere with GAS [52–55]. Certain types of GAS are also known to produce bacteriocins which inhibit the growth of other GAS strains, viridans and group B streptococci [54, 55]. It has not been reported in the literature whether there are GAS strains that produce bacteriocin that interfere with group C and G. The observation of the present study, Schwartz and Shulman [29] and Strömberg and colleagues [51] suggest a possibility that GAS may exert negative pressure on the other beta-haemolytic streptococci.

The occurrence of both ARF and APSGN throughout the year observed in the present study is in agreement with a report from another tropical country, Trinidad [41, 42]. In contrast, Bisno and colleagues [56], in Memphis, found APSGN associated with warm weather and to occur mainly during the summer, while ARF occurred mainly during the winter season. In the present study both diseases showed a similar monthly variation in incidence as was observed in Trinidad except during epidemics of APSGN [41, 42]. Also similar to the report from Trinidad, the monthly fluctuation of ARF did not correlate with the throat isolation rate of GAS from schoolchildren. In the present study both diseases showed two peaks, one during the dry season and the other in the middle of the rainy season. The incidence of both diseases was low during September and October. Our study covers only one year. Longer observation would be required to confirm this periodicity.

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