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A. Braito, MD., I. Galgani, MD., The Clinic of Infectious Diseases, Department of Molecular Biology, University of Siena, Italy, M. R. Mohammed, MD., Public Health Laboratory Ivo de Carneri (PHL-IdC), Wawe( Chake, Pemba), C. Iozzi, MD., The Clinic of Infectious Diseases, Department of Molecular Biology, University of Siena, Italy, S. M. Ame, Biologist, Public Health Laboratory Ivo de Carneri (PHL-IdC), Wawe (Chake, Pemba), H. S. Haji, Biologist, A. Zanchi, PhD., The Clinic of Infectious Diseases, Department of Molecular Biology, University of Siena, Italy

Request for reprints to: Dr. A. Braito, Clinic of Infectious Diseases, Policlinico Le Scotte, Viale Bracci 16, 53100 Siena, Italy

# EPIDEMIOLOGY OF STREPTOCOCCUS GROUP A IN SCHOOL AGED CHILDREN IN PEMBA

A. BRAITO, I. GALGANI, M. R. MOHAMMED, C. IOZZI, S. M. AME, H. S. HAJI and A. ZANCHI

## ABSTRACT

***Background:*** **In Pemba (Zanzibar) all the risk factors which favour Group A Streptococci spreading, infections and late sequelae are present, though GAS epidemiology is unknown.**

***Objective:*** **To determine the prevalence of GAS pharyngeal carriers among school-aged-children.**

***Design:*** **Community-based cross sectional study, carried out at the end of the dry season (January-February 2001).**

***Setting:*** **Eight primary schools over the four Pemba districts were included in the study.**

***Subjects and methods:*** **Two thousand two hundred and eighty six children aged 7-10 years were selected by random sampling and submitted to throat-swab after informed consent. Swabs were processed according to the "Lennette Manual of Clinical Microbiology" 7th Ed. Isolated were tested for antibiotic susceptibility toward penicillins, erythromycin, clindamycin, josamycin, cloramphenicol, levofloxacin, rifampin and tetracyclines.**

***Results:*** **Twenty seven point six percent of school-aged children harboured ß-haemolytic Streptococci in their pharynx; most of the isolates were serologically identified as non Group A streptococci group C and G represented more than 70% of all strains, 38.8% of whom were identified as group G; the prevalence of group A streptococci carriers among healthy children all over the island was 8.6%; group A streptococci isolates were sensitive to all the antibiotic tested, except tetracyclines, towards which 83.2% of strains were resistant.**

***Conclusion:*** **This is the first research in the field of bacteriology carried out in Pemba. According to the epidemiology of group A streptococci and to the environmental and underlying factors which predispose to late group A streptococci sequelae, we suggest to consider antibiotic treatment for children presenting with sore throat with fever and swollen cervical lymphonodes without cough or coryza.**

## INTRODUCTION

Rheumatic fever and rheumatic heart diseases were major health problems worldwide in the past decades and continue unchanged in developing countries, where 12 millions people are affected and 400,000 die each year. Rheumatic heart disease prevalently affects children and young people and is the result of repeated exposures to group A streptococci (GAS); risk factors are also undernutrition and low socio-economic level.

The disease control in western hemisphere has been reached by two forms of prophylaxis :antibiotic treatment of GAS pharyngitis and prevention of colonization with GAS by periodical treatment with penicillin in individuals who have already had a previous attack of rheumatic fever(1,2).

Group A streptococci epidemiology and its consequences are unknown in Pemba island. However, teenagers with endomyocardiopathy clinically compatible with rheumatic heart disease seem to be occasionally admitted to the local hospital, although not well documented.

The overall objective of this field research was to evaluate GAS circulation on the Island and its relationship with late, non suppurative sequelae.

*Specific aims:* To study the GAS pharyngeal carriage among healthy school-aged children, in order to achieve information about GAS circulation on the Island, to collect retrospective data on the frequency of hospitalization at Chake-Chake Hospital (the main town of the Island) for heart diseases of probable rheumatic origin, as diagnosed on clinical and electrocardiographic ground.

We couldn't get information on hospital admission due to heart diseases because of three under reported reasons. (i) Recording of inpatients and their diagnosis is not performed at Chake-Chake Hospital, (ii) Most of young people living at village level probably escape the diagnosis lacking health facilities, (iii) Cardiac heart diseases mainly observed in adulthood are better investigated outside Pemba, at Stone Town Hospital (Zanzibar). The above unexpected problems probably request an active surveillance on the occurrence of rheumatic valvulopathies, which was not part of our programme at that time and led us to miss the second aim of this research.

## MATERIALS AND METHODS

Pemba, approximately 35 miles northeast of Zanzibar and 45 miles off the mainland Tanzania Coast, is an Island 42 miles long and 14 miles wide; it is very densely populated (population 350,000-400,000); the island is divided into four districts (Mkoani, Chake, Wete, Micheweni) that differ each other in some geographical and economical characteristics.

This study was carried out on January-February 2001, at the Public Health Laboratory Ivo De Carneri, located in Wawi, (Chake, Pemba); final identification of ß-haemolytic streptococci was performed at the Reference Laboratory (Clinic of Infectious Diseases, Department of Molecular Biology, University of Siena, Italy). The field research took place on children living in all the Districts, in order to evaluate GAS circulation uniformly on the whole territory. The plan of action was designed as follows:

(i) *Preliminary approach:* the head master and the teachers of each selected school were informed about the aim of the study and the way it would be performed, in order to obtain the consent.

(ii) *Population selection:* Children aged 7-10 years, attending at eight primary schools (two for each district), were enrolled and 50% of them were randomly selected for GAS carriage evaluation.

(iii) Anagraphical data of the selected children were registered.

*Specimen collection and cultivation:* Throat swabs were performed according to the technique reported in the literature (3) and immediately inoculated onto Columbia CNA blood agar plates.

*Specimen processing:* Once carried out to the Ivo de Cameri Public Health Laboratory (PHL-Idc) plates were incubated at 37°C for 24 hours in $CO_2$ 5% atmosphere. ß-haemolytic colonies showing morphologic characteristics of streptococci were re-picked for bacitracin susceptibility, according to the conventional criteria (filter paper disks impregnated with 0.04 mg of bacitracin; inhibition zone>=12 mm) (4); ,B-haemolytic colonies showing doubtful morphologic aspect were also submitted to catalase reaction. ß-haemolytic catalase-negative specimens, classified as GAS if bacitracin sensitive or non-GAS if B acitracin resistant, were stored at 4°C and successively transferred to the Reference Laboratory.

The differentiation of ß-haemolytic Streptococci into the Lancefield groups was performed through a co-agglutination technique (Phadebact Streptococcus test). The samples serologically classified as GAS were further submitted to the PYR test which detects pyrase activity of *Streptococcus pyogenes* (Oxoid Biochemical Identification System).

*Testing for antibiotic sensitivity:* Specimens definitively classified as GAS were tested for sensitivity towards penicillin, erythromycin, clindamycin josamycin, chloramphenicol, levofloxacin, rifampin and tetracyclines. Sensitivity test was performed as a disk diffusion method (Kirby-Bauer) with filter paper disk impregnated with a specific amount of antimicrobial agent applied to the surface of Mueller-Hinton blood agar medium, inoculated with the test organism. The diameter of inhibited bacterial growth allows categorization of bacterial isolates as susceptible, resistant or intermediate. Criteria currently recommended for interpreting zone diameters are published by the National Committee for Clinical Laboratory Standards (NCCLS). Isolates with doubtful diffusion profile were re-tested with a gradient diffusion method (E-test) (4).

*Evaluation of the results:* Data were reported and handled with an Epidemiology Programme, EPI INFO 6, which can be freely copied from the net.

## RESULTS

Four thousand seven hundred and thirty six children attending the primary schools were enrolled in this study; 2368 were randomly selected to investigate pharyngeal GAS carriage. Anagraphical information and geographical distribution are summarised in Table l.

**Table 1**

*Distribution for age, sex and district of 2368 children selected for GAS carriage*

| District | School | No. of selected children | Average age | Male (%) | Female (%) |
|---|---|---|---|---|---|
| | Mkanyageni | 124 | 11 | 50.8 | 49.2 |
| Mkoani | | | | | |
| | Mtambile | 196 | 9.5 | 46.9 | 53.1 |
| Chake | Pujini | 325 | 10.3 | 48.6 | 51.4 |
| | Ziwani | 272 | 9.6 | 44.5 | 55.5 |
| Wete | Ole | 276 | 9.6 | 50 | 50 |
| | Minungwini | 342 | 9.8 | 49.1 | 50.9 |
| | Wingwi | 428 | 9.6 | 52.6 | 47.4 |
| Micheweni | | | | | |
| | Konde | 405 | 10.3 | 45.7 | 54.3 |

Five children (0.2%) refused throat swabs; 19 samples (0.8%) were lost after the inoculation of the swab on CNA agar plates; 58(2.4%) inocula were not considered because of contamination of plates by environmental fungi: on the whole, during the first steps of this study, GAS carriage couldn't be studied in 82(3.4%), children. We also kept in consideration the geographical distribution of missed samples in order to evaluate the daily standard of the work, potentially influenced from environmental and climate conditions, number of samples to be taken and other factors (Table 2).

Two thousand two hundred and eighty six throat swabs could be evaluated for ß-haemolytic Streptococci at the PHL-IdC; (Table 3). At IdC PHL, 588 specimens were presumptively diagnosed as GAS or non-GAS according to Bacitracin susceptibility test; the specimens were stored at 4°C until transported to Italy. Further sample processing and identification were carried out at the Reference Laboratory. On the re-picking of colonies 18 strains didn't grow: the remaining 570 specimens were analyzed according to the previously reported criteria.

All the specimens were confirmed to belong to ß-haemolytic streptococci; by means of serological grouping, PYR reactivity and biochemical characteristics, they were identified (Table 4). 184/187 GAS isolated were definitively diagnosed as S. *pyogenes* and tested for antibiotic susceptibility; all the isolates were sensitive to all the antibiotics tested except to tetracyclines which showed an inhibitory effect in only 31 of them. The children harbouring B-haemolytic streptococci in the pharynx were widely distributed all over the island districts (Table 5); the overall prevalence of GAS carriers is 8.6%; higher in the Centre and Southern areas.

**Table 2**

*Distribution by district and school of 82 children excluded from the study*

| District | School | No. of Selected * | No. specimens lost | contaminated | Total (%) missed |
|---|---|---|---|---|---|
| Mkoani | Mkanyageni | 124 | 2 | - | 2 (1.6) |
| | Mtambile | 196 | - | - | - |
| Chake | Pujini | 324 | 3 | 3 | 6 (1.8) |
| | Ziwani | 273 (1) | - | 6 | 7 (2.5) |
| Wete | Ole | 276 | 9 | 14 | 23(8.3) |
| | Mimmgwini | 342 (2) | - | 5 | 7(2) |
| | Wignwi | 428 | 4 | 23 | 27(6.3) |
| Micheweni | Konde I | 242 (2) | 1 | 5 | 8 (3.3) |
| | Konde II | 163 | - | 2 | 2 (1.2) |
| | Total | 2368(5) | 19 | 58 | 82 |

* In brackets the number of children who refused throat swab

**Table 3**

*Bacteriological studies on 2286 throat swab specimens (IDC PHL, Pemba)*

| Category | No. of specimens | (%) |
|---|---|---|
| Non-haemolytic bacteria<br>ß-haemolytic-catalase | 1473 | 64.8 |
| Positive bacteria<br>*(Staphylococci)*<br>ß-haemolytic-catalase | 182 | 7.8 |
| Negative bacteria *<br>ß-haemolytic catalase | 43 | 1.8 |
| Negative-bacitracin sensitive bacteria**<br>ß-haemolytic-catalase | 254 | 11.1 |
| Negative-bacitracin resistant bacteria *** | 334 | 14.6 |
| Total | 2286 | 100 |

* These samples were collected during the final stage of the study, when bacitracin tablets were no more available, ** Presumptively diagnosed as GAS *** Presumptively diagnosed as non GAS

**Table 4**

*Identification of 13-haemolytic streptococci isolated from pharyngeal swabs of 570 children (Infectious Diseases Laboratory, University of Siena)*

| Category | No. of specimens | (%) |
|---|---|---|
| Group A: | 187 | 32.8 |
| PYR neg | 3 | 1.6 |
| PYR pos *(S. pyogenes)* | 184 | 98.4 |
| Group B | 10 | 1.75 |
| Group C | 139 | 24.3 |
| Group G | 218 | 38.2 |
| Others * | 16 | 2.8 |
| Total | 570 | 100 |

**S. equisimilis, S. intermedius/costellatus, S. salivarius, Aerococcus SSP*

**Table 5**

*Geographical distribution of 13 haemolytic streptococci isolated from throat swabs of primary school children*

| District | School | No. of throat swabs | ß-haemolytic streptococci carriers* | Unidentified ß-haemolytic streptococci* | GAS* | Group B* | Group C* | Group G* | Others* |
|---|---|---|---|---|---|---|---|---|---|
| | Mkanyageni | 122 | 40 | - | 15 | 1 | 13 | 9 | 2 |
| Mkoani | | | (32.8) | - | (12.3) | (0.8) | (10.6) | (7.3) | (1.6) |
| | Mtambile | 196 | 47 | - | 17 | 1 | 7 | 22 | - |
| | Pujini | 318 | 24 | - | (8.7) | (0.5) | (3.5) | (11.2) | |
| | Ziwani | 266 | (23 3) | (0.7) | (7) | | (4 .1) | (11.6) | (0 7) |
| Chake | | | 62 | 2 | 18 | - | 11 | 31 | 2 |
| | Ole | 253 | 77 | 1 | 17 | | 27 | 27 | 4 |
| Wete | | | (30.4) | (0.3) | (6.7) | - | (10.6) | (10.6) | (1.5) |
| | Minungwini | 335 | 96 | 2 | 24 | 2 | 33 | 34 | |
| | | | (28.7) | (0.5) | (7.1) | (0.5) | (9.8) | (10.1) | (0.2) |
| | Wingwi | 401 | 110 | 5 | 42 | 18 | 18 | 40 | 3 |
| Micheweni | | | (27.43) | (1.2) | (10.4) | (4.4) | (4.4) | (9.9) | (0.7) |
| | Konde I** | 234 | 56 | - | 17 | 8 | 8 | 29 | - |
| | | | (24) | (7.2) | (3.4) | (3.4) | (12.3) | | |
| | Konde 11 ** | 161 | 51 | 51 | - | - | - | - | - |
| | | | (31.6) | (31.6) | | | | | |
| Total | | 2286 | 631 | 61*** | 187 | 10 | 139 | 218 | 16 |

* In brackets the percentage

** This was the last school where our research was carried out during two different days: on the second one the work couldn't be fully concluded due to supplies exhaustion or blood agar plate damage

*** In this category are enclosed 43 ß-haemolytic catalase negative strains for which bacitracin susceptibility test couldn't be evaluated, 13 bacitracin resistant and 5 bacitracin sensitive Streptococci

## DISCUSSION

This is the first research in the field of bacteriology carried out in Pemba. An overall of 143 specimens (6.5%) were missed throughout the duration of the study; in our opinion, the performance of the work can be considered satisfactory, taking into account that many external factors as climatic and environmental conditions, political events, temporary black out, the distance from the schools to the Idc-PHL and the exhaustion or damage of equipment may have negatively influenced the daily course of the research in the field. It is not surprising that most of the lost or contaminated specimens were collected during and immediately after a serious political event or in a high windy environment.

Lancefield grouping performed at the Reference Laboratory confirmed that 187 among 254 strains, previously classified as GAS according to the bacitracin sensitivity test, belonged to GAS. Among the factors that can erroneously induce non GAS to be overdiagnosed as GAS, a small size of inoculum may play an important role (4).

*ß-haemolytic Streptococci circulation:* In Pemba 23.3-32.8% (average 27.6%) of school-aged children harbour, ß-haemolytic Streptococci in their pharynx, according to the different areas of the Island; the circulation is higher in the Centre-Southern. GAS carriage, which varies from 6.7% to 12.3% has a similar geographical distribution. The difference may be related to some circumstances as socio-economic and crowd conditions and other operational factors. This study was carried out at the top of the dry season, circumstance which can underestimate the Streptococcal circulation all over the year, as reported in the literature. This is particularly true for temperate climates, while is not confirmed in tropical zones(1,5).

The number of children carrying ß-haemolytic streptococci is lower in our study than that reported in the literature(1). Most of the isolates were serologically identified as non Gas; group C and G are predominant, representing more than 70% of all strains, 38.8% of whom were identified as Group G streptococci, confirming data obtained in other tropical areas (l). It has been postulated that these groups can cause pharyngitis, but their role still remains controversial.

*Prevalence and antibiotic susceptibility of Group A Streptococci:* About one third of ß-haemolytic Streptococci were identified as almost exclusively belonging to S. *pyogenes;* the overall prevalence of GAS carriers among school-aged children all over the island is 8.6%. It appears to be worldwide significant geographical differences in GAS circulation among asymptomatic individuals. It seems to be higher in urban environment of temperate areas especially during the cold season, when GAS represent as many as 50-60% of all ß-haemolytic streptococci. The prevalence also depends on socioeconomical and environmental conditions, is higher in primary school children and in overcrowded conditions; yet, as recently reported, investigations on the epidemiology of GAS circulation/ infections and its sequelae are scanty and controversial (6-8) to our knowledge, there are no reports from the east African countries.

In our study GAS isolates were sensitive to all the antibiotics tested, except tetracyclines, towards which 83.2% of strains were resistant. This percentage is one of the highest reported in the literature; though not recommended as treatment for pharyngitis, tetracyclines are largely prescribed in Pemba in the adults both for diarrhoea and skin infections. A rise in the rate of GAS resistant to tetracycline has been found during the last decades in many other geographical areas especially in developing countries where this antibiotic is widely used both in human and veterinary infections, leading to a high consumption level(9-11).

The presence of GAS in the upper respiratory tract may reflect either acute infection or a carries state. It is currently accepted that while the patient with true infection is at risk of developing rheumatic fever-rheumatic/ heart diseases, the GAS carrier is unable to trigger late non suppurative sequelae. However, the concept of healthy carrier still remains one of the most unclear aspects of the interaction between the host and the bacteria. In our study, Pemban children harbouring GAS in their pharynx were considered as "healthy carriers", even though it was frequently difficult, if not impossible, to differentiate the true health carrier from individuals with GAS infection. GAS pharyngitis is apparently milder or asymptomatic in developing countries, where the diffusion of other severe diseases as malaria, diarrhoea, pneumonia may lead to underestimate the frequency of pharyngotonsillitis and its sequelae.

It is now well recognised that certain GAS strains may be more invasive depending on the M protein, a major surface antigen and virulence factor; among more than 80 M serotypes, now identified in the U.S.A., certain of them, (particularly M1 and M3) seem to be associated with severe streptococcal diseases(12-14). Unfortunately, typing of M proteins is seldom performed in the geographical areas where late sequelae of GAS infections are prevalent, thus a clear correlation with circulation of more virulent GAS strains is not clearly demonstrated in poorest countries. Although molecular studies on virulence factors of our isolates was out of the aim of our study, this aspect is now considered and under evaluation.

Factors other than M serotypes may contribute in developing non suppurative sequelae after GAS exposure. It has been suggested that the peculiar age distribution pattern of patients with late sequelae of GAS infections can be explained by the necessity of "priming" the immune system by repeated GAS exposures, which are more prominent in developing countries; furthermore, an impaired immunological response to repeated streptococcal stimuli must be considered as a consequence of concomitant diseases and undernutrition.

In our opinion, the results of this research may be considered both of epidemiological and clinical concern. GAS are spread from person to person; pharyngeal carriers can successfully disseminate GAS by droplets. Living in poverty and unsanitary overcrowded conditions may facilitate GAS spreading. In a recent review it was pointed out that inadequate prevention of streptococcal infections and deprivation of children have led to very high rates of rheumatic fever(13). In Pemba all the above risk factors are undoubtedly present.

According to the conditions mentioned above and the frequency of upper respiratory-tract infections in Pemban children, as reported by local health workers, we are inclined to recommend antibiotic treatment for children suffering from pharyngotonsillitis, even in the absence of information on the prevalence of rheumatic fever, studies have demonstrated that appropriate antibiotics given early in the course of GAS infections essentially prevent the subsequent development of rheumatic fever.

GAS can be suspected as the aetiological agent of pharyngitis both on epidemiological and clinical ground. GAS is by far the commonest bacterial cause of acute pharyngitis, accounting for approximately 15-30% of cases in children and 5-10% in adults(15). It is often difficult to differentiate clinically between streptococcal pharyngitis and infections due to viruses. In western countries, laboratory confirmation by throat cultures or by rapid antigen detection test can eliminate unnecessary antibiotic use; yet, in most places where the burden of streptococcal infections is higher, there are no accessible laboratory facilities(16). Numerous attempts have been made to identify those signs and symptoms most likely to differentiate true streptococcal pharyngitis from infections due to other agents: several algorithms and clinical guidelines have been proposed, with controversial results(1,6,17-19).

Some respiratory complaints as cough and coryza may represent key symptoms to differentiate upper respiratory diseases of viral origin from GAS pharyngitis(20). They are by far less likely to be present in the latter condition. We agree with this clinical approach and suggest to consider antibiotic treatment for pharyngotonsillitis in Pemban children if cough or coryza are absent and the following signs and symptoms are present: (a) sore throat, (b) fever, (c) tonsil erythema or exudates (d) anterior cervical adenopathy Experience gained so far both in developing and western countries shows that the two main factors responsible for the decrease of rheumatic heart disease are better housing conditions and penicillin treatment of streptococcal pharyngitis.

Penicillin is the first choice treatment. It is effective against streptococcal infections. All strains of GAS isolated to date worldwide have been sensitive to concentration of penicillin easily achievable with commonly used dosages; this antibiotic has proven safety and of low cost. Pharyngitis can be treated efficaciously with a single intramuscular injection of penicillin G benzathine: orally administered penicillins are equally active, but request a four times daily dosing schedules and compliance may be low.

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