Differences between Belgian and Brazilian Group A Streptococcus Epidemiologic Landscape

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Background. Group A Streptococcus (GAS) clinical and molecular epidemiology varies with location and time. These differences are not or poorly understood. Methods and Findings. We prospectively studied the epidemiology of GAS infections among children in outpatient hospital clinics in Brussels (Belgium) and Brasília (Brazil). Clinical questionnaires were filled out and microbiological sampling was performed. GAS isolates were emm-typed according to the Center for Disease Control protocol. emm pattern was predicted for each isolate. 334 GAS isolates were recovered from 706 children. Skin infections were frequent in Brasília (48% of the GAS infections), whereas pharyngitis were predominant (88%) in Brussels. The mean age of children with GAS pharyngitis in Brussels was lower than in Brasília (65/92 months, p<0.001). emm-typing revealed striking differences between Brazilian and Belgian GAS isolates. While 20 distinct emm-types were identified among 200 Belgian isolates, 48 were found among 128 Brazilian isolates. Belgian isolates belong mainly to emm pattern A–C (55%) and E (42.5%) while emm pattern E (51.5%) and D (36%) were predominant in Brasília. In Brasília, emm pattern D isolates were recovered from 18.5% of the pharyngitis, although this emm pattern is supposed to have a skin tropism. By contrast, A–C pattern isolates were infrequently recovered in a region where rheumatic fever is still highly prevalent. Conclusions. Epidemiologic features of GAS from a pediatric population were very different in an industrialised country and a low incomes region, not only in term of clinical presentation, but also in terms of genetic diversity and distribution of emm patterns. These differences should be taken into account for designing treatment guidelines and vaccine strategies.

INTRODUCTION

The incidence of Group A Streptococcus (GAS) diseases vary with location and time. Rheumatic fever is still a major cause of cardiovascular morbidity and mortality in developing countries [1,2], while it has become uncommon in the industrialized world. Pharyngitis occurs predominantly during the cold season in temperate climates and is less frequent in tropical regions [3]. By contrast, skin infections are described at a much higher rate in developing countries than in rich western nations [3,4]. These epidemiological differences are not or poorly understood.

Molecular GAS typing (emm typing) is based on the 5′ end sequence of the emm gene that encodes the hyper variable amino-terminal part of the M protein. This surface protein is a major virulence factor and acts as a protective antigen [5]. Currently, more than 170 recognized emm-types, constituting more than 750 sub-types, have been described (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.htm). Molecular epidemiological data are available for Western countries where a limited number of specific emm-types are predominant. Although few comprehensive and prospective studies have been performed in developing countries [1], a high diversity of emm-types has been described in certain locations [6–9].

The emm pattern serves as a genotypic marker to classify GAS strains according to their tissue tropism (skin or throat). It is based on the organization of emm and emm-like genes located in the mga locus [10,11]. The emm pattern A–C strains are usually recovered from the throat isolates, whereas strains presenting an emm pattern D are most often isolated from the skin. “Generalist” emm pattern E strains are associated with both tissue sites. Rheumatic fever has been historically associated with strains belonging to emm pattern A–C [12]. However, the correlation between emm pattern and site of isolation has not always been confirmed in developing countries, where rheumatic fever is still endemic [9,11]. In Nepal, for instance, A–C strains were preferentially recovered from skin infections [9]. These data indicate no definitive association between GAS emm pattern groups and the site of infection and/or the ability to cause a specific disease.

These epidemiological differences prompted us to conduct a comparative study between an industrialized country (Belgium) and a developing country (Brazil).

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RESULTS

1) Clinical Epidemiology

706 children (360 in Brussels, 346 in Brasilia) with various streptococcal manifestations were included in the study. No significant difference in the mean number of children under the same roof was observed between Brasilia (2.2 children) and Brussels (2 children). 334 GAS isolates (204 in Brussels, 161 of which were reported from the laboratory and clinical data completed retrospectively; and 130 in Brasilia) were recovered from the 706 children. In Brussels, 94% of the GAS isolates were recovered from the throat or external ear (pharyngitis, scarlet fever and otitis) while cutaneous infections were rare (3%). We observed two GAS invasive infections. In Brasilia, GAS were isolated equally from cutaneous infections and pharyngitis. There were 5 GAS invasive infections and 1 sequela (post-streptococcal glomerulonephritis).

Risk factors for pharyngitis and impetigo  The percentage of clinically diagnosed pharyngitis associated with GAS positive culture was higher in Brasilia (26%) than in Brussels (20%) (p = 0.13). In Brasilia, the percentage of positive culture varied with the age of children: 32%, 18% and 0% of the children older than 59 months, from 36 to 59 month old and under 36 month old presented a GAS pharyngitis respectively (p = 0.003) (Figure 1). In Brussels, the prevalence of GAS pharyngitis was unchanged the age of children: 32%, 18% and 0% of the children older than 59 months, from 36 to 59 month old and under 36 month old presented a GAS pharyngitis respectively (p = 0.13). Nutrition indices, crowding, sex or previous antibiotherapy did not constitute a risk for GAS pharyngitis in both Brussels and Brasilia. 61% of impetigo were associated with a GAS positive culture in Brasilia. These streptococcal skin infections were not related to age, sex, crowding, nutrition indices and previous antibiotic treatment during the last six months. The small number of impetigo in Brussels did not allow any statistical analyses.

2) Molecular Epidemiology

emm-types and emm-patterns distribution  The emm-type was successfully determined for 200 of the 204 Belgian isolates and 128 of the 130 Brazilian ones. Among the 200 isolates recovered in Brussels, 20 different emm-types were identified (figure 2 and table S1). A predominance of 6 emm-types, which accounted for 75% of the isolates, was observed in Brussels (emm 6 (20%), emm 1 (14%), emm 12 (12%), emm 89 (11%), emm 4 (11%) and emm 3 (8%)), 55% and 42.5% of the GAS isolates were classified into emm patterns A-C and E respectively, whereas only 0.5% presented an emm pattern D.

Among the 128 isolates recovered in Brasilia which were typed, 48 different emm-types were identified (figure 2 and table S2). None of these emm-type was predominant. The most frequent emm-type (emm 53) represented only 8.5% of the Brazilian isolates. The 6 most prevalent emm-types (emm 53, emm 22, emm 49, emm 58, emm 83, emm 8) among the Brazilian GAS isolates accounted for only 36% of the total number of GAS isolates and nearly half of the emm-types (22/48) were represented by a single isolate. emm patterns E, D and A-C accounted for 51.3%, 36% and 9.5% of the Brazilian isolates respectively.

15 emm-types (emm 1, emm 2, emm 3, emm 4, emm 6, emm 11, emm 12, emm 28, emm 44/61, emm 58, emm 64, emm 75, emm 87, emm 94 and emm st1815) were recovered from children in both Brussels (15/20 emm-types, 84% of the isolates) and Brasilia (15/48, 27% of the isolates).

New emm sub-types 8 new emm sub-types were identified. The 6 Brazilian new emm sub-types represented 7% of the isolates. They differed from the reference strains by a single nucleotide change. In Brussels, we found 2 new sub-types. One differed by a single substitution and the other by a 7 nucleotides change from the reference strains.

emm-types, emm-pattern, clinical presentation and site preference  emm pattern A-C strains are commonly found in the throat, whereas emm pattern D strains are most often isolated from skin lesions. emm pattern E is considered as “generalist”: It is readily found at both tissue sites. Among the Brazilian isolates, the relative risk for emm pattern A-C to be a throat isolate rather than a skin isolate was 1.68 (95% CI, 1.04–2.20). emm pattern D isolates had a relative risk to be recovered from skin rather than from throat of 2.16 (95% CI, 1.53–3.08). However, more emm pattern D than A-C were isolated from the throat of Brazilian children. A comparison of the emm pattern displayed by pharyngeal isolates recovered in both capitals is showed in Figure 3. The distribution of emm-pattern among Brazilian skin isolates showed a classical predominance of emm pattern D (55%) and E (42%) with few A-C (3%). In order to compare the emm pattern from the most relevant isolates from the two cities, the emm-types that were represented by a minimum of 3 isolates were selected (18/48 Brazilian emm-types...
and 16/20 Belgian ones). The proportion of these emm-types that was recovered from both cutaneous and pharyngeal sites was 66% (n = 12) in Brasília and 31% (n = 5) in Brussels. These emm-types were constituted mainly by emm pattern E (Brasília and Brussels, n = 7 and 5 respectively), but also emm pattern D (only in Brasília, n = 4) and A–C (in Brasília and Brussels, n = 1 and 3 respectively).

The 2 cases of invasive infections in Brussels were caused by emm 1 and 3 strains belonging to the emm pattern A–C. By contrast, invasive infections in Brasília were associated with different emm-types (1, 49, 80, 87, st213 and st 6735), belonging to the three different emm pattern (E (n = 4), D (n = 1) and A–C (n = 1)).

**DISCUSSION**

This comparative study illustrates how GAS epidemiology features vary greatly in clinical and molecular aspects between two cities with different history, climate and socio-economical situation, although both performed in underprivileged populations attending public hospitals.

No GAS was isolated in children under 3 year-old in Brasília, while in Brussels, the GAS prevalence was similar in the 1 to 3 year-old group and in older children [14]. This contrasts with previous studies showing that the incidence of GAS pharyngitis was lower in children younger than 5 year-old than in older children [15]. Moreover, Belgian children with GAS pharyngitis were younger than Brazilian ones. Differences in day care attendance, administrated vaccines and antibiotics consumption in the first years of life might contributed to the variations we observed, as these factors can modify the naso-pharyngeal flora.

In the Belgian children, GAS infections were mostly attributable to emm pattern A–C and E strains. In Brasília, the emm pattern A–C was rarer than in Brussels while emm pattern D was much more frequent. The predominance of emm pattern D strains in tropical
epidemiology has already been described [16]. However, the Brazilian emm pattern D strains, which classically have a skin tropism, presented interesting characteristics: (i) emm pattern D strains were recovered in one fifth (18.5%) of the isolates collected from the throat of Brazilian children. (ii) one third of the “non unique” Brazilian emm-types (which were represented by a minimum of 3 isolates) recovered from both throat and skin sites belonged to emm pattern D. (iii) emm pattern D strains (emm 53.0) were simultaneously isolated from the skin and the throat of two children presenting a sore throat associated with a skin infection. Some emm pattern D could therefore be considered as “generalist” with variable skin tropism. Similar findings were found in Ethiopia [7] and Nepal [9], where the emm pattern D strains were equally isolated from the skin and the throat. Previous studies showed that emm pattern D strains presented (with emm pattern E) the higher level of emm-type and multilocus sequence type (ST) diversity among the different emm pattern [10]. Recombination events appeared to be the most frequent in emm pattern D strains and included replacement of the emm-type locus [10]. Similarly, multilocus sequence typing identified substantial genetic recombination between the so-called skin and throat strains as well as various new combinations of emm and housekeeping genes in a small community with high rates of rheumatic fever and GAS impetigo [17]. Some emm pattern D strains might have evolved to acquire the capacity to colonize and/or infect both cutaneous and pharyngeal environment and therefore cause rheumatic fever. In Brasília, where rheumatic fever is endemic (Rheumatic fever was responsible of one third of the Brazilian heart surgeries in 2004; data from the Brazilian ministry of Health, Sistema Único de Saúde (SUS) - Ministério da Saúde), GAS skin infections were frequent and emm pattern D strains were more “generalist” than previously thought. Moreover, the A-C strains (classically associated with rheumatic fever) were rarely isolated. These findings are in agreement with the possible link between cutaneous strains and rheumatic fever in some epidemiological contexts. Indeed, if in temperate regions, rheumatic fever appears to be associated exclusively with throat infections, recent data suggested that GAS impetigo strains are probably involved directly or indirectly in acute rheumatic fever in particular communities [11,16,17]. In Ethiopia, emm pattern D strains were over represented (57%; 4/7) in the isolates associated with rheumatic fever [7]. Skin infections might constitute the GAS reservoir which triggers auto-immune reaction with or without secondary throat colonisation.

The high rate of rheumatic fever in Brasilia could also be associated with the impressive emm-types diversity we observed (48 emm-types over 128 isolates). This wide variety of emm-type circulating in Brasilia is probably responsible for a great diversity of anti-M proteine antibodies, hence increasing the risk for autoimmune response. Reports of GAS epidemiology outside Western nations are scarce but contradictory. Some of them (Korea, Mexico and aboriginal Australian) show the presence of a few predominant emm-types [11,18,19]. In other countries (India, Ethiopia and Nepal), a high diversity of emm-types was reported [6-9], although they were different from one location to the other. The studies showing a highly polyclonal distribution were performed in locations where massive and recent human migrations occurred. The city of Brasilia was built in a poor deserted region 45 years ago. The population of Brasilia, which has become one of the most developed city of the country, is now about 2 millions and immigrated from the whole country. The resulting diversity of hosts might contribute to the diversity of emm-types. By contrast with the Brazilian data, the emm-type distribution in Brussels was oligoclonal as typically seen in western countries [20].

This comparative study suggests that differences in the selective pressure between industrialised and low incomes regions of the world could favour the genetic evolution of GAS emm-types and therefore their emergence and/or persistence. Further studies are needed to clarify these differences and their impact for the prevention of GAS infections and sequelae. The theoretical coverage of the 26 valent GAS vaccine [21] would be 76% in Brussels whereas in Brasilia, it would be as low as 32%. This low theoretical coverage is similar to that reported in the studies from India, Ethiopia and Nepal. This is of particular relevance as these countries are those who could most benefit from a GAS vaccine.

MATERIALS & METHODS

We prospectively studied the epidemiology of GAS infections from February 1 to October 31, 2004 in children from Brussels and Brasilia.

Patients

Patients 0–15 year old attending 3 public hospitals of Brasilia (Hospital Universitário de Brasília, Hospital Regional da Asa Sul and Unidade mista de Saúde de São Sebastião with 250, 180 and 40 beds respectively) and one public hospital of Brussels (Hôpital Universitaire des Enfants Reine Fabiola with 170 beds) were included in this study. This study was approved by the ethical board of all participant hospitals. An informed consent was obtained from each child’s parent or guardian. Inclusion criteria were any infections with clinical suspicion of GAS aetiology or GAS isolation by the laboratory. Exclusion criterion was a current antimicrobial treatment. A pre-designed form was filled out by the pediatricians with personal, epidemiological and clinical data. GAS pharyngitis, cutaneous infections and otitis were defined as a positive GAS culture associated with symptoms of sore throat, impetigo and discharge from ear respectively. Scarlet fever was considered as clinically distinct from pharyngitis. Invasive infections were defined by a GAS isolation from a normally sterile body site. Post-streptococcal glomerulonephritis and acute rheumatic fever were defined according to published criteria [13]. Physicians were trained to swab with a standardised technique. No clinical follow-up was achieved.

Collection and isolation

Samples were plated on blood agar (5%) and were incubated at 37°C for 24–48H in the presence of 5% CO₂. Bloodcultures were incubated (Bactec®, Becton Dickinson, Franklin Lakes, United States) at 37°C for seven days. Beta haemolytic streptococci were phenotypically identified by beta haemolysis on blood agar, colony morphology, gram stain, catalase reaction and sensitivity to 0.04 U bacitracin disk. GAS identification was performed with a positive latex agglutination test containing group A specific antiserum (SlideX; Biomérieux, Paris, France).

emm typing

emm-typing of all isolates was performed according to the protocol described by the Center for Disease Control (CDC; http://www.cdc.gov/ncidod/biotech/strep/protocols.html). Primer 1 (5‘-TAT TGG CTT AGA AAA TTA A-3’) and primer 2 (5‘-AGT TCT TCG CTT TGC TT-3’) or primer MF2 (5‘-GGA TCC ATA AGG AGC ATA AAA ATG GCT A-3’) and MR1 (5‘-TGA TAG CTT AGT TTT CTT CTT TGC GTC TT-3’) were used to amplify the 5’ region of the emm gene as described by the CDC. emmseq2 primer (5‘-TAT CTT AGA AAA TTA AAA ACA GG-3’) and MF2 were used for sequencing. The sequences were compared to those in the databases available on the CDC.
emm pattern

GAS isolates within a given emm type usually display the same emm pattern [10]. The emm pattern for each isolate was predicted according to McGregor et al., 2004 [10].

Statistical Analysis:
Bilateral chi-square and student t test (SPSS 11.0, Gaussian Statistical Analysis:

**SUPPORTING INFORMATION**

**Table S1** emm-types, sub-type and pattern by clinical presentation in Brussels.REA: rearranged, TSS: toxic shock syndrome, Cell: cellulitis

**Table S2** emm-types, sub-type and pattern by clinical presentation in Brasília.REA: rearranged, TSS: toxic shock syndrome, Cell: cellulitis

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