Interpersonal relationships and group A streptococcus spread in a Mexican day-care center

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Abstract

Objective. To study the effect of different degrees of centrality on the carrying of identical group A streptococcus (GAS) clones in the nasopharynx of children from a Mexican public day-care center. **Material and Methods.** Nasopharyngeal cultures were performed in children from rooms B (RB) (n = 35) and C (RC) (n = 37). The Restriction Fragment Length Polymorphism (RFLP) patterns were compared among GAS isolates. A social networks questionnaire was filled out for each child and 10 classmates. Structure coefficients were compared among children with and without GAS. **Results**. Four GAS clones were identified; clone I in five children from RC; clone II in two from RC and one from RB; clone III in one from RB and one from RC; and clone IV in one from RC. Social network structure: Density of RB and RC = 0.40 (± 0.87) and 0.35 (± 0.80), respectively. In RB, the homophily pattern of interaction was different in carriers (0.00), non-carriers (0.47) and both (0.47) p = 0.35. In RC, the homophily pattern was also different in carriers (0.46), non-carriers (0.68) and mixed (0.19), p = .001. In 4/5 with clone I, the values of degree, closeness and betweenness were above the group mean. In 3/3 with clone II, the values of degree and betweenness were also above the mean. In contrast, in those with clone III and IV, the values of degree, closeness and betweenness were below the group mean. **Conclusions.** The spread of specific GAS clones was associated with groups of children having a high proportion of ties and a high centrality level. This is evidence that spread of GAS strains among children attending day-care centers is not random but dependent on the degree of communication and physical contact between pairs.

Keywords: *Streptococcus pyogenes*; networks; child day-care centers; Mexico

Villaseñor-Sierra A, Quiñonez-Alvarado MG, Caballero-Hoyos JR. Relaciones interpersonales y diseminación del estreptococo del grupo A (EGA) en una guardería de México. Salud Publica Mex 2007;49:323-329.

Resumen

Objetivo. Evaluar el efecto de grados diferentes de centralidad con la presencia de clonas idénticas de estreptococo del grupo A (EGA) en la nasofaringe de niños de una guardería pública de México. **Material y métodos.** Se realizaron cultivos nasofaríngeos en niños de los salones B (SB) (n = 35) y C (SC) (n = 37). El patrón de polimorfismos de longitud de fragmento por restricción (Restriction Fragment Length Polymorphism, RFLP) fue comparado entre aislamientos de EGA. Un cuestionario de redes sociales fue llenado para cada niño y 10 compañeros. Los coeficientes de estructura fueron comparados entre niños con y sin EGA. **Resultados.** Se identificaron cuatro clonas de EGA. Clona I en cinco niños del SC; clona II en dos del SC y en uno del SB; clona III en uno del SB y uno del SC; y clona IV en uno del SC. Estructura de redes sociales: Densidad SB y SC = 0.40 (± 0.87) y 0.35 (± 0.80), respectivamente. En SB, el patrón de homofilia de la interacción fue distinto en portadores (0.00), no portadores (0.47) y ambos (0.47) p = 0.35. En SC, el patrón de homofilia fue distinto en portadores (0.46), no portadores (0.68) y mixto (0.19), p = .001. En 4/5 con la clona I, los valores de grado, cercanía e intermediación estuvieron por arriba de la media grupal. En 3/3 con la clona II, los valores de grado e intermediación estuvieron por arriba de la media grupal. En contraste, en los niños con clonas III y IV, los valores de grado, cercanía e intermediación estuvieron por debajo de la media grupal. **Conclusiones.** La diseminación de clonas específicas de EGA se asoció a grupos de niños con gran proporción de lazos entre ellos y un alto nivel de centralidad. Esto evidencia que la transmisión de EGA entre niños de guardería no ocurre al azar sino que depende del grado de comunicación y contacto físico entre éstos.

Palabras clave: *Streptococcus pyogenes*; relaciones interpersonales; guarderías; México

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Group A Streptococcus (GAS) is an important cause of mild, moderate and severe human infections worldwide.[1,2] In day-care centers, GAS is responsible for nasopharyngeal, perianal and respiratory infections in children, along with impetigo, outbreaks of scarlet fever, and invasive infections associated with varicella.[3-7]

Several studies have determined the prevalence of GAS carriers and the spread of the bacteria during outbreaks among children attending day-care centers.[4,8-10] Healthy GAS carriers play an important role as potential sources of spread of GAS strains (with diverse degrees of virulence) between individuals.[11] Results from these studies showed that some risk factors related to the spread of virulent GAS strains are: a) sharing the index case's room, and b) the time in hours (more than 24 hours) that the "contacts" spend with the index case during a seven day period. A deeper approach for studying the relationships and interactions between groups of people, and how an action of one may affect others, is called the Social Network Analysis (SNA).[12] This approach makes it possible to identify the "most important or central" actors in a group.[13] SNA has been used to study the spread of diseases[14,15] and thus can be applied to help understand the effect of different degrees of communication and physical interaction patterns on the spread of a particular GAS strain among children. Common SNA measurements distinguish a specific actor based in their centrality in the network. Centrality measures include degree, closeness, and betweenness, which quantify the prominence of an individual actor embedded in a network.[13,16]

The aim of the study was to determine the relationship between sharing the same GAS clones and the interpersonal communication structure in children from a day-care center in Mexico.

Materials and Methods

A microbiologic survey was conducted from October 31st to November 7th, 2001, to detect nasopharyngeal carriers of GAS among children 3 to 4 years old, from two rooms (room B; *n* = 35 and room C; *n* = 37) at a day-care center of the Instituto Mexicano del Seguro Social (Mexican Institute of Social Security). Children attending the day-care center were from a middle socioeconomic stratum of Guadalajara, Mexico. Signed parental consent and approval of the Local Research and Ethics Committee (1305) of the Instituto Mexicano del Seguro Social were obtained prior to the survey, Samples were taken from the nasopharynx using a sterile cotton swab, transported to the microbiology laboratory in Stuart's medium, and cultured in 5% sheep blood agar within two hours of obtaining the specimen. Agar plates were incubated at 37 °C and 5% CO_2 for 18 hours. Beta hemolytic colonies were isolated and plated on a 5% sheep blood agar to test the sensitivity to bacitracin (TAXO A, BBL, Becton Dickinson. Sparks, MD, USA). Sensitive strains were subjected to latex agglutination (Strepto-Kit, bioMerieux. Marey, France) to confirm their identity as group A streptococci.

Because a history of antimicrobial consumption could affect the rate of GAS isolation in the nasopharynx cultures, parents of each child were asked about antibiotic use during the last two months. The type of antibiotic and the time elapsed since the last dose were recorded.

The polymorphisms of DNA from GAS isolates were analyzed by comparing the RFLP patterns of the VIR regulon by the method described by Gardiner et al[17] and modified by Hartas et al.[18] Briefly, one colony of a GAS strain was cultured overnight (ON) in Todd Hewitt Broth supplemented with yeast extract and glycine (THBYG), at 37 °C and 5% CO_2. After a purity check was performed, a 10% volume of the ON culture was sub-cultured in fresh warm THBYG and incubated under the same conditions for two hours to obtain a logarithmic phase culture. The culture was then centrifuged and the bacterial pellet washed twice with Tris-HCl pH 8.2. Bacterial lysis was performed by adding polyethylene-glycol (PEG) and lysozyme, and incubating the mix at 37 °C in an ON water bath. The sample was then centrifuged, the pellet re-suspended in Tris-HCl, EDTA, Proteinase K and SDS, and incubated in water bath at 37 °C. After ON incubation, NaCl was added and the sample centrifuged. The supernatant was discarded and the resulting DNA pellet was washed twice with ethyl alcohol and air dried overnight. The DNA was incubated at 37 °C in TE buffer until dissolved. A sample of DNA was run in a 1% agarose gel and stained with ethidium bromide to visualize the DNA. The DNA was then quantified and the Vir regulon amplified using primers VUF and SBR with the following PCR conditions: one cycle at 95 °C (1'), 30 cycles at 95 °C (15"), one cycle at 60 °C (2') and one cycle at 68 °C (6'). The amplified products were verified by running the samples in a 2% agarose gel. The DNA was then digested adding either *Hae*III or *Hinf*I and incubated in a water bath for two hours. The digested products were run in a 2.5% agarose gel and the bands obtained between 200-4000 bp were compared visually among each GAS strain. We considered a strain to be a distinct clone if at least one band was different.

Social networks evaluation was performed by one investigator blinded to the culture result. This investigator separately interviewed the two persons in charge of the children of each room by asking the

question "with whom does each child have the most communication?" The question was applied to a list of approximately ten classmates of each child. Frequency of communication with each classmate was ranged as extensive, some, or little. Because information regarding the communication profiles of each child was obtained from two persons in charge of the child, discrepancies did arise. These were resolved by re-questioning the caregivers until complete agreement was obtained.

Degree is a term used to describe the extent to which persons are tied to others in the "network". The closeness is a measure of how close or integrated is an actor to an interaction pattern of a local network of actors. An actor is central if he or she has a better efficiency in the connection to others, due to a more direct communication path. The betweenness refers to how a particular actor might be able to control interactions between pairs of other actors in the network. Density is the proportion of ties in a social network. Finally, homophily is a pattern of communication between actors who share some similar attributes. Measures of centrality (degree, closeness and betweenness) were compared among children with and without GAS. Central actors have a maximal centrality index.

Statistical analysis. Descriptive analysis was performed calculating frequencies, means and standard deviation. Comparative analysis included comparison of proportions among independent groups.

Results

GAS isolates and antimicrobial usage. GAS was detected in 2/35 (5.7%) and in 9/37 (24.3%) of the children from rooms B and C respectively. The percentage of children having used antimicrobials during the last two months was as follows: Group B, 12/35 (34.3%); Group C, 11/37 (29.7%). There were no differences in the history of antimicrobial usage between children harboring GAS and those without GAS from either group B (Chi square 1.38, d.f. = 1; p = .43) or group C (Chi square 0.32, d.f. = 1; p = .69). The time elapsed since the last antibiotic dose ranged from 1 to 60 days (median = 5) in the children from group B, and from 2 to 30 days (median = 12) in the children from group C. The antimicrobials most frequently prescribed for those from group B were β-lactams such as ampicillin or amoxicillin (50%), cephalosporins (25%), macrolides (16.6%) and sulfonamides (8.4%); whereas those used by group C were ampicillin/amoxicillin (54.5%), sulfonamides (27.3%), cephalosporins (9.1%) and macrolides (9.1%) (tables I and II).

GAS isolation in children with and without symptoms of upper respiratory infection. Information regarding symptoms of upper respiratory tract infections in the last 15 days was available in 29/35 (83%) children of group B and in 34/37 (92%) of group C. Among these children, 14/29 children of group B had symptoms of an upper respiratory tract infection; of those, 2 had a GAS isolated (2/14, 14%) and 12 were negative for GAS (Fisher's exact test, 2.30 d.f. 1; p = .22). In children of group C, upper respiratory symptoms were present in 23/34; of these, GAS was isolated in 7 (7/23, 30%) (Fisher's Exact Test 0.57, d.f. 1; p = .68).

Identification of GAS clones. The RFLP patterns of the various GAS isolates were heterogeneous and four different clones were identified and named I to IV according to the prevalence of the clones. Clone I was isolated in five children of group C; clone II in two children of group C and in one of group B; clone III was isolated in one child of group B and in one of group C; clone IV was isolated in a single child of group C (figure 1).

Partnership among children of group C with identical GAS clones. Among the 5 children harboring clone I, the degrees of communication between each other was: "extensive" (n = 2), "some" (n = 1), "little" (n = 1) and none (n = 1). The 2 children harboring clone II shared "some" degree of communication. Although children harboring clone III belonged to different rooms, they shared a single dining room at lunchtime.

The social network analysis. The density coefficient values of groups B and C were 0.40 (± 0.87) and 0.35 (± 0.80). This means that in group B the proportion of ties among the group was 40%, whereas in group C the proportion was 35%. The homophily pattern of interaction in group B was different in carriers (0.00), non carriers (0.47) and both groups together (0.47), (p = .35). The homophily pattern in group C was also different in carriers (0.46), non-carriers (0.68) and both groups together (0.19), (p = .001).

The centrality data showed that in 4/5 children with clone I, the values of degree, closeness and betweenness were higher than the group mean. In 3/3 children with clone II, values of degree and betweenness were also higher than the group mean. On the other hand, in the two children with clone III and the one with clone IV (the less frequently isolated clones), the values of degree and betweenness were lower than the group mean. The most widely distributed clones (I and II) were associated with those children having the highest centrality levels (figures 2 and 3).

Discussion

Group A Streptococcus was found in the nasopharynx of children from a day-care center in Mexico, and the clonal diversity between the isolated strains as well as

Table I

Centrality values of the 35 children attending room B of a day-care center of Guadalajara in 2001, with or without a group A streptococci nasopharyngeal isolate

Children with a nasopharyngeal culture negative for S. pyogenes

ID	*Gender*	*Degree*	*Closeness*	*Betweenness*	*Antimicrobials Last two Mo.*	*Days since last doses*	*Antimicrobial*
6	M	*50.00	*66.67	*4.53	No	-	None
8	F	26.47	57.63	1.44	No	-	None
12	F	32.35	59.65	0.93	No	-	None
17	M	20.59	55.74	0.39	No	-	None
24	F	20.59	53.97	0.70	Yes	1	β-lactamic
29	M	20.59	54.84	0.76	No	-	None
30	F	23.53	55.74	0.79	No	-	None
37	M	*64.71	*73.91	*8.40	No	-	None
39	M	11.76	52.31	0.07	No	-	None
41	F	*61.76	*72.34	*5.41	Yes	5	Macrolide
43	M	*35.29	*60.71	1.54	Yes	3	Cephalosporin
46	F	*44.12	*64.15	*2.27	Yes	35	Macrolide
50	M	23.53	56.67	0.29	No	-	None
51	M	14.71	52.31	0.50	No	-	None
55	M	26.47	57.63	0.72	No	-	None
56	M	29.41	58.62	0.73	Yes	2	β-lactamic
69	F	*38.24	*60.71	1.43	No	-	None
98	M	*41.18	*62.96	1.69	No	-	None
105	M	*67.65	*75.56	*8.98	No	-	None
111	F	29.41	58.62	1.26	No	-	None
153	M	*47.06	*65.38	*3.54	No	-	None
174	M	*38.24	*61.82	1.71	Yes	1	β-lactamic
176	F	23.53	55.74	0.92	No	-	None
181	M	26.47	56.67	0.71	No	-	None
183	F	32.35	59.65	1.04	Yes	-	None
186	M	23.53	56.67	0.82	No	-	None
196	F	*52.94	*68.00	*5.09	No	-	None
207	F	*47.06	*65.38	*4.11	Yes	1	β-lactamic
208	M	20.59	53.13	0.27	Yes	9	β-lactamic
209	F	32.35	59.65	1.02	Yes	60	β-lactamic
212	M	26.47	57.63	0.69	No	-	None
213	M	*38.24	*61.82	*3.49	Yes	8	β-lactamic
214	F	26.47	57.63	0.81	No	-	None
* Above mean:		13 of 33	13 of 33	9 of 33			

Children with a nasopharyngeal culture positive for S. pyogenes

CLONE	*ID. No.*	*Gender*	*Degree*	*Closeness*	*Betweenness*	*Antimicrobials Last two Mo.*	*Days since last doses*	*Antimicrobial*
III	78	F	29.412	58.621	1.233	Yes	10	Sulfonamides
II	82	F	*35.294	59.649	*3.208	Yes	22	β-lactamic
Mean (group)			33.78	60.23	2.04			
SD (group)			13.71	5.77	2.19			
* Above mean:			1 of 2	0 of 2	1 of 2			
			‡*P* = .66	*P* = .71	*P* = .91			

‡ Comparison of proportions among children with and without a GAS isolate

Table II

CENTRALITY VALUES OF THE 37 CHILDREN ATTENDING ROOM C OF A DAY-CARE CENTER OF GUADALAJARA IN 2001, WITH OR WITHOUT A GROUP A STREPTOCOCCI NASOPHARYNGEAL ISOLATE

Children with a nasopharyngeal culture negative to S. pyogenes

ID	*Gender*	*Degree*	*Closeness*	*Betweenness*	*Antimicrobials Last two Mo.*	*Days since last doses*	*Type of Antimicrobial*
1	M	*36.11	55.38	1.35	Yes	6	Cephalosporins
4	M	22.22	55.38	0.62	No	-	None
26	M	*52.78	*67.92	*6.13	No	-	None
27	M	22.22	50.00	0.14	Yes	20	Sulfonamides
40	M	27.78	52.17	0.47	No	-	None
45	F	25.00	51.43	0.38	No	-	None
47	M	25.00	54.55	0.49	No	-	None
57	M	30.56	58.06	1.51	No	-	None
58	F	*41.67	*62.07	*3.32	No	-	None
59	M	16.67	48.65	0.00	No	-	None
70	M	25.00	57.14	0.55	Yes	30	β-lactamic
74	F	*55.56	*69.23	*9.26	No	-	None
79	M	*63.89	*73.47	*7.24	No	-	None
80	M	*52.78	*67.92	*5.28	Yes	22	β-lactamic
83	F	22.22	51.43	0.21	No	-	None
85	F	*47.22	*65.45	*5.01	Yes	15	β-lactamic
86	F	19.44	48.65	0.02	No	-	None
103	M	27.78	56.25	0.52	No	-	None
109	M	22.22	56.25	0.77	No	-	None
143	F	*33.33	54.55	0.59	No	-	None
157	M	19.44	50.70	0.05	Yes	5	β-lactamic
159	F	22.22	56.25	0.66	Yes	3	Sulfonamides
162	F	30.56	56.25	1.16	Yes	12	Macrolide
166	M	22.22	52.94	0.95	No	-	None
187	F	*50.00	*66.67	*7.22	No	-	None
189	M	*41.67	*63.16	1.84	No	-	None
198	M	19.44	55.38	0.24	No	-	None
200	M	25.00	52.17	0.21	Yes	3	β-lactamic
* Above mean:		10 of 28	8 of 28	7 of 28			

Children with a S. pyogenes positive nasopharyngeal culture

CLONE	*ID. No.*	*Gender*	*Degree*	*Closeness*	*Betweenness*	*Antimicrobials Last two Mo.*	*Days since last doses*	*Antimicrobial*
II	7	F	*33.33	*60.00	*2.32	No	-	None
I	20	M	*36.11	*61.02	*2.61	No	-	None
III	61	F	19.44	55.38	0.80	Yes	30	Sulfonamides
I	81	M	*44.44	*61.02	*3.20	No	-	None
I	94	M	*63.89	*73.47	*7.49	No	-	None
I	161	F	27.78	52.17	0.27	Yes	2	β-lactamic
I	188	M	*38.89	*62.07	*2.83	No	-	None
IV	190	M	27.78	52.94	0.48	No	-	None
II	197	F	*41.67	*61.02	*2.39	No	-	None
Mean (group)			33.33	58.07	2.12			
SD (group)			13.09	6.71	2.53			
* Above mean:			6 of 9	6 of 9	6 of 9			
			‡*P* = .21	*P* = .09	*P* = .06			

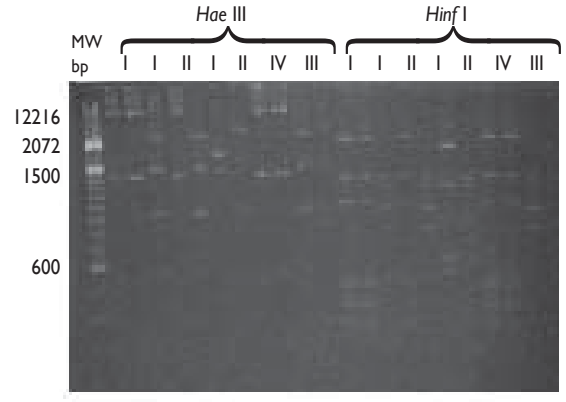
‡ Comparison of proportions among children with and without a GAS isolate

the degree of physical interaction between pairs was evaluated. We found that individuals having a high level of centrality in the interaction shared a common GAS isolate.

The prevalence of GAS isolated from the nasopharynx of the children studied was similar to reports from other day-care centers.[4,9] In contrast with the frequent isolation of a single clone of GAS in outbreaks of pharyngitis and other day-care associated infections, multiple clones GAS have been described in healthy GAS carriers,[19] even among those sharing the same space. Indeed, in our study there were four different GAS clones isolated simultaneously from healthy children sharing the same room.

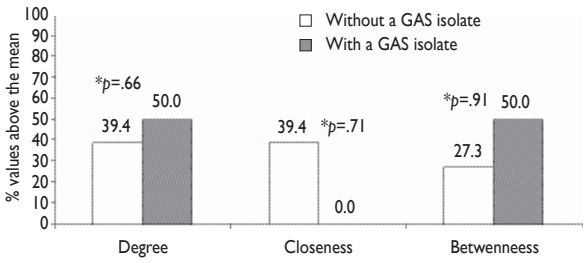
Regarding the measurement of interaction among the children, the density values of the groups showed an expected proportion of ties. On the other hand, the homophily pattern clearly defined two groups of children, i.e., those with and without a GAS isolate. Belonging to any of these groups integrated by some particular characteristics (i.e. age, gender) could facilitate the natural spread of strains of GAS once it has been introduced into one particular group.

Genetic analysis of the isolated bacteria was crucial for demonstrating epidemiological patterns. For example, of the five children carrying the most widespread strain (clone I), four had values of ties with others (degree), mediation between pairs (betweenness), and efficient communication actors (closeness) that were superior to the mean group values. The next most widespread strain (clone II) was isolated in three children whose values of degree and betweenness were also above the mean of the group. Finally, in the two children with clone III and in the one child with clone IV, the values of degree and betweenness were below the group mean.



The first column in the left shows the nucleic acid markers (100 bp DNA Ladder), followed by the RFLP patterns of each of the clones identified (clones I-IV). Two different restriction enzymes (*Hae*III and *Hinf*I) were used to corroborate the consistency of the vir typing method

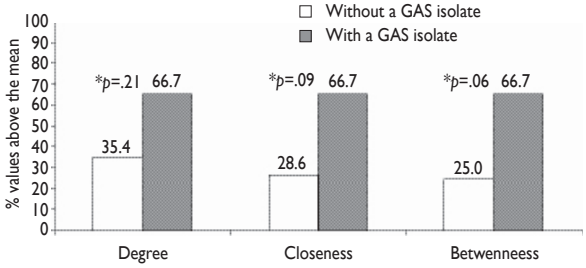
Figure 1. RFLP patterns of the vir regulon from 11 GAS strains isolated from children of a day-care center



* Comparison between proportions

The figure shows the percentage of children from "group B" with centrality values (degree, closeness and betweenness) above the group mean, with and without GAS isolate. Differences between group percentages was considered statistically significant when $p \leq .05$

Figure 2. Comparison of centrality values among children from group B, with and without a Group A streptococcus nasopharyngeal isolate



* Comparison between proportions

The figure shows the percentage of children from "group C" with centrality values above the group mean, with and without a GAS isolate. Differences in the percentages between groups were considered statistically significant when p value $\leq .05$

Figure 3. Comparison of centrality values among children from group C, with and without a Group A streptococcus nasopharyngeal isolate

Our results provide strong evidence that the horizontal transmission of GAS strains between carriers in children attending day-care centers is predictable. Specifically, we found that the children from this day-care center tend to group by some common characteristics. That grouping is usually associated with a high proportion of ties among their members, and may facilitate the spread of GAS strains. Transmissibility of GAS in daycare centers has not been previously quantified. This evidence could be helpful to target children at highest risk of infection by virulent GAS clones acquired from index children with invasive infections. It also provides guidance regarding the use of nasopharyngeal cultures or prophylactic antimicrobial treatment.

In conclusion, the GAS strains isolated simultaneously from children sharing the same room were genetically diverse. The sharing of specific GAS clones between carriers was not random but was associated with natural formation of groups based upon some particular characteristic, high proportion of ties among them and a high level of communication centrality.

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