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Brief report

Characterization of group A beta-haemolytic streptococcus with mucoid phenotype isolated in a tertiary hospital☆

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ABSTRACT

Introduction: The objective of this study is to characterize *Streptococcus pyogenes* isolates with a mucoid phenotype and to compare them with non-mucoid isolates obtained between April and August 2016.
Material and methods: Identification and antimicrobial susceptibility were performed in all isolates. The *emm* type and exotoxin genes *speA*, *speB*, *speC*, *speF*, *speG*, *speH*, *speJ*, *speZ* and *ssa* were analyzed. Clinical and demographic data were collected.
Results: From 96 isolates analyzed, 47% had a mucoid phenotype and 95.5% of them presented *speA-speB-speF-speG-ssa* genes and *emm3* genotype. The main clinical manifestation was pharyngotonsillitis (77.1%) evolving to scarlet fever in 67.5% of the cases.
Conclusion: This study describes the circulation of a mucoid phenotype strain with a *speA-speB-speF-speG-ssa* toxin profile and *emm3*.1 genotype considered one of the most frequent and virulent of SGA.

Caracterización de estreptococos beta-hemolíticos del grupo A con fenotipo mucoide aislados en un hospital de tercer nivel

Palabras clave:
Streptococcus pyogenes
Escarlatina
Mucoide
Serotipo *emm*3

RESUMEN

Introducción: El objetivo de este estudio es la caracterización de cepas de *Streptococcus pyogenes* con fenotipo mucoide y su comparación con las cepas no mucoides aisladas entre abril y agosto de 2016.
Material y métodos: Se llevó a cabo la caracterización y el estudio de sensibilidad antimicrobiana de todos los aislados. Se determinó el tipo *emm* y se analizaron los genes de exotoxinas *speA*, *speB*, *speC*, *speF*, *speG*, *speH*, *speJ*, *speZ* y *ssa*. Se recogieron datos clínicos y demográficos.
Resultados: De 96 aislados analizados, el 47% presentaron un fenotipo mucoide, y de estos últimos, el 95,5% presentaron los genes *speA-speB-speF-speG-ssa* y genotipo *emm*3. La principal manifestación clínica entre todos los pacientes fue faringoamigdalitis (77,1%) que evolucionó a escarlatina en el 67,5% de los casos.
Conclusión: Se describe la circulación de una cepa de aspecto mucoide con perfil de toxinas *speA-speB-speF-speG-ssa* y genotipo *emm*3.1 considerado de los más frecuentes y más virulentos de SGA.

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Introduction

Streptococcus pyogenes or group A beta-haemolytic streptococcus (GAS) causes numerous infectious processes that range from pharyngitis or skin infections to invasive diseases such as bacteraemia, septic arthritis, pneumonia, toxic shock syndrome or puerperal sepsis[1].

The M protein, encoded by the *emm* gene, has been described as the main virulence factor of GAS, and is used to classify strains into various genotypes[2]. Other determinants of virulence are streptococcal pyrogenic exotoxins, encoded by the *spe* genes, and the expression of hyaluronic acid capsular polysaccharide, which confers a mucoid appearance to the colonies[3].

In April 2016, a sudden increase in *S. pyogenes* isolates with a mucoid phenotype was observed in the samples received at the Microbiology Service of the Hospital Universitario Reina Sofía [Reina Sofía University Hospital] (HURS) in Cordoba. The objective of this study was the epidemiological and molecular characterisation of these strains with mucoid phenotype and their comparison with non-mucoid strains isolated in the same period of time.

Material and methods

GAS isolates obtained from clinical samples received at the HURS Microbiology Service between April and August 2016 were studied. The samples were seeded on blood agar plates with a bacitracin disk and incubated for 48 h in an atmosphere enriched with 5% CO_2 at 35 ± 2 °C. Identification and antimicrobial susceptibility was carried out by microdilution with panels of the semi-automated Wider system (Panel 94B MIC/ID, Francisco Soria Melguizo SA, Madrid, Spain). The results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Isolates resistant to erythromycin were classified into three phenotypes based on the sensitivity pattern shown in the double-disk test with erythromycin (15 μg) and clindamycin (2 μg): M phenotype (erythromycin resistant and clindamycin sensitive), constitutive MLS_B phenotype (erythromycin and clindamycin resistant) and inducible MLS_B phenotype (erythromycin resistant and inducible clindamycin resistance detected by flattening of the clindamycin inhibition halo near the erythromycin disk).

Molecular characterisation of the strains was carried out at the Carlos III Health Institute. The *emm* genotype of all isolates was determined by PCR amplification and subsequent sequencing of the *emm* gene following the Centers for Disease Control and Prevention (CDC) protocol (https://www.cdc.gov/streplab/protocol-emm-type.html). The exotoxin genes *speA*, *speB*, *speC*, *speF*, *speG*, *speH*, *speJ*, *speZ* and *ssa* were analysed by PCR[4]. Clinical and demographic data of the patients in whom the studied strains were isolated were collected. The data were analysed in the SPSS® programme, version 19.0.

Results

A total of 96 GAS isolates obtained from pharyngeal exudate (74; 77.1%), sputum (4; 4.2%), vaginal discharge (8; 8.3%), wound exudate (3; 3.1%), blood (2; 2.1%), ear discharge (1; 1%), bronchial aspirate (1; 1%), lymph node biopsy (1; 1%), pleural fluid (1; 1%) and lochia (1; 1%). The samples came from 14 primary healthcare centres (PHCs) (70; 72.9%), paediatric emergency departments (9; 9.4%), specialised clinics (10; 10.4%), internal medicine (4; 4.2%), the intensive care unit (2; 2.1%) and thoracic surgery (1; 1%). The mean age of the study patients was 16 years (range: <1–76 years); 61.5% were women and 38.5% were men.

Colonies from 45 (46.9%) of the isolates had a mucoid appearance and 51 (53.1%) had the classic appearance of shiny flat colonies.

In the set of 96 isolates, 13 genotypes were identified, the most frequent type being *emm*3 (49.4%), followed by *emm*4 (10.4%), emm*6* (6.3%) and emm*12* (5.2%). A new *emm*3 subtype, classified by the CDC as *emm*3.166, was identified in 11 patients. Among the isolates with a mucoid phenotype, 44 (97.8%) belonged to the *emm*3 type, with the majority being the *emm*3.1 (75.6%) and *emm*3.166 (15.6%) subtypes. The other mucoid isolate corresponded to the *emm*77 type. In all, 31.4% of the non-mucoid isolates presented the *emm*3 genotype, including emm*3.1* (23.5%) and *emm*3.166 (7.8%), as well as 10 other genotypes, the most frequent being *emm*4 (19.6%), *emm*6 (11.8%) and *emm*12 (9.8%).

19 different toxin profiles were identified. The most common *spe* genes were *speB* (92.7%), *speF* (91.6%) and *speG* (86.5%). A total of 95.5% of the strains with a mucoid phenotype presented the *speA-speB-speF-speG-ssa* genes. In strains with a non-mucoid phenotype, the most common toxin profiles were *speA-speB-speF-speG-ssa* and genotype *emm*3 (25.5%), followed by *speB-speC-speF-speG-speH* and genotype *emm*6 (21.6 %).

All isolates were sensitive to penicillin, ampicillin, clindamycin and vancomycin. Three isolates showed resistance to erythromycin with the M phenotype of macrolide resistance, presenting the genotypes *emm*77 with a mucoid phenotype, *emm*3 with a mucoid phenotype, and *emm*94 with a non-mucoid phenotype.

The main clinical manifestation was pharyngotonsillitis in 77.1% of the cases, which progressed to scarlet fever in 67.5% of them. Some 65% of these cases were in children under 5 years of age. No significant differences ($p = 0.220$) were observed between the mucoid and non-mucoid phenotypes regarding the development of scarlet fever. Of the scarlet fever-producing isolates, 67.3% presented the *emm*3 type, although this trend did not reach a statistically significant association ($p = 0.17$).

Six invasive diseases were diagnosed, four of them (one bacteraemia, one mediastinitis, one knee arthritis and one cervical adenopathy) caused by isolates with a mucoid phenotype and *emm*3.1 genotype, with no statistically significant association ($p = 0.316$). Two of these patients died. The other two invasive diseases (a bacteraemia that ended in death and a puerperal sepsis) were caused by isolates with a non-mucoid phenotype with genotypes *emm*87 and *emm*89. Another death occurred in a patient with lung cancer in whom GAS (non-mucoid phenotype with genotype *emm*1) was isolated in a sputum sample. The rest of the clinical manifestations were vaginitis (eight cases), pneumonia (three cases), wound infection (two cases) and ear infection (one case).

Table 1 shows the distribution of isolated cases by phenotype, sample type, syndrome, *emm* genotype and exotoxin gene profile.

Discussion

The most prevalent *emm* genotypes of GAS globally are *emm*1, *emm*28, *emm*3, *emm*89 and *emm*4. This distribution varies significantly according to geographic region and clinical manifestation, with the *emm*1, *emm*3, *emm*28 and *emm*18 types being the ones most associated with invasive disease[5,6]. In Spain, the most common *emm* genotypes are *emm*1, *emm*3, *emm*4, *emm*12, *emm*28 and *emm*89, with *emm*1 and *emm*3 being the ones mainly associated with invasive disease[7,8].

Of the six invasive diseases observed in this study, four of them were caused by the *emm*3 type with a mucoid phenotype, showing a non-statistically significant trend probably due to the low number of isolates producing invasive diseases obtained in this series.

One case of puerperal sepsis was detected due to the *emm*89 genotype, previously associated with this disease[3].

Table 1
Relationship between phenotype, sample type, syndrome, *emm* genotype and exotoxin gene profile in 96 group A streptococcus isolates.

Phenotype (n)	Sample type (n)	Syndrome (n)	*emm* genotype (n)	Exotoxin profile (n)
Mucoid (45)	Pharyngeal exudate (34)	Pharyngotonsillitis (9)	3.1 (6)	A,B,F,G,J,*ssa* (2)
				A,B,F,G,*ssa* (4)
			3.93 (1)	A,B,F,G,*ssa* (1)
			3.166 (1)	A,B,F,G,*ssa* (1)
			77 (1)	B,C,F (1)
		Scarlet fever (25)	3.1 (18)	A,B,F,G,J,*ssa* (4)
				A,B,F,G,*ssa* (13)
				A,G,*ssa* (1)
			3.166 (6)	A,B,F,G,*ssa* (6)
			3.93 (1)	A,B,F,G,*ssa* (1)
	Sputum (2)	Pneumonia (1)	3.1 (1)	A,B,F,G,J,*ssa* (1)
		Bronchiectasis (1)	3.1 (1)	A,B,F,G,*ssa* (1)
	Pleural fluid (1)	Mediastinitis (1)[a]	3.1 (1)	A,B,F,G,*ssa* (1)
	Vaginal discharge (4)	Vulvovaginitis (4)	3.1 (3)	A,B,F,G,J,*ssa* (1)
				A,B,F,G,*ssa* (1)
				G,*ssa* (1)
			3.24 (1)	A,B,F,G,*ssa* (1)
	Ear discharge (1)	Middle ear infection (1)	3.1 (1)	A,B,F,G,J,*ssa* (1)
	Blood (1)	Bacteraemia (1)[a]	3.1 (1)	A,B,F,G,*ssa* (1)
	Purulent discharge (1)	Knee arthritis (1)[a]	3.1 (1)	A,B,F,G,*ssa* (1)
	Lymph node biopsy (1)	Cervical adenopathy (1)[a]	3.1 (1)	A,B,F,G,*ssa* (1)
Non-mucoid (51)	Pharyngeal exudate (40)	Pharyngotonsillitis (16)	1.0 (1)	A,B,F,G,J,Z (1)
			3.1 (5)	A,B,F,G,J,*ssa* (1)
				A,B,F,G,*ssa* (3)
				B,F,G,*ssa* (1)
			3.166 (1)	A,B,F,G,*ssa* (1)
			4.0 (4)	B,C,F,J,Z,*ssa* (1)
				B,C,F,Z,*ssa* (2)
				B,C,F,G (1)
			6.0 (1)	B,C,F,G,H (1)
			12.0 (2)	Negative (1)
				B,C,F,G (1)
			82.0 (1)	A,B,C,F,G,H (1)
			89.0 (1)	B,F,G (1)
		Scarlet fever (24)	1.0 (1)	A,B,F,G,J,Z (1)
			3.1 (6)	A,B,F,G,*ssa* (6)
			3.166 (3)	Negative (1)
				A,B,F,G,*ssa* (2)
			4.0 (6)	C,Z,*ssa* (2)
				B,C,F,Z,*ssa* (4)
			6.0 (3)	B,C,F,G,H (3)
			12.0 (2)	B,C,F,G,H (2)
			21.0 (1)	B,C,F,G (1)
			87.0 (1)	B,C,F,G,J,Z,*ssa* (1)
			94.1 (1)	B,C,F,G,H (1)
	Sputum (2)	Pneumonia (1)	1.0 (1)	Negative (1)
		Bronchiectasis (1)	6.0 (1)	B,C,F,G,H (1)
	Bronchial aspirate (1)	Pneumonia (1)	12.0 (1)	B,C,F,G,H (1)
	Blood (1)	Bacteraemia (1)[a]	87.0 (1)	B,F,G,J,Z,*ssa* (1)
	Vaginal discharge (4)	Vulvovaginitis (4)	2.0 (1)	B,C,F,G,J (1)
			3.1 (1)	A,B,F,G,*ssa* (1)
			6.0 (1)	B,C,F,G,H,J (1)
			12.36 (1)	B,C,F,G,H (1)
	Purulent discharge (2)	Groin wound (1)	75.0 (1)	B,C,F,G (1)
		Pressure ulcer (1)	6.4 (1)	B,C,F,G,H (1)
	Lochia (1)	Puerperal sepsis (1)[a]	89.0 (1)	B,C,G (1)

[a] Invasive diseases.

In other parts of the world, such as in the US, mucoid strains are widely distributed, but there are few studies on this phenotype in Spain. Several works associate this virulence factor with the *emm*3 genotype[9,10]. A total of 97.8% of the isolates with a mucoid phenotype in this study presented the *emm*3 genotype (*emm*3.1, *emm*3.24, *emm*3.93 and the newly described *emm*3.166), with the majority being *emm*3.1 with a *speA-speB-speF-speG-ssa* toxin profile. The non-mucoid population presented up to 11 different *emm* genotypes, with *emm*3.1 being one of the most frequent, as well as different toxin profiles.

Some 51% of the patients developed scarlet fever, 65% being under five years of age. This could make it difficult to differentiate this condition from viral infections more typical of this age. These data coincide with those obtained by studies in other countries that have observed an increase in the rate of scarlet fever in recent years[11]. In Spain, as scarlet fever is not a notifiable disease, it is difficult to determine its real incidence.

Some authors associate the production of *speA* pyrogenic exotoxin with the appearance of scarlet fever[12]. In this study, 69% of the scarlet fever-producing isolates carried the *spe*A gene compared to 60% of the pharyngitis-producing isolates, with no significant differences observed ($p = 0.419$) between both groups. These results coincide with those obtained by other authors where no differences were observed[13].

The three isolates resistant to erythromycin (*emm*77, *emm*3 and *emm*94) presented the M phenotype, this being the most frequent in GAS isolated in Spain. Antibiotic resistance in GAS is concentrated in some genotypes, with *emm*77 being one of the most resistant

emm types. The *emm*94 genotype is less frequent in Spain, but also shows high resistance to erythromycin.

The circulation of a mucoid strain with a *speA-speB-speF-speG-ssa* toxin profile and *emm*3.1 genotype, considered to be one of the most frequent and most virulent GAS strains, has been described[3,5].

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Cunningham MW. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev. 2000;13:470–511.
2. Griffith F. The serological classification of *Streptococcus pyogenes*. J Hyg (Lond). 1934;34:542–84.
3. Luca-Harari B, Darenberg J, Neal S, Siljander T, Strakova L, Tanna A, et al. Clinical and microbiological characteristics of severe *Streptococcus pyogenes* disease in Europe. J Clin Microbiol. 2009;47:1155–65.
4. Schmitz FJ, Beyer A, Charpentier E, Normark BH, Schade M, Fluit AC, et al. Toxin-gene profile heterogeneity among endemic invasive European group A streptococcal isolates. J Infect Dis. 2003;188:1578–86.
5. Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR. Global emm type distribution of group A streptococci: systematic review and implications for vaccine development. Lancet Infect Dis. 2009;9:611–6.
6. Lamagni TL, Darenberg J, Luca-Harari B, Siljander T, Efstratiou A, Henriques-Normark B, et al. Epidemiology of severe *Streptococcus pyogenes* disease in Europe. J Clin Microbiol. 2008;46:2359–67.
7. Rivera A, Rebollo M, Miro E, Mateo M, Navarro F, Gurgui M, et al. Superantigen gene profile, emm type and antibiotic resistance genes among group A streptococcal isolates from Barcelona, Spain. J Med Microbiol. 2006;55:1115–23.
8. Montes M, Ardanuy C, Tamayo E, Domenech A, Linares J, Perez-Trallero E. Epidemiological and molecular analysis of *Streptococcus pyogenes* isolates causing invasive disease in Spain (1998–2009): comparison with non-invasive isolates. Eur J Clin Microbiol Infect Dis. 2011;30:1295–302.
9. Tamayo E, Montes M, Garcia-Medina G, Garcia-Arenzana JM, Perez-Trallero E. Spread of a highly mucoid *Streptococcus pyogenes* emm3/ST15 clone. BMC Infect Dis. 2010;10:233.
10. Sierra JM, Sanchez F, Castro P, Salvado M, de la Red G, Libois A, et al. Group A streptococcal infections in injection drug users in Barcelona, Spain: epidemiologic, clinical, and microbiologic analysis of 3 clusters of cases from 2000 to 2003. Medicine (Baltimore). 2006;85:139–46.
11. Watts V, Balasegaram S, Brown CS, Mathew S, Mearkle R, Ready D, et al. Increased Risk for Invasive Group A streptococcus disease for household contacts of scarlet fever cases, England, 2011–2016. Emerg Infect Dis. 2019;25:529–37.
12. Tyler SD, Johnson WM, Huang JC, Ashton FE, Wang G, Low DE. Streptococcal erythrogenic toxin genes: detection by polymerase chain reaction and association with disease in strains isolated in Canada from 1940 to 1991. J Clin Microbiol. 1992;30:3127–31.
13. Wu PC, Lo WT, Chen SJ, Wang CC. Molecular characterization of Group A streptococcal isolates causing scarlet fever and pharyngitis among young children: a retrospective study from a northern Taiwan medical center. J Microbiol Immunol Infect. 2014;47:304–10.