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Barcelona
29 y 30 Septiembre 2017

¿Qué hay de nuevo en la endocarditis por *Streptococcus* de grupo viridans?

SEICAV
Sociedad Española de Infecciones Cardiovasculares

Dr. Txema Marimón
Hospital Universitario Donostia
Donostia-San Sebastián
CIBER de enfermedades respiratorias

Endocarditis por Streptococus viridans

128 *Memórias do Instituto Oswaldo Cruz* **41**, (1) 1944

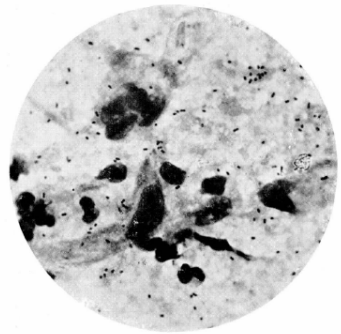
Endocardites sépticas

(Contribuição etiopatogênica)

por

J. Guilherme Lacorte e Mario Santos

(Com 40 figuras no texto)



Considerações gerais

O estudo bacteriológico das endocardites adquiriu extraordinária importância desde o momento em que Schottmueller descreveu, como agente etiológico da forma lenta, o *Streptococcus viridans*, assim como emprestou à doença o caráter de entidade mórbida autônoma. Antes dêsses trabalhos, realmente

Streptococcus:

Beta hemolíticos: piogénicos

Alfa hemolíticos (viridans): anginosus, bovis, mitis, mutans, sanguinis y salivarius

S. infantis, S. mitis, S. oralis , S. pneumoniae, S. pseudopneumoniae

International Journal of Systematic and Evolutionary Microbiology (2012), 62, 2941–2945 DOI 10.1099/ijs.0.038299-0

Streptococcus tigurinus sp. nov., isolated from blood of patients with endocarditis, meningitis and spondylodiscitis

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azbinden@imm.uzh.ch

Four Gram-stain-positive, catalase-negative, coccus-shaped bacterial strains were isolated from multiple blood cultures of patients with endocarditis, meningitis and spondylodiscitis. The isolates were tentatively identified as viridans streptococci on the basis of phenotypic characteristics. Comparative 16S rRNA gene sequencing studies showed that the organisms were members of the *Streptococcus mitis* group but did not correspond to any recognized species. The nearest phylogenetic relative was *S. mitis* ATCC 49456^T, with 98.6 % sequence similarity. The representative strain AZ_3a^T showed less than 96.8, 97.8, 94.5 and 95.5 % similarity to the phylogenetically most closely related species by *recA*, *rpoB*, *sodA* and *groEL* gene sequence analysis, respectively. DNA–DNA hybridization analyses showed a low reassociation value of 32.2 % between strain AZ_3a^T and *S. mitis* DSM 12643^T. Reassociation values with members of other *S. mitis* group species ranged from 27.3 to 49.7 %. The G+C content of the DNA was 40.0 mol%. Based on our biochemical and molecular analyses, the isolates represent a novel species, for which the name *Streptococcus tigurinus* sp. nov. is proposed. The type strain is AZ_3a^T (=CCOS 800^T =DSM 24864^T).

Accurate identification of bacteria within the *Streptococcus mitis* group remains a challenge, in particular for *Streptococcus mitis*, *S. pneumoniae*, *S. pseudopneumoniae* and *S. oralis*. Conventional phenotypic methods are limited in providing an accurate identification (Arbique *et al.*, 2004), and sequence analysis of the 5′ part of the 16S rRNA gene is not sufficiently discriminative to differentiate these species, because the sequence similarity is >99 % (Arbique *et al.*, 2004; Kawamura *et al.*, 1995). Several other target genes such as *sodA* (Kawamura *et al.*, 1999; Poyart *et al.*, 1998), *rpoB* (Drancourt *et al.*, 2004) and *groEL* (Glazunova *et al.*, 2009) have been investigated for species differentiation, primarily using type strains, and we recently proposed *recA* as an alternative target to differentiate *S. pneumoniae* from other viridans streptococci (Zbinden *et al.*, 2011). Since commensal species such as *S. oralis* and *S. mitis* have been recognized as important agents of endocarditis (Douglas *et al.*, 1993; Spellerberg & Brandt, 2011), accurate identification of these bacteria is important.

Recently, we isolated a viridans streptococcal organism from a 74-year-old patient with endocarditis that was present in six out of six blood cultures and was designated strain AZ_3a^T. Molecular analyses by 16S rRNA gene sequencing were performed for accurate identification. An identical sequence was also detected in an aortic valve specimen of the patient by direct 16S rRNA gene broad-range PCR (Bosshard *et al.*, 2003). BLAST analysis of the 16S

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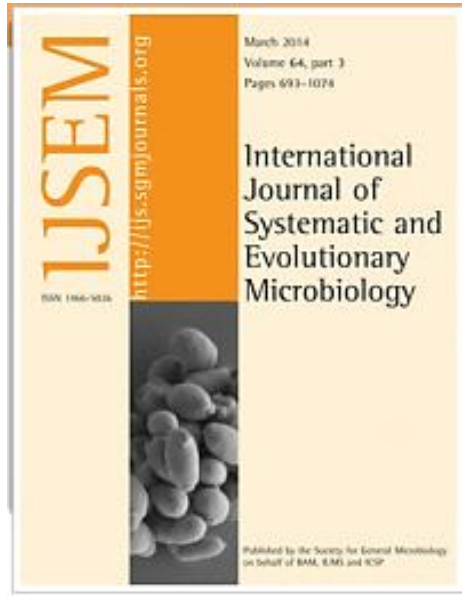
Abbreviation: MALDI-TOF, matrix-assisted laser desorption ionization-time of flight.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *recA*, *rpoB*, *sodA* and *groEL* gene sequences of strain AZ_3a^T are JN004270, JN004271, JQ085955, JQ085956 and JQ085957, respectively.

Five supplementary figures are available with the online version of this paper.



Universität Zürich[UZH]

Institut für Medizinische Virologie

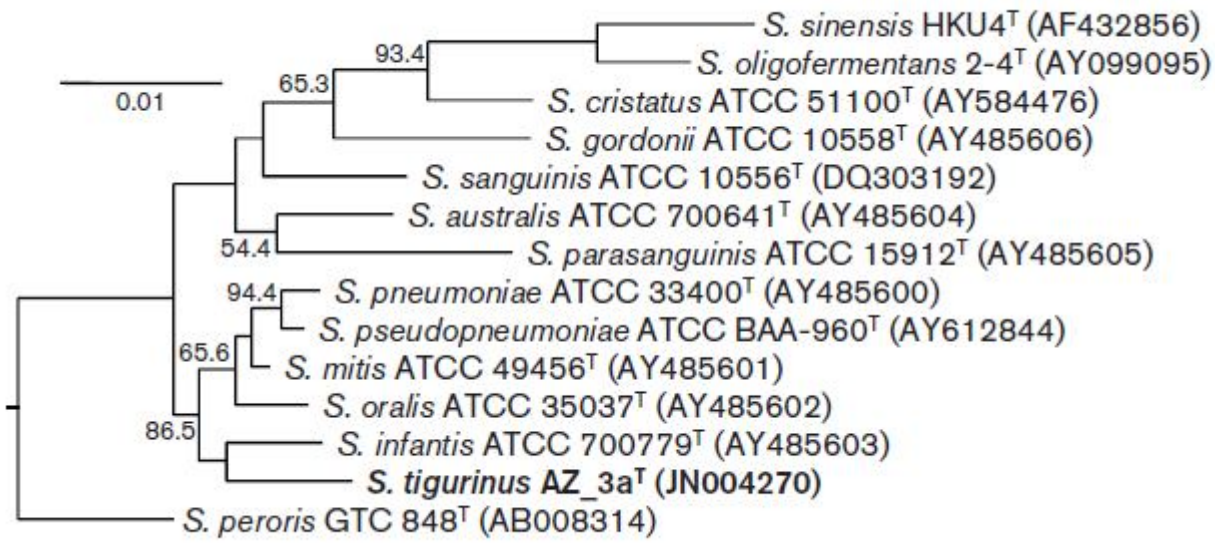
Andrea Zbinden, PhD.

Similitud con otras especies relacionadas de *Streptococcus*

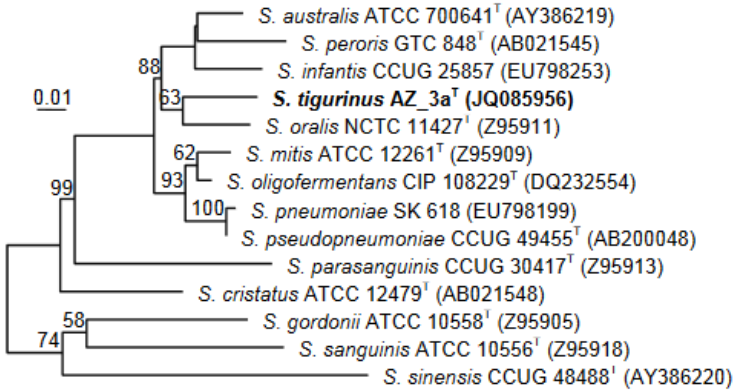
Gen 16S rRNA (>1300 bp)

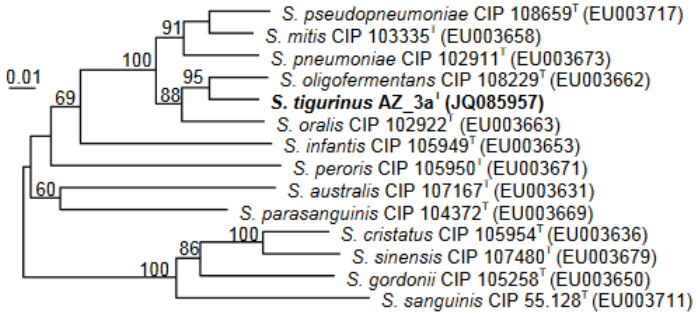
S. mitis (98.6%), *S. infantis* (98.5%), *S. pseudopneumoniae* (98.3%), *S. pneumoniae* (98.2%), *S. oralis* (98.1%).

Neighbour-joining phylogenetic tree based on partil 16S rRNA genes sequences



Neighbour-joining phylogenetic tree based on partial *sod*A *and gro*EL genes sequences





*rpo*B, *sod*A y *gro*EL: rama diferenciada de otras especies del grupo *S. mitis*

Hibridación DNA–DNA (% reasociación) :

S. oralis - 49.7% ±2.2%
S. pneumoniae - 40.9% ±4.2%
S. pseudopneumoniae - 35.4% ±4.6%
S. mitis - 32.2% ±4.2%
S. infantis - 27.3 ±4.0%

Nueva especie:

Streptococcus tigurinus

(adj. *tigurinus* perteneciente a **Tigurum**, distrito de Helvetia, actual Zurich)

JCM
Journals.ASM.org

Streptococcus tigurinus, a Novel Member of the *Streptococcus mitis* Group, Causes Invasive Infections

Andrea Zbinden,[a] Nicolas J. Mueller,[b] Philip E. Tarr,[c] Gerhard Eich,[d] Bettina Schulthess,[a] Anna S. Bahlmann,[a] Peter M. Keller,[e] and Guido V. Bloemberg[a]

Hosp. Univ. Zurich
2002-2013 EI: 48 casos por SGV

Especie	n	%
S. mitis/S. oralis	13	27%
S. sanguinis	11	23%
*S. tigurinus**	7	14.5%
S. gordonii	6	12.5%
Otros S. viridans	11	23%

* Inicialmente identificados como *S. mitis*

Reservorio. Microbiota oral??:

Saliva de 31 voluntarios: 0/608 alfa-hemolíticos estudiados (A. Zbinden et al, JCM 2015)

27/51 (53%) de los casos de periodontitis y de los controles (A. Zbinden et al, BMC Microbiol 2016)

23/35 (66%) en saliva, con una proporción 0.01 - 12.5% del total de bacterias (G. Conrads et al. JOM 2017)

Tracto gastrointestinal: fluido peritoneal, absceso perianal... (Bourasssa et al. JCM 2015)

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Brushing teeth could prevent meningitis: research

Brushing one's teeth and flossing regularly could help prevent meningitis, a study that identifies a link between a common type of mouth bacteria and the disease suggests.

Researchers have found a type of oral bacteria that they have linked to meningitis. Photo: GETTY

By Stephen Adams, Medical Correspondent
7:00AM GMT 22 Feb 2012

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Researchers in Zurich found the new unidentified bacterium *Streptococcus tigurinus* in the blood of patients with meningitis.

They also found it in patients with spondylodiscitis, or inflammation of the spine, and a type of heart disease called endocarditis.

Dr Andrea Zbinden, who led the study, said the bacterium "seems to have a natural potential to cause severe disease".

It could get into the bloodstream through bleeding gums, she added, although she noted its "specific risk" had yet to be determined. The study is published today (Wednesday) in the *International Journal of*

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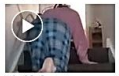

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Groźna bakteria z ust wywołuje zapalenie opon mózgowych

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23.02.2012, 09:07 | Aktualizacja: 23.02.2012, 09:09

1 | 2 | 0 | 5

Streptococcus tigurinus kolonizuje jamę ustną i może wywołać zapalenie opon mózgowych / Shutterstock

Naukowcy zidentyfikowali w jamie ustnej nowy typ bakterii, który może zaatakować mózg i wywołać śmiertelną

Najczęściej czytane

12 sposobów zmiany stylu życia, by zmniejszyć ryzyko zachorowania na raka

Witamina D działa podobnie jak testosteron. Ważna dla sportowców

Sąd w Inowrocławiu nie ograniczył władzy rodzicom, którzy nie zaszczepili dziecka

Erozja szkliwa, czyli co zjada twoje zęby?

Gdy masz za dużo potasu w organizmie. Czym jest hiperkaliemia?

Daily Mail

Lethal bug lurking inside the mouth could kill if it enters bloodstream, say scientists

By DAILY MAIL REPORTER
UPDATED: 08:49 BST, 22 February 2012

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A bacteria living in the mouth could cause serious disease or even kill if it enters the bloodstream, a new study warns.

Researchers have identified the bacterium, called Streptococcus tigurinus, for the first time and found it could pass into the bloodstream through bleeding gums into the bloods.

Scientists carried out tests, isolating the bacterium from the blood of patients suffering from endocarditis (inflammation of the heart), meningitis and spondylodiscitis (inflammation of the spine).

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Brush teeth 'to stave off meningitis'

Swiss researchers found a link between a common type of mouth bacteria and meningitis.

elCOLOMBIANO

Bacteria bucal que daña el corazón

29. feb / biología Salud / No hay comentarios

International Journal of Systematic and Evolutionary Microbiology (2016), 66, 4803–4820

DOI: 10.1099/ijsem.0.001433

Re-evaluation of the taxonomy of the Mitis group of the genus *Streptococcus* based on whole genome phylogenetic analyses, and proposed reclassification of *Streptococcus dentisani* as *Streptococcus oralis* subsp. *dentisani* comb. nov., *Streptococcus tigurinus* as *Streptococcus oralis* subsp. *tigurinus* comb. nov., and *Streptococcus oligofermentans* as a later synonym of *Streptococcus cristatus*

Anders Jensen, Christian F. P. Scholz and Mogens Kilian

Department of Biomedicine, Aarhus University, Wilhelm Meyers Allé 4, Aarhus 8000, Denmark

Correspondence
Anders Jensen
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The Mitis group of the genus Streptococcus currently comprises 20 species with validly published names, including the pathogen *S. pneumoniae*. They have been the subject of much taxonomic confusion, due to phenotypic overlap and genetic heterogeneity, which has hampered a full appreciation of their clinical significance. The purpose of this study was to critically re-examine the taxonomy of the Mitis group using 195 publicly available genomes, multilocus sequences designated type strains for phylogenetic analyses based on core genomes, including and 16S rRNA gene sequences, combined with estimates of average nucleotide identity (ANI) and *in silico* and *in vitro* analyses of specific phenotypic characteristics. Our core genomic phylogenetic analyses revealed distinct clades that, to some extent, and from the clustering of type strains represent known species. However, many of the genomes have been incorrectly identified adding to the current confusion. Furthermore, our data show that 16S rRNA gene sequences and ANI are unsuitable for identifying and circumscribing new species of the Mitis group of the genus Streptococci. Based on the clustering patterns resulting from core genome phylogenetic analysis, we conclude that *S. oligofermentans* is a later synonym of *S. cristatus*. The recently described strains of the species *Streptococcus dentisani* and *S. tigurinus* form referred to as '*S. mitis* biovar 2'. Together with *S. oralis*, *S. dentisani* and *S. tigurinus* form subclusters within a coherent phylogenetic clade. We propose that the species *S. oralis* consists of three subspecies: *S. oralis* subsp. *oralis* subsp. nov., *S. oralis* subsp. *tigurinus* comb. nov., and *S. oralis* subsp. *dentisani* comb. nov.

INTRODUCTION

The Mitis group of the genus *Streptococcus* currently comprises 20 species with validly published names (*Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus pneumoniae*, *Streptococcus pseudopneumoniae*, *Streptococcus dentisani*, *Streptococcus tigurinus*, *Streptococcus infantis*, *Streptococcus peroris*, *Streptococcus sanguinis*, *Streptococcus parasanguinis*, *Streptococcus gordonii*, *Streptococcus cristatus*, *Streptococcus oligofermentans*, *Streptococcus australis*, *Streptococcus massiliensis*, *Streptococcus sinensis*, *Streptococcus rubneri*, *Streptococcus lactarius*, *Streptococcus parodentis*). Although some-times giving rise to infections like septicaemia and subacute endocarditis in predisposed individuals, they are most generally regarded to be commensal inhabitants of the oral cavity and upper respiratory tract (Doern & Burnham, 2010). The exception is *S. pneumoniae*, which is one of the leading bacterial pathogens on a global scale, causing infections like

Abbreviations: ANI, average nucleotide identity; ANIb-values, ANI based on blastn; MLSA, multilocus sequence analysis.

Two supplementary figures and four supplementary tables are available with the online Supplementary Material.

INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

Official publication of the ICSP and the BAM Division of the IUMS

Noviembre de 2016

AARHUS UNIVERSITY

Århus · Department of Biomedicine (Denmark)

Anders Jensen, Dr.

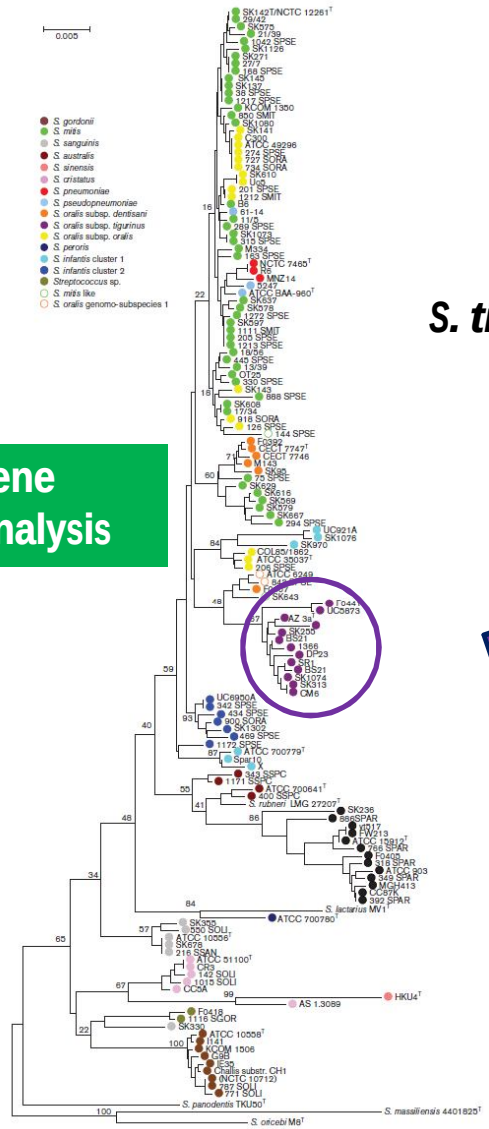

Fig. 6. Minimum evolution tree, based on partial 16S rRNA gene sequences of all twenty type strains belonging to the Mitis group of the genus streptococci combined with 130 16S rRNA gene sequences extracted from the genomes included in the study. The tree is based on 1317 bp; colouring is based on the clustering found from the core genome phylogeny, as shown in Figs 2, 3 and 4. Numbers at major branches represent bootstrap values based on 500 replications.

16S rRNA gene sequence analysis

S. tigurinus

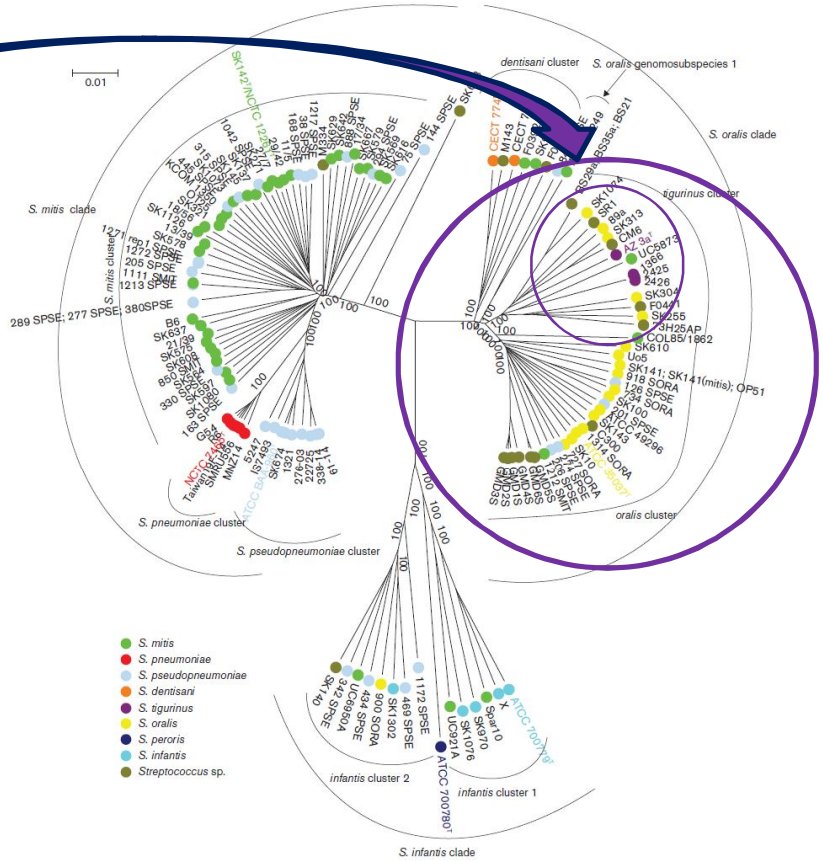

Fig. 3. Minimum evolution tree based on the core genome sequences of 135 genomes from GenBank belonging to the *S. mitis*, *S. oralis* and *S. infantis* clades, as defined in Fig. 2. The tree is based on 802 323 bp strains, which are coloured according to previous species annotation in the GenBank database. Type strains are colour-highlighted. Numbers at major branches represent bootstrap values based on 500 replications.

WGS analysis

J Infect Chemother 22 (2016) 762–766

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

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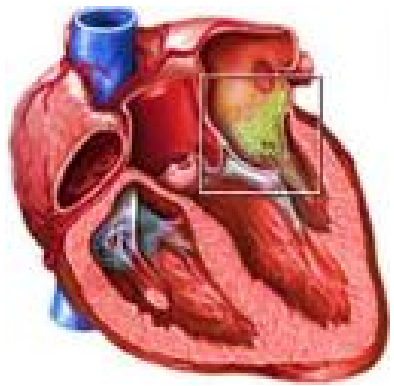
Case report

Bacteremia due to *Streptococcus tigurinus*: A case report and literature review

Jun Hirai [a, b, c], Daisuke Sakanashi [b], Mao Hagihara [b], Shusaku Haranaga [c], Kohei Uechi [c], Hideo Kato [b], Hiroyuki Hamada [b], Naoya Nishiyama [a, b, c], Yusuke Koizumi [a, b], Hiroyuki Suematsu [b], Yuka Yamagishi [a, b], Jiro Fujita [c], Hiroshige Mikamo [a, b, *]

[a] Department of Clinical Infectious Diseases, Aichi Medical University Hospital, Japan
[b] Department of Infection Control and Prevention, Aichi Medical University Hospital, Japan
[c] Department of Infectious Diseases, Respiratory, and Digestive Medicine, Graduate School of Medicine, University of the Ryukyus, Japan



33 casos (7 artículos):

11 endocarditis	33,3%
5 bacteriemia	15,2%
4 infección protésica	12,1%
3 infección tejido blando	9,1%
3 espondilodiscitis	
2 peritonitis	6,1%
2 osteomielitis	
1 empiema torácico, meningitis, periodontitis	

*Cartas científicas / Enferm Infecc Microbiol Clin. 2015;**33(8)**:569–576*

Endocarditis por *Streptococcus tigurinus*: a propósito de un caso y revisión de la literatura

Asier Michelena*, Claudia Bonavila, Beñat Zubeltzu y Miguel Angel Goenaga

Unidad de Enfermedades Infecciosas, Hospital Universitario Donostia, San Sebastián, Guipúzcoa, España

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Table 3
English and Japanese published literature related to infection with *Streptococcus tigurinus* between 2012 and 2015 including the present case.

Case	Age	Sex	Diagnosis	Underlying disease	Possible source	Treatment	Operation	Outcome	Ref#
3	74	F	IE	Heavy alcohol use	Oral	CVA/AMPC and then PCG and GM	Unknown	Recovered	2
4	37	F	IE	None	Unknown	PCG and GM and then CTRX	Unknown	Recovered	2
8	64	M	IE	None	Unknown	CVA/AMPC and GM and then PCG	Unknown	Recovered	2
9	65	M	IE	None	Unknown	CTRX and then CVA/AMPC and GM	Unknown	Recovered	2
13	47	M	IE, focal encephalitis	None	Unknown	CTRX and AMPC	Unknown	Recovered	2
14	57	M	IE	None	Unknown	PCG and GM and then PCG	Unknown	Recovered	2
15	21	F	IE	None	Unknown	PCG and GM	Unknown	Recovered	2
29	58	M	IE	Mitral regurgitation, hypertension	Unknown	CTRX + GM	Yes	Recovered	10
30	75	M	IE	Unknown	Unknown	CTRX + GM	Yes	Recovered	10
31	40's	M	IE	Dental problem	Oral	CTRX	Yes	Recovered	11
32	79	M	IE, spondylitis	Unknown	Unknown	CTRX and then PCG	Yes	Recovered	12

Factores de virulencia y biomarcadores de EI para *S. tigurinus*

Factores virulencia

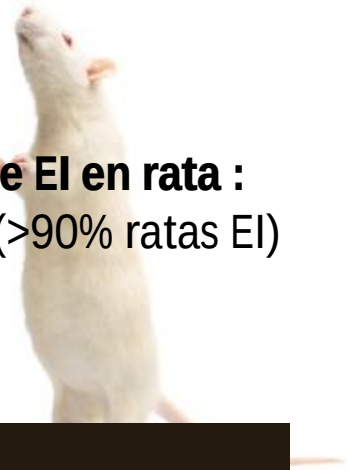
Toxina exfoliativa

Proteína de unión de fibronectina

(Y Gizard et al. 2013)

Autolysin LytA, cholin-binding protein CbpG, hyaluronidases HylA/HysA

(G. Conrads, 2017)



Virulencia. Modelo experimental de EI en rata :

10^4 ufc/mL *S. tigurinus*/ *S. aureus*: (>90% ratas EI)

Otros SGV: 10^5-10^7 ufc/mL

(TR Veloso et al. 2013)

Biomarcadores de endocarditis

No hay ninguno definitivo

Candidatos: NT-proBNP, Cys C, LBP, troponins, AQP9, S100A11, E-selectin, VCAM-1, IL-6.

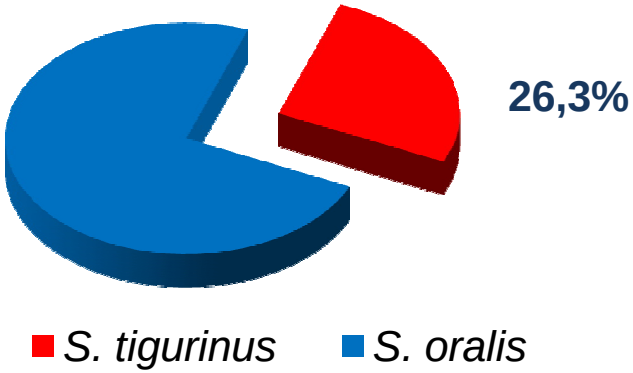
(MG Snipsøyr et al. Int J Cardiol 2016)

Presencia de *S. tigurinus* en HU Donostia

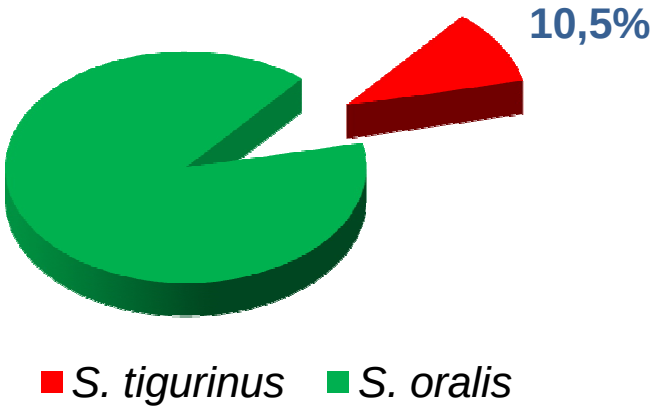
5/19 *S. oralis* aislados de hemocultivos

2/19 de muestras respiratorias

Hemocultivos



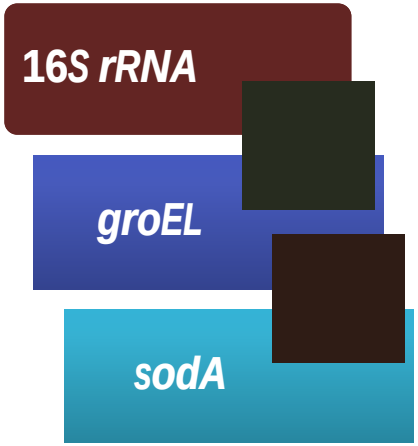
Respiratorio



Endocarditis (9) → ***S. tigurinus* (4)** → **Exitus (1)**

Spanish group of Infective endocarditis (GAMES)

Resultados preliminares
Identificación de EI por SGV estudio GAMES



Especie	Número de pacientes (n=50)
S. oralis subsp. tigurinus	15
S. oralis	15
S . mitis	13
S. parasanguinis	2
S. sanguinis	2
S. infantis	1
S. infantarius subsp. infantarius	1
S. anginosus	1

Ramón y Cajal: 1/3
Puerta de Hierro: 0/2
Gregorio Marañón: 2/5
Valdecilla: 2/6
Clínico BCN: 6/19
Costa del Sol: 0/1
HU Donostia: 4/14

Bellvitge: 3/17

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Oral Streptococcus MLST Databases

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ST65, ST30

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Search Oral Streptococcus locus/sequence definitions database by combinations of loci

Schemes

Please select the scheme you would like to query:

S. oralis MLST | Select

Isolate fields									S. oralis MLST							
id	isolate	aliases	species	country	year	source	carious	serotype	aroE	ddl	gdh	gki	hexB	recP	xpt	ST
88	SB3A		S. oralis	UK	2006	supra-gingival plaque	caries free		39	33	19	2	37	8	12	65

Analysis tools:

Breakdown: Fields | Two Field | Combinations | Polymorphic sites | Schemes/alleles | Publications

Analysis: Codons | Presence/Absence

Export: Dataset | Sequences

Please enter your allelic profile below. Blank loci will be ignored.

aroE	ddl	gdh	gki	hexB	recP	xpt
39	33	19	2	37	8	12

ST: 65 | Autofill

Options

Search: Exact or nearest match

Order by: ST | ascending

Display: 25 records per page

Action

Reset | Submit

Search or browse Oral Streptococcus isolates database

Profile information for ST-30 (S. oralis MLST)

ST	aroE	ddl	gdh	gki	hexB	recP	xpt
30	10	11	8	10	8	9	11

form parameters to filter or enter a list of values.

Allele designations/scheme fields

ST (S. oralis MLST) | = | 30

3 records returned. Click the hyperlinks for detailed information.

Isolate fields									S. oralis MLST							
id	isolate	aliases	species	country	year	source	carious	serotype	aroE	ddl	gdh	gki	hexB	recP	xpt	ST
11	13C2		S. oralis	UK	2001	supra-gingival plaque	root caries lesions		10	11	8	10	8	9	11	30
20	18A2		S. oralis	UK	2001	supra-gingival plaque	root caries lesions		10	11	8	10	8	9	11	30
21	18B2		S. oralis	UK	2001	supra-gingival plaque	root caries lesions		10	11	8	10	8	9	11	30

Analysis tools:

Breakdown: Fields | Two Field | Polymorphic sites | Combinations | Schemes/alleles | Publications

Analysis: BURST | Codons | Presence/Absence | PhyloTree | PhyloViz

Export: Dataset | Sequences

Susceptibilidad antibiótica de 15 aislamientos de *S. oralis* subsp. *tigurinus*

Estudio GAMES	*Identificación definitiva*	Peni	Ampi	Cefotax	Eritro	Clinda	Tetra	Levoflox	SXT
92	Valdecilla	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	≤1	2	≤0.5/9.5
178	Valdecilla	≤0,03	≤0,12	≤0,06	>0,5	≤0,25	≤1	2	≤0.5/9.5
86	Ramón y Cajal	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	>4	2	≤0.5/9.5
14	Clinic i Provincial	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	≤1	2	≤0.5/9.5
21	Clinic i Provincial	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	≤1	2	≤0.5/9.5
22	Clinic i Provincial	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	≤1	2	≤0.5/9.5
34	Clinic i Provincial	0,12	0,25	0,25	≤0,25	≤0,25	≤1	2	≤0.5/9.5
210	Clinic i Provincial	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	>4	2	≤0.5/9.5
260	Clinic i Provincial	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	≤1	2	≤0.5/9.5
240	Gregorio Marañón	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	≤1	2	≤0.5/9.5
256	Gregorio Marañón	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	>4	2	≤0.5/9.5
1203224635	**HUD**	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	>4	2	≤0.5/9.5
325949	**HUD**	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	≤1	2	≤0.5/9.5
326654	**HUD**	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	≤1	2	≤0.5/9.5
72503953	**HUD**	≤0,03	≤0,12	≤0,06	≤0,25	≤0,25	>4	2	≤0.5/9.5

Resumen

- La recientemente descrita especie (subespecie) de SGV *S. tigurinus* es un patógeno relevante para el ser humano y parece ser una causa frecuente de endocarditis

- A pesar de su virulencia el pronóstico de la EI por *S. tigurinus* parece mejor que la causada por otras especies bacterianas

- Estudio GAMES: se encuentra ampliamente distribuido entre pacientes con EI: se detectó en 5/7 hospitales y en 15/50 muestra de EI por SGV

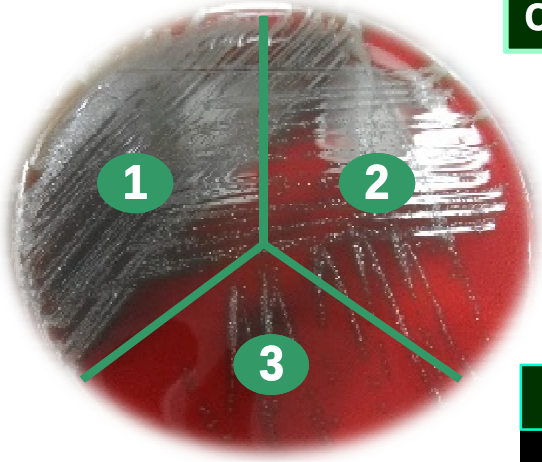
- Genéticamente es muy heterogéneo y en general sensibles a beta-lactámicos

Protocolo de trabajo

Hisopos con medio de cultivo

Coger una colonia aislada

Conservación de las bacterias



Extracción de ADN

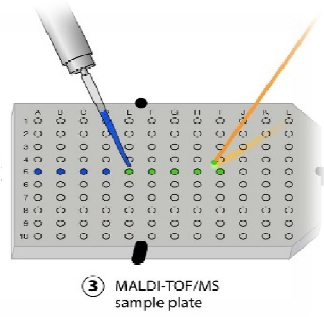
Coger una colonia aislada

Cultivo directo en placa TSA

Extracción con ácido fórmico y acetonitrilo

Peak Score

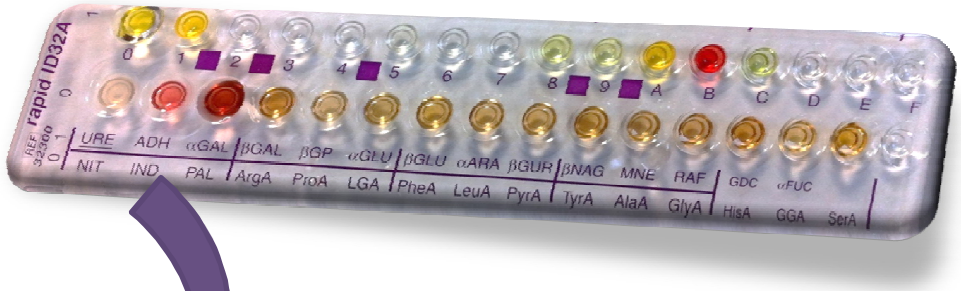
Extracción con etanol, ácido fórmico y acetonitrilo



S .oralis
hemocultivo
1998 - 2017

19 invasivas

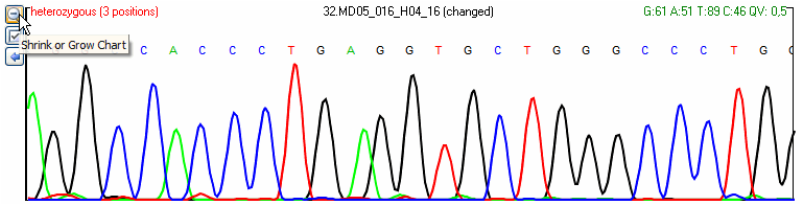
19 No invasivas



sodA, groEL, gyrB

Proximal Cluster ['Streptococcus_oralis_subsp._tigurinus JQ085957']
The closest sequence based on patristic distances is Streptococcus_oralis_subsp._tigurinus JQ085957 and belongs to the proximal cluster

Streptococcus tigurinus strain AZ_3a heat shock protein (groEL) gene, partial cds



QBPP
Quick Bioinformatic Phylogeny of Prokaryotes

Nucleotide BLAST