

Usefulness of fatty acid analysis for identification of nutritionally variant streptococci

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Introduction

Nutritionally variant streptococci (NVS) have been described as a major causative agents of septicaemia in neutropenic cancer patients and accounted for serious cases of infective endocarditis. Patients with endocarditis due to NVS (Figure 1 A, B, C) are more difficult to treat than those infected with other gram-positive catalase negative cocci, formerly often called "viridans streptococci" (Figure 1 D). In recent years, changes in taxonomy based on the results of a new techniques of molecular biology made an identification of NVS complicated (Table 1).

In clinical laboratories, the identification of NVS often remains dependent upon their phenotypic characterization. An overlap in physiological characteristics between NVS and other gram-positive catalase negative cocci is known.

Our study examined if it is possible to confirm preliminary phenotypic identification as NVS by analysis based on whole cell fatty acids.

TABLE 1

1961	"viridans streptococci" (Frenkel and Hirsch)	1995	<i>Abiotrophia</i> gen. nov. (Kawamura et al.)	2000	<i>Granulicatella</i> gen. nov. (Collins and Lawson)
	descriptoin of satellitism behaviour		biochemical characteristics and DNA homology		phylogenetic distances of 16S rRNA gene sequence
1989	<i>Streptococcus defectivus</i> sp. nov. → <i>Streptococcus adiacens</i> sp. nov. → (Bouvet et al.)	1995	<i>Abiotrophia defectiva</i> comb. nov. → <i>Abiotrophia adiacens</i> comb. nov. → <i>Abiotrophia elegans</i> sp. nov. → (Roggenkamp et al.)	2000	<i>Granulicatella adiacens</i> comb. nov. <i>Granulicatella elegans</i> comb. nov.
		2000	" <i>Abiotrophia para-adiacens</i> " (Kanamoto et al.)	1999	<i>Granulicatella balaenopterae</i> sp.nov. (Lawson et al.)

Methods

Two years of study on gram-positive catalase negative cocci recovered from blood cultures (BacT/Alert, BioMérieux) yielded 9 isolates of NVS. API 20 Strep (BioMérieux) was used for biochemical characterization. Final identification was realized by the analysis of partial sequence of 16S rRNA gene in German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) - isolates were identified as *Granulicatella adiacens* (7) and *Abiotrophia defectiva* (2).

Analysis of fatty acid methyl esters (FAME) was performed using MIS Sherlock (MIDI, Inc., USA). For extraction of FAME the strains were cultivated on a modified Brain Heart Infusion Agar (Becton Dickinson, USA) at 35 °C, 48 h under anaerobic conditions (BHIBLA library, version 3.8).

NVS reference strains (*A. defectiva* CCM 4672^T, *G. adiacens* CCM 4671^T, *G. elegans* CCM 4945^T) and isolates, which phenotypically resembled NVS and which were confirmed as *Streptococcus* spp. by restriction fragment length polymorphism analysis of PCR-amplified 16S rRNA genes were included in the study. The obtained FAME profiles were also compared with the phenotypically similar *Gemella morbillorum*.

Results

Streptococcus spp. phenotypically resembling NVS (Figure 2 E) significantly differed from other strains tested by lower amount of both C16:1 ω9c and C18:1 ω9c fatty acids.

From all other isolates, only *G. elegans* (Figure 2 C) showed unique fatty acid composition - the amount of C18:2 ω6,9c was almost 3 times higher and C16:1 ω9c was not detected at all.

The most related seems to be *A. defectiva*, *G. adiacens* and *G. morbillorum* (Figure 2 A, B, D), in which only slight differences of the amounts of three fatty acids were chosen to distinguish them - C16:0, C18:1 ω9c and C18:2 ω6,9c.

In *A. defectiva* was detected that the amount of C16:0 was lower than 30% and simultaneously the amount of C18:1 ω9c was higher than 20%, which is in contrast to *G. adiacens* and *G. morbillorum*.

G. adiacens differed from *G. morbillorum* by the amount of C18:2 ω6,9c being lower than 3%.

FIGURE 2

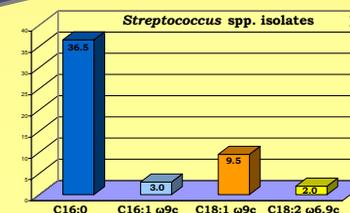
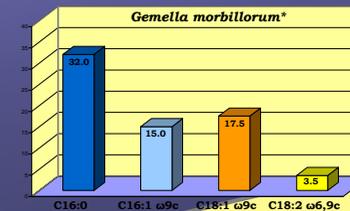
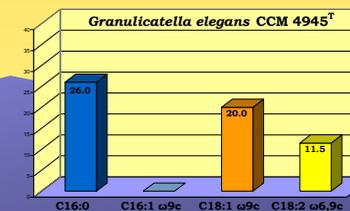
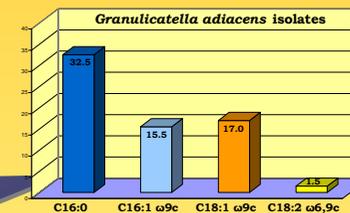
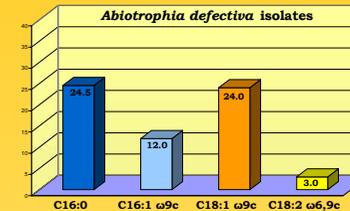
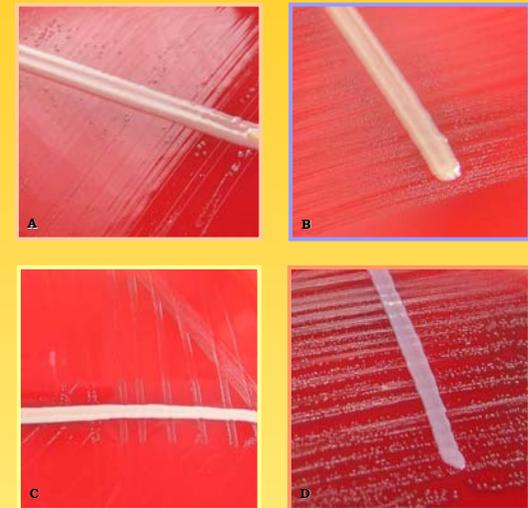


FIGURE 1



Conclusion

- the fatty acid composition of all tested NVS significantly differs from phenotypically similar *Streptococcus* spp.
- fatty acid analysis could be a useful tool for the discrimination of *G. elegans* from *G. adiacens* and *A. defectiva*
- in case of failure of satellitism behaviour, the founded differences in fatty acid composition could help to distinguish *G. morbillorum* from NVS
- a majority of the NVS recovered from blood cultures comprised *G. adiacens*, no isolate was identified as *G. elegans*

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