

ANAEROBIC COCCI IN HUMAN HEALTH AND DISEASE

Essay

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ABBREVIATION

G.P.A.C	: gram positive anaerobic cocci
G.N.A.C	: gram negative anaerobic cocci
C.D.S.	: Center disease control
An B.A.P.	: anaerobic blood agar plate
PEA medium	: Anaerobic Penamycin - Vancomycin blood agar
A.P.V.	: Anaerobe paromomycin - Vancomycin blood agar
T H I O	: thioglycolate medium
V. F. A	: Volatile Fatty acid
G. L.C	: gas liquid chromatography
PPV	: Proteose peptone-yeast extract broth
C. M.B	: Columbia agar base
G. L. C.	: gas - liquid - chromatography
PPV.G	: proteose peptone yeast Glucose broth
L. D.	: Lombard & Dowell media
St.	: Streptococcus
Staph	: Staphylo coccus
P	: Pepto Coccus
P st.	: Pepto strepto coccus
M. I. C.	: Minimal Inhibitory Concentration
A.T system	: Anaerobic Tek
P.A. G.E	: polyacrylamide . gel electro phoresis

INTRODUCTION

Anaerobic gram positive cocci were found to be part of the normal indigenous Flora of the intestinal tract in healthy adult (19.6%) but were also isolated from healthy neonates (6.1%) (Bezintzoglou E & Romond 1991). Anaerobic gram-negative cocci which were found to be of the normal Flora of urogenital system and intestinal tract of young children were found also in female genital system. They were also found as normal Flora of the buccal cavity, teeth, respiratory tract, nasopharyngeal cavity (Prevot 1966) .

Anaerobic gram-negative cocci were obtained from faeces using very strict anaerobic techniques as 10^7 - 10^8 /g wet faeces (Sussman 1974). Anaerobic cocci were found as subgingival microflora in children of early childhood, school age and circumpuberty (Nakagawa et al 1991).

It seems to be generally appreciated that anaerobic cocci are almost always involved in infections associated with liver abscess, appendicitis, intraperitoneal infection, perineal, perirectal infection (Sussman 1974) .

Streptococcus intermedius were found to be associated with other anaerobic bacteria in intracranial suppuration (Merad 1989) .

The most common bacteria isolated from aspiration of exudate in children with chronic suppurative otitis media were anaerobic gram positive cocci (Brook I, Vocump 1989) .

Recent studies suggest that the microbial Flora of acute dento-alveolar abscess is involving CO_2 dependent organisms which are strictly anaerobic gram positive cocci (Lewis et al 1990). Also they were predominant (45.9 %) cause in wound sepsis (surgical, non surgical) (Arora et al 1990) they found that the most pyogenic liver abscess are caused by gram negative cocci associated with other anaerobes (Sussman 1974) .

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CHAPTER 1

CLASSIFICATION OF ANAEROBIC COCCI

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CLASSIFICATION OF ANAEROBIC COCCI

Anaerobic cocci was first described in the late 19th century by Veillon, who isolated such an organism, named by him "Micrococcus" foetidus, from a case of Bartholinitis. Other workers soon recorded the isolation of anaerobic cocci from a variety of infections, for example, gas gangrene, puerperal sepsis, periurethral abscess and otitis media. Anaerobic cocci form part of the commensal flora of various sites in man, including the mouth, intestine, male and female genital tracts and skin (Eschenebach E.A 1982); they are frequently isolated from human and animal samples, often in association with infection, but usually in mixed culture with aerobic or other anaerobic organisms Barnes et al 1977, Perry et al 1982.

There is confusion surrounding the nomenclature of these organisms. Until recently, classification schemes for anaerobic cocci relied on morphological characters that were inconsistent and depended on the type of culture media; these schemes also included strains that were not obligate anaerobes, confusion arose with strains that were apparently obligate anaerobes on primary culture but grew on subculture in 10% CO₂ in air. Such strains are capnophilic and will grow on primary isolation within 7 days on chocolate blood agar in 10% CO₂ in air (Walt and Jack 1977). Although they may well be clinically significant, they should not be considered as "true anaerobic cocci". More recent studies have resulted in classification schemes based on more reproducible criteria. (Allen et al 1985) a Anaerobic cocci can be divided into 2 groups: gram positive anaerobic cocci and gram negative anaerobic cocci (Kronvall et al 1977) .

CLASSIFICATION OF GRAM POSITIVE ANAEROBIC COCCI

The gram positive anaerobic cocci have traditionally been divided into 2 genera, *peptostreptococcus* and *peptococcus*, on the basis of morphological characteristics i.e the tendency of *peptostreptococcus* to form chains and *peptococcus* to form clumps [Perkins et al 1975].

It has long been recognized that differentiation on morphological grounds is unreliable and greatly influenced by variables such as the composition of the medium [Holdman et al 1974]

Several workers earlier in this century attempted to divide these organisms into groups, notably Prevot in 1948, who divided the anaerobic cocci into 8 genera according to their microscopic appearance and then differentiated strains within these genera into species on the basis of colonial appearance and the results of tests such as indole production, liquefaction of gelatin and growth in litmus milk. Moore and his colleagues in 1969 put forward an alternative scheme, in which anaerobic cocci were divided into 6 (later 10) groups on the basis of fermentation of carbohydrate substrates. In 1974 Rogosa assigned the gram positive anaerobic cocci to a new family, *peptococcaceae*, containing several genera, of which *peptococcus* and *peptostreptococcus* were the most important as caused of human and animal infections. This was the basis used for subsequent classification schemes. [Dowell and Hawkin 1974, Sutter et al 1975, Holdeman et al 1977]

In recent years, workers have applied a variety of modern analytical techniques to the classification of anaerobic cocci, including determination of the DNA Guanine + cytosine (g+C) content, DNA - DNA homology studies, analysis of cell wall fatty acids and peptido-

glycan structure, and polyacrylamide - gel electrophoresis (PAGE) of proteins (Koneman, et al 1983)

Such studies have highlighted the inadequacies of previous classification schemes. Ezaki & Coworkers in 1983 propose that the majority of species comprising the genus *peptococcus* in the previous schemes (*Peptococcus magnus*, *Peptococcus indolicus*, *Peptococcus prevotii* and *Peptococcus saccharolyticus* be reassigned to the genus *peptostreptococcus* and that the organisms previously grouped under the name *GAFFKYA anaerobia* be renamed *peptostreptococcus tetradius*.

These proposals have now been accepted (Moore, et al 1986) . Together with the transfer of organism previously known as *peptostreptococcus intermedius* (Holdeman and Moore 1974) and *peptostreptococcus* (Cato, 1983) and *peptococcus saccharolyticus* to the genus *staphylococcus* also *peptostreptococcus morbillarum* and *peptococcus constellatus* were transferred to *streptococcus* (Kilpper - Balz and Schleifer 1981).

More recently ezaki and Yabuuchi in 1986 have been found that the rumen organism *peptococcus heliothrinreducens* has been transferred to the genus *peptostreptococcus* and *peptococcus glycinophilus* to *peptostreptococcus micros*.

Anaerobic gram positive cocci of several genera may be encountered in man and animals including *Ruminococcus* (Rogers 1974), *Coprococcus* (Holdeman and Moore 1974) and *Sarcina* .

The Taxonomic status of these organisms, and their relatedness to *peptostreptococcus* and *peptococcus* remains to be elucidated.

It may be that a few strains of streptococci are true anaerobes including streptococcus hansenii (Holdeman and Moor 1974) from human faeces.

PREVIOUS CLASSIFICATION OF GRAM POSITIVE ANAEROBIC COCCI.

PEPTOCOCCUS

SARCINA

PEPTOSTREPTOCOCCUS

RUMINOCOCCUS

GAFFKYA trevisan

Species of peptococcus	Species of peptostreptococcus	Species of Gaffkya
<i>P. niger</i>	<i>Pst. anaerobius</i>	<i>Gaffkya</i>
<i>P. Saccharolyticus</i>	<i>Pst. parvulus</i>	<i>anaerobia</i>
<i>P. asaccharolyticus</i>	<i>Pst. intermedius</i>	
<i>P. heliothrinreducens</i>	<i>Pst morbillorum</i>	
<i>P. indolus</i>		
<i>P. magnus</i>		
<i>P. glycinophilus</i>		
<i>P. prevotii</i>		
<i>P. constellatus</i>		

(quoted from Pier et al 1972)

NEW CLASSIFICATION OF GRAM POSITIVE ANAEROBIC

COCCI

<i>Peptostreptococcus</i>	<i>Sarcina</i>
<i>Peptococcus</i>	<i>Ruminococcus</i>
<i>An. Streptococcus</i>	<i>Coprococcus</i>
<i>An. Staphylococcus</i> (<i>staph Saccharolyticus</i>)	

Species of peptococcus	Species of peptostreptococcus	Species of anaerobic streptococcus
<i>P. niger</i>	<i>Pst. anaerobius</i> <i>Pst. asaccharolyticus</i> <i>Pst. heliotrinreducens</i> <i>Pst. indolicus</i> <i>Pst. magnus</i> <i>Pst. micros</i> <i>Pst. prevotii</i> <i>Pst. productus</i> <i>Pst. tetradius</i>	<i>Streptococcus parvulus</i> <i>Strep. intermedius</i> <i>Strep. hansenii</i> <i>Strep. morbillarum</i> <i>Strep. constellatus</i>

P. peptococcus

Pst. peptostreptococcus

quoted from Holdeman et al 1977, Skerman et al, 1980, Klippel -blaz
and Schleifer 1981, Cato 1983, Ezaki et al 1983 .

Reason for taxonomic realignment, the G+c content of the DNA of *P. niger*, the type species of the genus *peptococcus* is 51% mol whereas that of *pst. anaerobius*, the type species of the genus *peptostreptococcus* is 33 mol %. The G+c content of *P. magnus*, *P. indolicus*, *P. prevotii* and *P. asaccharolyticus* ranges from 29 to 34 mol %. Also the cellular fatty acids profiles of these species more closely resemble the profile of *Pst. anaerobius* than *P. niger*. For these reasons Ezaki & Coworkers in 1983 proposed that these species be transferred to the genus *peptostreptococcus*. *P. saccharolyticus* was shown on the basis of nucleic acid studies to be more closely related to the genus *staphylococcus* than to *peptococcus* and has therefore, been transferred to the genus *staphylococcus* (Kilpper - Balz and Schleifer 1981) .

Similarly, the Saccharolytic species *Pst. parvulus* was transferred to the genus *streptococcus* because it produces large amounts of lactic acid from carbohydrate fermentation (Cato, 1983) .

CLASSIFICATION OF GRAM NEGATIVE ANAEROBIC COCCI

Veillon and Zuber in 1898 first described gram - negative anaerobic cocci isolated from a human appendix. They named the organism *staphylococcus parvulus* but it was renamed *Veillonella parvula* (*V. parvula*) by Prevotii in 1933, who added a second species, *V. Alcalescens*, to the genus. After exhaustive studies of the cultural, biochemical and serological characters of a large number of isolates, Rogisa in 1974 described the division of these two species into 7 subspecies, which corresponded to serotypes were *V. parvula* sub sp. *parvula*, *rodentium*, *atypica*, *V. alcalescens* subsp. *alcalescens*, *ratti*, *criteria* and *dispar*.

However DNA; DNA pairing by Mays & his colleagues in 1982 suggested that the genus *Veillonella* consisted of at least 8 distinct groups which with one exception, corresponded to *Rogosa* serotypes.

V. alcalescens was considered synonymous with *V. parvula*, the remaining subspecies, *V. caviae*, was proposed, comprising strains isolated from the mouth of guinea. pigs. According to this study most strains isolated from the human oral cavity belong to the species *V. atypica*, *V. parvula* and *V. dispar*

GRAM-NEGATIVE ANAEROBIC COCCI

VEILLONELLA	MEGASPHAERA	ACIDAMINOCOCCUS
<i>V. parvula</i>	<i>M. elsdenii</i>	<i>A. fermentans</i>
<i>V. atypica</i>		
<i>V. ratti</i>		
<i>V. criceti</i>		
<i>V. caviae</i>		

CHAPTER 2

ANTIGENIC PROPERTIES OF ANAEROBIC COCCI