Received Date : 04-Apr-2013

Revised Date : 21-Jun-2013

Accepted Date : 22-Jun-2013

Article type : Original Article

Corresponding author E-mail ID : ogarner@mednet.ucla.edu

Multi-Center Evaluation of Mass Spectrometric Identification of Anaerobic Bacteria Using the VITEK® MS System

Submitting Author:

Garner, Omai Brant
UCLA - Pathology and Laboratory Medicine
10833 Le Conte Ave Brentwood Annex Los Angeles California 90095
United States
T: 310 794-8748
F: 310 794-2765

Authors & Institutions:

Mochon, A. Brian
UCLA - Pathology and Laboratory Medicine
Los Angeles, California
United States

Branda, John A
Massachusetts General Hospital and Harvard Medical School - Pathology
Boston, Massachusetts
United States

Burnham, Carey-Ann D
Washington University School of Medicine - Pathology & Immunology

St Louis, Missouri
United States

Bythrow, Maureen
North Shore-LIJ Health System Laboratories - Division of Infectious Disease Diagnostics
Lake Success, New York
United States

Ferraro, Mary Jane
Massachusetts General Hospital and Harvard Medical School - Pathology
Boston, Massachusetts
United States

Ginocchio, Christine Cesarsky
North Shore-LIJ Health System - Pathology and Laboratory Medicine
Lake Success, New York
United States

Jennemann, Rebecca
Barnes Jewish Hospital - Pathology
St Louis, Missouri
United States

Manji, Ryhana
North Shore-LIJ Health System - Pathology and Laboratory Medicine
North Shore-LIJ Laboratories 10 Nevada Drive , Lake Success, New York 11042
United States

Procop, G.W.
Cleveland Clinic - Clinical Pathology
Cleveland, Ohio
United States

Richter, Sandra S
Cleveland Clinic - Clinical Pathology
Cleveland, Ohio
United States

Rychert, Jenna A
Massachusetts General Hospital and Harvard Medical School - Pathology
Boston, Massachusetts
United States

Sercia, Linda
Cleveland Clinic - Clinical Pathology
Cleveland, Ohio
United States

Westblade, Lars F
Hofstra North Shore-LIJ School of Medicine - Pathology and Laboratory Medicine
Hempstead, New York
United States

Lewinski, Michael A
UCLA - Pathology and Laboratory Medicine
Los Angeles, California
United States

Introduction

Anaerobic bacteria constitute a major component of the microbial flora found within the oral cavity and the gastrointestinal tract. Under most conditions anaerobic bacteria have a saprophytic relationship with the human host. However, these same organisms are capable of causing devastating infections and intoxications. Most anaerobic infections/intoxications develop in areas of devitalized tissue secondary to surgical and traumatic wounds, bites, and ischemic extremities (e.g., arteriosclerosis and diabetes mellitus) or are acquired by consumption of a toxin or toxin-producing organisms. Often times, anaerobic infections are polymicrobic, contributing to difficulty in medical management (1) therefore, rapid species identification of anaerobes can be critical to successful treatment.

The identification of anaerobes has classically relied upon phenotypic assays such as Gram staining, growth characteristics and biochemical reactivity patterns. These methodologies can be both time consuming and laborious, hindering the selection of appropriate therapy. Many anaerobic species are not very biochemically active, thus a large quantity of anaerobe is necessary for successful identification by commercially available biochemical kits. Anaerobes typically exhibit very slow doubling times

contributing to the extended length of time required for correct identification with phenotypic-based methods. Therefore, classical phenotypic anaerobic bacteriology in the clinical lab exists as a confirmatory science due to extended turnaround times that can be multiple days long, as opposed to being truly diagnostic (2). Nucleic acid sequencing provides a more reliable identification, but is currently too expensive, technically complex, and labor intensive for routine identification of all clinically isolated anaerobic bacteria.

The implementation of Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry in the routine clinical laboratory provides the opportunity for inexpensive, rapid and accurate identification of anaerobes. Mass spectrometry uses an ionization source to charge and separate ionized particles according to their mass-to-charge ratio (m/z). A detector and mass analyzer then determine the relative abundance of each molecular fragment by their m/z ratio and generate a mass spectrum. MALDI-TOF MS produces and detects unfragmented large proteins/peptides from whole cells that generate spectral profiles that are reproducible and specific to bacterial species. These stable mass spectral fingerprints are then compared with reference mass spectra of well characterized strains to produce a reliable identification. The aim of this multi-center study was to evaluate the performance of the VITEK® MS MALDI-TOF and VITEK® MS v2.0 strain database in the identification of clinically relevant anaerobic bacteria in a clinical microbiology laboratory.

Materials and Methods

Clinical Evaluation Sites: Five clinical sites within the United States participated in the evaluation of the VITEK® MS system including the UCLA Health System (Los Angeles CA), North Shore LIJ Hospital (Lake Success, NY), Barnes Jewish Hospital (St. Louis MO), Cleveland Clinic (Cleveland OH), and Massachusetts

General Hospital (Boston, MA). Proficiency testing was performed at all of the clinical trial sites following training on the instrumentation by a bioMérieux representative.

Bacterial Strains: 339 anaerobic strains were derived from clinical specimens collected from the five clinical sites. 312 frozen isolates were provided by bioMérieux in order to expand the analysis to include additional organisms. A total of 651 clinically relevant anaerobic isolates were tested. Each of the five clinical testing sites tested a subset (at least 100) of organisms between January 2012 and August 2012. All cultures were incubated under anaerobic conditions for a minimum of 24 h and a maximum of 72 h after visible growth at 35°C. Frozen isolates were subcultured twice before analysis. Anaerobic isolates were all cultivated on Brucella blood agar plates (BBL™, Sparks, MD).

Sample Preparation: Pure cultures of anaerobic bacteria were applied to the VITEK® MS-DS target slide using a 1µl loop. Typical application involved more than one isolated colony due to the small size of anaerobic isolates. A thin layer of organism was applied to the center of the well. One microliter of VITEK® MS CHCA (bioMérieux) matrix solution (α-cyano-4-hydroxycinnamic acid) was overlaid and allowed to air dry completely. Isolates from the same plate were selected for Gram stain analysis and subcultured for shipment to the reference testing facility (**MIDI, Inc.,** Newark, DE) for 16S rRNA gene sequencing analysis.

Calibration and Quality Control: *Escherichia coli* (ATCC 8739) was used for system calibration. A fresh (18-24h) isolate was applied to the designated wells on the target plate. Proper spectral acquisition of the calibrator was necessary for analysis of the other bacterial wells. A panel of four organisms was used for positive control strains and fresh isolates were tested by VITEK® MS each day of testing. These organisms included *Staphylococcus aureus* (ATCC 29213), *Klebsiella oxytoca* (ATCC 13182),

Pseudomonas aeruginosa (ATCC 10145), and *Enterobacter aerogenes* (ATCC 13048). The negative control consisted of 1 µl of matrix alone.

VITEK® MS Organism Identification: For each target well, 100 mass spectra profiles were generated within a range of 2 to 20 kilodaltons. The laser frequency was 50 Hz and was recorded in a linear positive mode. The mass profiles were averaged to produce a single, composite mass spectrum. Analysis of a composite mass spectrum for accurate identification used the VITEK® MS v2.0 database. This database is not a library of spectra, but uses a bin matrix. The mass peaks between 2 and 20 kilodaltons are placed into 1300 separately analyzed bins. The bin matrix consists of a table of specificity values for mass peaks per bin for each species that is present in the database. The mass peaks found in the composite spectra are compared to the bin matrix, and the peak intensity of each mass signal allows for the calculation of a composite score and probability for each species. A probability score between 60% to 100% represents a high discrimination value and a reliable identification. A probability score that is lower than 60% is found in a low discrimination identification that consists of a list of two to four choices for an identification match. A report of no identification is produced when either no match is found for the composite spectra, or not enough spectral peaks were obtained in the analysis. Isolates that yielded no identification results were redeposited to the target plate and reanalyzed.

Confirmation of Isolate Identification: 16S rRNA gene sequencing served as the reference standard for identification of anaerobic bacteria and was performed at an outside reference laboratory. Sequencing of a 527-bp region within the 16S rRNA gene was performed using universal 16S primers at positions 0005F and 0531R. Isolate identification was determined using the MicroSeq® system (Applied Biosystems, Foster City, CA) and the Sherlock® DNA data analysis software (MIDI, Inc.)

Results

A total of 651 anaerobic bacterial isolates were analyzed by the VITEK® MS, representing 11 genera and 26 separate species. A total of 91.2% (594/651) of the isolates were correctly identified to species as confirmed by 16SrRNA gene sequencing (Table 1). An additional eight isolates were identified correctly to the genus bringing the total number of isolates correctly identified to the genus-level to 92.5% (602/651). Ten isolates gave a result of no identification on the first spot but gave a correct identification on the repeat spot. Forty-nine of the anaerobic isolates tested were unable to be identified by the VITEK® MS system. Thirty-six isolates produced a result of no identification. The additional 13 isolates displayed results as mixed genera (Table 2).

Gram-positive isolates

Eight genera of Gram-positive anaerobes (265 isolates) were assessed by the VITEK® MS system (Table 1) consisting of *Actinomyces*, *Clostridium*, *Finegoldia*, *Mobiluncus*, *Parvimonas*, *Peptoniphilus*, *Peptostreptococcus*, and *Propionibacterium*. The VITEK® MS was able to identify 91.7% to the species-level and 92.5% of the isolates to the genus-level. Five of the 265 isolates were defined as mixed genera identifications, whereas 15/265 (5.7%) had no identification. Three different species of *Actinomyces* were evaluated. As a genus, 74.1% (20/27) of isolates were identified correctly to species. *Actinomyces odontolyticus* showed 85.7% correct identification to species while *A. meyeri* and *A. neuii* showed 75% and 66.7% correct identification to species with a concurrent 12.5% and 33.3% (one mixed genera result) no identification, respectively. Four different species of *Clostridium* were evaluated. As a genus, 96.3% (104/108) of isolates were correct to species. *Finegoldia magna, Parvimonas micra, Peptoniphilus asaccharolyticus*, *and Peptostreptococcus anaerobius* all showed a correct to species identification above 95%. *Mobiluncus curtisii* had a species-level identification of 75% with the remaining isolate having a mixed genera result consisting of *Bifidobacterium* spp. and *Vibrio alginolyticus* in addition to *M.*

curtisii. Propionibacterium acnes showed a correct to species identification of 82.7% (43/52). One of the *Propionibacterium* isolates was correct to the genus-level only. Two of the 52 isolates had mixed genera results and consisted of *Clostridium bifermentans* and *Parvimonas micra* as well as *P. acnes*.

Gram-negative isolates

Three hundred eighty-six isolates of Gram-negative anaerobes were also evaluated by the VITEK® MS system (Table 1). The isolates tested were from three genera: *Bacteroides*, *Fusobacterium*, and *Prevotella*. The VITEK® MS was able to identify 90.9% to the species-level and 92.5% of the isolates to the genus-level. Eight of the 386 isolates were defined as mixed genera identifications, whereas 21/386 (5.4%) had no identification. Six different species of *Bacteroides* were evaluated. As a genus 92% (242/263) of isolates were identified correctly to species. An additional three isolates were identified correctly to the genus-level. *Bacteroides caccae, B. fragilis, B. thetaiotamicron*, and *B. vulgatus* all showed a greater than 93% correct to species identification. *Bacteroides ovatus* was 85% correct to the species-level while *B. uniformis* was only 73.3% correct to species. A total of 26.7% (eight isolates) of *B. uniformis* were not identified including one that gave mixed genera results. Two different species of *Fusobacterium* were evaluated. As a genus, 81.8% (27/33) of isolates were identified correctly to species. *Fusobacterium necrophorum* showed a 92.3% correct to species identification while *F. nucleatum* only showed a 42.9% correct to species identification with a 57.1% no identification including one that gave mixed genera results. Five different species of *Prevotella* were evaluated. As a genus, 91.1% (82/90) isolates were identified correctly to species. An additional three isolates were identified correctly to genus-level. *Prevotella bivia, P. buccae, and P. denticola* all showed 100% correct identification to species. A total of 81.3% of *P. intermedia* were identified correctly to species while only 54.5% *of P. melaninogenica* were correct to species. Three strains (27.3%) of *P. melaninogenica* were not identified including two that gave mixed genera results.

Discussion

In this study, we evaluated the performance of the VITEK® MS v2.0 MALDI-TOF system in the identification of clinically relevant anaerobic bacteria. Overall, the performance of this system was highly accurate (91.2% correct to species-level identification compared to 16S rRNA gene sequencing.) Less than 8% of the total tested isolates were not identified or gave mixed genera identification results.

Prior to this evaluation, most MALDI-TOF studies showed varied rates of identification for anaerobes. As an example, 1660 aerobic and anaerobic bacterial isolates were evaluated in parallel using MALDI-TOF MS and conventional phenotypic identification tests (3). MALDI-TOF correctly identified 95.4% of isolates to at least the genus-level. However, the anaerobe group contained the highest number of either no identifications or misidentifications. MALDI-TOF only identified 2 of 30 (6.7%) *Bacteroides* spp., 1 of 5 (20%) *Fusobacterium* spp., 1 of 2 (50%) *Lactobacillus* spp., 27 of 60 (45%) P*ropionibacterium* spp. to the genus-level (3). The current evaluation showed an increased ability for accurate identification of most of the aforementioned anaerobes, except *Fusobacterium nucleatum*. This species was only correctly identified 42.9% of the time.

A separate study looked specifically at the discriminating power of MALDI-TOF MS to identify clinical isolates of *Bacteriodes*. Their data showed that this technique unequivocally identified 97.5% of the strains tested (270/277). The authors also showed that the rate of detection could be increased when previously sequenced strains were added to the MALDI-TOF database (4). This highlights the point that extending database depth with the mass spectra of additional reference strains or sequenced clinical isolates improves the identification capacity of the method. The VITEK® MS system was also able to identify of a wide range *of Bacteroides* species with a correct species identification of 92% (242/263).

In a study by La Scola *et al*. 544 anaerobic clinical isolates consisting of 79 species were evaluated by MALDI-TOF and 16S rRNA gene sequencing. Using MALDI-TOF MS the authors were only able to identify 61% of the isolates. The authors were able to confirm 100% of isolates identified as *Clostridium perfringens*, *Bacteroides fragilis* and other common *Bacteroides*. In contrast, other common anaerobes such as *Propionibacterium* species, *Finegoldia magna*, most *Fusobacterium* species, and *Prevotella* species were identified at levels only above 50% by MALDI-TOF MS. The authors concluded that the poor bacterial identification was mostly due to insufficient spectra within the database (5). Clearly, the VITEK® MS v2.0 system shows database improvement for these anaerobes. *Prevotella* species were identified correctly at 91.1% and all but 6 out 26 species tested showed a correct to species identification above 80%.

Additional studies looking at anaerobic identification by MALDI-TOF have shown improved results (6 - 12). A very recent study (14) looking at 14 different species of anaerobes showed a 100% identification rate of 274 isolates with the VITEK-MS. In the presented work, the VITEK-MS and VITEK MS version 2.0 database shows the highest accuracy for the identification of the genus and species of the tested isolates. That said, this system still shows some areas of weakness in identification. *Actinomyces neuii, Fusobacterium nucleatum*, and *Prevotella melaninogenica* all showed a correct to species identification of less than 70%. This is either due to difficulty in producing robust spectra, or deficiencies in the number of confirmatory isolates found in the database. Of the 36 isolates that gave a result of no identification, 20 produced insufficient spectra for analysis while 16 isolates had no match in the database. As the database improves, and more labs adopt MALDI-TOF as a method for analysis, the percentage of correct identification is predicted to increase.

The strengths of this study include the inclusion of a large number of isolates for most of the tested species. These isolates come from a broad geographic range within the United States, represented by the five clinical testing sites. This variability demonstrates the breadth of spectra representing each species found within the Vitek MS database. This study is notably limited by the lack of some very important, clinically relevant anaerobes including members of the genus *Porphyromonas, Bilophila, Eggerthella*, and an expanded list of *Clostridium* including *C. innocuum, C. septicum, and C. novyi*. Schmitt et al (15) recently showed that MALDI-TOF is an accurate system for a broad array of anaerobes, even though the isolate numbers per species found in this paper were very low.

The VITEK-MS tests as an accurate system for identifying clinically relevant anaerobic bacteria. The implementation of this technology in the clinical microbiology lab will lead to decreased turn-around times for identification. MALDI-TOF can also be used in addition to traditional methods (colony morphology and gram stain) for organisms that are difficult to identify. This represents a significant shift in the laboratory diagnosis of anaerobic bacterial infections and will allow for improved patient care.

References

1. Brook, I. 2011. Anaerobic infections in children. Adv Exp Med Biol 697:117-52.
2. Citron, D. M. 2012. Pre-molecular identification: ignorance was bliss? Anaerobe 18:189-91.
3. Seng, P., M. Drancourt, F. Gouriet, B. La Scola, P. E. Fournier, J. M. Rolain, and D. Raoult. 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 49:543-51.
4. Nagy, E., T. Maier, E. Urban, G. Terhes, and M. Kostrzewa. 2009. Species identification of clinical isolates of Bacteroides by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry. Clin Microbiol Infect 15:796-802.
5. La Scola, B., P. E. Fournier, and D. Raoult. 2011. Burden of emerging anaerobes in the MALDI-TOF and 16S rRNA gene sequencing era. Anaerobe 17:106-12.

6. Bizzini, A., C. Durussel, J. Bille, G. Greub, and G. Prod'hom. 2010. Performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. J Clin Microbiol 48:1549-54.

7. Cherkaoui, A., J. Hibbs, S. Emonet, M. Tangomo, M. Girard, P. Francois, and J. Schrenzel. 2010. Comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. J Clin Microbiol 48:1169-75.

8. Justesen, U. S., A. Holm, E. Knudsen, L. B. Andersen, T. G. Jensen, M. Kemp, M. N. Skov, B. Gahrn-Hansen, and J. K. Moller. 2011. Species identification of clinical isolates of anaerobic bacteria: a comparison of two matrix-assisted laser desorption ionization-time of flight mass spectrometry systems. J Clin Microbiol 49:4314-8.

9. Risch, M., D. Radjenovic, J. N. Han, M. Wydler, U. Nydegger, and L. Risch. 2010. Comparison of MALDI TOF with conventional identification of clinically relevant bacteria. Swiss Med Wkly 140:w13095.

10. van Veen, S. Q., E. C. Claas, and E. J. Kuijper. 2010. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J Clin Microbiol 48:900-7.

11. Veloo, A. C., M. Knoester, J. E. Degener, and E. J. Kuijper. 2011. Comparison of two matrix-assisted laser desorption ionisation-time of flight mass spectrometry methods for the identification of clinically relevant anaerobic bacteria. Clin Microbiol Infect 17:1501-6.

12. Nagy, E., S. Becker, M. Kostrzewa, N. Barta, and E. Urban. 2012. The value of MALDI-TOF MS for the identification of clinically relevant anaerobic bacteria in routine laboratories. J Med Microbiol 61:1393-400.

13. Fedorko, D. P., S. K. Drake, F. Stock, and P. R. Murray. 2012. Identification of clinical isolates of anaerobic bacteria using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Eur J Clin Microbiol Infect Dis 31:2257-62.

14. Jamak, W. Y., M. Shahin, and V.O. Rotimi. 2013. Comparison of two matrix-assisted laser desorption/ionization- time of flight (MALDI-TOF) mass specrometry methods and API 20AN for identification of clinically relevant anaerobic bacteria. J Med Microbiol 62: 540-44.

15. Schmitt, B.H., S. A. Cunningham, A.L. Dailey, D. R. Gustafson, and R. Patel. 2012. Identification of anaerobic bacteria by Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry with on-plate formic acid preparation. J Clin Microbiol 51: 782-86.

Table 1. Identification of Clinically Relevant Anaerobes by the VITEK® MS

Anaerobe isolates (651)	**Genus-level (%)**	**Species-level (%)**	**Mixed Genera (%)**	**No ID (%)**
	602 (92.5)	594 (91.2)	13 (2)	36 (5.5)
Gram-positive species (265)	245 (92.5)	243 (91.7)	5 (1.9)	15 (5.7)
Actinomyces meyeri (8)	7 (87.5)	6 (75)	0	1 (12.5)
Actinomyces neuii (12)	8 (66.7)	8 (66.7)	1 (8.3)	3 (25)
Actinomyces odontolyticus (7)	6 (85.7)	6 (85.7)	0	1 (14.3)
Clostridium clostridioforme (7)	7 (100)	7 (100)	0	0
Clostridium difficile (30)	27 (90)	27 (90)	0	3 (10)
Clostridium perfringens (61)	60 (98.4)	60 (98.4)	1 (1.6)	0
Clostridium ramosum (10)	10 (100)	10 (100)	0	0
Finegoldia magna (24)	23 (95.8)	23 (95.8)	0	1 (4.2)
Mobiluncus curtisii (4)	3 (75)	3 (75)	1 (25)	0
Parvimonas micra (10)	10 (100)	10 (100)	0	0
Peptoniphilus asaccharolyticus (4)	4 (100)	4 (100)	0	0
Peptostreptococcus anaerobius (36)	36 (100)	36 (100)	0	0
Propionibacterium acnes (52)	44 (84.6)	43 (82.7)	2 (3.8)	6 (11.5)
Gram-negative species (386)	357 (92.5)	351 (90.9)	8 (2.1)	21 (5.4)
Bacteroides caccae (30)	29 (96.7)	28 (93.3)	0	1 (3.3)
Bacteroides fragilis (71)	70 (98.6)	70 (98.6)	1 (1.4)	0
Bacteroides ovatus (40)	35 (87.5)	34 (85)	2 (5)	3 (7.5)
Bacteroides thetaiotaomicron (51)	49 (96.1)	48 (94.1)	0	2 (3.9)
Bacteroides uniformis (30)	22 (73.3)	22 (73.3)	1 (3.3)	7 (23.3)
Bacteroides vulgatus (41)	40 (97.6)	40 (97.6)	1 (2.4)	0
Fusobacterium necrophorum (26)	24 (92.3)	24 (92.3)	0	2 (7.7)
Fusobacterium nucleatum (7)	3 (42.9)	3 (42.9)	1 (14.3)	3 (42.9)
Prevotella bivia (34)	34 (100)	34 (100)	0	0
Prevotella buccae (23)	23 (100)	23 (100)	0	0
Prevotella denticola (6)	6 (100)	6 (100)	0	0
Prevotella intermedia (16)	14 (87.5)	13 (81.3)	0	2 (12.5)
Prevotella melaninogenica (11)	8 (72.7)	6 (54.5)	2 (18.2)	1 (9.1)

Table 2. Mixed Genera Results for Anaerobic Bacteria from the VITEK® MS

16S rRNA Gene Sequencing Identification (n)	**VITEK® MS - Mixed Genera Results**
Actinomyces neuii (1)	***Actinomyces neuii***, *Haemophilus influenzae*
Bacteroides fragilis (1)	***Bacteroides fragilis***, *Shewanella algae*
Bacteroides ovatus (2)	***Bacteroides ovatus***, *Citrobacter amalonaticus*, *Staphylococcus auricularis*, *Bacteroides vulgatus*, *Microbacterium paraoxydans*, *Bacteroides thetaiotaomicron*
Bacteroides uniformis (1)	***Bacteroides uniformis***, *Trueperella bernardiae*, *Bacteroides caccae*
Bacteroides vulgatus (1)	***Bacteroides vulgatus***, *Staphylococcus cohnii* spp. *urealyticus*, *Enterococcus casseliflavus*
Clostridium perfringens (1)	***Clostridium perfringens***, *Aeromonas hydrophila/caviae*
Fusobacterium nucleatum (1)	***Fusobacterium nucleatum***, *Aerococcus viridans*
Mobiluncus curtisii (1)	***Mobiluncus curtisii***, *Bifidobacterium* spp, *Vibrio alginolyticus*
Prevotella melaninogenica (2)	***Prevotella melaninogenica***, *Pediococcus acidilactici* *Streptococcus gallolyticus* spp. *gallolyticus* *Corynebacterium pseudodiphtheriticum*, *Streptococcus constellatus*
Propionibacterium acnes (1)	*Clostridium bifermentans*, ***Propionibacterium acnes***, *Parvimonas micra*