Available online at http://www. journalcra. com

International Journal of Current Research
Vol. 9, Issue, 11, pp.60697-60700, November, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

VITEK 2 ANC CARDS – A STEP AHEAD IN MICROBIOLOGICAL ANALYSIS - A CLINICAL REPORT OF TWO CASES

[1,*]Singh Soundarya and [2]Sharma Anamika

[1]Periodontist, Lecturer, LLRM Medical College, Meerut, Uttar Pradesh, India
[2]Prof. and Head, Department of Periodontology, Subharti Dental College and Hospital, Meerut, Uttar Pradesh, India

ARTICLE INFO

Article History:
Received 29th August, 2017
Received in revised form
04th September, 2017
Accepted 14th October, 2017
Published online 30th November, 2017

Key words:
Chronic Periodontitis,
Anaerobic Bacteria,
Vitek 2 ANC cards.

ABSTRACT

Background: Periodontitis has been defined as an inflammatory disease of supporting structures of teeth, of specific bacterial origin which progress with attachment loss. The etiology of the disease is multi factorial and bacterial deposits play an essential role in the pathogenesis. The bacteria that are involved in periodontitis accumulate in the sub-gingival plaque that comprises predominantly of Gram-negative strict anaerobic rods. Among them *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Bacteroides spp., Selenomonas spp***.** Predominate accurate identification of numerous bacterial species is nowadays possible with highly automated systems that are increasingly used in clinical laboratories because of their cost effectiveness, practicability, and ability to provide rapid turnaround time. It is now well established that anaerobes may be involved in numerous infections, including severe infections. Until recently, however, identification of anaerobes in clinical laboratories relied mainly on the use of time-consuming and lab or-intensive conventional methods or of manual commercial systems, the performances of which are quite variable at the species level. BioMe´rieux (Marcy, France) has recently developed a new colorimetric identification card (ANC card) which, in conjunction with the Vitek 2 system, permits this automated and widely distributed identification system to identify 63 taxa, including 49 taxa of anaerobic bacteria.

Method: The aim of the present case reports is the identification of anaerobic bacteria using Vitek 2 ANC cards in Chronic Periodontitis patients**.**

Results: The study showed that anaerobic organism identified were: *Parvimonas micra, Fusobacterium species, Actinomyces species, Veillonella, peptostreptococcus* in subjects with chronic periodontitis having probing depth ≥ 5mm taking GCF as a sample.

Conclusion: The Vitek 2 ANC card provides a reliable identification for a limited number of relevant anaerobic bacteria species in a routine-diagnostic setting.

Citation: Singh Soundarya and Sharma Anamika, 2017. "Vitek 2 ANC cards – a step ahead in microbiological analysis - a clinical report of two cases", *International Journal of Current Research*, 9, (11), 60697-60700.

INTRODUCTION

Periodontitis is a disease of the tooth supporting structures (alveolar bone and the periodontal ligament) in which there is destruction of these structures ultimately leading to tooth loss. Dental plaque is the primary etiology for the disease; however there are other factors that contribute to the progression of this disease. Complexes of oral anaerobic bacteria and perhaps viruses are thought to interact with risk factors, such as smoking and diabetes, to create the conditions which make a person susceptible to periodontitis. The patient's immuno-inflammatory response to the bacteria causes the tissue destruction which occurs in chronic periodontitis.

****Corresponding author:*** **Singh Soundarya,**
Lecturer, LLRM medical College, Meerut, Uttar Pradesh, India.

Chronic gingivitis is the very common inflammatory reaction occurring in the gingival tissues in response to the accumulation of dental plaque. It usually precedes the development of periodontitis, but chronic gingivitis does not inevitably progress to periodontitis. The clinical appearance of gingivitis may be modified by systemic factors such as poorly controlled diabetes, which can significantly accentuate the gingival tissues response to dental plaque (Hussian, 2011). The etiology of the disease is multi factorial and bacterial deposits play an essential role in the pathogenesis. The bacteria that are involved in periodontitis accumulate in the sub-gingival plaque that comprises predominantly of Gram-negative strict anaerobic rods (Van Winkelhoff *et al*., 2002). Among them *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Bacteroides spp., Selenomonas spp*. have been associated with chronic or refractory periodontitis

However, some anaerobic, Gram positive microorganisms such as *Peptostreptococcus micros and Eubacterium species* have recently been implicated in chronic periodontitis (Mutters, 2001). The involvement of anaerobes in numerous and severe clinical infections has been reported. The differences in antimicrobial susceptibility and the development of resistance to antimicrobial drugs among anaerobic bacteria have been documented. Traditional methods for the identification of anaerobic pathogens are not always available in clinical bacteriology laboratories and are often laborious and time-consuming. Therefore; the need for a rapid and accurate method for the identification of anaerobic pathogens is highly desirable for appropriate treatment.

In the last decades, different commercial enzyme kits for the identification of clinically relevant anaerobe isolates have been developed and evaluated, e.g., the RAPID-ANA II panel, the Minitek systems, the Vitek ANI card, the BBL Crystal ANR ID kit, the API rapid ID 32 A system, and the API 20 A system. The new Vitek 2 ANC card (bioMe´rieux, Marcy l'Etoile France) is designed to provide clinical laboratories with the capability for the rapid and accurate identification of clinically relevant anaerobic bacteria and *Corynebacterium* species. The card contains 64 microwells with 36 colorimetric enzymatic tests. The ANC database comprises 63 taxa of anaerobic bacteria and corynebacteria. Twenty genera are listed in the Vitek 2 ANC database: *Actinomyces*, *Arcanobacterium*, *Bacteroides* (*Parabacteroides*), *Bifidobacterium*, *Clostridium*, *Collinsella*, *Corynebacterium*, *Eggerthella*, *Eubacterium*, *Finegoldia*, *Fusobacterium*, *Lactobacillus*, *Microbacterium*, *Parvimonas* (formerly *Micromonas*), *Peptoniphilus*, *Peptostreptococcus*, *Prevotella*, *Propionibacterium*, *Staphylococcus*, and *Veillonella*. The system provides only a genus-level identification for *Bifidobacterium* sp. and *Veillonella* sp. Of the 36 biochemical profiles, 13 are fermentation tests, 17 are glycosidase and arylamidase tests, 2 are alkaline reactions, and 4 are other biochemical tests. Additional simple off-line tests, including cell morphology, Gram stain characteristic, and aerotolerance testing, are required to complete the identifications.

In order to assess the accuracy of the ANC card in a "real life" setting, unknown clinical isolates of anaerobic bacteria were used, regardless of whether the species are present in the Vitek database. This is in contrast to the methods used in two previously reported studies. The identification obtained by use of the Vitek 2 ANC system was compared with that obtained by use of 16S r RNA gene sequencing (Lee *et al*., 2011).

MATERIAL AND METHODS

The aim of the study was the identification of anaerobic bacteria using Vitek 2 ANC cards in Chronic Periodontitis patients. Subjects with chronic periodontitis were selected between the age of 35-55 years with probing depth ≥5mm in at least eight sites with minimum of twenty teeth from the OPD of Department of Periodontology, Subharti Dental College and Hospital. Subjects were excluded for the following reasons: Smokers, carious lesion, on medication since last six months, presence of orthodontic appliance, partial dentures fixed or removable appliance, diseases of soft tissues, patient diagnosed of generalized aggressive periodontitis, history of pregnancy, and uncooperative patients.

Pre–operative recording: Probing depth (PD), Clinical attachment levels (CAL), Gingival Bleeding Index (Ainamo and Bay 1975), Plaque Index (PI) (Silness and Loe 1964), and Bleeding on probing (BOP) were recorded. Taking all aseptic measures the area was dried and a standardized volume of 5µl of gingival crevicular fluid (GCF) was collected from three deepest probing pocket depth using 1 to 5 calibrated volumetric micro capillary pipettes. Each sample collection was allotted a maximum of 10 minutes (Pradeep *et al*., 2009) (Figure 1).

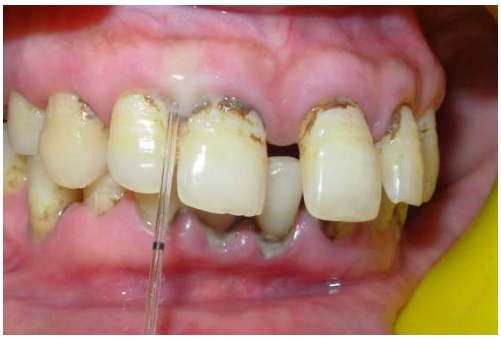

Figure 1. Sample (GCF) collected

Figure 2. Sample collected in RCM and kept in water bath

The fluid was immediately immersed in Robertson Cooked Meat (RCM) and kept in water bath for 15 mins (figure 2). Then it was allowed to cool and vertexed for 10 seconds, 10 fold dilutions were made 4-5 times and subculture was performed on blood agar (Aero tolerance testing). Colonies were made on the agar plate.

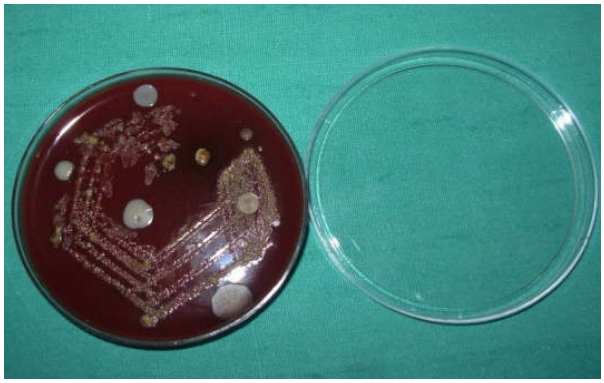

Figure 3. Colonies made on agar plate

The agar plates were kept in anaerobic gas chamber and left for 48 hrs. The colonies suspected to be of anaerobic bacteria on colony morphology, were identified (Koneman *et al*., 2006) (Figure 3). For identification upto the species level, the inoculum suspensions was prepared with 0.45% aqueous NaCl until a turbidity between 2.70 and 3.30 McFarland standards was reached by using calibrated Vitek 2 Densichek instrument

and Vitek 2 card (Figure 4) (BioMeriux, Marcy I'Etoile, France) (Lee *et al*., 2011) (Figure 5).

Figure 4. Vitek 2 ANC card

RESULTS

Two patients with generalized periodontitis having probing depth ≥ 5m participated in the study. After following the procedure mentioned above following anaerobic bacteria's were identified.

Case 1: *Veillonella, Peptostreptococcus*

Case 2: *Parvimonas micra, Fusobacterium Species, Actinomyces species.*

DISCUSSION

In this study, we assessed the reliability of the Vitek ANC card system for the identification of anaerobic bacteria isolated from clinical materials. In a recent study by Blairon *et al*. (2010) 196 clinical isolates were tested, some of which were not included in the Vitek ANC database. Those authors reported correct species- and genus-level identifications for 51.5% and 70.9% of isolates, respectively. Lee *et al*. (2011) using Vitek 2 ANC cards (bioMe´rieux, Marcy l'Etoile, France) with 301 anaerobic isolates. Each strain was identified by 16S r RNA gene sequencing, which is considered to be the reference method. The Vitek 2 ANC card correctly identified 239 (79.4%) of the 301 clinical isolates to the genus level, including 100 species that were not represented in the database. Correct species identification was obtained for 60.1% (181/301) of the clinical isolates. For the isolates not identified to the species level, correct genus identification was obtained for 47.0% of them and 16 were accurately designated not identified. Although the Vitek 2 ANC card allows the rapid and acceptable identification of the most common clinically important anaerobic bacteria within 6 h, improvement is required for the identification of members of the genera *Fusobacterium*, *Prevotella*, *Actinomyces* and certain Gram-positive anaerobic cocci (GPAC). In contrast to other validations of the ANC card Mory *et al*. (2009) and Rennie *et al*. (2008) previously reported correct species level identifications for 86.5% and 95.1% of isolates, respectively. However, species and genera not present in the database had been eliminated from their study isolates. Mory *et al*. (2009) evaluated 261 anaerobic clinical isolates tested with the new Vitek 2 ANC card, 257 (98.5%) were correctly identified at the genus level. Among the 251 strains for which identification at the species level is possible with regard to the ANC database, 217 (86.5%) were correctly identified at the species level. Two strains (0.8%) were not identified, and eight were misidentified (3.1%). Of the 21 strains (8.1%) with low-level discrimination results, 14 were correctly identified at the species level by using the recommended additional tests. This system is a satisfactory new automated tool for the rapid identification of most anaerobic bacteria isolated in clinical laboratories.

Conclusion

A high inoculums density is required for the inoculation of the Vitek 2 ANC card. Fast-growing bacteria are good candidates for Vitek 2 ANC identification. However, fastidious anaerobes such as *C. ureolyticus* and *B. wadsworthia* require several agar plates to obtain sufficiently large inoculums. Cell morphology and Gram stain characteristics provide useful information to avoid a misidentification of the microorganism. Especially, the Gram stain characteristics of Gram-negative staining isolates should be confirmed by use of special-potency disks. The Vitek 2 ANC card provides a reliable identification for a limited number of relevant anaerobic bacterial species in a routine-diagnostic setting. The system performs inadequately concerning species not present in the database. For certain species not included in the database, the system would benefit from limiting the identification to the genus level. Improvement and extension of the database may result in more accurate identifications.

REFERENCES

Blairon L, Maza M.L, Wybo I, Pierard D, Dediste A, Vandenberg O. 2010. Vitek 2 ANC card versus BBL crystal anaerobe and RAPID ANA II for identification of clinical anaerobic bacteria. Anaerobe,16:355-361.

Hussian AM. 2011. The relationship between diabetes mellitus and periodontitis. *Al Ameen J Med Sci.,* 4 :84-86.

Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Win WC 2006. Jr. Identification of Bacteria. In: Color Atlas and textbook of diagnostic microbiology, 6th ed;878-939.

Lee EHL, Degner JE, Welling GW and Veloo ACM. 2011. Evaluation of the Vitek 2 ANC Card for identification of clinical isolates of anaerobic bacteria. *J Clin Microbiol,* 49:1745-1749.

Mory F, Alauzet C, Matuszeawski C, 2009. Riegel P and Lozniewski A. Evaluation of the new Vitek 2 ANC Card for identification of medically relevant anaerobic bacteria. *J Clin Microbiol,* 47:1923-1926.

Mutters CNR, Jacoby LFD. 2001. Microbiological characteristics of sub gingival micro biota in adult periodontitis localized juvenile periodontitis and rapidly progressive periodontitis subjects. *Clin Microbial Infect.,* 7:213-217.

Pradeep AR, Daisy H, Hadge P, Garg G, and Thorat M. 2009. Correlation of Gingival Crevicular Fluid, Interleukin-18 and Monocyte Chemo attractant Protein-1, Levels in Periodontal Health and Disease. *J Periodontol,* 80:1454-1461.

Rennie RP, Brosnikoff C, and Krilcich A. Multicenter Evaluation of the Vitek 2 Anaerobe Van Winkelhoff AJ, Loss BG, Van Der Reijden WA, Van Der Velden U. 2002. Porphyromonas gingivalis, Bacteroids for sythus and other periodontal pathogens in subjects with and without periodontal destruction. *J Clin Periodontol.,* 29:1023-1028.

******