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Use of Fourier Transform Infrared (FTIR) Spectroscopy for Typing Vancomycin Resistant Enterococci (VRE)

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Abstract

The present study is dedicated to the development of a new experimental technique for the characterization of Enterococci bacteria. Fourier transform infrared spectroscopy was used in studying Enterococci bacteria for the first time. The results had shown that Fourier transform infrared (FTIR) spectroscopy has been successful in characterizing the VRE genotypes (Van-A, Van-B, and Van-A plus Van-B) and other Enterococcus clinical specimens of different patients obtained from various wards from Al-Makassed hospital, Jerusalem / Palestine. FTIR initial spectra were obtained in the range 4000-500 cm^{-1}. Recognitions and separations between Enterococci species were revealed by spectra analysis. Out of 33 isolates a total of 11 groups were identified; out of which Van- A have been divided into three groups, Van-B two groups and Van-A plus Van-B one group. The comparisons of the whole cell of the Enterococcus have been done in the fingerprint regions in the range 900-700 cm^{-1} and presented using their values of absorbance peaks, i.e. FTIR absorbance spectra. We demonstrate that FTIR spectroscopy and VRE genotypic results presented a good correlation. Moreover, spectroscopy measurements were simpler and easier to perform than using PCR method. It has been shown that using FTIR spectroscopy is more effective in differentiating Enterococci species which were useful in creating spectral library for vancomycin resistant Enterococci (VRE).

ملخص:

هذه الدراسة تهدف إلى أ ابتكار طريقة جديدة والتي يمكن أن تستخدم في التعريف والتمييز بين أنواع البكتيريا ألمسماه (Enterococcous) وبالتحديد ألأنواع المقاومة للمضادات الحيوية (Vancomycin Resistance Enterococci) والخطيرة على صحة ألإنسان. حيث تم استخدام جهاز (FTIR spectroscopy) ولأول مرة في هذه الدراسة.

أن النتائج التي تم الحصول عليها من هذه الدراسة قد أثبتت نجاح هذاالطريقة الجديدة والتي من خلالها تم التعريف والتمييز بين أكثر أنواع هذه البكتيريا إمراضا وخطورة وهي (VanA, VanB, VanA+B) وكذلك ألأنواع ألأقل خطورة.

لقد تمت دراسة هذا النوع (Enterococcous) في فلسطين وفي مدينة القدس. ومن الوحدات المختلفة في مستشفى المقاصد تم عزل عينات مختلفة من هذه البكتيريا لأغراض البحث العلمي. حيث تم التميز بين أنواع (Enterococcous) باستخدام أساليب بيولوجية مثل (PCR) والذي يستخدم المواد الكيماوية في أجراء هذا الفحص. وقد كانت نتائج هذا الفحص أنه كشف وجود(5) أنواع فقط في 33 عينة بكتيرية معزولة من وحدات مختلفة في مستشفى المقاصد.

أما الطريقة الجديدة في التعريف والتمييز والتي تعتمد على استخدام ألأشعة التحت الحمراء في أل (Mid Range Infrared) ومن خلال استخدام جهاز (FTIR spectroscopy) فقد أستطاع الكشف عن(11) نوع بكتيري موجودة في أل (33) عينة والتي تشمل ألأنواع ألأكثر خطورة والأقل خطورة. لقد أثبتت النتائج أن هذا الأسلوب الجديد (الطريقة) المستخدم (FTIRs) أكثر دقة من أسلوب أل (PCR) مع أن العلاقة التعاونية بينهما ضرورية.

لقد أعتمد أسلوب (FTIRs) على دراسة كامل محتويات الخلية (Whole Cell) وفي منطقة البصمة (Fingerprint region) والذي يتراوح مداها بين 700 – 900 / سم وكذلك تم استخدام جداول تحتوي على قمم ألامتصاص (Absorbance Peaks) متبوعة بشكل طيف ألامتصاص (Absorbance Spectrum) لكل عينة في منطقة البصمة وذلك بهدف التعريف بالعينة. وأما أغراض المقارنة والتميز بين ألأنواع ألمختلفة فقد تمت عن طريق البرامج المحوسبة (Origin Software) كما تشير له النتائج.

وبسبب أن أوجه الشبه بين أنواع هذه البكتيريا كبير وبصعوبة يمكن التميز بينهم فقد تم استخدام قطعة (Silicon Window) لأول مرة والتي تمتاز بخصائص متميزة لإعطاء نتائج أكثر دقة وكذلك سهولة في تحضير العينات (Sample Preparation) .

لقد أثبتت النتائج أن استخدام أسلوب (FTIRs) في التعريف والتميز بين أنواع البكتيريا (Enterococcous) يوفر الوقت والتكلفة المادية، كما أن نتائجه دقيقة جدا مقارنة مع غيره ، وأن أجراء هذا الفحص لا يحتاج إلى شخص متخصص وعلى درجة كبيرة من التدريب.

لذلك يمكن اعتباره أسلوبا جديدا، كما ويمكن استخدامه بسهوله في دراسة الكثير من الكائنات الحية الدقيقة للحصول على مجموعة من أطياف الامتصاص، كما يمكن أيضا عمل مكتبة أطياف (Spectral Library) لهذه الكائنات المفحوصة.

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Introduction

There are many spectroscopical techniques such as: NMR spectroscopy, Raman spectroscopy (RS) is a unique tool for probing or mapping, nanophases dispersed in a matrix (e.g. pigments in a ceramic glaze (Colomban et al, 2001). On the other hand, infrared spectroscopy (IR) also is an important and popular tool for structural elucidation and compound identification since it can be used to determine the chemical functional groups in the sample. Consequently, the spectroscopical techniques that have been used offer many useful applications.

The present work is aimed at the use of FTIR spectroscopy technique which proved its success in many fields (Zhu et al, 2001). Many researchers (Amiel et al, 2000; Curk et al, 1994; Lefier et al, 2000; Mariey et al, 2001; Naumann et al., 1991) have recognized Fourier transform infrared spectroscopy (FTIR) as a method fulfilling these needs such as compositional compounds chemistry, medicine, etc. The application of vibrational spectroscopic techniques (Fourier transform-infrared [FTIR]) is such an approach, which may provide a potential alternative to conventional methods. It has been shown that FTIR spectroscopy is very sensitive to small changes in the composition of cells (Galiche et al,2001; Galiche et al, 2000). These techniques are rapid because little biomass is needed, significantly reducing culturing time. FTIR Spectroscopes are also easy to use and may

become very cost-effective, because they enable considerable reduction in sample handling and use of reagents and do not require highly skilled personnel. This method allows the discrimination of intact microbial cells without their destruction and produce complex biochemical fingerprint-like spectra, which are reproducible and distinct for different microorganisms. It can measure molecular vibrations based on the absorption (IR) or scattering of radiation interacting with a sample. The observed microbial are a complex composition of many different vibrational modes of all the cell components, i.e., DNA, RNA, proteins, and membrane and cell wall components. The applicability of FT-IR spectroscopy in the field of microbiology has already been persuasively demonstrated (Goodacre et al, 1996; Helm et al, 1991; Helm, Labischinski, Schallehn, Naumann 1991; Naumann et al, 1991; Naumann et al, 1996). Various studies have shown that vibrational spectroscopy provides sufficient resolution power to distinguish microbial cells at different taxonomic levels, even at the strain level i.e genetic variant types of the same species (Helm et al, 1991). The Extensive reference libraries containing thousands of spectra of well-characterized microorganisms that can be used for the rapid identification of unknown isolates at the species as well as at the strain level (Ku¨mmerle et al, 1998; Maquelin et al, 2003; Oberreuter et al, 2002).

The purpose of this study was to evaluate the discriminatory power of a novel phenotypic approach based on infrared absorption spectroscopy to the typing of enterococci isolates over one-year period from patients of different wards of a hospital in Palestine. Here, PCR antimicrobial susceptibility testing and FTIR spectroscopy analysis were compared. The application of FTIR study special type characterization of bacteria i.e. enterococci is not well known yet. Enterococci are opportunistic human pathogens, the two most important species, *E. faecalis* and *E. faecium*, which are considered part of the normal intestinal flora, are among

the leading causes of nosocomial infections (second most common bacteria responsible for nosocomial infections) and may cause severe infections, including endocarditis, septicemia, intra-abdominal, pelvic and urinary tract infections, (Monstein et al,1998; Schaberg et al ,1991). These infections are often difficult to treat due to the increased antibiotic resistance associated with this organism (Buschelman et al, 1993; Tsakris et al, 1998; Vincent et al, 1991). The recent increase of vancomycin-resistant *E. faecium* (VRE) strains in clinical isolates is especially a cause of serious concern, because this glycopeptide-type antibiotic often remains the last treatment available in life-threatening infections (Wilke et al, 1997). The situation is further complicated by the fact that Enterococci have developed a number of mechanisms for the transfer of resistance genes (Bodnar et al, 1996). Therefore, perhaps the greatest threat posed by VRE comes not from these organisms themselves but from the potential that they could transfer their resistance genes to other more pathogenic gram-positive bacteria, thus creating a highly dangerous pathogen difficult to treat with currently available antibiotics (Moellering, 1998). Furthermore, the recent studies have revealed that the incidence of more unusual species such as *E. durans, E. hirae, E. gallinarum and E. casseliflavus* has increased significantly in clinically isolated enterococci (Willey et al, 1999). Overall, this has resulted in an increased need for rapid and accurate identification of Enterococci at the species and subspecies level as a means of effectively assisting infection control and epidemiological studies. For most clinical microbiological laboratories, the primary method of identifying Enterococcus strains relies on phenotypic characterization. However, various studies have shown that an unequivocal species identification of enterococci by phenotypic means is a challenging procedure that can take several days to accomplish because of the phenotypic and biochemical similarities between many enterococci

(Cheng et al, 1997). In addition, the automated systems currently in use often fail to accurately identify rare species (Bryce et al, 1991; Devriese et al, 1995; Iwen et al, 1996; Sader et al, 1995; Tritz et al, 1990). Molecular genetic techniques, such as randomly amplified polymorphic DNA analysis, PCR ribotyping, or other PCR-based methods targeting various genes, have been successfully used to identify enterococci at the species level (Devriese et al, 1995; Jones et al, 1997; Ke et al, 1999; Roger et al, 1999). Although these techniques are specific and sensitive, it is difficult to adapt them for use in routine laboratories due to their high costs and the requirement for highly skilled personnel. Infection control and epidemiological studies primarily require rapid and simple means of identifying and typing clinical isolates. Consequently, a variety of approaches have been developed.

The present work has new specific goals, in which Fourier Transform Infrared spectroscopy [FTIRS] will be used to distinguish between the most pathogenic Enterococci species that were isolated in Palestine. A new method of preparing samples is targeted allowing a characterizing process and creating spectral libraries for different microorganisms.

Chapter one of this thesis deals with theoretical concepts of atomic bond, IR radiation and principals of FTIRS. The new technique of preparing samples was discussed in chapter two. The experimental results will be presented in chapter three and discussed in chapter four. Finally, conclusions and suggested further work are given in chapter five that is followed by spectral library and a list of references.

Chapter five

Conclusions and further work

5.1 Conclusion:

The results of this study confirm that FITR spectroscopy has discriminated between eleven groups of Enterococci isolates, i.e. it posses a good discriminatory power, the least it is far more comparable to that of genetic type method like PCR which has revealed only four groups of Enterococci isolates in the same group of samples. Since FTIR spectroscopy is a physicochemical methodology that fingerprints the whole cell and is able to detect subtle compositional changes that cannot be revealed by PCR method. It should be concluded that the comparison between PCR and FTIR procedures shows that, the first methodology depends less on preparation conditions, although its large of protocol steps influences reproducibility and rapidity furthermore, intensities of band pattern are sometimes variable, and the interpretation analysis of result become difficult. On the other hand, using FTIR spectroscopy is much easier to carry out than using PCR method and allows the analysis of large number of strains, although it requires a high degree of standardization and complex data processing. In addition, the differences between PCR types are due to differences in generated DNA fragment and sometimes these are due to methodological variability or strain modification.

Enterocccal isolates were carried out by PCR method in Jaber study, and then characterized by FTIR method, this can be concluded that the second method is able to find out the differences of Enterococci characterizations resulted from the first one, since FTIR method has accurate analysis. It is also important to consider FTIR spectroscopy limitations, because it does not reflect changes in key molecules and there is not an understandable connection between the differences in the spectra and the changes in biochemical composition. The main conclusion from this study demonstrates that the obtained results by using FTIR spectroscopy in typing and identification of strains belonging to the most pathogenic species of Enterococci Van A and Van B of *E. faecalis* is more accurate and easy than using PCR.

5.2 Further work

The present method of using FTIR spectroscopy in this study opens an era for further studies in identifying different types of microorganisms, a part from the three genotypes which were identified in the present study namely: VanA, VanB and VanA plus B. This method can help deepen investigation in identifying genes that can be transferred from Enterococci to other bacteria and in finding out the influence of temperature and time on cultures and sample preparation procedure for repeatability of FTIR measurements. Consequently, the previous developed method for measuring FTIR spectra and a strategy for their analysis can be used in creating spectral library.