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Some Principal Issues of Molecular Techniques in Health Research: Facilitators and Barriers, Advantages and Disadvantages.

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ABSTRACT

The development of molecular technology and DNA analyzing methods are used as tools. However, modern diagnostic methods examine the bacterial isolates at a molecular level to detect of bacterial genotypes and subtypes and also can explore epidemiology and biology of infectious diseases. Today the detection of pathogen nucleic acids (DNA and RNA) is applied by Molecular methods. So the finding of contamination in clinical samples and the food is performed with high selectivity and rapidity. The molecular techniques provide the new alternative in molecular epidemiology. Molecular methods besides of traditional diagnostic methods have done in routine pathogen detection and may be replaced in near future. Molecular methods were developed to reduce the traditional methods problems. Despite the benefits of molecular techniques, some barriers restrict the application of molecular diagnostics. In the present study, we discussed part of them without proposing on a special method or comparing of the molecular techniques. We have reviewed current and new molecular methods for examining finding in clinical research, aiming to give an overview of their advantages and disadvantages. In this review, we discussed facilitators and barriers of molecular tools in clinical research in order to find the health research benefits.

Keywords: Molecular Techniques, Facilitators, Advantages, Disadvantages

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INTRODUCTION

Digital technology" changing human's behavior; "molecular biotechnology" changing human's lifestyle. The structure of DNA was discovered in 1953, human genome project begun in 1990 and the genomic research have been started since 2002 [1]. In Twenty years period, molecular biology and other fields of biology were progressed, and led to data improvement. A large amount of data was associated with a development of molecular methodologies, and DNA sequencing methods helped us to complement of phenotypic identification, keeping and analyzing them [2].Recently molecular or genotypic techniques have used more interesting because of analyzing epidemiologic interrelationships [3]. The application of new technique of molecular biotechnology is applied in the various field similar to medical science, pharmaceutics, environmental protection, the epidemiology of infectious mediators and basically concentrating on the pathogens themselves [1, 4]. Pathogen phenotype is the basis of the traditional diagnostic methods e.g. features of growth on a certified medium, metabolism of chemical composition, etc. has been used for classification. The appropriate antibodies against membrane proteins are used for the exact classification of a serotype, or with serotype-specific bacteriophages. The origin of pathogens and contaminants should be isolated by rapid and secure methods because of taking 2-3 days for correct assessment. It is essential that the pathogen detection techniques develop [5]. An important advantage for many diagnostic intentions is able to access to fast and similar methods. These diagnostic methods will provide food safety [6]. In addition, other benefits of molecular biotechnology really make the great changes in our natural world and living life such as environmental monitoring and scrutinizing, pollutant removal, soil and groundwater remedy, molecular nanotechnology and green energy. Molecular biotechnology serves as a tool to improve our environment via contamination avoidance [1]. Molecular techniques are used for investigating microbial diversity in soil communities and provide new information on the distribution and diversity of organisms [7, 8]. Therefore new techniques are able to resolve the old problems and hence the new problems are required to figure out the answers [1].In clinical analyses, molecular assays have been improved to detect the presence of mRNA species of viable pathogenic microorganisms which used to assess the efficacy of antimicrobial therapy and persistence of infection during treatment [9]. Molecular tools applied in public health and researchers will explore possible ways to be used in future research [2].Applied deliberation this subject correlates to practical matters and relates to the routine result measurement in practice [10]. Although objectives of research programs are identifying possibilities, facilitating feasibilities and determining research barriers, the essential observation of molecular research is their weak and strong points [11-13]. The molecular methods are focused on the benefits and challenges of applying available techniques to infectious agents [4, 14].Some barriers and facilitators of molecular measurement have already been conducted in research programs [10]. Category of Barriers and facilitators is based on personal, resources, and access and administration groups [12]. The impact of technique interventions is usually poorly represented in public health research, In this review we have presented current and recent molecular typing methods for detection of epidemiological surveillance of bacterial pathogens in clinical practice in public health research areas, in order to give an overview of their specific advantages and disadvantages [15, 16].The structure consists categorization of the benefits and problems molecular research in health research and the description of the points are involved in this theme. Most barriers and facilitators noted in this study which indicated the sections of the possibility of high impact on public health research. In this basis, facilitators and barriers of molecular techniques have studied in clinical research in order to find their health research benefits.

METHODS AND DATA COLLECTION

For the literature review, standard search approaches using the inquiry of two online databases MEDLINE , GOOGLE SCHOLAR (1966–2010) and keywords were used by evaluation of the bibliographies of relevant articles, for exploring published work PubMed Web sites and reference lists provided by our expert research to find appropriate methods in public health using a combination of specific keywords to regain the relevant articles without any limitation on the time of publication and also the literature review was developed methods that included: inclusion and exclusion criteria to recognize potentially related articles, search plans to retrieve articles, abstract review papers, and a usage of scoring published studies for completeness. Additional articles were found by hand searching from the reference lists of papers which included in the review. Several topics available were not of direct relevance. Comprised papers were first categorized into following: special research, general research, directly related subject, indirect topic, mixed methods research.. Their content was

quantitative and qualitative research; key factors extracted from each paper into a summary. All issues were then compared with each other to identify higher level themes.

Molecular tools and technique roles

Combining molecular approaches with traditional approaches is an imperative stage which can help cross-check the accuracy of results, reveal the relative qualities of each approach. Combined methods provide significantly more information than either approach alone. Molecular methods should be truly experimented and compared with existing standard methods other than using traditional methods versus new molecular methods. According to this information, we focus on molecular biology which presents the new techniques that could improve management of environmental resources. Molecular biology techniques have become increasingly incorporated into the study of infectious disease epidemiology [17]. Molecular techniques may be applied to the measurement of host or reason factors and exposures of these features. When they applied the study of disease, the resulting measurement enhanced our ability to more reliably detect relations. Molecular techniques can also stratify and refine data by supplying more sensitive and specific measurements, facilitating epidemiologic activities. They include disease inspection, occurrence investigations, recognizing transmission patterns and risk factors among apparently different cases, distinguishing host-pathogen interactions, detecting uncultured organisms. All of them give clues for possible causes of cancer and other chronic diseases, and providing the better finding of disease pathogenesis at the molecular level. Molecular techniques could not be replaced with conventional methods. They refer epidemiologic problems that cannot be approached or would be more intensive, expensive endeavor and/or time wasting to refer to conventional techniques. Currently molecular technique can become tomorrow's conventional diagnostic tool or eventually be outdated. The recognition and amplification of nucleic acids are based on rapid diagnostic methods. Similar detection can recognize the nucleic acids of all organisms. The same techniques apply in clinical diagnosis via identity of food-borne pathogens and GMOs. The amplification and tracing of very small quantities of nucleic acids have found for many years, but these methods have used in diagnostics in 10- 15 years ago. In addition, available data of the amount of nucleic acid sequence of several organisms such as the whole genome sequence of a large number of pathogens has supported favorite situation for DNA/RNA-based tests. Molecular methods recognize faster and more sensitive diagnostic compare with traditional immunoassays and culture techniques. In spite of the absolute advantages molecular methods, they have replaced in a few of analyses [18].

Molecular tools and microorganisms

Study of heterogeneous organisms with molecular techniques application improves epidemiologic researches and enhances our ability to sub classify these organisms into meaningful groups, This ability help us for recognition of sporadic cases and also detection of disease incidence that may be undetected. The new identified genes can be used to further characterize the epidemiology. Training dedicated staff to master the molecular methods is not time-consuming, thus skilled personnel can develop new methods and related theories [4].Needs of the molecular marker to track the transmission of specific strains of infectious organism is useful in molecular epidemiology of infectious disease. Commonly during epidemic, fingerprint as a marker varied greatly [19]. Molecular methods have augmented speed, sensitivity, and specificity. DNA and RNA have been successfully analyzed through polymerase chain reaction (PCR), reverse transcriptase PCR (RT- PCR) and nucleic acid sequence- based amplification (NASBA), as routine molecular techniques [9]. DNA sequences also provide genotypic information in clinical microbiology laboratories [2]. While rDNA and rRNA are generally used as characters in phylogenetic analysis. For microorganisms, molecular data often provide the greatest wealth of information because a microorganism such as bacteria apparently does not have the diversity of structure to make useful morphological characteristics in phylogenies. The use of SSU, rRNA or rDNA sequences, combined with fluorescent oligonucleotide probes offers a powerful technique for studying soil microorganisms that may not be amenable to the current culturing system [7, 20]. In addition, molecular methods could significantly affect the validity of the viability assay via the choice of target and sensitivity of the method. Determination of viability is essential for the analysis of pathogens detection in environmental or clinical specimens. PCR analysis shows the targets and mRNA analysis displays target transcript. The quantitative approach with array-based detection systems to allow the simultaneous analysis of multiple targets can provide an effective monitoring system for the identification of viable cells [9, 11, 21]. The enormous range of new molecular techniques causes to investigate Metagenomics, Biological Science diversity

and genetic material characterization from complex microbial environments. Anyway, some barriers have caused that the molecular diagnostic assays have not replaced the pathogen's phenotype completely. Interfering factors lead to the plenty of false positives and false negatives. The presence of DNA in the environment and in the laboratory instruments makes the reaction mix with the false detection of a pathogen. The cell contaminations can be easily wiped out from surfaces and lab equipment while DNA is not removed easily. The samples contain chemical compounds able to interfere with the enzyme activity. Enzyme inhibitors also are effective the false negative results. In many experiments, the host genomic DNA of the organism in PCR Molecular method can intervene with the detection procedure by competing for the probe and primer annealing. His interference occurs even with a few host cells because of the genome of vertebrates or plants by an average of 103-104 folds larger than bacteria. This problem can solve by genome complexity reduction through eliminating the repetitive sequences of the genome [18]. Competitors or abundant inhibitors are necessary to make bacteria enrichment through culture [22, 23]. In many situations, molecular methods can detect the contaminations. Redundant probes or primers that performlong probes and less severe hybridization situations or formulate enormously unseemly the concurrent variation of every target sequences can be overcome this problem [24].

Illustration of Molecular technique

The benefits of DNA-based assays contain to detect viable and nonviable pathogens. Non-cultivable pathogens are an advantage but there are some problemssuch as the genomic DNA of death pathogenic organisms that still exist in the sample, can show a false positive result. RNA-based techniques, like NASBA or RT-PCR, are suitable methods since the RNA is less stable than DNA [25].Fluorescent in situ hybridization (FISH) or quantitative real-time polymerase chain reaction (q PCR) are two molecular options that include specific fluorescence probes (in situ hybridization) or primers that help us for quantitative analyses of organisms that previously have been identified in the metagenomic library. Rapid analysis of whole bacterial populations in human health and disease are achieved with the application of microarray technology (metagenomics) and molecular techniques.

The advantages as follow:

1- High throughput and short learning time.
2- Anaerobic DNA can be transported easily between laboratories.
3- Uncultivable species are detectable.
4- Quantification of next to one molecule of target DNA.

The hybridization microarray is able to complete in less than 1 h, instead of several hours [26-28]. Microfluidics provides high sensitivity and improving experiment manufacture with the use of small volumes and sample amounts. To limit the sample leftover can reduce the risk of contamination. Procedures of Lab-on-chip are more easily standardized and done in one device. Portable devices enable the pathogens diagnosis in limiting the laboratory work, sample handling, and transportation with miniaturizing and standardizing techniques. NASBA technology (Nucleic acid sequence based amplification) amplifies nucleic acids without the use of a thermal cycler [29]. A few RNA molecules often make available many copies of RNA by this method. Also, quick amplification of expressed genes is an advantage of the Bacteria detection which their enterotoxin is known resistant [30] and also NASBA is low-cost the lab equipment than PCR. The initial denaturation step is carried out a water bath or an isothermal simply. The use of NASBA could provide the portable and suitable method even for less well-equipped laboratories [31] .

Disadvantages are:

1-Standardisation of extraction of genetic material from each similar species is so difficult.
2- In the mixed population, we have severed paradox to introduce in detection.
3- Selection of specific primers and probes are an important matter and also some techniques are not quantitative to analysis and very insensitive.

Further analysis of gene expression in hard to find organisms, directly from their natural environment can reveal any bias introduced through manipulation and repeated culture passage [32, 33]. Molecular testing

is a necessary tool which is cost- effective in medical labs as important screening techniques [3].Accordingly, modern methods can isolate at a molecular level to differentiate among bacterial types and subtypes. This has resulted in better achievement of infection control programmers.

PFGE has been a primary typing tool to analyze transmission events, and it has been used successfully in large-scale epidemiological investigations which allowed fast detection of emerging clones and monitoring of the spread of pathogenic bacterial strains through different regions or countries. AFLP analysis enables the determination of genetic relatedness among studied bacterial isolates [34-38]. Application of AFLP is restricted because of intensive work (a typical analysis takes about three days) and expensive cost of the extraction kits for total DNA, fluorescence detection system, Enzymes, and adopters. Primers that hybridize to noncoding intragenic recurring sequences scattered across the genome were done in the rep- PCR method. Lack of reproducibility is important limitation role to rep- PCR combined with electrophoresiswhich produces variable reagents and gel electrophoresis systems. The main disadvantage of arrays is the inability to identify the sequences which are not included in the array. The Certain problem in these newer methods implies the expensive equipment, hence the traditional methods are replaced with newer ones easily at the local stage rather than in large national or international laboratories (with different staff and budgets) where the same new typing method are implemented and all participants are trained in its standardized application. It is noticeable to understand that a newly offered method must be determined its validity of potential typing by various independent laboratories and this procedure takes years rather than months and also during the validation process a particular unequivocal appellation must be developed and improved for a new method [15, 39, 40]. Obviously, bioinformatics tools have enhanced the public health impacts of the extensively applied typing methods.

Environmental benefits of the molecular biotechnology are as follow:

1- Environmental monitoring and inspecting.
2- Environmental management and risk evaluation.
3- Soil and groundwater treatment.
4- Molecular nanotechnology.
5- Green Technology Examination.
6- Responsibility of environment disputes.

A cytogenetic technique called FISH (Fluorescent in situ hybridization) can detect and localize specific DNA sequences on chromosomes. Fluorescence microscopy is able to find the fluorescent probe bounded to the chromosome. The towering technique will provide the environmental monitoring and inspecting easily. Another type of new technology for DNA microarray is gene chip in which analyzes the gene expression in a whole bunch of genes. This method is contrary to traditional methods which are very expensive and spend a lot of time to inspect a lot of microorganisms in order to confirm the pollutants. In contrast, it can reduce the number of biological experiments and testing time. Recently most of the environmental toxicity examinations apply this technology to decrease the time of the biological test. Both of nice techniques for inspecting environmental detection are DNA – chip – PCR and genotoxic Effects. Therefore these technologies can solve a great number of environmental management problems and reduce environmental risk factors [1].

Molecular Method description

DNA arrays advanced the techniques of nucleic acid detection. The first versions were known as microarrays that included the collections cDNA probes, each targeting a different gene and then spotted on a nitrocellulose membrane. In addition to improvement of the array, similar steps keep for macro and microarrays. The typical experimental procedure includes labeling mRNA, cDNA, PCR products or genomic DNA of the sample under investigation with a dye (e.g. a fluorescent or radioactive dye) then denaturing to produce single strand fragments and finally hybridizing to the array. However, its problems are pin clogging and cross contamination of probes give non-specific signals and missing spots result [41] .It is important the detection of the pathogen is done carefully because of Coexistence inoffensive and harmful bacteria present in the product. False positives or false negatives result by mistake recognition. The right diagnosis is affected by contaminated product of the wide range of pathogens. To solve this problem microarrays method to miniaturize different pathogen with specific probes that supports sensitivity and specificity of them. The LDR-

UA technique with the probe sets that make a very sensitive reaction to mismatches [42]. This results in a high discriminatory power system to use in diagnostics.

Molecular investigator ideas

The molecular researchers opinions in some molecular centers in a less resource country comprise: less skilled technicians and inadequate facilities , modern instruments and budget, lack of teamwork between research labs and clinical centers, lack of comprehensive courses in health research , reduction of attention to health research themes, performing and making non-essential research in this issue, lack of support and maintenance instruments , molecular techniques and associated materials are more expensive, insufficient examination location, and molecular updates. In close future in this setting, achievable microarrays can present to choose the pathogen detection and diagnostics. Lin et al. [18] set up a diagnostic test to detect from 23 bacteria and viruses.

CONCLUSION

The current review identified the studies that discuss the barriers and facilitators to use the molecular methods in practice. The findings of this review highlight the approach that likely impact on the results of laboratory methods in practice. Some of the examples cited above are illustrative of molecular methods benefit and weakness. The wide range of represented problems makes many specific usages of new molecular techniques into molecular research management. The presence of molecular techniques in the field of molecular researches will be fundamental to solve previous problems .Molecular testing will continue as a critical tool, for the cost-effective and medically desires. Molecular typing is a powerful tool in the examination for hostility the spread of problem microorganisms in the hospital environment. To use molecular methods, such as pulsed-field gel electrophoresis (PFGE) to allow the microorganism typing to extend beyond bacterial disease. They identify the organisms that are difficult or impossible to culture while the analyzing gene expression in these organisms is possible; therefore the choice of an appropriate molecular method to provide convincing effects in clinical subjects.

DNA sequences and DNA microarray profiles allow easier and faster laboratory assessments since speed is important for controlling local disease occurrence. The PCR-based method with high indicative power makes well can facilitate the isolation. Notably, some of the newer methods, such as SSU, Rt-PCR, qPCR, SNP or DNA microarray analysis, provide the isolates precisely and urgently needed results can be achieved in shorter periods of time. Molecular technology would employ original definitive processes to obtain definitive results. It is important that a newly initiated method must be well validated by different independent laboratories to determine its appropriate application. Hence, the replacement of an old well- and widely established method with a new one must be accomplished gradually to avoid the loss of precious historic information produced over many years. Furthermore, these achievements will be put to clinical use not only in industrial countries but also in less-resourced countries. Present study showed that molecular techniques could solve the plenty of problems in traditional methods but these methods are not used for all clinical diagnosis because of some barriers. Pervious study confirmed the usage of the special methods which faced with some experiment restriction. The objectives were not discussed commonly. In brief, the molecular techniques provide marvelous facilitators in health research but requiring expensive equipment, training staff especially limited the use of them in less-resourced countries in medical diagnosis and health research. In the future molecular tools create a revolution in the results of molecular research particular in health programs.

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