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EXCELLENCE OF MODERN METHODS OVER CONVENTIONAL METHODS FOR BACTERIAL IDENTIFICATION

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ABSTRACT
Laboratory identification of human pathogen is a great diagnostic challenge using traditional methods. Proper identification of microbes is always considered as an important part of any research. For all kinds of research related to microbiology, there is a demand for establishing rapid and accurate methods of identifying causative agent. This kind of approach alleviates researcher to do further study using identified microbes. The bacteria that can be identified by phenotypic methods can be identified by genotypic methods also but all bacteria identified by the genotypic method cannot be identified by the phenotypic methods. In this aspect, evaluation of 16S rRNA has been thought to be a great hope for proper identification and characterization of isolated strain. Similarly, use of mass spectroscopy for bacterial identification, especially 'matrix associated laser desorption ionization time of flight' have been reported as rapid, precise and cost- effective method for bacterial identification. The modern identification methods have hence thought to help us in acquiring more applicable identity of bacteria of different species and diagnosing more upcoming species causing threat to human life. More research is required to validate existing methods and make an effective strategy for rapid identification of clinical isolates to carryout effectual therapy.

KEYWORDS: Traditional methods, Phenotypic methods, Genotypic methods, Ionization Time Of Flight, Mass spectroscopy, 16S rRNA, Matrix associated Laser Desorption.

INTRODUCTION
Humankind has always been kin in discovering new organisms. Out of all these organisms, bacteria has been found to be the main fruit of interest because of its saprophytic, pathogenic, spoiler and other characteristics. It is the most infectious organism as compared to fungus, viruses and protozoans which has the capability to cause wide spread diseases. Starting from the milk we drink to the feces we secrete everywhere bacteria has its noteworthy presence(Daliri 2015). By increasing development and modernization in the research as well as therapeutical medicine, there is also the emergence of new and mutated bacterial species, which are even more harmful (Woodford 2007). So rapid and accurate identification is very necessary for the welfare of overburdened healthcare units. The identification of bacteria can be done by phenotypic as well as genotypic method (Donelli 2013). Initially, the bacterial identification was done by phenotypic methods which identify bacteria on the basis of shape, size, structure, presence or absence of flagella or number of flagella, culture, growth and colony characteristics, biochemical tests etc of bacterial species (Busch 1999). But due to some drawbacks of the traditional methods the laboratory scientists have to opt for modern techniques which involve identification on basis of genotypic method which also called modern methods. The major advantages of this method include producing quick and accurate results, evaluating large number of samples, identifying samples that are non-cultivable, minimizing human errors and being stable with environmental changes to give reliable and meticulous results. The genotypic method involves identification on the basis of gene extraction, amplification, and sequencing. Combination of traditional and modern techniques helps us to develop the rapid diagnostic assay. A sequence analysis of the 16S rDNA, protein profiling using 'matrix-assisted laser desorption - ionization time-of-flight' (MALDI-TOF), metabolic finger profiling using BIOLOG, rapid identifications as Vitek 2 API System (Weisburg 1991, Janda 2007).

TRADITIONAL METHODS TOOLS
1. MICROSCOPIC METHODS
a. MICROSCOPY
Optic microscopy is the most premature technique of identification. Further the invention of compound microscopes made it easier in studying little about

cellular organism. Additional innovations in microscopy brought phase contrast, fluorescence and many other microscopes and now we have highly advanced one called electron microscope which can magnify million times and gives better resolution.

b. STAINED MICROSCOPY

Concept of staining gave contrast picture of the microbes with the aid of microscopes and provided a clear observation with high resolution (Walker 1994, Surman 1996). Simple stain consists of a single dye, helped to know the actual morphology of bacteria. Further advancement in staining with differential stain helped to differentiate between Gram positive or negative bacteria, acid fast and non-acid fast cells and to visualize internal constituents of microbial cells including endospores, capsule, flagella and metachromatic granules (Harlow 1988, Cowan 2004).

2. CULTURING TECHNIQUES

Culturing is an important methods for bacterial cultivation as well as identification where bacteria uses a nutritional media for growth and providing special indicator along with special selective components helps to identify bacteria from other one such as MacConkey agar, Xylose lysine deoxycholate agar, etc (Cashion 1997).

3. **BIOCHEMICAL TESTS**

These tests fetched another advancement in the traditional methods which was used in confirmation as well as characterization of bacteria after the study of culturing and microscopy. The various tests used are Amylase production test, Cellulose production test, Gelatinase test, Urease test, IMVic test, etc.(Mac Faddin 1976)

MODERN METHOD TOOLS

1. **RAPID DIAGNOSTIC TESTS**

These tests has increased effeciancy of results, minimized the human errors and produced rapid results for quick diagnosis and treatment. Numerous rapid tests have been discovered these days like detection of many fatal diseases such as- Malaria, Dengue, Syphilis, Typhoid, Human Immunodeficiency Virus using card and strip method, etc (Chan 2009).

2. **POLYMERASE CHAIN REACTION**

This method incorporated automation in amplifying specific DNA sequences which is a rapid methods (Saiki 1985).

3. **HYBRIDIZATION**

This method allowed accurate identification of specific proteins and nucleic acid present in bacterial colonies. These procedures have increased the specificity in rigorous detection and treatment accordingly. The evolved methods are Northern blotting, Southern blotting, Western blotting, Dot blotting and Colony and Plaque blotting. This processes have increased the efficacy and specificity of the tests for detection of microscopic organism (Grunstein 1975).

4. **MASS SPECTROSCOPY**

This method identifies the molecular or atomic species on the basis of their ionic charge and mass even from complex mixtures and associates it to the phenotypic characters. It further helps in identifying drug targets and typing microorganisms on the basis of the mass of the proteins and peptides present in them (Claydon 1996).

5. **PHYLOGENETIC ANALYSIS**

It is part of evolutionary biology that catalogues microorganisms on the basis of their species, traits, race and helps in differentiation and proper identification and confirms the specific identity (Amann 1995).

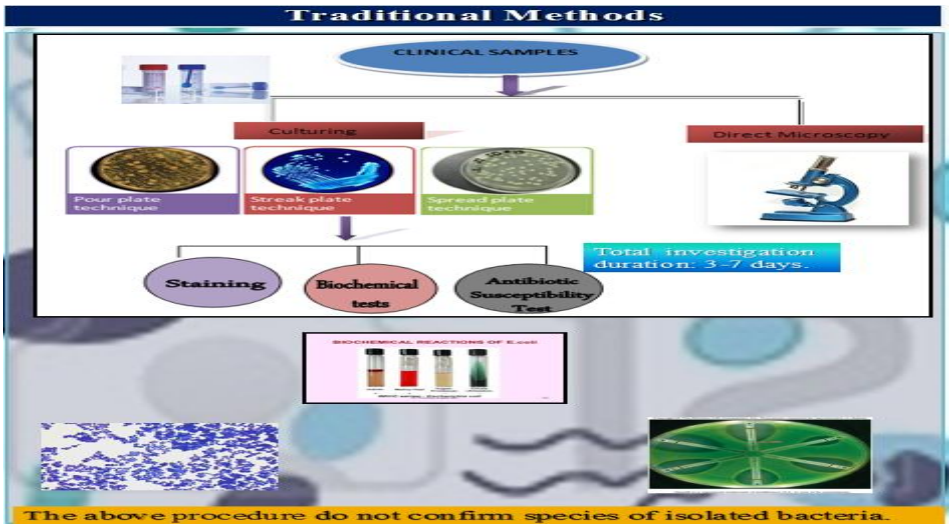

Fig. 1: Various traditional methods

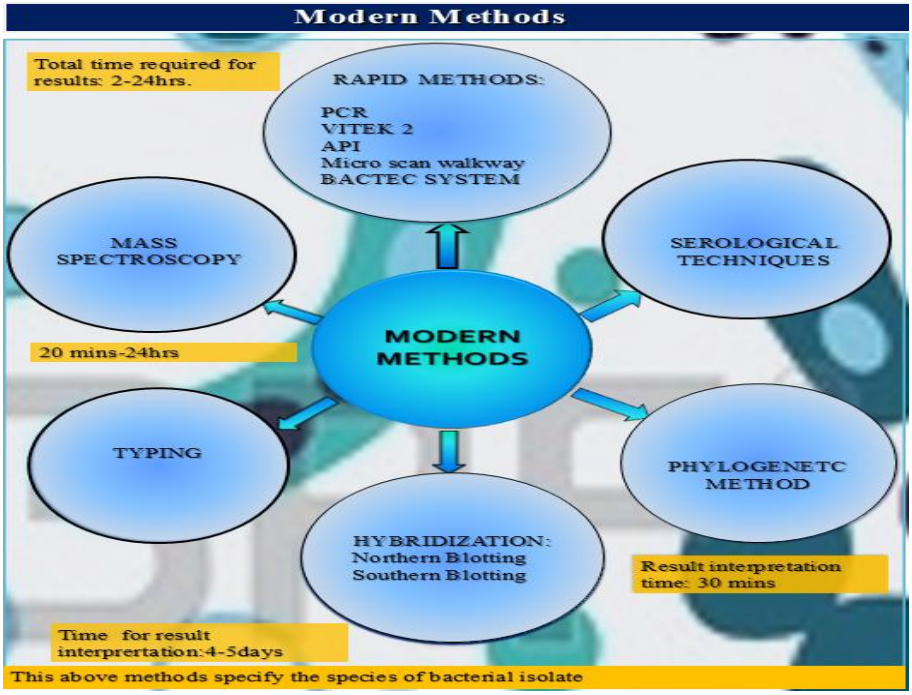

Fig. 2: Various modern methods

DISCUSSION

The conventional methods used for bacterial identification, does not provide us specific identification of the bacterial isolate. The result interpretation takes a long time may extend up to week. The techniques such as- culturing, biochemical testing, antibiotic susceptibility testing succeeded by microscopy can be used whereas, the Modern methods are mostly based on genomic studies and can help us in the specific identification of the bacterial species, within a reduced time span. The modern methods include Rapid methods which are capable of interpreting results in few hours. The use of Modern methods have sensitized the bacterial identification. The species that remained unidentified for years can be detected easily now. The serotypes of bacteria species may also be studied which helps us in determining the pathogenicity of the bacterial isolate (Friedman 1976). The modern methods are proved to be more helpful to the humankind due to its fastidious, accurate and efficient result interpretation.

CONCLUSION

Modern methods are hence proved to be more sensitive towards bacterial species identification over the conventional methods due to their ability to produce fastidious, accurate and effective results. Some species remain unidentified by conventional methods, may be either due to their varying chemical reactions or mutations in their genes sequence or adaptations by the bacteria but modern methods are capable of identifying such bacteria on basis of their genomic study.

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