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Editorial

Microbial Identification and Classification-From Phenotypic Evaluations to Molecular Characterization

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Since the invention of the microscope and the discovery of microorganisms in the 17th century [1], the study of microorganisms and the field of microbiology research have come a long way from the traditional observations of their phenotypic properties/characteristics. Phenotypic characteristics of interest in the characterization and identification of microbes include morphologic characteristics of both individual cells and groups of cells /colony (cell size, shape and structures), growth characteristics, cellular metabolism and biochemical characteristics [2]. Advances in the techniques used to identify microorganisms have often led to improved microbial classification in research and diagnosis [3,4].

Initial identification and classification of microbes were based on the morphologic appearance of the cells [2]. On the basis of cell morphology and structure, microorganisms were then classified as unicellular or multicellular, and subsequently as prokaryotes and eukaryotes depending on whether or not the cells have cell nucleus and other membrane-bound organelles. Eukaryotic microorganisms include many protists, fungi and some micro-animals such as microscopic arthropods, crustaceans and nematodes [5]. The prokaryotes include bacteria and archaea. However, research and studies in the field of microbiology has been concentrated on bacteria, archaea, fungi, protozoa, and viruses (although some people consider viruses to be non-living [6], as they cannot survive/multiply on their own). The studies of these groups of microbes form the various branches of microbiology. Nevertheless, bacterial studies pre-dominate, with more emphasis on pathogenic species and strains.

Based on individual cell shapes, bacteria were characterized and identified as either cocci (spherical) or bacilli (rod-shaped). Additionally, groups of cells display characteristic patterns/ morphologic arrangements which are used to further classify/identify bacteria as streptococci/streptobacilli (forming chains) or staphylococci (forming clusters/bunch) [7]. The development of the Gram staining technique in the 19th century, as well as other staining techniques (such as Ziehl–Neelsen staining) has also aided more accurate characterization and identification of bacteria based on their cell wall structure/characteristics [8]. Furthermore, morphologic characteristics of the colony such as colony shape and size also helped to identify bacteria more accurately [7].

Basic diagnostic identification of bacteria could be performed by a combination of morphological characterization and gram staining. However, the need to specifically and rapidly identify groups of related bacteria and some uncommon/atypical species further led to the development of specific growth assays and biochemical tests to aid in more efficient identification and diagnosis. These tests aim to characterize bacteria on the basis of their specific nutritional requirements for growth, and the measurable outcomes of their cellular metabolism [9]. Hence, a wide range of biochemical tests were developed to measure various metabolic processes and enzymatic activities such as glucose/carbohydrate fermentation and utilization, oxidation, reduction of chemical compounds (nitrates, sulphates, carbon, etc), and activities of enzymes such as catalase, coagulase, oxidase, etc. Various biochemical test kits were made available commercially for rapid identification of bacteria based on their established biochemical profiles [10]. However, the number of tests required for adequate identification of organisms and accurate diagnosis vary from one group of organisms to the other, and can be quite extensive in some cases, with the associated cost implications. In addition, pathogenic or epidemic strains need to be further characterized on the basis of

their pathogenicity for therapeutic and epidemiologic purposes. This is often achieved by serotyping and antibiotic susceptibility/inhibition patterns, thereby increasing both cost and time required for diagnosis.

The need to quickly initiate appropriate treatment and/or control disease outbreaks underscores the importance of rapid, accurate and sensitive identification/characterization of pathogenic microbes in clinical laboratories [11]. Other than the cost implications, most clinical laboratories are able to achieve this routinely in most typical/common cases, using phenotypic techniques/procedures. However, with atypical strains, and in emerging and re-emerging disease conditions, samples are often sent to reference laboratories that perform highly specialized test [12], thereby considerably increasing time and cost of diagnosis and initiation of appropriate response. In many of such atypical cases, morphologic and biochemical tests are inadequate for a specific identification of the organisms, leading to misidentification and misdiagnosis [13]. Also, inappropriate or inadequate handling/storage of both specimen and test kits also often lead to misdiagnosis even for routine or typical strains and cases. In addition, many of the easily accessible tests are only able to identify bacteria up to specie level or serotypes, but are neither able to distinguish between virulent and avirulent strains nor detect strains with modified/irregular phenotypes from other closely related bacteria [2]. Furthermore, bacterial identification and classification based on phenotypic characteristics are also prone to technical errors by other factors that affect phenotypic expressions such as environmental/growth conditions (e.g. incubation temperature, pH), substrate availability and cell permeability, mutations, presence of plasmids, etc. Hence, modern molecular techniques have been developed to identify and classify microbes on the basis of their genetic and biomolecular relatedness [14]. This phylogenetic approach to microbial classification and identification has greatly enhanced the accurate and rapid identification of microbes [2], especially in the diagnosis and control of emerging and re-emerging diseases.

Various molecular techniques have been developed for the characterization and identification of microbes. These include nucleic acid amplification and detection methods such as PCR/RT-PCR, nucleic acid electrophoresis, hybridization, RNA microarray, restriction endonuclease analysis of whole-cell or plasmid DNA, gene sequencing, etc [15,16]; and protein detection techniques such as SDS PAGE/protein electrophoretic patterns, immunoblotting, etc [4]. These techniques are based on the identification of specific biological macromolecules (DNA, RNA and protein) peculiar to the organism for rapid and accurate identification of the organism. Specific genetic probes are now being made available commercially for microbial identification. These molecular techniques have the advantages of being sensitive and specific [13], as well as being easily applicable for atypical strains and organisms that are difficult to culture [17]. Sometimes, they can even be applied directly to clinical samples [15]. However, its major limiting factor remains the use of complex and costly equipments that often require delicate laboratory conditions.

Identification of bacteria and other microbes using molecular characteristics (genomics, RNA profiling, proteomics, plasmid characterization) is becoming increasingly popular, and in many cases mandatory for accurate identification [18]. This is due to their rapidity, sensitivity, specificity, ease of reproducibility and high-throughput capacity in clinical diagnosis and research. Further development of rapid molecular diagnostic kits that can be applied on fresh samples in field conditions would indeed help to harness the full potentials of molecular identification of microbes for rapid and accurate diagnosis and response, as well as improve research on emerging and re-emerging infectious diseases.

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