genomic segment 1 of influenza virus isolate A/PR/8/34 (H1N1), prevented disease caused by a second infection with a heterologous IBV. Protection against IBV was partially alleviated in mice that did not express a functional type I IFN receptor. Furthermore, a first infection with influenza A-based defective interfering virus also protected mice against a second infection with pneumonia virus, a genetically unrelated respiratory virus.

Conclusions. Recent viral infections of the respiratory tract might induce a refractory period during which the host is less likely to be infected by another respiratory virus. This viral interference requires closely spaced virus co-exposures, implying that both viruses share common ecologic conditions (e.g., cold weather). Factors that could predict an interference between respiratory viruses include the capacity of the interfering virus to induce a rapid IFN response; the degree of susceptibility of the second virus to immune mediators; the extent to which the different viruses counteract the induction and antiviral effects of IFN; and the differential innate immune response patterns triggered by each viruses in the upper and lower respiratory tracts.

BIOLOGICAL METHODS TO IDENTIFY MICROORGANISMS

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Introduction. Two factors determine the potential use of microorganisms in biotechnological processes, and also the pathogenicity of other strains are their genetic features and biochemical abilities. In the near future, industrial application as well as treatment of infection, will be possible after characterization, identification, and following taxonomic classification of the biological material. It is necessary to emphasize that taxonomy and systematics, very often used interchangeably, are in fact two different terms. Although systematics deals with the diversity of organisms, relationships, and possible interactions, taxonomy is a classification of organisms in a hierarchical structure of homogeneous groups that consist of descendants of the nearest common ancestor. Despite a high degree of phenotypic similarity, every assemblage of an individual shows some degree of phenotypic diversity due to genotypic variation. The greater the differences at the genetic level, the farther the related organisms are. Commonly known and used examples of hierarchical classification are the kingdom, division, class, family, genus, species, and finally, strain. Research works in the field of classification, systematics, and identification of microorganisms are interconnected and have an impact on each other. Accurate identification affects taxonomic classification of microorganisms as well as their systematics, and vice versa. Therefore, the broader the research aimed at the characterization of an individual microorganism, the more precise its identification, and thus the classification and systematics. Accordingly, the

"polyphasic" methodology is centered on morphological and biochemical data complemented with molecular techniques data. The combination of the classical approach together with 16S rRNA genes, molecular fingerprinting techniques, and/or other molecular markers is considered an extremely important foundation for the identification and classification of microbes.

The accurate identification of microbes is essential for scientists involved in many areas of applied research and industry which ranges from clinical microbiology to food production. There are many criteria for the division of the abundance of methods used in the area of the identification of microorganism, however, generally they can be assigned to direct and indirect techniques.

Materials and Methods. A literature search was conducted in Google Scholar, PubMed, Scopus and Web of Science databases.

Results and Discussion. It is not possible to focus on microorganism identification without a reference to taxonomy, as it is a common idea that "identification is a part of taxonomy". The word taxonomy comes from the Greek words taxis (arrangement) and nomos (law) and it is the science of the description, classification, and inventory of life. Taxonomy dates back to ancient Greece, when Aristotle proposed the first classification of living organisms, and modern taxonomy was created by Linnaeus, who introduced the binomial classification which is still used today and has been most responsible for the most recent taxonomy classifications.

In microbiology, traditional identification methods rely mainly on cultivation proceedings employing various media to enumerate, isolate, and identify specific microorganisms. For many years these methods were employed extensively and they continue to be used nowadays, especially in some laboratorial routines where a particular type of microorganism has to be identified rapidly (for example, in a medical diagnostic for the detection of a particular pathogen). Although being inexpensive and allowing both quantitative and qualitative information about the diversity of microorganisms present in a sample, however, these methods are laborious and time consuming (media preparation, dilution, plating, incubation, counting, isolation, and characterization), and results are only observed after several days, and frequently false positives are obtained especially when considering similar microbial species. Another problem associated with culture-based methods is the fact that they cannot identify non-culturable cells.

Phenotypic identification methods usually incorporate reactions to different chemicals. One of the traditional methods most used is a simple visual detection of growth of the tested organism in the presence of a substrate by increased turbidity. Results are determined by comparing the microbial under analysis with a control test, and a Wickerham card is used to read the turbidity. This type of reaction may be difficult to read and always involves a minimum of an overnight incubation. Immunoassays such as the enzyme-linked immunosorbent assay (ELISA), although

efficient, are expensive and are designed for only some bacterial species. A typical model is the analytical profile index (API), where standard methods are integrated into miniaturized reaction couples, scored as "positive" and "negative" and finally matched to a scoring system on the basis of "best fit", to create an analytical profile. For many years researchers have used API 20E testing apparatus, which consisted of a plastic piece with 20 cupules that contain pH-based substrates allowing the identification of almost 100 taxa. Until 1992, this method was considered the" gold standard" especially in clinical microbiology. A significant advantage of this method was the availability of an extensive database, although it had a major disadvantage associated with it being time-consuming. Other automatic methods started to appear in this decade including improvements of the API 20E system, in an attempt to reduce the time needed for the procedure using recurring automations. The BBL Crystal is also a variation of the API system. An automated version of the API is the Vitek system, first developed in the 1970s, which eliminated the subjectivity of the reading of test reactions. The Vitek system is useful for simultaneous bacterial identification and antimicrobial susceptibility testing (AST) profiles from isolated patient samples. The system uses a totally automated broth microdilution technique that applies attenuation of light measured by an optical scanner for growth or no growth detection (it is crucial that the samples in the cards are pure isolates). The device regularly monitors growth over a period of 18-24 h for bacteria and 36 h for yeast. Although it is versatile, there are some microorganisms that lead to correct MIC reports or yield unreliably (e.g., pseudomonas). A variation of the Vitek is the Biolog OmniLog System. It is a rapid, standardized, method for determining bacterial oxidation (tetrazolium redox dye) of different and simultaneous carbon sources (sugars, carboxylic acids, amino acids, and peptides, where 71 are carbon sources and 23 are chemical assays counting pH, salt tolerance, and chemical sensitivity tests). The results obtained are compared to a database (through an analysis of the obtained "yes-no" reactions). It is available for the phenotypic identification of bacteria and fungi (filamentous and yeasts).

With the advancement of biochemistry knowledge and the appearance of more robust instrumentation, these methods started to be used less and more modern biochemical methods were developed, with numerous advantages over conventional culture-based methods, such as short analysis times and the ability to simultaneously determine many microorganisms, while retaining accuracy of the results.

The advent of the "molecular biology age" has provided a plethora of tools and techniques for the detection, identification, characterization, and typing of bacteria for a range of clinical and research purposes. Previously, the identification and characterization of bacterial species was largely done by phenotypic and biochemical methods, which relied on preliminary isolation and culture. While these methods continue to hold place in certain settings, molecular-based techniques have provided unprecedented insights into bacterial identification and typing. To name a few examples,

genotypic methods have enabled the identification of a large diversity of previously unknown taxa, the characterization of uncultivable bacteria, and facilitated metagenomics studies on large and diverse bacterial communities. Both clinical and research setting have provided in depth insights into bacterial virulence, pathogenesis, antibiotic resistance, and epidemiological typing, as well as identification of novel, emerging, and re-emerging species. In addition, the widespread use and availability of molecular tools for bacterial genotyping has resulted in high throughput analysis, more sensitive and discriminatory results, and rapid turn-around-times, which are only likely to get better with automated tools and data analysis pipelines. Most molecular methods for bacterial identification are based on some variation of DNA analysis, either amplification or sequencing based. These methods range from relatively simple DNA amplification-based approaches (PCR, real-time PCR, RAPD-PCR) towards more complex methods based on restriction fragment analysis, targeted gene and whole-genome sequencing, and mass spectrometry. In addition to this, approaches based on unique protein signatures such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and similar variations have also been explored. While the advantages and limitations of these approaches vary, the choice of the technology employed depends on several factors including sample type (clinical or research, single-species or mixed-species), depth and accuracy of results generated, resources and cost factors, as well as the turn-around-times expected. Given that the present "molecular biology revolution" is resulting in a larger number of laboratories, including small-scale and resource-limited setups, having access to genomic approaches, it is imperative to understand the fundamental principles of these techniques, their applications, and their limitations.

Conclusions. The tools for the determining the identity of a microbial sample have been emerging in the last decades. Although having limitations, culture and microscopy are still two of the most utilized techniques. PCR and other genetic approaches are particularly important for nonculturable microorganisms and MS has been shown to be useful, quick, and easy for the identification of microbial samples and detection of microbial threats. However, it is reserved for pure isolates and cannot be used for complex samples, since they may promote interference in the background. This may be simplified through the use of chromatography-based methods.

In the future, development of the detection limits for microorganisms will continue to be a key assignment in clinical microbiology. The combination of these methodologies and instrumentation will surely improve the skills for the detection of pathogens.