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Anti bacterial properties test on bacteria: A review

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Abstract
In recent years there has been a growing interest in researching and developing new antimicrobial agents from various source to combat microbial resistance. Therefore, a greater attention has been paid to the antimicrobial activity screening and evaluating methods. Several bioassays such as disk diffusion, well diffusion are well known and commonly used, but other such as flow cytofluorometric and bioluminescent are not widely used because they require specified equipment□s and further evaluation for reproducibility and standardization, even if they can provide rapid results of the antimicrobial agents effects and a better understanding of There impacts on the viability and cell damage inflicted to the tested microorganism.in this review article, and exhaustive list of *in vitro* antimicrobial sustainability testing method and detailed information on their advantages and limitations are reported.

Keywords: thin-layer chromatography (TLC) bio autography, time kill test, antimicrobial gradient method, agar diffusion method, bioluminescent, cytofluorimetric

Introduction
Anti-microbial sustainability testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome.in this review, we focused on the use of antimicrobial testing methods for the *in vitro* investigation of extracts and pure drugs as potential antimicrobial agents.
After the revolution in the golden era when almost all groups of important antibiotics were discovered and the main problem of chemotherapy were solved in 1960s.the history repeats itself nowadays and these exciting compounds are in danger of losing thee efficiency because of the increase in the microbial resistance. Currently its impact is considerable with the treatment failures associated with multidrug-resistant bacteria and its has become a global concern to public health.
For this reason, discovery of new antibiotics is an exclusively important objective. Natural products are still one of the major new drug molecules today. Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. The fact that a plant extract exhibits antimicrobial activity is of interest, but this preliminary part of data should be trustworthy and allow researchers to compare result, avoiding work in which researcher use the antimicrobial activity investigation only as a complement to a phytochemical study.

Method
A variety of laboratory methods can be used to evaluated or screen the *in vitro* antimicrobial activity of an extract or a pure compound. The most known and basis methods are the disk-diffusion and broth or agar dilution methods. Other methods are used especially for antifungal testing, such as poisoned food technique. To further study the antimicrobial effect of an agent in depth, time kill test and flow cytofluorometric methods are recommended which provide information on the nature of the inhibitory effect and the cell damage inflicted to the test microorganism.

Owing to the new attraction to the properties of new antimicrobial products like antimicrobial products like combating multidrug-resistant bacteria, it is important to develop a better understanding of the current methods available for screening and quantifying the antimicrobial effect of its applications in human health, agriculture and environment.

Standardisation and Methodology
Agar Disk Diffusion Method
Agar disk diffusion testing developed in 1940 is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the clinical and laboratory standards institute(CLSI) for bacteria can be tested accurately by this method, the standardization has been made to test certain fastidious bacterial pathogen like streptococci, haemophiles, parainfluenza etc. agar plates are inoculated with a standardization inoculum of the test microorganism. Then filter paper discs containing the test compounds at a desired concentration, are placed on the agar surface.

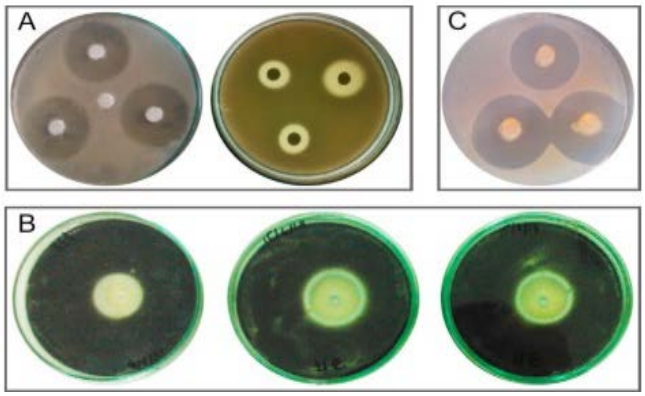

Fig 1

A. Disk diffusion method of microbial extract using.
B. Agar well diffusion method of essential oil using Aspergillus niger as test microorganism
C. Agar plug diffusion method of Bacillus sp. Against C. albicans.

Antimicrobial Gradient Method

The antimicrobial gradient method combines the principle of dilution methods with that of diffusion methods in order to determine the MIC value.it is based on the possibility of creating a concentration gradient of the antimicrobial agents tested in the agar medium. This method is used for the MIC determination of antibiotics, antifungals and antimycobacterial. MIC value is determined at the intersection of the strip and the growth inhibition ellipse.

Thin Layer Chromatography Bioautography

Good all and Levi combined paper chromatography method with contact bioautography to detect different penicillin for their determination. This technique combines TLC with both biological and chemical detection methods.

Dilution Methods

Dilution methods are the most appropriate ones for the determination of MIC values, since they offer the possibilities to estimate the concentration of the tested antimicrobial agents in the agar.

Time Kill Test

Time kill test is the most appropriate method for determining the bactericidal or fungicidal effect. It is a strong tool for obtaining information about the dynamic interaction between the antimicrobial agents and the microbial strains. The time kill test revels a time dependent or a concentration dependent antimicrobial effect.

ATP Bioluminescence Assay

ATP bioluminescence assay is based on the capacity to Measure adenosine triphosphate produced by bacteria or fungi. As ATP is the chemical form of energy of all living cells, it is present in more or less a constant amount in a cell. Bioluminescence assay has a large range of applications, such as cytotoxicity test *in situ* evaluation of the impact of biofilms *in situ* and drug screening on Leishmania

Flow Cytoflurometric Method

Several years ago, the usefulness of flow cytometry for susceptibility testing of microorganism was suggested. Thus, many authors investigated the antibacterial and antifungal activities of many drugs using this methodology. The rapid detection of damaged cells by t is approach depends on the use of appropriate dyes staining. Therefore, propidium iodine, a fluorescent and intercalating agent, is widely used as DNA stain.

Conclusion

Currently, microbial infections have become an important clinical threat, with significant associated morbidity and mortality resistance to the existing antimicrobial agents. Therefore, methods for antimicrobial susceptibility testing and discovering novel antimicrobial agents have been extensively used and continue to be developed.

Moreover, if we consider the use of solvents that may affect the growth of the microorganism tested, we can say that making minor methodological adaptations to standardization protocols can be a solution to ensure accurate experimental approach and allow other researchers to compare results.

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