Review on experimental parameters of antimicrobial susceptibilities

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Susceptibilities are studied in bacterium, viruses and animal cells. It often refers to whether a desired outcome is achieved for specific compounds. In antimicrobial susceptibilities, parallel views agree on a single method of analysis, yet, this leads to pertinent information loss. This article therefore provides new strategies for antimicrobial analysis and experimental design. The combination of these strategies, and approaches, are to be able to accurately report antimicrobial susceptibilities factoring issues concerning experiments and the bacterium. Bacteria vary in structure between species and among genera, and, thus, antimicrobial susceptibility problems crop up due to this. The same applies to viruses, with great exceptions for animal cells. Experimental parameters are the core discussion of this review, and while it provides a current and pertinent understanding, a laboratory analysis on susceptibility often mimics optimum growth conditions. Although this is true, in multicellular organisms a far differed response to chemicals for bacteria is possible.

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1. What is antimicrobial susceptibility?

Antimicrobial susceptibility is a concept that is known in microbiology. It requires two elements to be successfully studied. These elements are a chemical, or compound, and a microorganism such as bacteria. The availability of the chemical compound to a microorganism is the main component for susceptibility to be achieved. It is therefore obvious that susceptibility pertains to the microorganisms sensitivity to a chemical compound (e.g. drugs), and that the role of such compounds, in general, like ampicillin, ciprofloxacin, cycloserine, etc, are to attempt arresting their future development in hosts [11, 13]. Though antimicrobial susceptibility tests are used to deduce information on chemical compounds in bacterium species, it is also a reflection on bacterium genetics, especially in the proteins controlling bacterium growth. When a microorganism is susceptible to chemicals, then, antimicrobial susceptibility is considered successful [4, 10, 12, 13].

2. How is antimicrobial susceptibility tested?

Antimicrobial susceptibility is tested using unspecific and combined methods [13]. There are three methods used to test antimicrobial susceptibility. These are disc diffusion, the agar well method, as well as the minimum inhibitory concentration (MIC) [6, 8]. These methods are self descriptive, but vary in time taken to complete each test. In the first two methods, the use of agar plates is mandatory, while to perform an MIC test, 24 or 96 well plates are used [11, 12].

The disc and well techniques are similar but are opposite in respect to the location of the chemical. Disc (cut filter paper) are saturated with chemicals placed on inoculated agar plates. Post incubation, the length of the inhibition zone indicates the effect of a chemical. The converse is that agar wells on plates are seeded with chemicals, and the length of inhibition zones of the inoculated plates after an incubation period is taken [13]. In all methods, the incubation used is microorganism specific, and for bacterium causing pathogenesis in humans it is body temperature (37^{o} C) [11]. In a MIC test, microorganisms are treated with chemicals and the effect of them is deduced by measuring the bacterium growth after 2 -4 weeks [8, 11]. This measurement is computational and the inoculum and chemical concentration dependent. The advantage of the MIC technique is the use of the same amount of bacterium cultures according to the requirements provided by MacFarland standards. This standard is also used when performing antimicrobial susceptibility tests on other bacterium species [11].

3. The barriers in antimicrobial susceptibility testing

There are bacterium physical barriers, as well as, technical barriers in antimicrobial susceptibility testing [11, 13]. The bacterium capsule is a physical structural trait that is the first entry point for chemicals into them [9, 14]. The polysaccharides (multiple simple sugar units) that comprise the capsule make the entry of chemicals sometimes difficult. The implication of this is that a susceptible result could be put down as resistant, and vice versa. This applied to bacterium inhibiting chemicals. For bacterium growth stimulants, whether they enter the bacterium culture or don't, if the growth conditions allow fission, they would multiply independently of the treatment [11]. It is still, and always, important to account for bacterium species that are resistant to chemicals [1, 3, 5, 15]. This takes into consideration resistance in antimicrobial susceptibility testing, and in this way, susceptibility wouldn't be recorded as resistant [13].

Pili and other surface structures on bacterium species (like Clostridium and TB genera) allow for movement of bacteria in media [13]. It is possible that the diversity, distribution and spacity of them is linked to the susceptibility profile of some bacteria. This arises because susceptibility testing is a human activity even though bacterium species have evolved to possess uniquely distinct characteristics. Bacterium proteins involved in cell wall degradation also affects antimicrobial susceptibility testing. As an obvious consequence, a chemical compound specific for stopping the growth of a bacterium wouldn't exert its effect with a wide, a direct, synergistic effect if fission-involving proteins are damaged [13]. Reasons are that if a fission-involving protein is damaged the first and second enzyme kinetics fail to reach equilibrium of products and reactants [12]. This relates to the chemical substances needed to produce bacterium fission proteins, such as mycobacterium proteins [11]. Depending highly on whether a chemical compound starts the induced fit or lock and key mechanism, it would succeed in action if the fission proteins are in its optimum inherent and organised form [13].

There are many technique aspects that must be considered in antimicrobial susceptibility testing [11]. A technique barrier in performing susceptibility tests is being able to inoculate inoculums with uniformity such that all wells, as well as, the agar gets an equal spread of bacterium culture simultaneously [2, 8, 10, 11, 13]. Although minor, the unparalleled time spread affects chemical entry to an extent, such that the mechanism for bacterium death begins at different times on treatment [11]. Other obscurities in antimicrobial susceptibility results arise due to saturated discs drying out before placing them on inoculated plates, the use of improperly dilutes chemicals in a series, the repeated use of frozen and dried out compounds, irregular-shaped wells with compounds on inoculated plates, and over vortexing of bacteria cultures while standardising them to the MacFarland standard [11, 12]. The latter is mentioned because overvortexing cultures are problematic in antimicrobial susceptibility tests for two reasons. The first is that vortexed cultures can be damaged prior to susceptibility tests, and the second being that compound resistance could be detected as being susceptible [11]. This implies that it is possible for false resistant and false susceptible bacterium species to be detected in antimicrobial susceptibility testing. Also certain bacterium colonies / inocula may naturally lose their viability because of vortexing.

4. A new strategy for analysing antimicrobial susceptibility profiling and experimental design

There is one correct way of analysing and making experimental designs for antimicrobial susceptibility [13]. This allows for accurate analysis of chemicals to be attained, but concurrently a substantial amount of pertinent information is lost. Although universal, the strategical approaches factor technical barriers, but little can be provided by the microorganism because the approaches resolve them at the microscopical level. There is however a new strategy of providing missing explanations in antimicrobial susceptibility testing. That strategy involves analysing susceptibility patterns relative to one and other, and controls, within data sets [11]. Often this strategy is different and possible, but, it requires that data sets are aligned in respect to defined criteria. In a few papers [11, 12], this strategy has been used. This strategy involves making use of a stepwise approach to deduce findings on antimicrobial susceptibility patterns.

First in this strategy the susceptibility readings of individual inoculums for bacteria species are compared between times and days. Any similarities between day readings are noted. Like in MIC tests, in this way any compound resistance is deduced, if, for instance, the MIC on two consecutive week readings are same, i.e., no shift is present in spite of a higher dose of compound treatment. The other part to this strategy is that inoculums absent of susceptibility is identifiable. This absence is true when growth is found in plates, or wells, before and after a reading day or time. This new strategy thus allows for comments on minor technique errors, like inoculum counts and pipetting [11]. In the agreeable strategies of analysis antimicrobial susceptibilities, this deduction can be made, but isn't often.

There are many approaches to antimicrobial profiling and experimental design. Antimicrobial profiling approaches, often, involve characterising compounds based on their action in bacteria. It could involve arranging microorganisms on the basis of the number of compounds they are affected by or the spectrum of compounds a single microbe may be affected by. An alternative approach for antimicrobial profiling is to arrange microbes according to a trend that has been observed in data sets. This allows for the experiment design to account for statistical tests [7], as well as, for a broad spectrum of comments in antimicrobial susceptibility profiling to be achieved. The experimental approach must therefore be simple, same as previous, but allow for more comparatives, like non-susceptibles, atypical inoculums and compounds. The discrepancies between data sets could prove positive in this manner. This would be the case possibly because the experimental inoculums are random, and atypical inoculums allow for irregularities to be factored [11].

Experimental designs for antimicrobial susceptibilities must have microbes arranged in order of responses to single compounds so that comparisons on susceptibilities can be made. The new strategy, together with this approach for antimicrobial profiling and experimental design is innovative and is definitely the key to being able to understand antimicrobial susceptibility in a variety of bacterium species.

5. Why is antimicrobial susceptibility testing significant in today's world?

Antimicrobial susceptibility is a widely researched area in microbiology, overlapping into fields like pharmacology, biochemistry and medicine is also known. This evidences its importance in today's world, i.e., to attempt to eradicate diseases, or the aggressive spread or virulence, and pathogenesis caused to *Homo* species by bacteria genera. It is a prerequirement to search novel compounds on panels of bacterium strains to check their bioactivity, and thus, their bioavailability. It is therefore significant, since it allows for commercially important chemicals to be marketed long- or short-term (trial phase studies) in combating bacterial diseases. In this way bacterium species affected by a compound, or mix of chemicals, can be noted as having a particular biological reaction with that substance/s for future studies.

Antimicrobial susceptibility testing in today's world is thus very significant in order to reduce the labour intense efforts by researchers to detect new compounds by making bacterium genera affected by compounds, as well as, by allowing new information about antimicrobials, potential antimicrobials and bacteria, to be accessible.

6. Perspectives and conclusions

Antimicrobial susceptibility is an area studied in microbiology for years. The advantages and downfalls of antimicrobial susceptibility are varied and, thus, difficult to pinpoint. There are, however, many perceptions on what a good antimicrobial susceptibility test is. For instance in many cases only one method of analysing antimicrobial susceptibility is performed. This means that the preparation of scientific knowledge on susceptibilities is consistent. The disadvantage is that, as a result of a single method of understanding susceptibilities being used, a more stringent approach has becoming appreciated. This is indicative that solutions to this problem are difficult to derive without conforming to traditional and conventional approaches. In terms of statistical parameters, a basic approach is often used since susceptible often, are reported as merely single across days and times. Although this is the situation, outcomes of such tests are reliable. The complications to antimicrobial susceptibilities therefore definitely are associated with viable, damaged and dead microorganisms [11]. An example often cited, in general, are viruses pertaining to sexually transmitted diseases. The situation with those viruses is that the replication proteins catalyse fission rapidly. This attribution to being unable to find suitable, and non-harmful, cures for the HIV/AIDS endemic, is definitely problematic in lab tests deriving possible chemical compounds against STIs. In this case, profound efforts are in place to treat bacterium species, and genera, with compounds directed to mutate replication proteins, a hugely laborious task. Often these chemicals are compounds known to cause genetic damage in bacteria. The major problem is, however, to tackle the physical barriers of bacterium species.

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