Review Article

General considerations on antimicrobial tests and conditions applicable in textile industry

Abstract

Due to the globally increasing need for utilizing antimicrobial materials, it is necessary to improve and develop newer methods and techniques for determining the antimicrobial properties of these materials qualitatively and quantitatively, especially in the medical field. Recently there has been a debate among scientists between the difference between antimicrobial test and sterility test. For antimicrobial and microbial resistance, the test is limited to the standard methods to determine the effect of the sample as an antimicrobial without paying attention to the fact that the sample is sterile or not, unless the sample was taken into sterile atmosphere and isolated with a protective suitable sterile package cover after treatment with the antimicrobial agent because the external atmosphere contains a lot of scattered types of bacterial and fungal microorganisms. In case of sterility test, the sample to be examined should be prepared, isolated and coated from the outside atmosphere, where the presence of microbes on the surface of the sample by standard methods is detected and in some sterilization tests it is prohibited to include an antimicrobial substance to the sample to be tested to avoid the interference with the test. In both cases the tests should be implemented in a sterilized room and conditions according to the recognized scientific principles. In conclusions, the antimicrobial test is used to make sure that the specimen is attained antimicrobial properties or not and the sterilization test is done by ensuring that the sample is free of contaminated microorganisms. This review poses on some factor and conditions affecting antimicrobial action and some standard test methods for determination of antimicrobial and sterility potential of materials.

Keywords: antimicrobial activity, sterility tests, mode of actions, precautions, conditions

Introduction

The performance of antimicrobial susceptibility testing is of great importance in clinical microbiology laboratories to determine the effect of drugs and antimicrobial agents against different pathogenic microorganisms and also to assure the degree of microbial susceptibility for particular infections. In this connection the antibacterial materials such cloths and fabrics are became important to avoid the deleterious effect of pathogenic microorganisms [1]. Various types of antibacterial compounds have been used for all types of fabrics and textiles to reduce and prevent infections especially in textiles of hygienic and medical use [2,3].

These methods include the disc agar diffusion method (CLSI-M44A) in which the zone of inhibition is estimated in millimeters at each center and Broth dilution tests in which two-fold dilutions of antimicrobial agents or antibiotics (eg, 1, 2, 4, 8, and 16 μg/mL) in a liquid growth medium dispensed in test tubes [4,5]. The tubes were inoculated, incubated at 37°C and then examined for visible bacterial growth as evidenced by turbidity and broth micro-dilution method (CLSI-M38A) in which the determination of minimum inhibitory concentration (MIC) or minimum effective concentration (MEC) is determined according to the categories:- susceptible (S), intermediate (I), or resistant (R). The tests for examination of antimicrobial effect and sterility are classified according to the following needs:-

- **Sterility of materials' surfaces.**
- **Type of material under investigation.**
- **Efficacy of antimicrobial treatment.**
- **Determination of MIC of antibiotics or antimicrobial agents.**
- **Ensuring fixing on or leaching of antimicrobial agents from treated materials.**
- **Investigation of the effect of washing cycles on antimicrobial textiles.**
- **Selection of the proper and potent antibiotics against infectious microorganisms that reside in human specimens.**

Factor affecting antimicrobial action

1. Type of growing microorganism: In general microorganisms are usually present in many forms. It was found that growing vegetative

forms are more susceptible than spore forms with varying degrees. Bacterial spores are more resistant than yeast and mold spores.

2. Concentration of microorganisms: Increasing number of microorganisms require more antimicrobial agent for killing.

3. Aggregation of microorganisms: Clumping of microbes such as in case of biofilms affect negatively the action of antimicrobial agent due to the failure of the agent to penetrate the depth of the biofilm. Biofim's cells are not very susceptible to many antimicrobial agents.

4. Physical characteristics of the antimicrobial agent used for destruction: Heat and its moist nature are more effective in more killing than dry heat.

5. Concentration of antimicrobial agent: The antimicrobial agent may be bactericidal at one concentration and bacteristatic at a lower concentration. Increasing the concentration of antimicrobial agents effectively reduces microbial contamination.

6. Chemical nature of antimicrobial agent: The chemical nature of antimicrobial agent is of importance in imparting antimicrobial properties. For example, sodium chloride is of no value as a disinfectant in this form; however the bleaching solution NaOCl is extremely effective. Surface tension of disinfectant solutions (surfactants) is another factor affects antimicrobial action. Low surface tension is preferable to increase the contact between antimicrobial agents and microbes.

7. Temperature: The efficiency of antimicrobial agents (disinfectants) can be increased by increasing temperature, as warm solution is more effective than a cold one. Warming the disinfectants lowers the surface tensions and consequently the heat increases the chemical reaction between agent and microbe.

8. Nature of organic material: Antimicrobial agents that coagulate protein should be avoided and replaced by disinfectants which have less coagulative properties or another suitable sterile technique, eg. blood, serum foodstuffs ...etc.

9. Combination of antimicrobial agents with extraneous organic material: This combination resulted to decrease the efficiency of disinfecting agent by neutralizing its effect by the formed material.

10. Time of application: The longer time an agent is applied the more killing of microorganisms. The duration time recommended were determined according to the nature of the material and/or to the decision to sterilize or kills spores.

12. pH value of antimicrobial solution: Acidity and alkalinity affect the efficiency of antimicrobial agent. Acidic bacteriocides (anionic) such as, phenol, organic acids,…, etc. are more effective at low pH values, and if heated tends to increase the dissociation of acids.

Mode of action of antimicrobial agents

Antimicrobial agents act in different ways, the mechanism through which antimicrobial agents depends mainly on the chemical nature of the antimicrobial agents, the degree of affinity to certain targets sites within microbial cells and the function that is affected by the agents [6] .Resistance of microorganisms towards antimicrobial agents described by two ways, the first category includes microorganisms that do not posses target sites for the agent and consequently the antimicrobial agent does not affect them (Intrinsic resistance) and the second category includes the susceptible microorganisms that do not be affected by the antimicrobial agent (Acquired resistance). The acquired microorganisms induce their effect either by reducing the uptake to the antimicrobial agent, presence of an enzyme that inactivates the antimicrobial agents, post-transcriptional or post-translation modification of the antimicrobial agent's target or active efflux of the antimicrobial agent. The inhibition mechanism of action is summarized in the following points:

- **Inhibition of the cell wall synthesis (Penicillin) [7].**
- **Inhibition of cell membrane and cytoplasm functions (Quaternary ammonium salts, phenol, soaps) [8].**
- **Inhibition of nucleic acid synthesis, ribosomal function and DNA replication (Fluoroquinolones) [9].**

- **Inactivation of antimicrobial agents by enzymes (cyanide, fluoride, halogens) [10].**
- **Inhibition of folate metabolism (Sulfonamides and trimethoprim) [11].**

Precautions required in implementing antimicrobial tests

1. **Avoid contamination and use safe and proper techniques in handling, plating and transferring of test materials.**
2. **Some qualitative and quantitative tests should be performed by trained personnel.**
3. **Some infectious microorganisms are allergic and pathogenic and capable of infecting humans in the laboratory and/or associated environment, therefore necessary precaution must be taken into consideration.**
4. **Wear protective clothing, sterile gloves, wear safety glasses and respiratory protection should be provided to the personnel working with microorganisms to prevent penetration of the spores.**
5. **All contaminated samples should be sterilized prior to disposal.**
6. **An eyewash/ safety shower should be supplemented for emergency use.**
7. **Avoid exposure to antimicrobial hazardous chemicals and control them below the recommended levels.**
8. **Accurate testing requires the selection of a pure, uncontaminated, non-mutant test culture of microorganism.**
9. **Some hydrophobic fabrics tests need the addition of surfactant to enhance wetting of these materials.**
10. **Glassware and particularly pipettes must be clean. Avoid a hang-up of droplets in sterile pipettes that interfere with the correct actual volume delivery.**
11. **For curl specimens, place sterile glass slides on the ends of the specimens to hold it in place to facilitate good contact with the inoculated surface.**

12. **Check culture purity by periodically making streak plates and making sure for a single species-characteristic type of colonies.**
13. **The antimicrobial or antibiotic discs package should be equilibrated to room temperature before opening by removing from the refrigerator or freezer two hours before.**
14. **Sterile plates should be used within seven days after preparation unless adequate precautions have been taken to decrease drying of the agar in these plates.**

Tests for evaluation of antimicrobial activity

The following techniques are some examples of the used tests for evaluation the antimicrobial activity of materials:-

Disc and well diffusion tests

The disc diffusion test is very simple and practical and is performed by applying a bacterial inoculum (about 10^8 CFU/ml) to the surface of Mueller-Hinton agar plate (150 mm diameter). Paper antimicrobial or antibiotic discs of known concentration are fixed on the inoculated agar surface (Figure 1). Plates are incubated for 24 hours at 37°C. For results determination, the zones of growth inhibition around each disc are measured in millimeter. The zone diameter is related to the susceptibility of the isolate and to the diffusion rate of the antimicrobial agent through the agar medium. The interpretation of the results was made using the Clinical and Laboratory Standards Institute (CLSI) or Food and Drug Administration (FDA), according to the categories:- susceptible (S), intermediate (I), or resistant (R). The "susceptible" (S) category indicates that the isolates are inhibited by the recommended concentrations of antimicrobial agent resulting in clinical efficacy, and the "intermediate" (I) category includes isolates with antimicrobial agent with minimum inhibitory concentrations (MICs) approach attainable blood and tissue levels and their response rates may be lower than for susceptible isolates and the "resistant" (R) category indicates that the isolates are not inhibited by the usually used antimicrobial concentrations. The Disc diffusion method is the least costly of all susceptibility methods and it is characterized by its simplicity and has been standardized for testing many infectious

bacteria including Streptococci through use of specialized media and incubation conditions. The amount of antimicrobial agents or antibiotics applied to discs in ug/ml is inversely proportional to the zone of inhibition diameter width, for some antimicrobial agents, the disc diffusion zone diameters do not correlate with minimum inhibitory concentration (MIC) values [12-16]. In the well diffusion test, bacterial inoculum culture to be tested was spread on nutrient agar plates with the aid of sterile swab and 10mm diameter were punched into the agar medium under aseptic condition and filled with about 100µl of the required concentration of extracts or antimicrobial agent and allowed to diffuse at room temperature for about one hour. The plates were then incubated at 37°C for 24 hours. Positive and negative controls should be applied at the same conditions. Zone diameter of inhibition was then measured in mm (Figure 2).

Fig.1: Disc diffusion test (antimicrobial zones of inhibition around treated discs).

Fig.2: Well diffusion test (antimicrobial agent and antibiotics show clear inhibition zones around wells).

Parallel streak method

The parallel streak method (AATCC 147-2004) is a relatively easily and quick qualitative executed method to determine

bacteriostatic antibacterial diffusible activity of treated antimicrobial textile materials. This method provides an evidence of antibacterial activity against Gram positive and Gram negative bacteria. It is useful also for obtaining a rough estimate of activity in that the growth of the inoculum microorganism decreases from one end of each streak to the other and from one streak to the next. This technique permits the estimation of antibacterial activity of textile materials after multiple washing cycles. Specimens of the test material along with the corresponding untreated control of the same materials are placed in contact with nutrient agar which has been previously streaked with inoculums of a test bacterial strain. After incubation for 24 hours at 37°C , the clear area appeared underneath and along the sides of the test specimen under evaluation indicates that the antibacterial character of the specimen (Figure 3). In this test *Staphylococcus aureus* ATTC 6538 and *Klebsiella pneumonia* 4352 are recommended as Gram positive and Gram negative bacteria respectively, other bacterial species can be used depending on the intended end-use of the test sample. The procedure is summarized in the preparation of agar plates containing nutrient agar, after cooling five streaks of diluted inoculum approximately 60 mm in length spaced 10 mm apart covering the central area of a Petri-dish were applied, then gently press test and control specimen transversely across the five inoculums streaks. Incubate at 37 ± 2°C. The clear zones of inhibition beneath the specimen and beyond its edge, indicates that the specimen has antibacterial properties against the tested bacterium. The average width of clear zone of inhibition (W) can be calculated as follows:-

W= T-D / 2 where T= total diameter of test specimen and clear zone in mm; D= diameter of the test specimen in mm [17-22].

(b) **(a)**

Untreated fabric sample **Treated fabric sample**

Fig 3: Parallel streak method (AATCC 147); (a) No indication about antimicrobial activity of untreated fabric sample (Blank); (b) Treated fabric sample shows clear zones indicating the presence of antimicrobial activity.

Shake flask method

This method (ASTM E2149 -2001) is used for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions to overcome difficulties in using classical test methods to evaluate substrate-bound antimicrobials (i.e. non-leaching antimicrobials). The activity of a substrate-bound antimicrobial is dependent upon direct contact of microbes with the active chemical agent. This method is performed by incubating the treated antimicrobial specimen in a diluted bacterial suspension under shake condition for one hour. The antimicrobial activity in the suspension is evaluated by calculating the percent reduction in the numbers of viable bacterial microorganisms (Colony Forming Units; CFU) as compared by appropriate untreated controls [23,24]. For example, the two flasks containing the same volume of the specified diluted sterilized medium (Figure 4) are supplemented with fabrics (a1: 40x40 mm untreated fabric sample; b1: 40x40 mm treated fabric sample with antimicrobial agent) and inoculated under aseptic condition with 1 ml (10^5 CFU/ ml) of a bacterium to be tested. The two flasks are then incubated under shaking in an incubator shaker (100 rpm) at 37^{o}C for 1 hour. After incubation, the absorbance of the broth of each flask is determined against the previously mentioned medium without inoculation (Blank) using a spectrophotometer at 660 nm. The percentage of Colony Forming Units (CFU) reduction is calculated according to the following equations:-

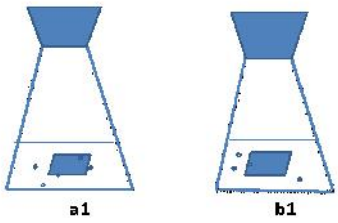
Total % CFU in flask b2 = OD_{b2}/ OD_{a2} x 100 …… (1)

%CFU Reduction in flask b2= 100 – Total % CFU in flask b2 ……..(2)

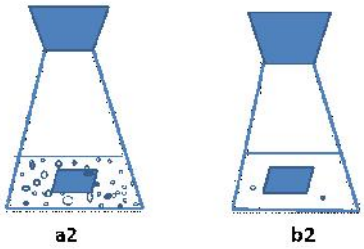
Flask b_1= containing the treated sample after incubation.

Flask a_1 = containing the untreated sample after incubation.

CFU= Colony Forming Units.



Before incubation under shaking condition



After incubation under shaking condition

Figure 4: Shake flask method: a1) Untreated sample. b1) Treated sample with antimicrobial agent(s) before incubation; a2) Untreated sample. b2) Treated sample with antimicrobial agents after incubation.

Conclusion

The antimicrobial susceptibility testing methods provide reliable results when used according to the previously mentioned standard procedures. As prevalence patterns of resistant microorganisms vary widely and change continually, regional and local resistance must be taken into consideration in the selection of suitable and appropriate antimicrobial agent to combat specific pathogens and to avoid misuse of antibiotics [25-28]. Recently, resistance to many classes of

antimicrobial agents and antibiotics is increasing, which indicates the fact that previous practices during the past period have not been sufficient to prevent the development and spread of resistant pathogens. Since antimicrobial resistance has become a public health concern throughout the world, there is an increasing need for development of new methods and automated instruments that could provide antimicrobial results in a short time and decrease the cost of analysis by reducing the labor requirements and reagent costs.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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