Development of in-vitro Sensitivity Testing for Pathogenic Bacteria

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Abstract
A new method developed for in-vitro susceptibility test in medical laboratories consist of micro tubes or gloves containing dehydrated tryptic soya broth, 5% glucose, 0.1% bromothymol blue and one type of antibiotics (ampicillin, tetracycline and chloramphenicol) with critical concentration MIC (minimum inhibitory concentration) for susceptibility. Standard quality control strains of bacterial (Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa) suspension were adjusted to 0.5 McFarland turbidity standard (1 × 10⁶ cell/mL) were used in inoculation the media and incubated two hours at 37 °C. The MIC of ampicillin against E. coli, S. aureus, and P. aeruginosa were 4, 32, and 256 µg/mL of the media for the bacteria respectively, while the MIC of tetracycline against bacteria were 512, 512 and 32 µg/mL respectively, the MIC of chloramphenicol were 512, 32 and 512 µg/mL, respectively. Where, the resistant bacteria to the antibiotics could grow and ferment glucose sugar producing a color change of the media from blue to yellow, while the sensitive bacteria do not grow or show no change in color. Our study result compared with common used antibiotic disk method obtaining similar results. This developed method characterized by fast (only two hours) and less cost in comparison to conventional technique. The new micro tube strip is highly stable (more than one year) with more sensitive in detection of variable pathogenic bacteria including standard bacteria strains compared with conventional technique.

Introduction
The clinical symptom of an infectious disease reflects the interaction of the pathogenic microorganism with the host. This interaction is affected by microbial virulence factors and the host immune status. The symptoms and signs are difference according to the site and severity of infection [1]. The diagnosis requires a composite of information including history, physical examination, radiographic findings, and laboratory data [2]. The determination of microbial susceptibility to antimicrobials is very important responsibility of the microbiology laboratory after microbial detection and isolation [3, 4]. The term susceptible means that the microorganism is inhibited by a concentration of antimicrobial agent that can be present in blood with the normally depended dose of the antimicrobial reagent and suggested that an infection occurred by this microorganism may be appropriately controlled with the antimicrobial agent. Microbial resistant indicates that the microorganism is resistant to concentrations of the antimicrobial agent that can be obtain with normal doses and implies that an microbial infection could not be successfully treated with this antimicrobial agent [5, 6]. Many bacteria have unpredictable susceptibilities to antimicrobial agents and their susceptibilities can be measured in vitro to help the choice of the most
appropriate antimicrobial agent. The widely used susceptibility testing methods are the disk diffusion and broth agar dilution tests. The MIC (minimum inhibitory concentration) of a particular drug to a organism can be quantitatively determined in-vitro through the broth agar or dilution test. These testing methods have been standardized and the NCCLS (National Committee of Clinical Laboratory Standards) provides susceptibility test guidelines [6-8]. In this study we tried to improve new technique for susceptibility test of pathogenic bacteria to the antibiotics.

**Materials and Methods**

**Measurement of MIC (Minimum Inhibitory Concentration)**

Stock solution: Ampicillin (Aldrich/ Sigma) (50 mg/mL), Tetracycline (Aldrich/ Sigma) (5 mg/mL), Chloramphenicol (Aldrich/ Sigma) (34 mg/mL).

**Agar Dilution**

The anti-bacterial agents was measured by using broad spectrum antibiotics (ampicillin, tetracycline and chloramphenicol) of different concentrations (0.5, 2, 4, 8, 16, 32, 64, 128, 256 and 512 µg/mL) and inoculated with standard tested organisms (E. coli, S. aureus and P. aeruginosa) which were prepared by mixing part of the growth from each of 5 similar colonies in saline and incubated at 37 °C for 2 hours.

Turbidity of the suspension was adjusted to 0.5 McFarland standards (BioMerieux, France) spectrophotometric ally at 600 nm wavelength (1 x 106 cell/mL) [9-11].

**Preparation of Susceptibility Strip Test**

The test strip consists of micro tubes containing dehydrated (lyophilized) media tryptic soya broth, glucose (5%) and bromothymol blue (0.1%) and broad-spectrum antibiotics in critical concentration tested for all antibiotics were equivalent to the MIC break point for susceptibility in sterile condition. The pH was adjusted to 7.4 (Alkaline, blue color).

**Application of Strip Test**

Bacterial suspension of particular microorganisms (E. coli, S. aureus and P. aeruginosa) were inoculated in tubes containing various antimicrobial agents. After incubation for 2 hours at 37 °C, results were reported (as a change in color of the media) by naked eye.

**Conventional Antibiotic discs Method**

Inoculums of the test organism were prepared as before. Sterile cotton swabs were depended in the test and control organisms separately. These swabs were used in inoculation of the specified areas of the Petri-dishes with test and control organisms.

Later flamed forceps used to apply Antibiotic discs with light pressure on the agar surface after the inoculums had dried. Finally, the Petri-dishes were incubated for 18-24 hours at 37 °C and the results were reported (radial width of the zones outside the antibiotic discs) by naked eye [11].

**Results and Discussion**

Table 1 show different concentrations of ampicillin tested against standard bacterial suspension of E. coli, S. aureus and P. aeruginosa were inoculated in the media. Tested critical concentrations in sterile condition for ampicillin were equivalent to MIC break point which were 4, 32, 256 µg/mL for tested bacteria, respectively. While, the average number of bacteria were 15.6 × 10⁶, 12.9 × 10⁶ and 2.1 × 10⁹ bacteria/mL, respectively. The E. coli was more sensitive to ampicillin followed by S. aureus and then P. aeruginosa.

Table 2 shows the results of testing the sensitivity of standard pathological bacteria (E. coli, S. aureus and P. aeruginosa) for tetracycline using different concentrations of the antibiotics. Where it was noted that the focus MIC to the bacteria were 512, 512, and 32 µg/mL, respectively, and the average number of bacteria were 16.2 × 10⁶, 16.2 × 10⁶, and 5.4 × 10⁶ bacteria per mL, respectively.

P. aeruginosa was seen to be more sensitive to tetracycline, whereas the bacteria S. aureus and E. coli had the same degree of sensitivity.
Whereas, Table 3 showed sensitivity test of different concentrations of chloramphenicol against the standard pathogenic strains (E. coli, S. aureus and P. aeruginosa). The MIC of the bacteria were 512, 32, and 512 µg/mL, respectively. The average number of bacteria were $4.2 \times 10^6$, $21.9 \times 10^6$ and $4.8 \times 10^6$ bacteria per mL, respectively. It was note that S. aureus was more sensitive to chloramphenicol, whereas the bacteria E. coli and Pseudomonas aeruginosa had the same degree of sensitivity. In addition to the MIC value, the MBC (minimum bactericidal concentration) value of antibiotics was estimated for all pathogenic strains as it is shown in Table 4. Though the values of the MBC of ampicillin were 32, 64, and 512 µg/mL for E. coli, S. aureus and P. aeruginosa, respectively, but MBC of tetracycline were 128, 16 and 16 µg/mL for standard bacteria, respectively. The MBC of chloramphenicol were 4, 512, and 128 µg/mL, respectively for tested bacteria.

In this study glucose sugar selected in the preparation of the new test strip because most types of bacteria containing the enzyme fermented glucose sugar. Bromothymol blue dye was the most suitable dye used to indicate fermentation process and can note the color change clearly with the naked eyes as it is in Fig. 1. The process of freeze drying had important role in maintaining culture media in the pockets of test strips and control small quantities used in addition to the ability to keep for a long time at low temperatures (4-6 °C).
We concluded from the results of the study provide a great opportunity to work in diagnostic laboratories, wherever they are located inside or outside the provinces. The implementation of potential sensitivity

![Micro tubes strip before inoculated bacterial growth](image1)

Fig. 1 Antibiotic susceptibility test performing micro tubes strip before (a) and after (b) inoculated bacterial growth.

Blue color: sensitive; Yellow: resistance bacteria.

testing and providing of best services, as well as reducing the cost and materials, all these required for economy support to achieve sensitivity test for bacterial types. Recognizable to tape record also reduced the period required to achieve the desired goal of 24 hours to 2 hours and this reduces the effort and increases the speed of the delivery of the required treatment to the patient. Finally, we recommend the Ministry of Health for the adoption of the way to ensure the test required in all diagnostic laboratories.

References
تطوير طريقة جديدة لفحص حساسية البكتريا المرضية لبعض للمضادات الحياتية

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الخلاصة
طُرّط طريقة جديدة لفحص حساسية البكتريا المرضية للمضادات الحيانية في المختبرات الطبية وذلك باستخدام شريط الفحص (المكون من أسيب أو جريد صغير لاصقة بالشريط) تحتوي في داخلها على الوسط الزرعي المجفف المكون من مرق صوصاً Ampicillin, Tetracycline, Chloramphenicol, و بتراكز نموذجي (التركيز الأدنى المطلوب للنمو MIC). استخدمت عالق العزلات البكتيرية القياسية (Pseudomonas aeruginosa و Staphylococcus aureus, Escherichia coli) في الاختبار وتلفيق الوسط، بعد أن ضبطت عقدة النقل مع عقدة حغو ثابت العقدة القياسى مكملدنالد 0.5 × 10^3 وحدة تكوين المستقبلات / لـل الواحد (CFU/ML) وحضنت لفترة ساعتين في 37 °C.

للحساسية القصوى، حددت التركيز الأدنى (MIC) لكل من البكتريا المرضية (Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa) والمضادات الحياتية Ampicillin, Tetracycline, Chloramphenicol، مما لوحظ أن العزلات البكتيرية المقاومة للمضادات الحياتية تمكنت من النمو وتخليص سكر الكوكس والذي سبق في تغيير لون الوسط من اللون الأزرق إلى الأصفر نتيجة تغير pH، بينما البكتريا الحساسة لم تنمو، إذا لم تحدث تغيير في اللون. فورت نتائج البحت مع طريقة أقراس المضادات الشائعه استخدامها وكانت النتيجة مطابقة.

تميزت هذه الطريقة الجديدة كونها سريعة (ساعتين فقط) وذات كلفة قليلة جدا مقارنة بالتقنيات التقليدية. كما يميز شريط الفحص المكون بالأسئل العامية (أكثر من سنه واحد) وحسابتهن في التشخيص العديد من البكتريا المرضية ضمن العزلات البكتيرية القصوى مقارنة بالطرق التقليدية.

الكلمات المفتاحية: الحساسية، MIC, المضادات، تقنيات المختبرات الطبية