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ORIGINAL ARTICLE

A comparison of antibiotic disks from different sources on Quicolor and Mueller-Hinton agar media in evaluation of antibacterial susceptibility testing

Neda Saffari[1], Siavosh Salmanzadeh-Ahrabi[2*], Ahya Abdi-Ali[2], Mohsen Rezaei-Hemami[3]

[1]Department of Biotechnology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran
[2]Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran
[3]Institute of Health & Wellbeing, Health Economics & Health Technology Assessment, University of Glasgow, Glasgow, Scotland

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ABSTRACT

Background and Objectives: Antibacterial susceptibility testing of clinical bacterial isolates through disk diffusion method plays a major role in antibacterial treatment. One of the main factors affecting the result of these tests is the type, structure and quality of the disks. The main objective of this study was to compare the agreement of antibiotic disks originated from three companies on Quicolor and Mueller-Hinton agar.
Materials and Methods: Quicolor and Mueller-Hinton agar media were used in disk diffusion method. Seventy clinical isolates from *Enterobacteriaceae* family (21 *Klebsiella spp.,* 36 *Escherichia coli,* 1 *Enterobacter spp*. and 12 *Shigella spp.*) were investigated in the study. After obtaining data, the results were interpreted as resistant, sensitive or intermediate. Kappa coefficient measured the agreement of two media. Coefficient of variation (CV) was also calculated for antibiotic disks.
Results: The kappa agreement values for three types of antibiotic disks on Quicolor and Mueller-Hinton agar plates were good or excellent for all the examined antibiotics. CV values were also very satisfactory in the majority of cases.
Conclusion: Antibiotic disks from three manufacturers can successfully be used on both Quicolor and Mueller-Hinton agar plates.

Keywords: Antibiotic disks, Disk diffusion, Quicolor medium

INTRODUCTION

Discovery of antibiotics was a tremendous help in quick and accurate treatment of infectious diseases (1). Disk diffusion method is widely used to detect the susceptibility of bacterial isolates to antibiotics. Colorimetric media have also been developed for rapid antibacterial susceptibility testing of bacteria instead of Mueller-Hinton agar plates. Quicolor (QC) (Salubris Inc., Massachusetts, USA) is a medium for colorimetric and rapid antibacterial susceptibility testing. It is based on a rapid culture medium that indicates early growth of bacterial through changes in the color. Since the results are available within 3.5-6 hours after inoculation, it may have a significant impact on reduction of hospitalization time,

*Corresponding author: Siavosh Salmanadeh-Ahrabi, PhD, Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran.
Tel: +989336159153
Fax: +98-21-88058912
Email: salmanzadeh1@yahoo.com

total medical costs and even rate of mortality. Quicolor is cost-effective, easy to use and do not require any special instruments. Use of antibiotic disks is necessary for both mentioned media. According to the Clinical and Laboratory Standards institute (CLSI) guidelines, interpretation of the results in standard disk diffusion method is on the basis of growth inhibition zone whereas for the Quicolor is based on the color changes according to the manufacturer's instructions. The Kirby Bauer technique (2) for disk susceptibility testing has been recommended by the CLSI (3) which has been approved by Food and Drug Administration (FDA) and is also recommended by WHO (4, 5). Thus, the antibiotic sensitivity test report can have a strong effect on antibiotic consumption and hence on the factors that facilitate the emergence of antimicrobial drug resistance. Therefore, the test should be highly standardized using standard reagents, disks and appropriate strains as the quality controls. The antibiotic disks themselves serve as key parameters in obtaining accurate and reproducible results (6-8).

Quality of disks and potency of their antibiotics, produced by different manufactures, must be approved through three FDA,WHO and the US Department of National Health and Welfare (DNHW) (9). Different levels of antibiotic saturation may be chosen by the manufactures since some disks may be impregnated with more than 100% of the stated content to compensate for loss of activity in the handling of disks (10).

There are three main international standards for potency of antibiotic in the disks and all the manufactures do not produce according to the same standards. Their specifications have been summarized as follows: FDA specification 67-150%, WHO specification 75-135% and DNHW 90-125% of the stated concentration (11, 12).

In this study, we investigated the quality and type of antibiotic disks from different origins and their effects on the results of antibacterial susceptibility testing.

MATERIALS AND METHODS

Bacterial isolates. Seventy clinical isolates from *Enterobacteriaceae* family (21 *Klebsiella spp.,* 36 *Escherichia coli,* 1 *Enterobacter spp* and 12 *Shigella spp.*) were obtained from Pasteur Institute of Iran isolated from blood or urine samples during the years 2009-2010. Strains were confirmed through biochemical tests and stored at -70 °C for future use. *E. coli* ATCC 25922 strain was used as a quality control strain.

Agar media. Dehydrated Quicolor ES agar medium (specific for *Enterobacteriaceae* and Staphylococci) containing carbohydrates, peptones, dye indicator, vitamins, salts as well as Quicolor enrichment supplement were used. Mueller-Hinton agar plates were also prepared according to the manufacturer's instructions.

Antibiotics. Antibacterial susceptibility testing was done by standard disk diffusion method on Mueller-Hinton agar and Quicolor according to CLSI or manufacturers guidelines using disks from three different manufacturers; Rosco, Mast and Padtan Teb companies. The results were interpreted as resistant, sensitive or intermediate. Five types of antibiotics were selected including ciprofloxacin (CIP, 5µg), cefotaxime (CTX, 30µg), cefazolin (CZ, 30µg), meropenem (MEM, 10µg) and cotrimoxazole (SXT, 25µg). Three batches were used for each of five types in one experiment, one for antibiotics from mast, antibiotic from Rosco company (NEO-Sensitabs-Rosco), and Padtan Teb.

Disk diffusion method. Antibiotic susceptibility test was done according to Kirby Bauer method (2) using 2 mentioned media. All procedures and conditions were the same to ensure even in the cases of different zone diameters there is no error. The influencing factors such as type, depth and pH of the medium, type of the clinical strains, using the same bacterial colony in order to make bacterial suspension, potency of antibiotic disks, accuracy of the cultivation procedure were considered in the experiments (13, 14). Zone of inhibition in Quicolor plates was measured after 4-6 hours incubation whereas inhibition zones in MHA plates was measured after 18 to 24 hours (7).

Statistical analysis. Statistical analysis was done by SPSS (v. 22) and Stata softwares. CV was calculated for each antibiotic disk on both Quicolor and Mueller-Hinton agar plates. Data have been reported after four repeats of disk diffusion test. Reproducibility of the results was considered unsatisfactory

if CV percentage of a disk was more than 5%. Subsequently Kappa values were calculated to investigate the agreement of the results on both media (Table 3).

Kappa values interpreted in four groups: weak agreement for values among 0 to 0.25, moderate agreement for 0.25 to 0.5, good agreement for 0.5 to 0.75 and excellent agreement for 0.75 up to 1. In the other way, we stated percentage of agreement with turning Kappa values to percentage from 0 to 100. Kappa +1 indicate that two cases that compare with another act similar to another completely.

RESULTS

Disk diffusion test was done for all strains separately. Every time for one test quality control experiment was done for validation of other tests, antibiotic disks, cultures and overall. The results were acceptable and diameter of inhibition zone was in its range (Table 1 and 2).

In this study, antibiotics originated from three manufactures, NEO-SENSITABS-Rosco (Denmark), Mast (UK) and Padtan Teb (Iran) were compared. Kappa values have been presented in Table 3 indicating all excellent or good agreements except for meropenem on MHA plates that showed moderate agreement. In the other way, we turn Kappa values to agreement percentage (Tables 4 and 5).

Four Mast disks, 3 Rosco tablets and 2 disks of Padtan Teb showed slightly higher CV values (greater than 5%) when Mueller-Hinton agar medium was used. In the case of Quicolor plates, one Mast disk, 2 Rosco-tablets and 6 disks from Padtan Teb company showed slightly higher CV values (higher than 5%).

Table 1. Quality control of antibiotic disks using *Escherichia coli* ATCC 25922 control strain

Antibiotics	Diameter of inhibition zone (mm)
Ciprofloxacin	30-40
Cefotaxime	29-35
Cefazolin	21-27
Meropenem	28-34
Cotrimoxazole	23-29

Table 2. Antibiotic susceptibility test on bacterial strains for three company

Antibiotics	Resistant	Intermediate	Sensitive (mm)
Ciprofloxacin	≤15	16-20	≥21
Cefotaxime	≤22	23-25	≥26
Cefazolin	≤19	20-22	≥23
Meropenem	≤19	20-22	≥23
Cotrimoxazole	≤10	11-15	≥16

Table 4. Percentage of agreement fortested antibiotic disks on Quicolor plates

Compared groups	CIP	CTX	CZ	MEM	SXT
Mast *vs.* Padtan Teb	100%	98%	100%	98%	98%
Rosco *vs.* Padtan Teb	100%	98%	100%	98%	98%
Rosco *vs.* Mast	100%	100%	100%	100%	100%

Table 5. Percentage of agreement for tested antibiotic disks on Mueller-Hinton agar plates

Compared groups	CIP	CTX	CZ	MEM	SXT
Mast *vs.* Padtan Teb	95%	97%	88%	94%	98%
Rosco *vs.* Padtan Teb	97%	97%	87%	94%	97%
Rosco *vs.* Mast	98%	98%	92%	97%	98%

Table 3. Kappa values for antibiotic disks on two agar plates

Disks	Quicolor plates					Mueller-Hinton agar plates				
	CIP	CTX	CZ	MEM	SXT	CIP	CTX	CZ	MEM	SXT
Mast *vs.* Padtan Teb	1	0.97	1	0.66	0.97	0.92	0.94	0.78	0.31	0.96
Rosco*vs.* Padtan Teb	1	0.97	1	0.66	0.97	0.94	0.94	0.75	0.31	0.93
Rosco*vs.* Mast	1	1	1	1	1	0.97	0.97	0.86	0.48	0.96

DISCUSSION

According to the results of the present study Quicolor medium can be used for rapid antibiotic susceptibility testing of *Enterobacteriaceae* in combination with standard disk diffusion method especially when urgent results are needed. Comparative statistical analysis indicated good to excellent agreements on both Quicolor and Mueller-Hinton agar plates when disks from different resources were used. CV values were satisfactory for the majority of cases using three types of disks on both media.

In the case of Quicolor medium, it should be mentioned that the plates should be stored in refrigerator at 4-8°C (15, 16). In the case of longer storage, plates should be kept in dark. Development of inhibition zones will take longer times if the depth of the agar is more than usual. Color of the agar medium is dark red at higher pH values and it takes a long time for developing yellow inhibition zones. Quicolor medium represents the results within 4 to 6 hours depending on special factors such as type of the strain. If diameter of the inhibition zone is not read within 8 hours, the zones will be disappeared. There are some observed unusual zones such as binary zones, zones with feather margins and some colonies within the zones which will be developed when antibiotic disks have been located very close to each other (17).

Quality of antibiotic disks from different manufacturers depends on several factors including quality of applied antibiotics, quality of paper or tablet disks which should be standardized (18, 19). Disks may occasionally contain antibacterial substances other than, or in addition to those quoted on the label (18) and the frequency of its occurrence is very difficult to estimate. Such disks would be rarely detected through inclusion of controls in disk sensitivity tests and potentially are more dangerous than non-reacting disks because they can report a resistant microorganism as a sensitive one.

Humidity and temperature affect the stability of antibiotics in disks (20-22). Consequently, disks should be stored in sealed containers, preferably containing a desiccant, at 4°C or below and should be allowed to warm up to room temperature before use.

On the other hand, in high pH values penetration of antibiotics increases which yield to larger inhibition zones. Thus, all the manufacturers should keep cold production chains (5, 13, 23, 24) indicating the importance of source and origin of antibiotic disks we use.

CONCLUSION

Commercially available disks are often designed for use in testing methods within the home country but they may be imported into the other countries where different methods are being used. Thus, it is important to know whether they can cause different interpretations in sensitivity tests. In order to obtain reliable interpretations, it is necessary for each country to have its national standard quality control laboratories.

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