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Quicolor: A novel system for rapid antibacterial susceptibility testing

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Abstract - Early determination of antibacterial susceptibility increases the success of therapy, decreases unnecessary use of antibacterials and side effects and lowers the overall healthcare costs. We have evaluated a rapid antibacterial susceptibility test, Quicolor (Salubris Inc., Massachusetts, USA), which is based on a rapid culture medium that indicates growth early by changing its colour. Quicolor proved to be a reliable rapid test for determining antibacterial susceptibility, having an overall agreement of 97.6% with the conventional CLSI disk diffusion susceptibility test results. Between two methods overall agreement was 96.7% for Enterobacteriaceae, 96.8% for staphylococci and 94.2% for non-fermentative bacteria. There was only 0.6% major discrepancy in Enterobacteriaceae, 1.7% in staphylococci and 0.9% in non-fermentative bacteria. Since the test provides results in 3.5-6 h, it can provide the means to choose the right treatment regimen the same day the infectious agent is isolated.

Key words: disk diffusion, susceptibility, rapid test, Quicolor.

INTRODUCTION

Early determination of antibacterial susceptibility of bacteria, isolated from cases like meningitis, bacteraemia and sepsis is very important for the selection of appropriate therapy as soon as possible. Even when there is no such urgent need, determination of antibacterial susceptibility on the same day the culture results are obtained, it is crucial for the selection of the right treatment regimen, for increasing the success of therapy, for lowering the rate of side-effects and mortality and for cutting down the healthcare costs (Granato, 1993; Doern *et al.,* 1994; Schifman *et al.,* 1997; Barenfanger *et al.,* 1999; Tunney *et al.,* 2004; Kanemitsu *et al.,* 2005). We have developed, produced and tested a media called Quicolor (Salubris Inc., Massachusetts, USA) for increasing efficiency in early determination of antibacterial susceptibility. Quicolor is a media that changes its colour due to metabolic activity of growing bacteria. Quicolor is poured in Petri dishes and used to determine susceptibility to antibacterials by disk diffusion method.

Quicolor has two types. Quicolor ES agar is used to determine the susceptibility of Enterobacteriaceae and staphylococci. This media changes its colour from red to yellow by bacterial growth and red circular inhibition zones are produced around disks containing antibacterials (Fig. 1A). Quicolor NF agar is used to determine the susceptibility of non-fermentative bacteria like *Pseudomonas* and *Acinetobacter* species and this media changes its colour from yellow to red producing yellow inhibition zones (Fig. 1B).

In this study, the susceptibility to various antibacterials of 177 clinical isolates grown from the clinical samples submitted to Clinical Pathology Laboratory of Hacettepe University, Medical School, was determined by disk diffusion method using Quicolor and Mueller Hinton agar and the results were compared.

MATERIALS AND METHODS

Clinical isolates. For evaluating Quicolor, all the isolates grown from clinical samples during a three-month period and 34 frozen strains of non-fermentative Gram-negative bacteria grown within the previous three months, were included in the study. Among the 177 isolates studied, 66 belonged to Enterobacteriaceae (45 *Escherichia coli*, 9 *Klebsiella pneumoniae*, 4 *Klebsiella oxytoca*, 3 *Serratia marcescens*, 2 *Proteus mirabilis*, 1 *Enterobacter cloacae*, 1 *Serratia liquefaciens*, 1 *Proteus vulgaris*), 41 staphylococci (34 *Staphylococcus aureus*, 6 *Staphylococcus epidermidis*, 1 *Staphylococcus caprae*) and 70 non-fermentative Gram-negative bacteria (45 *Pseudomonas aeruginosa*, 14 *Acinetobacter baumannii*, 5 *Acinetobacter lwöffii*, 6 *Stenotrophomonas maltophilia*).

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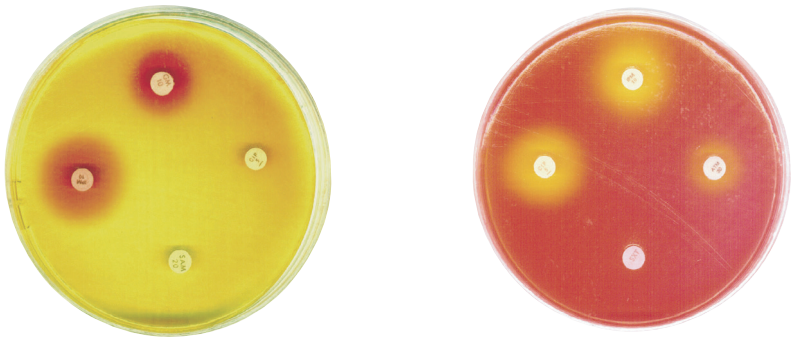

A B

FIG. 1 - A: Quicolor ES agar, inoculated with enteric bacteria, turned the colour of the media to yellow, producing red inhibition zones around the antibacterial containing disks that the bacteria are susceptible. B: Quicolor NF agar, inoculated with non-fermentative Gram-negative bacteria, turned the colour of the media to red, producing yellow inhibition zones around the antibacterial containing disks that the bacteria are susceptible.

Susceptibility determination by Quicolor. Bacterial suspensions were prepared from overnight cultures of clinical samples, using the Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, USA), to produce a turbidity of 0.5 Mc Farland. The suspension was spread on the agar surface of Quicolor ES agar, for Enterobacteriaceae and staphylococci, and Quicolor NF agar for non-fermentative Gram-negative bacteria. Disks containing antibacterials (BBL Microbiology Systems) were placed and the plates were incubated at 35 °C until inhibition zones in colour became apparent (Fig. 1A and 1B). The inhibition zone diameters was measured and interpreted according to the "Easy Read Chart" (a transparent card with circles that show the breakpoints for susceptibility and resistance for each antibacterial) provided by Salubris Inc. *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* 1951 high ESBL producing strain, *K. pneumoniae* 1204 low ESBL producing strain, *S. aureus* ATCC 25923, methicillin resistant *S. aureus* 27R strain, and COL strain, *S. epidermidis* glycopeptides intermediate strain which is one of the challenge strains and numbered as WHO-6 by Tenover *et al*. (2001) were also evaluated as quality control strains.

Susceptibility determination by CLSI disk diffusion method. From the bacterial suspensions prepared for Quicolor, bacteria were spread on the surface of Mueller-Hinton agar (BBL Microbiology Systems) and paper disks containing the same antibacterials tested in Quicolor were placed. The plates were incubated for 18-24 h at 35 °C. The inhibition zone diameters were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) (formerly the NCCLS) criteria (CLSI, 2005).

Evaluation of the results. Quicolor was evaluated by comparing the susceptibility results obtained from this and the CLSI disk diffusion test. If the test results were the same, either susceptible or resistant by both tests, it was defined as "agreement". If the result was susceptible or resistant by one test and intermediate with the other, this was called "minor discrepancy". If the result was susceptible by one test and resistant by the other it was called "major discrepancy".

RESULTS

The susceptibility results of isolates belonging to Enterobacteriaceae, with Quicolor ES agar were obtained in 4 h for the majority of the strains, the range being from 3.5 to 5 h. The average incubation time required for obtaining the susceptibility results of staphylococci and non-fermentative bacteria by Quicolor ranged between 4 and 6 h, but the majority of strains produced results in 4.5 h.

The agreement between the susceptibility results obtained by Quicolor and CLSI disk diffusion test, for Enterobacteriaceae, staphylococci and non-fermentative bacteria (*Pseudomonas* and *Acinetobacter* species) are shown in Tables 1, 2, and 3 respectively. The total agreement between two tests was 96.7% for Enterobacteriaceae, 96.8% for staphylococci and 94.2% for non-fermentative bacteria. Out of 3.3% total discrepancy only 0.6% was classified as major discrepancy for Enterobacteriaceae. The major discrepancy in staphylococci was 1.7% and in Gram-negative non-fermentative bacteria 0.9%.

DISCUSSION

Clinical and financial benefits of early reporting of antibacterial susceptibility results have been shown in many studies (Granato, 1993; Doern *et al.,* 1994; Schifman *et al.,* 1997; Barenfanger *et al.,* 1999; Tunney *et al.,* 2004; Kanemitsu *et al.,* 2005). Barenfanger *et. al*. (1999) reported that early reporting of antibacterial susceptibility test results decreased the length of stay in the hospital by 2.0 days and the average total cost for patient by $ 2395. In another group of patients Doern *et al*. (1994) reported a cost saving of $ 4194 per patient and additionally a statistically significantly lower mortality rate in rapid antibiotic susceptibility test group. In recent years, major technological advances have been made in clinical microbiology that have resulted in rapid reporting of antimicrobial susceptibility results that many regard as the most important information generated by the microbiology laboratory (Granato, 1993). Although several automated systems aiming to provide early antibacterial susceptibility results became available,

TABLE 1 - The percentage of agreement between Quicolor and CLSI disk diffusion susceptibility results for Enterobacteriaceae (n = 66)

Antibacterial	Minor discrepancy (%)	Major discrepancy (%)	Total discrepancy (%)	Total agreement (%)
Amikacin	0	1.5	1.5	98.5
Ampicillin	4.5	0	4.5	95.5
Cefazolin	4.5	0	4.5	95.5
Cefoperazone	4.5	0	4.5	95.5
Cefotaxime	4.5	0	4.5	95.5
Ceftazidime	3.0	0	3.0	97.0
Cefuroxime	0	0	0	100
Ciprofloxacin	1.5	1.5	3.0	97.0
Gentamicin	1.5	2.0	4.5	95.5
Imipenem	0	0	0	100
Meropenem	0	0	0	100
Piperacillin	1.5	0	1.5	98.5
Ampicilllin/sulbactam	7.5	0	7.5	92.5
Trimethoprim/sulfamethoxazole	4.5	3.0	7.5	92.5
Total	2.7	0.6	3.3	96.7

TABLE 2 - The percentage of agreement between Quicolor and CLSI disk diffusion susceptibility results for staphylococci (n = 41)

Antibacterial	Minor discrepancy (%)	Major discrepancy (%)	Total discrepancy (%)	Total agreement (%)
Cefazolin	0	4.8	4.8	95.2
Ciprofloxacin	4.8	0	4.8	95.2
Clindamycin	4.8	0	4.8	95.2
Erythromycin	7.1	2.4	9.5	90.5
Fusidic acid	0	0	0	100
Oxacillin	0	0	0	100
Penicillin	0	0	0	100
Rifampicin	0	0	0	100
Ampicillin/sulbactam	0	4.8	4.8	95.2
Cefotaxime	2.4	4.8	7.2	92.8
Teicoplanin	0	0	0	100
Trimethoprim/sulfamethoxazole	0	4.8	4.8	95.2
Vancomycin	0	0	0	100
Total	1.5	1.7	3.2	96.8

Table 3 - The percentage of agreement between Quicolor and CLSI disk diffusion susceptibility results for non-fermentative Gram-negative bacteria (n = 70; *Pseudomonas, Acinetobacter, Stenotrophomonas* species)

Antibacterial	Minor discrepancy (%)	Major discrepancy (%)	Total discrepancy (%)	Total agreement (%)
Amikacin	7.0	1.4	8.4	91.6
Aztreonam	7.0	0	7.0	93.0
Ceftazidime	2.8	1.4	4.2	95.8
Ciprofloxacin	2.8	0	2.8	97.2
Gentamicin	2.8	0	2.8	97.2
Imipenem	9.9	0	9.9	90.1
Meropenem	7.0	1.4	8.4	91.6
Piperacillin	0	2.8	2.8	97.2
Total	4.9	0.9	5.8	94.2

only limited information about the accuracy and especially the speed of these systems can be found in literature. In comparative evaluations of susceptibility testing procedures, very major errors should occur in < 1.5% of all tests, and the overall agreement between tests and the reference method should be 95% (Nolte *et al.,* 1986).

Vitek (bioMerieux, NC, USA) and MicroScan Walkaway (Diamond Diagnostics, MA, USA) are two of the most commonly used automated antimicrobial susceptibility test systems. A study evaluating susceptibility of Gram-negative bacilli to eleven antibacterials using MicroScan Rapid Neg MIC/Combo panels (Diamond Diagnostics) and autoSCAN-W/A (Baxter MicroScan, West Sacramento, CA) showed that the results were available between 3.5 and 7.0 h in 92.7% of the isolates and overall agreement with the standard test was 94% with a 3.4% major error rate (Godsey *et al.,* 1991). Ribeiro *et al*. (1999) reported eight *Staphylococcus aureus* strains initially identified by Vitek GPS-BS or GPS-SA cards as resistant to oxacillin to be found on further testing to be susceptible to oxacillin.

Another study evaluating susceptibility of Gram-positive bacteria to 26 antibacterials using MicroScan Rapid Pos MIC/Combo panels and autoSCAN-W/A (Baxter), showed that the results were available between 3.5 and 15 h in 98% of the organisms with an overall 96% agreement with standard MIC panels (Bascomb *et al.,* 1991). McGregor *et al.* (1995) evaluated MicroScan and found out very major or major discrepancies in 2% and minor discrepancies in 8% of Gram-negative susceptibility tests, the results being available in 7 h for 93% of the isolates; in Gram-positive susceptibility tests major and minor discrepancies with the standard test were 1% and 7% respectively. Comparison of Vitek and Cobas Micro Systems, (Roche Diagnostics, Basel, Switzerland) with a semi automated conventional microsystem MIC2000 (Dynatech, McLean, Va., USA), for susceptibility testing of Gram-negative bacilli revealed 86% overall agreement with 3% major discrepancies for Vitek and 90% overall agreement with 2% major discrepancies for Cobas Micro systems (Simoons-Smit *et al.,* 1994). Evaluation of 500 Gram-negative isolates to determine the number of major susceptibility interpretation discrepancies between the Vitek and MicroScan Walkaway for 9 antimicrobial agents revealed only 1.06% discrepancies between these tests (Rittenhouse *et al.,* 1996).

Ling et al had compared susceptibility testing results of 228 various members of the Enterobacteriaceae, *Pseudomonas aeruginosa* and other Gram-negative bacteria, obtained with the Vitek 2 AST-No. 12 cards with those obtained by the broth microdilution method. They have reported 0.5% major errors (resistant with the Vitek 2 system but sensitive by the broth microdilution method) and 0.4% very major errors (sensitive with the Vitek 2 system but resistant by the broth microdilution method) (Ling *et al.,* 2001).

In this study, Quicolor proved to be reliable rapid test for determining antibacterial susceptibility the former having an overall agreement of 96.7% with CLSI disk diffusion test results for Enterobacteriaceae, 96.8% for staphylococci and 94.2% for non-fermentative bacteria. In Quicolor only 0.6% was classified as major discrepancy for Enterobacteriaceae, 1.7% in staphylococci and 0.9% in non-fermentative bacteria. Since the test makes the results available between 3.5 and 6 h, it may have a significant impact on lowering length of stay in the hospital, total cost for patient care and even mortality by providing the means for choosing the right treatment regimen the same day the infectious agent is grown. In serious cases the susceptibility results may be confirmed by conventional standard tests.

Quicolor was previously used successfully in rapid determination of extended spectrum beta-lactamases (ESBL) in Enterobacteriaceae using both double disk diffusion and E-test (Sancak *et al*., 2005; Kocagöz *et al*., 2006).

This is the first study evaluating Quicolor for its speed and efficiency in correctly identifying antibacterial susceptibility. Further studies are needed in different settings to reveal if this novel rapid system can be used as a reliable method.

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