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Dio-sensimedia: a novel culture medium for rapid detection of extended spectrum β-lactamases

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Abstract

Background: Resistance to contemporary broad-spectrum β-lactams, mediated by extended-spectrum β-lactamases (ESBL), is an increasing problem worldwide. Many of the emerging antimicrobial resistance problems of this decade have been characterized by difficulty in the recognition of resistance in the laboratory, particularly by rapid susceptibility test methods. The plasmid-encoded ESBL represent such a resistance phenomenon that is difficult to recognize.

We compared Dio-Sensimedia-ES (DSM-ES; Diomed, Istanbul, Turkey) and Mueller-Hinton (MH) agar in the double-disk synergy test (DDST) as a novel rapid system for detecting ESBL directly from bacterial culture.

Methods: Sixty ESBL-producing *Klebsiella pneumoniae* isolates cultured from blood (30), endotracheal aspirates (20), urine (5) and pus (5), as well as 40 *Escherichia coli* isolates cultured from endotracheal aspirates (15), urine (10), blood (8) and pus (7) were studied. Isolates positive for ESBL by the combined disk tests were tested with the DDST using MH and DSM-ES agar to detect ESBL-mediated resistance in *K. pneumoniae* and *E. coli*. DSM-ES agar was also used to determine the susceptibility of *Enterobacteriaceae* and staphylococci.

Results: Among 60 ESBL-producing *K. pneumoniae* isolates, 59 (98.3%) were identified as ESBL-positive by the DDST using MH, and 58 (96.6%), using DSM-ES agar. Of 40 ESBL-producing *E. coli* isolates, 38 (95%) were ESBL-positive by the DDST on MH agar, and 37 (92.5%), on DSM-ES agar. The average incubation period required for ESBL detection by the DDST on DSM-ES agar was 4 hours.

Conclusions: Since the DDST results were available within 4 hours when DSM-ES agar was used, the use of this media may significantly lower the length of hospital stay, the total cost for patient care and even the mortality rate by fascilitating early treatment against ESBL-producing organisms.

Background

Microbial resistance through extended-spectrum β-lactamases (ESBL) was first reported in Europe, and subsequently in the United States. Today this resistance mechanism has been recognized globally [1–6]. Many clinical microbiology laboratories have problems to detect ESBL-mediated resistance. Controversy exists regarding the clinical importance of such resistance, the choice of optimal laboratory methods to detect it, and surveillance of ESBL-producing organisms. Failure to detect

ESBL-mediated resistance has contributed to uncontrolled spread of ESBL-producing organisms and related treatment failures.

Among the genera of *Enterobacteriaceae*, ESBL are most commonly produced by *Klebsiella* spp. and *Escherichia coli* [7]. In addition, these enzymes have been isolated from other Gram-negative bacilli including *Pseudomonas aeruginosa* and *Acinetobacter* spp. [8]. Most ESBL are mutants of TEM and SHV ß-lactamase types [9,10]. Unlike these parent enzymes, ESBL hydrolyse penicillins and oxyimino-aminothiazolyl cephalosporins including cefuroxime, ceftriaxone, ceftizoxime, ceftazidime, cefpirome, cefepime, but not cephamycins [10–13].

The ESBL-producing Gram-negative bacilli possess genes encoding more than one type of the ESBL and enzymes that are responsible for resistance to other antibiotics such as aminoglycosides and fluoroquinolones that are active against Gram-negative bacilli [14,15]. The emergence of multidrug resistance in these virulent pathogens has significantly hampered the efforts to devise effective empiric or directed antibiotic treatment regimens [16].

ESBL-mediated resistance may be determined by several laboratory methods, including the combined disk methods (Oxoid "Combination disks" and Mast "MAST DD"), the double-disk synergy test (DDST), the three-dimensional agar test, the investigational Vitek ESBL cards (bioMérieux, Hazelwood, Mo., USA), and the ε-test ESBL strip (AB Biodisk, Solna, Sweden; Cambridge Diagnostic Services, Cambridge, UK) [17–19].

In our study, we report a novel rapid system for detecting the presence of ESBL directly from microbiological cultures. DSM (Diomed, Istanbul, Turkey) enables observation of the inhibition zones within 4 hours by changing its color in response to the metabolic activity of growing bacteria, even before the bacterial layer has had an opportunity to grow. DSM is an antibacterial susceptibility testing agar media, which is poured in Petri dishes and used to determine susceptibility to antibacterials by the disk diffusion method. The use of this medium in disk diffusion technique is analogous to an improved, chromogenic version of the Kirby-Bauer disk diffusion method.

Methods

Bacterial strains and culture media

Sixty ESBL-producing *K. pneumoniae* isolates cultured from blood (30), endotracheal aspirates (20), urine (5), and pus (5) as well as 40 *E. coli* isolates cultured from endotracheal aspirates (15), urine (10), blood (8), and pus (7) were studied. The isolates were non-repetitive. All isolates were cultured from patients hospitalized in an intensive care unit at Istanbul Faculty of Medicine, Istanbul University from January 2000 through December 2001. Identification of the isolates was performed by API 20E (bioMérieux, Hazelwood, Mo., USA). *E. coli* ATCC 25922 and *K. pneumoniae* K6 (ATCC 700603) were used as reference strains.

Screening and confirmatory tests to detect ESBL-mediated resistance were performed according to the National Committee for Clinical Laboratory Standards criteria [20]. Modified criteria, i.e., a diameter of <22 mm for ceftazidime; <25 mm, for ceftriaxone; and <27 mm, for cefotaxime or aztreonam were used for interpretation of Kirby-Bauer disk zone diameters. In this study, a combination of ceftazidime (30 μg) and clavulanic acid (10 μg) was used. Zones around *K. pneumoniae* or *E. coli* colonies, which were larger than that around the disk containing ceftazidime-clavulanic acid, were deemed to indicate ESBL-positivity.

DMS-ES agar was also used to determine susceptibility of *Enterobacteriaceae* and staphylococci. This media changes its color from red to yellow as bacteria grow and red circular inhibition zones forms around disks containing the antibacterial agent. All isolates that were positive for ESBL by the combined disk tests were tested with the DDST on Mueller-Hinton (MH) and DSM-ES agar to detect ESBL-mediated resistance. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 13883 were used as negative controls.

Double-disk synergy test

The detection of ESBL-mediated resistance by the DDST was performed according to a published protocol [11,12]. Bacterial suspensions were prepared from overnight cultures of clinical isolates to produce a turbidity of a 0.5 McFarland standard. These suspensions were then spread on the surface of MH and DSM-ES agar plates as in the Kirby-Bauer disk diffusion technique. Antibiotic susceptibility disks containing amoxicillin (20 μg) plus clavulanate (10 μg) were placed on the centre of Petri dishes containing the two different media. Ceftazidime (30 μg), ceftriaxone (30 μg), cefpodoxime (10 μg), aztreonam (30 μg), cefotaxime (30 μg) disks were placed 25–30 mm apart circularly around the co-amoxiclav disk. The MH agar plates were incubated for 24 hours at 35°C. DSM-ES agar that is originally red, changed its color to yellow as bacterial culture grew (Fig. 1). DSM-ES agar plates were incubated at 35°C until red inhibition zones became apparent. Red inhibition zones around disks containing antibacterials were visually observed and measured. When the disk containing co-amoxiclav extended to any of the other antibiotic disk inhibition zones, ESBL production was inferred [11,12] (Fig. 2).

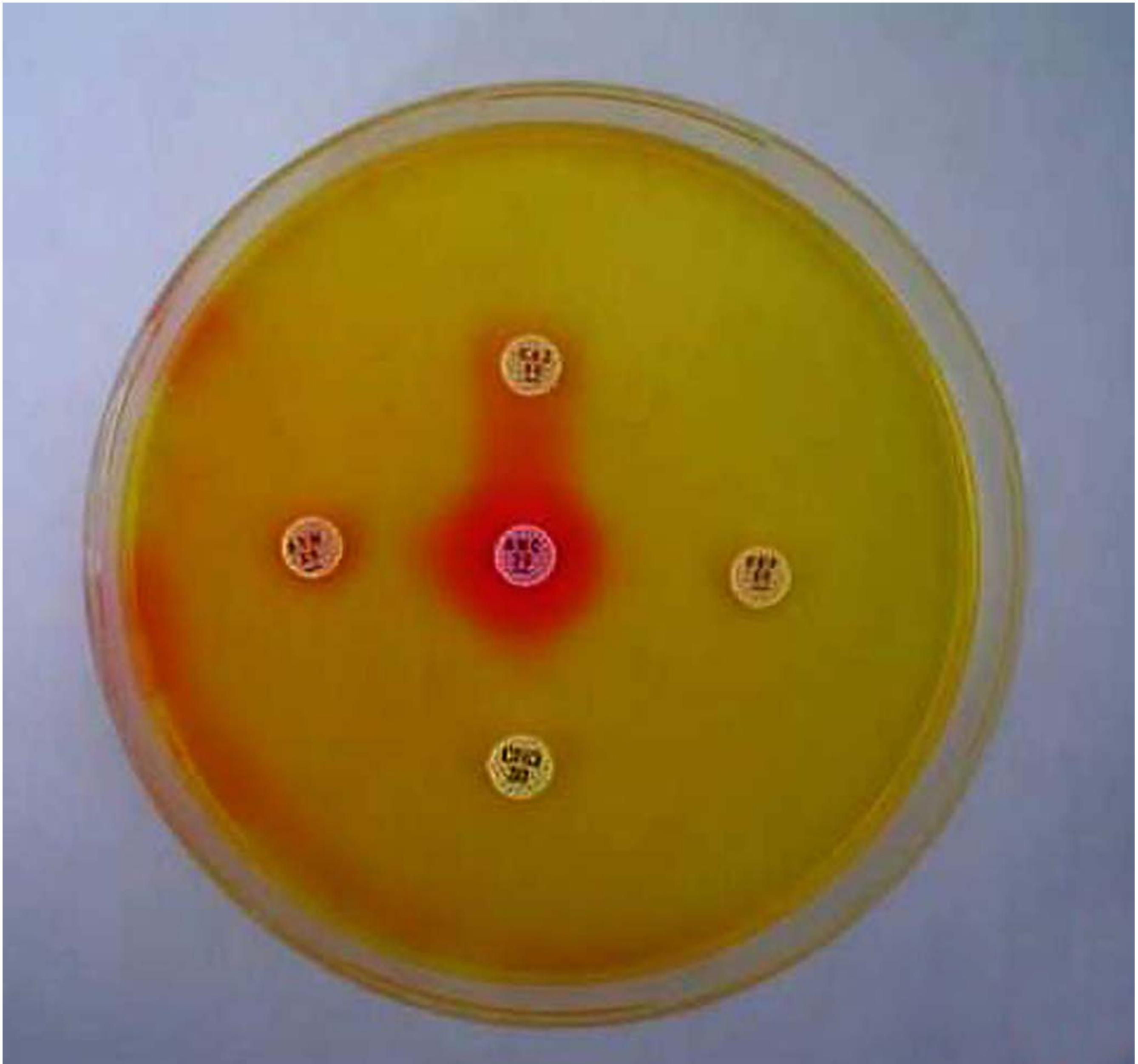

Figure 1
Red circle inhibition zone around the disk on the left, and at the top. Disks: centre, amoxycillin+clavulanate 20 + 10 μg; right, cefepime 30 μg; left, aztreonam 30 μg; top, ceftazidime 30 μg; bottom, ceftriaxone 30 μg.

Evaluation of the susceptibilities by two methods

Determination of ESBL production using was done by using both DSM-ES and Mueller-Hinton agar. The results of the DDST using the two media were compared.

All the test results were read and interpreted by one of the authors [AAC], who was blinded to the results of microbiological typing.

Results

Among 60 ESBL-producing *K. pneumoniae* isolates, 59 (98.3%) were identified as ESBL-positive by the DDST on MH agar; and 58 (96.6%), on DSM-ES agar (Table 1). The average incubation period required for detecting ESBL-mediated resistance when using DSM-ES agar was as short as 4 hours.

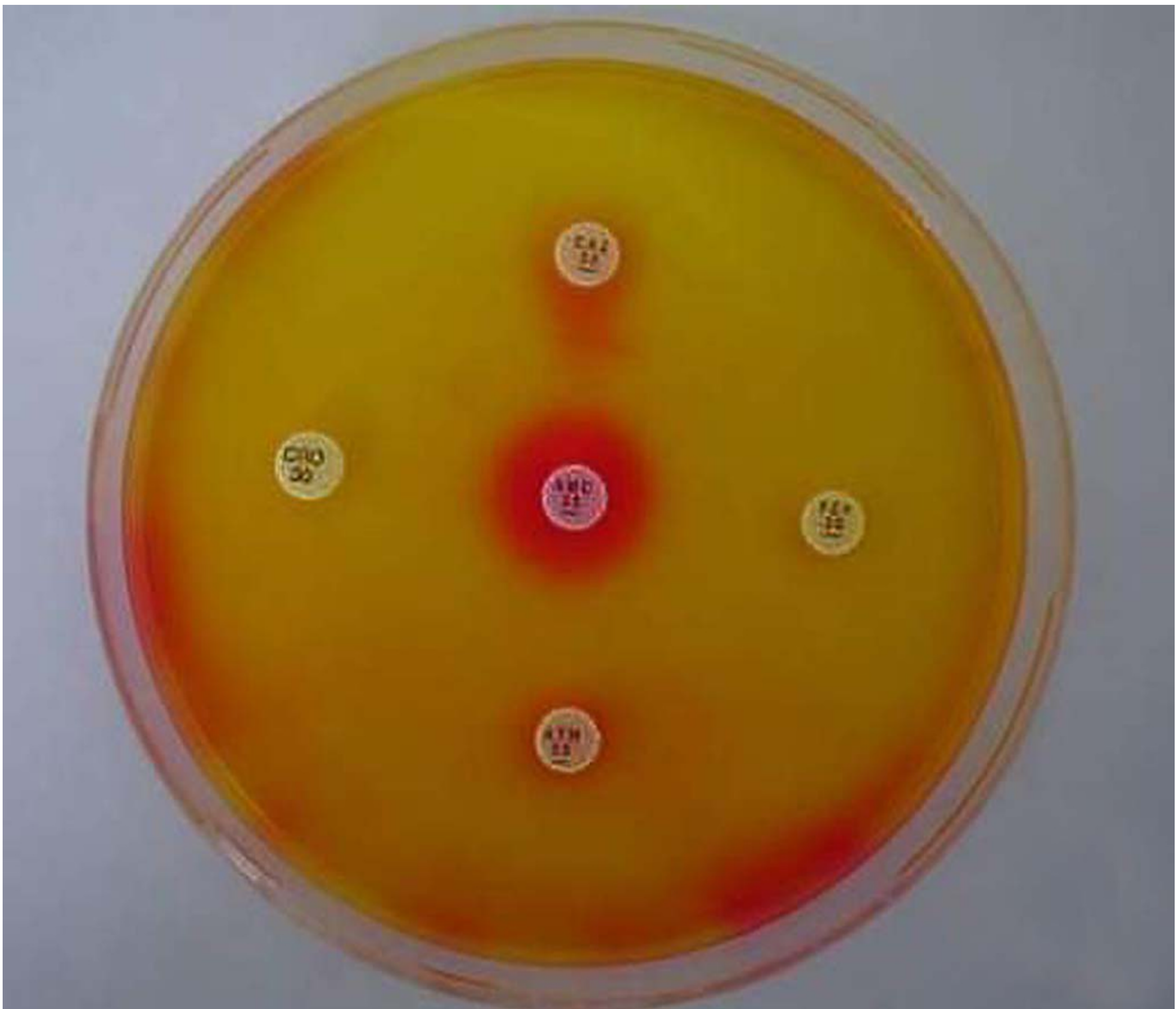

Figure 2
Detection of ESBL production by the double disk test on DSM-ES agar. Disks: centre, amoxycillin+clavulanate 20 + 10 μg; right, cefepime 30 μg; left, ceftriaxone 30 μg; top, ceftazidime 30 μg; bottom, aztreonam 30 μg.

Table 1: Percentage of ESBL-positive Isolates as Determined by DDST either on MH or DSM-ES Agar

	Combined Disk Test	DDST on MH Agar	DDST on DSM-ES Agar
K. pneumoniae (n = 60)	60	59 (98.3%)	58 (96.6%)
E. coli (n = 40)	40	38 (95%)	37(92.5%)

Of 40 ESBL-producing *E. coli* isolates, 38 (95%) were positive on MH agar; 37 (92.5%), on DSM-ES agar (Table 1). The average incubation period required for detecting ESBL-mediated resistance by the DDST on DSM-ES agar was 4 hours.

Discussion

ESBL are encoded on conjugative plasmids, tranposons or integrons. These mobile genetic elements are readily spread under selective antibiotic pressure [21]. Increased clinical use of antibiotics, access to antibiotics without a doctor's prescription in many countries, international travel, and uncontrolled use of antibiotics in the environment and by meat-producers have led to an increase in antibiotic resistance in many bacterial species [22]. Early determination of ESBL-mediated resistance is clinically crucial in cases like meningitis, bacteremia and sepsis in order to start appropriate therapies as early as possible. Even in less emergent situations, early determination of antibacterial susceptibility is important to select the appropriate treatment regimens and increase the success rate of the therapy, lower the rate of side-effects of antibiotics, and decrease health-care costs.

Clinical and financial benefits of early determination of antibacterial susceptibility have been shown in many studies [23]. Barenfarger *et al* [23] reported that early reporting of antibacterial susceptibility tests resulted in a decrease in the length of hospital stay by 2.0 days and in the average total cost per patient by US$ 2395. Doern *et al* [24] reported a saving of US$ 4194 per patient in addition to a statistically significant lower mortality rate when a rapid antibiotic susceptibility test was used.

The detection of ESBL-mediated resistance in Gram-negative bacilli is one of the major problems in a clinical microbiology laboratory [13]. The techniques recommended for this purpose by the NCCLS guidelines is not easily performed in routine clinical practice. Several automated systems such as Vitek (bioMerieux, NC, USA) and MicroScan Walkaway (Diamond Diagnostics, MA, USA) aimed to provide early results have recently become available and approved by the Food and Drug Administration. Another commercial assay used for ESBL detection, the ε-test is available from two manufacturers (AB Biodisk, Solna, Sweden; and Cambridge Diagnostic Services, Cambridge, UK). These commercial assay kits have a sensitivity of >90% to detect ESBL-mediated resistance.

Published data on the accuracy and the speed of such systems are limited. McGregor *et al* [25] evaluated MicroScan to test susceptibility of Gram-negative bacteria, and found major and minor discrepancies in 2% and 8% of the samples assayed, respectively, as compared to a standard test. When testing susceptibility of Gram-positive bacteria, major and minor discrepancies occured in 1% and 7% of the samples assayed, respectively. In this study 93% of the results were available within 7 hours. Ling *et al* [26] compared the Vitek 2 AST-No. 12 cards and the broth microdilution method to test susceptibility of 228 isolates, including various members of the *Enterobacteriaceae, Pseudomonas aeruginosa* and other Gram-negative bacteria. They reported major discrepancies (resistant by the Vitek 2 system but sensitive by the broth microdilution method) in 0.5 %, and very major discrepancies (sensitive by the Vitek 2 system but resistant by the broth microdilution method) in 0.4% of the tests.

A phase-three trial compared the disk diffusion method, which is recommended by the NCCLS guidelines, with an automated growth-monitoring system (Vitek ESBL). In this trial, the two methods were found to be comparable in both sensitivity (98% for disk diffusion vs. 99.7% for Vitek ESBL) and specificity (99.4% for disk diffusion and 100% for Vitek ESBL) [18]. Cormican et al. compared the ε-test with the disk diffusion method. ε-test was found to be more sensitive than the disk diffusion method (100% vs. 87%), whereas both methods were comparable in their specificity [27].

Although the DDST detected *K. pneumoniae* more frequently than *E. coli*, this test was equally efficent to detect ESBL-mediated resistance in both species. The average incubation period required to detect ESBL-mediated resistance in both species was approximately 4 hours. In this study, the DDST on DSM-ES agar proved to be a reliable, rapid and cost-effective test for detecting of ESBL in *K. pneumoniae* and *E. coli*. Since the DDST results can be obtained within 4 to 6 hours when DSM-ES agar is used, the use of this media may significantly lower the length of hospital stay, the total cost for patient care and even the mortality rate by fascilitating early treatment.

Competing interests

A. Atahan Cagatay: None declared.

Tanil Kocagoz is the head of the Research and Development Laboratory at DIOMED Ltd. Co, Istanbul, Turkey.

Haluk Eraksoy: None declared.

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