

## Evaluation of a chromogenic medium for rapid detection of extended spectrum $\beta$ -lactamase producing *Salmonella* spp.

Sesin Kocagöz, Fatma Budak\* & Deniz Gür\*\*

*Yeditepe University School of Medicine, Department of Clinical Microbiology, Istanbul, \*Kocaeli University School of Medicine, Department of Microbiology, Kocaeli & \*\*Hacettepe University School of Medicine Children's Hospital, Clinical Microbiology Laboratory, Ankara, Turkey*

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**Background & objectives:** *Salmonella* spp. resistant to second- and third-generation cephalosporins and related antibiotics by production of various extended-spectrum  $\beta$ -lactamases (ESBLs) are on the rise in Turkey. Early detection of ESBL producing *Salmonella* is important to institute appropriate treatment in time. In this study rapid detection of ESBL production among clinical isolates of *Salmonella* was evaluated using double-disk synergy test in a new chromogenic medium. The colour of the medium changes from red to yellow with bacterial growth and red circular inhibition zones are produced around disks containing antibacterials.

**Methods:** A total of 182 clinical isolates of *Salmonella* were evaluated in this study. The presence of ESBLs in clinical isolates was determined by double-disk synergy test using Mueller-Hinton (MH) agar and Quicolor E&S agar plates.

**Results:** Six isolates were shown to harbour ESBL enzymes with double disk synergy test by Mueller Hinton agar. The same results were obtained using Quicolor E&S agar after 4-6 h by changing its colour in response to the metabolic activity of growing bacteria.

**Interpretation & conclusion:** Our findings showed that with this new medium, the results can be evaluated rapidly within 4-6 h and the enhancement of inhibition zones can be easily detected with the colour changes thus enabling the treating physician to institute the right treatment regimen immediately.

**Key words** ESBL - medium - Quicolor E&S agar - *Salmonella*

Salmonellosis ranges clinically from the *Salmonella* gastroenteritis to enteric fevers which are life-threatening febrile systemic illness requiring prompt antibiotic therapy. It is endemic in many developing countries with poor sanitary conditions,

but emerges sporadically as a serious public health threat in developed countries<sup>1-3</sup>. Extended spectrum  $\beta$ -lactamase (ESBL) producing organisms are among the growing problems in the area of infectious diseases such as Salmonellosis. *Salmonellae* can be

the primary ESBL gene recipients, as transient or persistent members of human intestinal flora or secondary recipients, since they can exchange plasmids with other *Enterobacteriaceae*. Resistance to extended spectrum  $\beta$ -lactam antibiotics in *Enterobacteriaceae* is often due to the presence of ESBLs<sup>4,5</sup>. ESBL production is also of particular concern, as expanded-spectrum cephalosporins are the drugs of choice for children because they cannot be treated with fluoroquinolones. Early determination of ESBL-mediated resistance is clinically crucial in serious infections in order to start be appropriate therapy as soon as possible. Even in less severe cases, 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.

In this study, we evaluated rapid detection of ESBL production among clinical isolates of *Salmonella* by using double-disk synergy test in a novel chromogenic medium. The colour of the medium changes according to the metabolic activity of growing bacteria, before the bacterial growth is visible. This method can be described as an improved, chromogenic version of the Jarlier's disk diffusion method<sup>6</sup>.

### Material & Methods

**Bacterial isolates:** *Salmonella* spp. that were isolated consecutively in Hacettepe University Pediatric Hospital's Microbiology Laboratory, Ankara, Turkey between October 1998 and January 2001 and Kocaeli University Medical Faculty, Central Laboratory, Kocaeli, Turkey were included in this study. A total of 182 *Salmonella* spp. were isolated from clinically significant samples including stool (179), urine (2) and blood (1). Only one isolate per patient was included. The isolates were subcultured on SS agar (*Salmonella* Shigella) and lactose negative colonies were examined in TSI agar (Triple Sugar Iron). The isolates were identified with specific antisera (Difco Laboratories, Detroit, USA) and Crystal test (Crystal Enteric Panel Becton Dickinson, USA).

**Antibiotic susceptibility testing:** Disk diffusion tests were performed on Mueller-Hinton agar (MH) with

commercial antibiotic discs (Oxoid Ltd. Basingstoke, UK) according to the recommendations of Clinical Laboratory Standards Institute (CLSI)<sup>7</sup>. The antibiotic disks ( $\mu$ g) were ampicillin (10), amoxicillin-clavulanic acid (20/10), trimethoprim-sulphamethoxazole (1.25/23.75), ciprofloxacin (5), chloramphenicol (30), amikacin (30), gentamicin (10), ceftriaxone (30), ceftazidime (30) (Oxoid, UK).

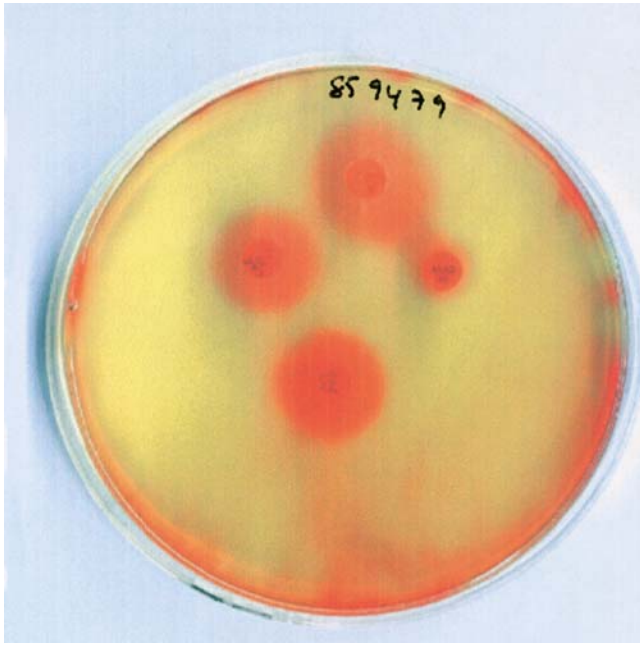
**Double-disk synergy test (DDST):** Disk approximation test was used in these isolates as a screening test for ESBL production using disks of amoxicillin-clavulanic acid, ceftriaxone and ceftazidime<sup>8</sup>. The 0.5 Mc Farland turbidity of bacteria were spread on the surface of MH and Quicolor E&S Agar (Salubris, Turkey) plates. DDST was performed by comparing the inhibition zones of disks containing an extended-spectrum cephalosporin with and without clavulanic acid.

The presence of ESBLs in clinical isolates was determined with screening and confirmatory tests according to CLSI criteria<sup>7</sup>. Quicolor medium is a red colored agar medium, which is poured in petri dishes and used to determine susceptibility to antibacterials with disk diffusion method. While MH agar plates were incubated at 35°C for 24 h, Quicolor E&S agar plates were incubated until inhibition zones became apparent which appeared within minimum 4, maximum 6 h of colour change. The red colour of the medium changes to yellow with bacterial growth, and red circular inhibition zones are observed visually around disks containing antibacterials, which enable ease in measuring.

When the zones were enlarged around the disk containing combination of clavulanic acid, these isolates were considered as ESBL-positive. The results of DDST using both Quicolor E&S and MH agar were compared. For negative control, *Escherichia coli* ATCC 25922 and for positive control *Klebsiella pneumoniae* ATCC 700603 were used (Bactrol Disks Set A QC, Raven Biological Laboratories, Microbiologics USA).

### Results

A total of 182 clinical isolates of 110 (60.4%) non-typhoidal *Salmonella* group D and 54 of (29.6%)



**Fig.** Appearance of inhibition zones on Quicolor E&S agar after incubation with ESBL producing *Salmonella* spp approximately in 4 h.

*Salmonella* group B were identified. Ten isolates were *Salmonella* group C1, 8 *Salmonella* group C2. The most frequent multiple resistance phenotype was ampicillin (Amp), chloramphenicol (Chl) and trimethoprim-sulphamethoxazole (Sxt) resistance which was observed in 11 isolates. Six isolates were resistant to ceftazidime (Caz) and ceftriaxone (Cro) and all of them were serogroup B except one which was serogroup D. These six isolates were also resistant to ampicillin and chloramphenicol.

All of six isolates which were resistant to ceftazidime and ceftriaxone were shown to produce ESBL with the double disk method (Table). Compared with MH agar, same results were obtained using Quicolor E&S agar. After 4-6 h following colour change in response to the metabolic activity of growing bacteria, enhancement of red circular inhibition zones were clearly visible in the agar plates (Fig.).

## Discussion

In recent years, non-typhi *Salmonella* spp. producing extended-spectrum  $\beta$ -lactamases have been reported from several countries<sup>9</sup>. These enzymes show resistance

to oxyimino-beta lactams such as cefotaxime, ceftazidime and aztreonam. As extended-spectrum  $\beta$ -lactamases are encoded on conjugative plasmids, transposons or integrons, they can readily spread under selective antibiotic pressure and constant antibiotic enables their transmission among patients<sup>10-12</sup>.

Several phenotyping methods have been suggested to augment the detection of resistance mechanism such as DDST, the 3-dimensional test, the Vitek test (Biomérieux, Inc. Hazelwood, Ma, USA), and the E test (AB Biodisk, North America). The DDST is considered the most cost-effective and, therefore, most widely used. The 3-dimensional test has not been adopted for routine use in the clinical laboratory because it is technically difficult to perform. Technically, the Vitek method is the least demanding method to perform, as it is an integral part of the routine susceptibility test card. E test strips are reliable, but are expensive of all the techniques used<sup>13</sup>. However, all of these are time consuming. On the other hand, molecular methods for ESBL determination are quick and reliable but are expensive and difficult to perform in all laboratories.

Clinical and financial benefits of early reporting of antibacterial susceptibility results have been shown in many studies<sup>14-17</sup>. Çağatay *et al*<sup>18</sup> reported that among 60 ESBL-producing *K. pneumoniae* isolates, 59 (98.3%) were identified as ESBL positive by DDST using MH agar, and 58 (96.6%) by Quicolor E&S agar. Of 40 ESBL-producing *E. coli* isolates,

**Table.** Resistance pattern to antibiotics in ESBL positive *Salmonella* serogroups

Serogroup (N)	Resistance pattern
<i>S. paratyphi</i> B	Amp, Chl, Tmp-Smx, Amc, Cro, Caz
<i>S. paratyphi</i> B	Amp, Chl, Gen, Cro, Caz
<i>S. paratyphi</i> B	Amp, Chl, Gen, Tmp-Smx, Amc, Cro, Caz
<i>S. paratyphi</i> B	Amp, Chl, Gen, Tmp -Smx, Amc, Cro, Caz
<i>S. paratyphi</i> B	Amp, Chl, Gen, Amc, Cro, Caz
<i>Salmonella</i> D	Amp, Chl, Amc, Cro, Caz

Amc, amoxicillin/clavulanic acid; Amp, ampicillin  
Chl, chloramphenicol; Caz, ceftazidim; Cro, ceftriaxone  
Gen, gentamicin; Tmp/Smx, trimethoprim/sulphamethoxazole

38 (95%) were ESBL-positive by the DDST on MH agar, and 37 (92.5%) on this agar<sup>18</sup>. The average incubation period required for ESBL detection by the DDST on this agar was 4 h.

With Quicolor E&S rapid and accurate inhibition zones in double disk synergy test can be detected among ESBL enzyme producing *Salmonella* spp. within 4 h. The zone of inhibitions around disks containing antibacterials are observed as red circular inhibition zones which make it easy to measure them visually.

Since with this medium the results are available rapidly, this may have a significant impact on reducing the duration of stay in the hospital, total cost for patient care and mortality by providing the means to select the right treatment regimen at an early date.

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