

Rapid detection of ESBL-producing gram-negative bacteria isolated from blood: a reasonable and reliable tool for middle and low resource countries[†]

Jennifer M. Cuellar-Rodríguez,* Alfredo Ponce-de-León,* Ruth Quiroz-Mejía,* Arturo Galindo-Fraga,* Ana L. Rolón-Montes-de-Oca,* Melissa Hernández-Durán,* Guillermo M. Ruiz-Palacios,* José Sifuentes-Osorio*

* Departamento de Enfermedades Infecciosas, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán.

ABSTRACT

Introduction. Delay in appropriate treatment in patients with bacteraemia can increase morbidity, mortality, and health expenditures. We compared the Rapid Direct Test (RDT) designed to detect ESBL producing gram negative bacteria (GNB) directly from positive blood cultures bottles, with two conventional ESBL detection tests: Screening and Confirmatory Disk Diffusion Assay (SC DDA) and an MIC Screening and ESBL E test (MIC/ET). **Material and methods.** We screened all blood cultures in a tertiary care facility from August to December 2005. We only included one positive bottle per patient in which GNB were observed. RDT: Blood from each bottle was inoculated on Mueller Hinton agar. Cefotaxime and cefotaxime disks with and without clavulanic acid were added and incubated at $35\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 24 h. Results were interpreted according to CLSI recommendations for the SC DDA and MIC/ET. All methods were performed simultaneously. Time for reporting as an ESBL producer and cost of the tests were recorded. **Results.** We isolated 124 GNB in 114 episodes of bacteraemia, 10 of them (8.8%) polymicrobial; 79 (63.7%) of the GNB were enteric bacteria, 44 (35.5%) glucose non fermenter GNB and one *Haemophilus influenzae*. The most common microorganism was *Escherichia coli* in 56 episodes (45.2%), followed by *Pseudomonas aeruginosa* in 24 (19.3%), and *Klebsiella pneumoniae* in 13 (10.5%). Of the 114 episodes, 41 (36%) had at least one GNB resistant to 3rd generation cephalosporins, and 25 (21.9%) were caused by an ESBL producing GNB. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the RDT were 96%, 98.9%, 96% and 98.9%, respectively. Agreement by kappa index between RDT and SC DDA was 0.95 and between the RDT and MIC/ET was 0.92. The RDT detected 24/

Detección rápida de los bacilos gramnegativos productores de BLEE aislados de sangre: un método razonable y confiable para países de recursos bajos e intermedios

RESUMEN

Introducción. El retraso en la administración del tratamiento adecuado en los pacientes con bacteriemia puede aumentar la morbilidad, la mortalidad y los costos en el cuidado médico. Comparamos la prueba directa y rápida (PDR) diseñada para detectar bacilos gramnegativos (BGN) productores de beta lactamasas de espectro extendido (BGN BLEE) directamente de los frascos de hemocultivo positivos, con dos métodos convencionales para la detección de BGN BLEE: el método de difusión de disco para escrutinio/confirmación (SC DDA) y el método de escrutinio por microdilución y confirmación por prueba E (MIC/ET). **Material y métodos.** Incluimos todos los hemocultivos tomados en un hospital de tercer nivel entre agosto y diciembre de 2005. Incluimos un frasco positivo a BGN por paciente. La PDR se realizó con un inóculo de 0.2 mL de cada frasco de hemocultivo; se sembró en agar Mueller Hinton; se agregaron discos de cefotaxidima y cefotaxima, con y sin ácido clavulánico y se incubó a $35\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ durante 24 hrs. Los resultados fueron interpretados de acuerdo con las recomendaciones de CLSI (SC DDA y MIC/ET). Todas las pruebas fueron realizadas simultáneamente y se analizaron el tiempo de ejecución y los costos de cada prueba. **Resultados.** Identificamos 124 BGN de 114 episodios de bacteriemia, 10 de ellos (8.8%) polimicrobianos; 79 (63.7%) de los BGN fueron enterobacterias, 44 (35.5%) BGN no fermentadores de glucosa y un *Haemophilus influenzae*. El microorganismo más frecuente fue *Escherichia coli* en 56 episodios (45.2%), seguido

[†] Preliminary results of this study were presented at the 44th Annual Meeting of IDSA, Toronto, Ontario, Canada. October 12-15, 2006.

25 ESBL producing bacteria. The mean time to detect an isolate as an ESBL producer after a positive blood culture bottle signal was 1.02 ± 0.19 days when using the RDT, and 3.40 ± 0.59 days when using any other method. The difference in reporting time was 2.38 ± 0.63 days ($p < 0.0001$). Our estimated cost per test was \$1.54 for RDT, \$2.32 for screening/confirmatory SC DDA, and \$49.65 for MIC screening and MIC/ET. **Conclusions.** The RDT is a rapid, reliable and easy analysis to perform, as well as cost effective.

Key words. Bacteraemia. Gram negative rods. ESBL detection. Rapid methods.

INTRODUCTION

Bacterial resistance to β -lactam antibiotics is an increasing problem worldwide. Extended spectrum β -lactamases (ESBL), enzymes capable of hydrolyzing third generation cephalosporins and monobactams, have disseminated worldwide since their first description in 1985.¹ Currently, over 130 TEM-type and more than 50 SHV-type β -lactamases have been identified, and recently, a new CTX-M type β -lactamase has been described. They have been predominantly detected in *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*,² but they may occur in other enteric bacteria and in some non-enteric organisms, such as *Acinetobacter species*.^{3,4}

The incidence of ESBL-producing enteric bacteria varies among different geographical regions. According to antimicrobial resistance surveillance studies such as the SENTRY⁵ and the MYSTIC,⁶ the highest rate of ESBL-producing *K. pneumoniae* was observed in South America and Eastern Europe (45-50%), whereas in North America the incidence was only about 8%. *E. coli* behaves similarly with incidences of 30% and 20% in Eastern Europe and South America, and 7% in North America. In Mexico, ESBL-producing *K. pneumoniae* has been reported in outbreaks within neonatal intensive care units.^{7,8} Our group has recently reported an intermediate prevalence of ESBL-producing *K. pneumoniae* in cases of bacteraemia (14%) during a long period (1993-2002) and even lower than previously reported in Latin America. Bacteraemia caused by ESBL-producing *K. pneumoniae* was not associated

por *Pseudomonas aeruginosa* en 24 (19.3%) y *Klebsiella pneumoniae* en 13 (10.5%). De los 114 episodios de bacteriemia, 41 (36%) tuvieron cuando menos un BGN resistente a cefalosporinas de 3a generación y 25 (21.9%) fueron ocasionados por un BGN BLEE. La PDR tuvo sensibilidad, especificidad, valor predictivo positivo y negativo de 96%, 98.9%, 96% y 98.9%, respectivamente. El índice de concordancia entre PDR y SC DDA fue de 0.95 y entre PDR y MIC/ET de 0.92. La PDR detectó 24/25 BGN BLEE. La mediana de tiempo para detectar un BGN BLEE a partir de la señal positiva en la consola de hemocultivos fue de 1.02 ± 0.19 días con PDR y de 3.40 ± 0.59 días con cualquiera de los otros métodos. La diferencia en el reporte del resultado positivo de una BGN BLEE fue de 2.38 ± 0.63 días ($p < 0.0001$). El costo estimado para PDR fue de \$1.54 dólares estadounidenses, de \$2.32 para SC DDA, y \$49.65 para MIC/ET. **Conclusiones.** El método de escrutinio para detección de BGN BLEE (PDR) fue rápido, confiable, de fácil ejecución y de costo menor que los métodos estándar.

Palabras clave. Bacteriemia. Bacilo gramnegativo. Detección de BLEE. Métodos rápidos.

with a worse clinical outcome and we were able to identify a plasmid-mediated horizontal dissemination during the study period.⁹

The current recommendations of the Clinical and Laboratory Standards Institute (CLSI) for the detection of bloodstream ESBL-producing organisms require: a subculture in solid media until a pure isolate is obtained, a screening susceptibility and a confirmatory ESBL detection test, and three to four days to deliver a final report following the current standard recommendations.¹⁰ Additionally, many clinical laboratories have problems detecting ESBL-mediated resistance, especially in low resource settings.

Since delay in appropriate antimicrobial treatment in patients with bloodstream infections can result in increased morbidity, mortality and health cost,^{11,12} a low-cost and rapid detection test of ESBL-producing enteric bacteria could help to diminish risks derived from suboptimal therapy and length of hospitalization, and eventually might help to contain costs of health care.

Recently, Navon-Venezia, *et al.*¹³ described a method, which allowed the early and accurate detection of ESBL-producing gram-negative bacteria in blood cultures 24 h after growth was detected. The aim of this report is to describe the performance of a rapid protocol for ESBL-detection, following the one described by Navon-Venezia, but sparing the time taken to observe macroscopic growth on culture media, after inoculating directly from blood cultures that automated systems detected as positive and with a positive identification of a gram-negative bacillus (GNB) upon smear examination. This proto-

col was then compared with two other tests currently recommended for ESBL-detection to assess the impact on reporting time and to compare the cost of this new method with our standard laboratory procedures. Our results show that this protocol is comparable to conventional methods but it has a clear impact on the time of detection and cost of the procedure.

MATERIAL AND METHODS

Setting

This prospective study was conducted in a 200-bed tertiary-care centre in Mexico City. All consecutive blood culture bottles obtained during a 5-month period (August to December 2005) were included for evaluation. The study was reviewed and approved by the Institutional Review Board.

Microbiological methods

Blood culture bottles were incubated in the BACTEC 9240 automated system (Becton Dickinson, Cockesville, MD, USA). We included all samples in which GNB were observed in the Gram stain, and excluded those with gram-positive organisms. The final identification of each blood isolate was determined by the VITEK system (bioMérieux, Lyon, France) using the VITEK GNI+ card (bioMérieux Inc., Durham, NC, USA); antimicrobial susceptibility was tested using the VITEK GNS-604 card (bioMérieux Inc., Durham) and confirmed by an in-house MIC determination, using broth microdilution following CLSI guidelines for MIC determination, which is the standard practice at our laboratory. The latter was considered the gold standard for susceptibility testing. An isolate was considered resistant to 3rd generation cephalosporins according to the MIC value ($\geq 8 \mu\text{g/mL}$ for ceftazidime or cefotaxime) and/or when it was an ESBL-producer.

ESBL detection

- **Rapid Direct Test (RDT).** Upon detection of a growth signal from the BACTEC system, and after its identification as an GNB by the Gram stain, 0.2 mL aliquots from the positive blood culture bottle mixture were inoculated into two Mueller-Hinton (MH) agar (Becton Dickinson) plates, as previously described.^{13,14} Four disks (Becton Dickinson, Le Ponte de Claix, France)

were used: ceftazidime (CAZ) (30 μg), cefotaxime (CTX) (30 μg), ceftazidime with clavulanic acid (CAZ/CLA) (30/10 μg) and cefotaxime with clavulanic acid (CTX/CLA) (30/10 μg). Plates were incubated at $35 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ for 18 hr. Results were interpreted as positive for ESBL production if the zone of growth inhibition for either antimicrobial agent tested in combination with CLA was $\geq 5 \text{ mm}$ than the zone of inhibition when the antimicrobial was tested without CLA; according to CLSI recommendations for the DDA.

- **Screening and Confirmatory Disk Diffusion Assay (SC-DDA).** Isolated colonies from an overnight agar plate were used for standard identification and susceptibility testing. Simultaneously a screening ESBL test using a standard DDA, with disks containing CAZ and CTX was performed. Isolates that were either, *E. coli*, *Klebsiella sp.*, or *Proteus sp.*, and that were considered suspicious of ESBL production according to CLSI guidelines, were then retested. Confirmatory testing required the use of both CAZ and CTX disks, alone and in combination with 10 μg of CLA. Plates were incubated at $35 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ for 18 hr. Results were interpreted according to CLSI recommendations.^{10,15}
- **MIC Screening and ESBL E Test (MIC/ET).** Isolated colonies from an overnight agar plate were used for identification and susceptibility testing. Simultaneously susceptibility testing using broth micro-dilution for MIC determination, which is routinely done on blood isolates in our laboratory, was carried out. Those isolates with a MIC $\geq 1 \mu\text{g/mL}$ for CAZ and/or cefotaxime were considered suspicious of ESBL production and confirmed with two E test ESBL strips (AB Biodisk, Solna, Sweden); one strip contained CAZ and CAZ with CLA, and the other CTX and CTX with CLA. Plates were incubated at $35 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ for 18 hr. The test was considered positive for ESBL production when the MIC for CTX was $\geq 0.5 \mu\text{g/mL}$ and the ratio of CTX/CTX-CLA was ≥ 8 and/or when the MIC for CAZ was $\geq 1 \mu\text{g/mL}$ and the ratio of CAZ/CAZ-CLA was ≥ 8 . If the MIC for CTX and CAZ alone and with CLA were all greater than the highest concentrations on the E test strips, or if the MIC for one of the drugs set were both greater than the highest concentration on the E test strip and the other drugs set was negative, the ESBL results were considered non determinable.^{16,17} All interpretations were done according to the package insert.

E. coli ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive controls, respectively, for ESBL production for all methods. Whenever there was a discordant result between the comparison methods, the E-test and the DDA were repeated. A bloodstream isolate was considered an ESBL-producer whenever the two tests (SC-DDA and MIC/ET) confirmed its presence. Time to reporting was recorded.

Cost evaluation

The cost of each test was estimated using the prices listed in the clinical microbiology laboratory of our institution and reported in US dollars. We estimated the cost of the tests needed to detect one blood culture with ESBL-producing GNB.

Statistical analysis

To calculate the sensitivity, specificity, positive and negative predictive values and for each method, a blood culture was considered to have ESBL producing organisms, whenever the E-test confirmed its presence; it was considered resistant to 3rd generation cephalosporins when it was according to the MIC value and/or when it was an ESBL producer. Differences between sensitivity, specificity, positive and negative predictive values were determined using the χ^2 test. The Cohen-kappa index was used to determine the agreement between the RDT and the other two methods. Time to detection of an ESBL producer was tested with the Wilcoxon Signed Rank Test. Values of $p < 0.05$ were considered as significant.

RESULTS

During the study period, 3,219 bottles of blood cultures (643.8 per month) were sent to the laboratory; 420 (13.0%) gave a positive signal. By Gram stain, growth of a GNB was observed in 246 (58.6%), a gram-positive bacteria (GPB) in 141 (33.6%), both, GPB and GNB, in seven (1.7%), a yeast or mould in 14 (3.3%), a yeast and GNB in four (1.0%), and mycobacteria in eight (1.9%). Those 246 bottles, which exclusively grew GNB, belonged to 114 patients, and we included only one bottle per patient for further analysis. Of the 114 episodes of GNB bacteraemia, 10 (8.8%) were polymicrobial and 104 (91.2%) were monomicrobial, in which we isolated 124 GNB: 79 (63.7%) enteric bacteria, 44 (35.5%) glucose non-fermenter GNB, and one *Haemophilus influenzae*, which was excluded from the analysis. The most common microorganism isolated was *E. coli* in 56 episodes (45.2%), followed by *Pseudomonas aeruginosa* in 24 (19.3%) and *K. pneumoniae* in 13 (10.5%). Of the 114 episodes of bacteraemia, 41 (36%) had at least one GNB resistant to 3rd generation cephalosporins and 25 (21.9%) were caused by an ESBL-producing GNB (Table 1).

The sensitivity, the specificity, the positive predictive value and the negative predictive value for detecting an ESBL-producing GNB using the RDT were 96%, 98.9%, 96% and 98.9%, respectively. No statistical difference was found when compared to the other two methods (Table 2). The agreement between RDT and SC-DDA was 0.95 and between the RDT and MIC/ET was 0.92. The RDT effectively detected 24/25 episodes of ESBL-producing GNB bacteraemia. The

Table 1. Proportion of ESBL producer and/or 3rd generation cephalosporin resistant gram-negative bacteria among patients in this study.

	Total n (%)	ESBL-producer n (%)	Resistant to 3rd generation cephalosporins n (%)
Patients with bacteraemia due to GNB*	114	25 (21.9)	41 (36)
Monomicrobial	104 (91.2)	–	–
Polymicrobial†	10 (8.8)	–	–
Gram-negative rods isolated	124	26 (21)	41 (33.1)
<i>Escherichia coli</i>	56 (45.2)	22/56 (39.3)	24/56 (42.9)
<i>Klebsiella pneumoniae</i>	13 (10.5)	3/13 (23.1)	3/13 (23.1)
Other glucose-fermenters‡	10 (8.1)	1/10 (10)	2/10 (20)
<i>Pseudomonas aeruginosa</i>	24 (19.3)	0/24	9/24 (37.5)
Other non-fermenters§	20 (16.1)	0/20	3/20 (15)
<i>Haemophilus influenzae</i>	1 (0.8)	0/1	0/1

* GNB: Gram-negative bacteria. † Each one of the episodes of polymicrobial bacteraemia had two different GNB, which accounts for the 124 gram-negative rods isolated. ‡ Other gram-negative bacteria: *Enterobacter cloacae*, *Klebsiella oxytoca*, *Salmonella* sp., *Serratia marcescens*, *Proteus mirabilis*, *Aeromonas* sp. § Other glucose non-fermenters GNB: *Acinetobacter* sp., *Pseudomonas* sp., *Stenotrophomonas maltophilia*, *Achromobacter* sp., *Burkholderia* sp., *Ralstonia* sp.

Table 2. Performance of three ESBL detection methods and resistance detection in gram-negative bacteria isolated from blood cultures.

	Sensitivity % (IC 95%)	Specificity % (IC 95%)	PPV % (IC 95%)	NPV % (IC 95%)
ESBL+ [†] (25/113)				
RDT*	96 (80-99.8)	98.9 (93.8-99.9)	96 (80.5-99.8)	98.9 (93.9-99.9)
SC-DDA	100 (84.1-100.0)	100 (94.8-100.0)	100 (84.1-100.0)	100 (94.8-100.0)
MIC/ET	96 (78.4-98.7)	100 (94.8-99.9)	100 (83.4-99.8)	98.9 (93-99.6)
Resistance to 3rd generation cephalosporins [‡] (41/113)				
RDT	100 (89.3-100.0)	87.5 (77.4-92.9)	82.0 (68.6-89.7)	100 (92.9-99.9)
SC-DDA	92.7 (80.6-97.5)	98.6 (92.5-99.9)	97.4 (86.8-99.9)	95.9 (88.7-98.6)
MIC/ET	97.5 (85.6-99.2)	100 (93.8-100.0)	100 (89.3-100.0)	98.6 (91.7-99.5)

* RDT: Rapid Direct Tests. SC-DDA: Screening and Confirmatory Disk Diffusion Assay. MIC/ET: MIC Screening and ESBL E-test. [†] No statistical differences were found between the three test concerning ESBL detection. [‡] Concerning resistance to 3rd generation cephalosporins there was a significant statistical difference between the three methods when comparing the sensitivity, specificity and PPV.

only bacteraemia not identified as an ESBL-producer was a polymicrobial episode (*P. aeruginosa* and *Serratia marcescens*), in which *P. aeruginosa* was resistant to 3rd generation cephalosporins and *S. marcescens* was an ESBL-producer; this blood culture was reported as resistant to 3rd generation cephalosporins. When using the RDT for the detection of resistance to 3rd generation cephalosporins, the test correctly identified 41/41 episodes of bacteraemia with resistant GNB; there were nine false positive and no false negative results. The sensitivity, specificity, positive and negative predictive values for detecting resistance to 3rd generation cephalosporins using the RDT were 100%, 87.5%, 82.0% and 100%, respectively (Table 2). The agreement between the RDT and SC-DDA was 0.76 and between the RDT and MIC/ET was 0.82, respectively.

The mean time to detection of a clinical isolate that produced an ESBL after the blood culture bottle signalled as positive was 1.02 ± 0.19 days when using the RDT, and 3.40 ± 0.59 days when using any of the other two methods. The difference in time to detection was 2.38 ± 0.63 days ($p < 0.0001$). The estimated cost per test for the RDT was \$1.54, for SC-DDA was \$2.32 and for MIC/ET was \$49.65. The cost per ESBL detected or cost-effectiveness (the cost of the total number of tests needed to detect one ESBL was \$7.43 for the RDT, \$5.80 for SC-DDA and \$199.22 for MIC/ET ($p < 0.0001$).

DISCUSSION

In this study we evaluated the usefulness of a rapid direct test for the detection of ESBL-producing microorganisms, its impact on time of detection and on cost. Several features of the current study are

worthy of note. First, the sensitivity, the specificity, the positive and the negative predictive values of the RDT are comparable to the disk diffusion assay currently recommended by the CLSI guidelines for detection of ESBLs, and to the ESBL MIC/ET, which has been extensively validated for this purpose.^{16,18} Second, this method allowed us to detect resistance to 3rd generation cephalosporins even in cases of polymicrobial infections, such as the mixed bacteraemia caused by *P. aeruginosa* and *S. marcescens*. It can be argued that this was an error of detection of an ESBL-producer in our hands, however, the majority of the authors conclude that the significance of this result is marginal and minor,^{14,19} if detection of the phenotypic trait (resistance to 3rd generation cephalosporins) was accomplished. In fact, we considered them in this way since they would not have had a major impact on the treatment of this patient. On the other hand, we did have one false positive result which turned out to be an infection caused by an *K. oxytoca* isolate; it was initially interpreted as an ESBL-producer with the CTX disks with the RDT, and as a non-producer with the CAZ disks, the SC-DDA and the MIC/ET. This result can be explained by several well-described mechanisms, such as a denser inoculum or a different substrate by a particular ESBL. However, we do not feel this is relevant in our setting.

Navon-Venezia, *et al.*¹⁴ reported sensitivity, specificity, PPV and NPV of 100%, 98%, 94%, 100%, respectively, but they excluded from the study all episodes of polymicrobial bacteraemia. Of all the episodes of bacteraemia included in this study, 8.8% were polymicrobial; for the RDT to be a useful screening tool in routine clinical practice, it is important to show its performance even in the face of

polymicrobial bloodstream infections. When evaluating the performance of the SC-DDA, all cases of ESBL-producing GNB were correctly identified. With the MIC/ET, which included MIC screening and an ESBL E-test, we had one false negative result; this isolate was an *E. coli* that had an MIC < 2 for CAZ and CTX. According to CLSI recommendations the isolate would not have been considered suspicious of ESBL production and would have been reported as susceptible to 3rd generation cephalosporins and not as a truly ESBL-producer. It is worth mentioning that all discordant results were subject to an ESBL E-test and all isolates were correctly identified as ESBL-producers by this test. We found an almost perfect agreement between the RDT and the other two methods, which suggests that the use of this test could be equivalent in detecting ESBL production.

Previous studies have shown the importance of a prompt and adequate antimicrobial therapy in different patients, hospital settings and co-morbid conditions, especially in infections caused by ESBL-producing organisms.^{11,12,20,21} The impact on morbidity, length of hospital stay and mortality are clear, therefore, every effort should be made to initiate adequate antimicrobial treatment in a timely manner and this could be accomplished by reducing the detection time of an ESBL-producer GNB. Current CLSI recommended methods, although sensitive and specific, are time consuming and may delay appropriate antimicrobial treatment.

When evaluating the capacity of the RDT to detect isolates resistant to 3rd generation cephalosporins, it correctly identified 100% of the resistant isolates. Although the agreement for detecting resistance to 3rd generation cephalosporins was lower than for detecting ESBL production, and its specificity was not as good (nine false positives), we consider this a minor problem, since we are currently proposing this method only for triage. As expected, the other tests had a very high specificity since they are standard tests to determine antimicrobial susceptibility.

In relation to the cost of each test, the RDT and the SC-DDA are similar; both are inexpensive and easy to perform. The MIC/ET is also easy to perform but it substantially adds up to the price of the test. In relation to cost-effectiveness, the price of the RDT is slightly higher than the combination of the screening and confirmatory SC-DDA, since the RDT had to be performed in all blood cultures which demonstrated GNB. The other two confirmatory tests were only performed on those GNB that could be expected to be ESBL-producers. Even with this

setback, the RDT effectively identified ESBL-production 2.4 days sooner than the other two tests. Based on previous studies in which mortality in patients with bacteraemia is directly related to the timing of appropriate treatment administration, being significantly higher in those who did not receive an adequate treatment during the first 48 hours of bacteraemia,^{11,12,20,22} we believe that the RDT may have a great impact on reduction of morbidity, mortality and length of hospital stay.

In conclusion, the RDT is an inexpensive test, easy to perform, and can be added as a routine screening procedure in laboratory practice in medical institutions located in countries where there is a high incidence of ESBL-producing GNB and limited economic and technologic resources.²³⁻²⁶

ACKNOWLEDGEMENTS

Beatriz R. Ruiz-Palacios, M.D. for reviewing the manuscript.

REFERENCES

1. Kliebe C, Nies BA, Meyer JF, Tolxdorff Neutzling RM, Wiedemann B. Evolution of plasmid coded resistance to broad spectrum cephalosporins. *Antimicrob Agents Chemother* 1985; 28: 302 7.
2. Bradford PA. Extended spectrum β lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 2001; 14: 933 51.
3. Poirel L, Gniadkowski M, Nordmann P. Biochemical analysis of the ceftazidime hydrolyzing extended spectrum β lactamase CTX M 15 and of its structurally related β lactamase CTX M 3. *J Antimicrob Chemother* 2002; 50: 1031 4.
4. Turner PJ. Extended spectrum B lactamases. *Clin Infect Dis* 2005; 41(Suppl. 4): S273 S275.
5. Biedenbach DJ, Moet GJ, Jones RN. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997 2002). *Diag Microbiol Infect Dis* 2004; 50: 59 69.
6. Turner PJ, Greenhalgh JM, Edwards JR, McKellar J. The MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) programme. *Int J Antimicrob Agents* 1999; 13: 117 25.
7. Silva J, Gatica R, Aguilar C, Becerra Z, Garza Ramos U, Velázquez M, et al. Outbreak of infection with extended spectrum β lactamase producing *Klebsiella pneumoniae* in a Mexican Hospital. *J Clin Microbiol* 2001; 39: 3193 6.
8. Alcántar D, Tinoco JC, Gayosso C, Carlos A, Daza C, Pérez Orado MC, et al. Nosocomial bacteraemia and urinary tract infections caused by extended spectrum β lactamase producing *Klebsiella pneumoniae* with plasmids carrying both SHV 5 and TLA 1 genes. *Clin Infect Dis* 2004; 38: 1067 74.
9. Mosqueda Gómez JL, Montañó A, Rolon AL, Cervantes C, Bobadilla del Valle M, Silva J, et al. Molecular epidemiology and risk factors of bloodstream infections caused by extended spectrum β lactamase producer *Klebsiella pneumoniae*. A case control study. *Int J Infect Dis* 2008; 12: 653 9.
10. Clinical and Laboratory Standards Institute/NCCLS (2008). Performance standards for antimicrobial susceptibility testing; 18th Informational Supplement. CLSI/NCCLS document

- M100 S18, Vol. 27, No. 1 [ISBN 1 56238 556 9] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 1898, USA.
11. Du B, Long Y, Liu H, Chen D, Liu D, Xu Y, et al. Extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* bloodstream infection: risk factors and clinical outcome. *Intensive Care Med* 2002; 28: 1718 23.
 12. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The Influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. *Chest* 2000; 118: 146 55.
 13. Navon Venezia S, Ben Ami R, Schwaber MJ, Leavitt A, Schwartz D, Carmeli Y. Protocol for the accelerated detection of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* strains from blood cultures. *Eur J Clin Microbiol Infect Dis* 2004; 23: 200 02.
 14. Navon Venezia S, Leavitt A, Ben Ami R, Aharoni Y, Schwaber MJ, Schwartz D, et al. Evaluation of an accelerated protocol for detection of extended spectrum beta lactamase producing gram negative bacilli from positive blood cultures. *J Clin Microbiol* 2005; 43: 439 41.
 15. Swenson JM, Hindler JF, Jorgensen JH. Special phenotypic methods for detecting antibacterial resistance. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Tenover FC, Tenover FC (eds.). *Manual of clinical microbiology*. 8th Ed. Washington D.C., US: ASM Press; 2003, p. 1178 95.
 16. Ozakin C, Sinirtas M, Sevçican E, Kazak E, Gedikoglu S. Comparison of the E test method with an automated bacterial identification and antimicrobial susceptibility detection system for screening extended spectrum beta lactamase producers. *Scand J Infect Dis* 2003; 35: 700 03.
 17. Pfaller MA, Segreti J. Overview of the epidemiological profile and laboratory detection of extended spectrum β lactamases. *Clin Infect Dis* 2006; 42(Suppl. 4): S153 S163.
 18. Vercauteren E, Descheemaeker P, Ieven M, Sanders CC, Goossens H. Comparison of screening methods for detection of extended spectrum β lactamases and their prevalence among blood isolates of *Escherichia coli* and *Klebsiella* spp. in a Belgian teaching hospital. *J Clin Microbiol* 1997; 35: 2191 7.
 19. Spanu T, Sanguinetti M, Tumbarello M, D'Inzeo T, Posteraro B, Santangelo R, et al. Evaluation of the new Vitek 2 extended spectrum beta lactamases (ESBL) test for rapid detection of ESBL production in Enterobacteriaceae isolates. *J Clin Microbiol* 2006; 44: 3257 62.
 20. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. *Clin Infect Dis* 2001; 32: 1162 71.
 21. Rodríguez Baño J, Navarro MD, Romero L, Muniain MA, Peñalva EJ, Perez Cano R, et al. Clinical and molecular epidemiology of extended spectrum β lactamase producing *Escherichia coli* as a cause of nosocomial infection or colonization: implications for control. *Clin Infect Dis* 2006; 42: 37 45.
 22. Schwaber MJ, Navon Venezia S, Kaye KS, Ben Ami R, Schwartz D, Carmeli Y. Clinical and economic impact of bacteraemia with extended spectrum beta lactamase producing Enterobacteriaceae. *Antimicrob Agents Chemother* 2006; 50: 1257 62.
 23. González Vértiz A, Alcántar Curiel D, Cuauhtli M, Daza C, Gayosso C, Solache G, et al. Multiresistant extended spectrum β lactamase producing *Klebsiella pneumoniae* causing an outbreak of nosocomial bloodstream infection. *Infect Control Hosp Epidemiol* 2001; 22: 725 8.
 24. Ndugulile F, Jureen R, Harthung S, Urassa W, Langeland N. Extended spectrum β lactamases among gram negative bacteria of nosocomial origin from an intensive care unit of a tertiary health facility in Tanzania. *BMC Infect Dis* 2005; 5: 86.
 25. Pokharel BM, Koirala J, Dahal RK, Mishra SK, Khadga PK, Tuladhar NR. Multidrug resistant and extended spectrum beta lactamase (ESBL) producing *Salmonella enterica* (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. *Int J Infect Dis* 2006; 10: 434 8.
 26. Kato Maeda M, Bautista Alavez A, Rolón Montes de Oca AL, Ramos Hinojosa A, Ponce de León A, Bobadilla del Valle M, et al. Increasing trend of antimicrobial drug resistance in organisms causing bacteremia at a tertiary care hospital: 1995 to 2000. *Rev Invest Clin* 2003; 55: 600 05.

Correspondence and reprint request:

José Sifuentes-Osornio, M.D.
 Head, Laboratory of Clinical Microbiology,
 Department of Infectious Diseases,
 Instituto Nacional de Ciencias Médicas y
 Nutrición Salvador Zubirán
 Vasco de Quiroga 15,
 Col. Sección XVI, Tlalpan
 14080, México, D.F.
 Tel.: +52 (55) 5487 0900, Ext. 2174
 Fax: +52 (55) 5513 3945
 Correo electrónico: jso@quetzal.innsz.mx

Recibido el 17 de febrero de 2009.
 Aceptado el 13 de mayo de 2009.