

Rapid detection of extended-spectrum- β -lactamase-producing *Enterobacteriaceae* in blood cultures using the ESBL NDP test in Cotonou, Benin

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Abstract

Purpose. Rapid and inexpensive tests for detecting extended-spectrum- β -lactamase (ESBL)-producing *Enterobacteriaceae* are needed, particularly in low-resource countries where infections with these bacteria constitute a major public health issue. The recently described ESBL NDP test performed well in developed countries. This study was designed to assess performance, cost and feasibility of this test in positive blood cultures, in Cotonou, Benin (West Africa).

Methodology. The test was performed on 175 positive Bactec broth blood cultures containing *Enterobacteriaceae*, and blindly compared with the double-disc synergy test (DDST) for the phenotypic detection of ESBL producers.

Results. There was a complete agreement between the ESBL NDP test and the DDST. On average, the time to give results was 37 min for a sample and the cost was US\$ 7.3.

Conclusion. The ESBL NDP test is rapid, relatively affordable and performed well in our setting.

INTRODUCTION

Beta-lactams are the most widely used family of antibacterial agents as a result of their cost-effectiveness, ease of use and good tolerability [1]. However, rapid development of various resistance mechanisms in certain bacterial species decreases the effectiveness of antibiotics [2]. Of these mechanisms, extended-spectrum β -lactamases (ESBLs) in *Enterobacteriaceae* represent a real threat as ESBL-producing *Enterobacteriaceae* (E-ESBL) are resistant to penicillins and cephalosporins including third-generation cephalosporins (3GCs). In addition, E-ESBL have been reported to often be co-resistant to other antimicrobial families such as aminoglycosides and fluoroquinolones [3]. In cases of severe infections such as sepsis due to E-ESBL, carbapenems remain the last-resort therapeutic option [3]. Their intensive use is likely to increase resistance (e.g. carbapenemase production) to these last-resort antibiotics. It is therefore important to rapidly rule out E-ESBL in cases of severe sepsis in order to justify use of carbapenems.

For detecting bacteria involved in sepsis, blood culture is the gold standard test [4]. However, if blood culture is positive, antimicrobial susceptibility testing using conventional methods requires 24 to 48 h to provide results. Such diagnostic delay can be fatal to the patient. To preserve carbapenems against unnecessary use and at the same time, avoid jeopardizing the patient's life with ineffective antibiotic therapy in such cases, rapid tests for detection of E-ESBL directly from positive blood cultures are required.

Recently, several phenotypic tests have been developed allowing the detection of E-ESBL in a few minutes [5, 6]. Among these tests, the ESBL NDP test, developed by a team of three researchers – Patrice Nordmann, Laurent Dortet and Laurent Poirel – offers many advantages in terms of performance compared to other rapid tests [6–8]. The ESBL NDP test was mainly evaluated in developed countries [7, 8], and to the best of our knowledge, technical performance and feasibility of the test have not been evaluated in a low-income country such as Benin, where E-ESBL infections are of major public health concern [9–11]. Furthermore, previous assessment has been

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Abbreviations: AST, antibiotic susceptibility testing; DDST, double-disc synergy test; ESBL, extended-spectrum- β -lactamase; E-ESBL, extended-spectrum β -lactamase-producing *Enterobacteriaceae*; 3GC, third-generation cephalosporin; GNB, Gram-negative bacilli.

done from positive blood cultures in the BacT/Alert system (bioMérieux), but not in the Bactec system (Becton Dickinson) [7]. Finally, the ESBL NDP test was reported to be cheap but without a clear study on its cost [7, 8].

In this study, we proposed to evaluate performance, feasibility and cost of the ESBL NDP test for rapid detection of E-ESBL in positive Bactec blood broth cultures at the Hubert Koutoukou Maga Hospiatal, a University Teaching Hospital in Cotonou, Benin.

METHODS

Samples

The study was done from February to October 2016 at the microbiology laboratory of the Hospital Hubert Koutoukou Maga, a 610-bed university teaching hospital in Cotonou, Benin. Positivity of blood cultures was detected by using the BD Bactec FX40 system (Becton Dickinson). After obtaining Gram-staining results, blood cultures positive for Gram-negative bacilli (GNB) were tested with the ESBL NDP test, and concurrently by another technician, identification with API 20E (bioMérieux) and antibiotic susceptibility testing (AST) was performed.

AST

AST was performed by disc diffusion method according to recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [12]. This includes the double-disc synergy test (DDST) for the phenotypic detection of ESBL producers [12]. Briefly, for each sample, a disc containing a 3GC (cefotaxime, cefixime, ceftazidime or cefepime), and a second disc containing amoxicillin-clavulanate or piperacillin-tazobactam were used. The DDST was also performed on Mueller Hinton agar plates containing cloxacillin (250 mg l⁻¹) to inhibit cephalosporinase activity of natural producers of those inducible cephalosporinases if no synergy was observed between 3GC and amoxicillin-clavulanate or piperacillin-tazobactam. AST results were available after 24 to 48 h.

ESBL NDP test

The test was performed as previously described [8]. Briefly, 0.5 ml positive blood culture was transferred to each of three Eppendorf tubes, size 1.5 ml (tubes A, B and C). After adding 50 µl of a 10% Triton solution, the mixture was vortexed, incubated for 5 min at room temperature, and centrifuged at 13 000 g for 2 min. The supernatant was discarded and the pellet re-suspended in 500 µl distilled water. The bacteria suspension was centrifuged again at 13 000 g for 2 min, the supernatant was discarded and the bacterial pellet re-suspended in 100 µl of 20 mmol l⁻¹ Tris-HCl lysis buffer (B-PERII, Bacterial Protein Extraction Reagent; Thermo Scientific). Thereafter, 10 µl of 40 mg ml⁻¹ tazobactam solution (Sigma-Aldrich) was added to tube C; 100 µl revelation solution [phenol red (0.5%, w/v); Merck KGaA] was added to tube A and 100 µl revelation solution supplemented with cefotaxime 6 mg ml⁻¹ (Claforan; Sanofi-Aventis) to tubes B and C. After 15 min incubation at 37 °C, the colour of solution in tubes was observed. A test result was

considered positive when the tube containing cefotaxime alone (tube B) turned from red to yellow/orange and the tube containing cefotaxime supplemented with tazobactam (tube C) remained red (unchanged colour). For interpretation, various modalities are shown in Fig. 1 (a–d).

The performance of the ESBL NDP test in comparison with the DDST (as reference method) was evaluated in terms of sensitivity (ability to detect a true ESBL) and specificity (ability to detect a true non-ESBL susceptibility) as well as negative and positive predictive values [13].

Time assessment

The time needed for each step of the ESBL NDP test was evaluated. These steps included extemporaneous preparation of reagents, the test itself and reading of results. A timer was started at the beginning of each step and then stopped at the end of the step. Finally, the sum of the duration of all steps was calculated. This procedure was followed for one, three and five samples at a time. For each, the test was repeated three times; the mean duration and standard deviation were then determined.

Cost of reagents

The cost of reagents used in the test was assessed. Only the cost of reagents (from a local supplier in Benin) was considered.

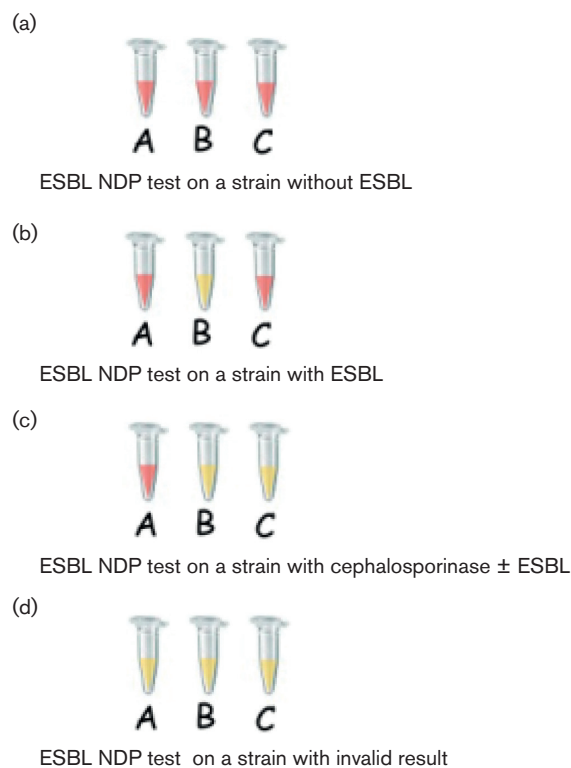


Fig. 1. Interpretation of ESBL NDP test. A, Bacteria suspension with revelation solution. B, Bacteria suspension with revelation solution + cefotaxime. C, Bacteria suspension with revelation solution + cefotaxime + tazobactam.

Equipment as well as consumables such as Pasteur pipettes, gloves, etc. were not included in the cost calculation.

RESULTS

In total, blood culture samples positive for GNB were obtained from 198 patients. In 20 samples, two different strains were isolated. At least one and up to two bacteria belonging to the family *Enterobacteriaceae* (193 strains) were isolated from 175 samples. The most affected age group was children under 15 years of age, accounting for 76.3 % of all patients in the study. Among these children, the most represented were newborns (0 to 1 month) at 80.1 %. In total, 110 patients were male (55.6 %) and 88 were female (44.4 %) giving a M:F ratio of 1.25 : 1.

Enterobacteriaceae isolates ($n=193$) accounted for 88.5 % of the strains isolated, while non-fermenting GNB accounted for 11.5 % (*Acinetobacter* sp., *Pseudomonas aeruginosa* and *Burkholderia cepacia*). Of the *Enterobacteriaceae* isolates, *Klebsiella pneumoniae* accounted for 68.9 %, *Enterobacter* sp. for 18.1 %, and *Escherichia coli* for 11.4 %, while *Providentia stuartii* and *Hafnia alvei* accounted for 1.6 %.

Using DDST, the prevalence of E-ESBL was 84.5 % and no high level cephalosporinase was observed. Resistance to the main drugs for E-ESBL as well as for non-E-ESBL is presented in Table 1. Antibiotics that remained significantly active against E-ESBL were imipenem and cefoxitin with 0.6 and 19.6 % resistance rate, respectively.

As shown in Table 2, there was complete agreement between DDST and the ESBL NDP test. Thus sensitivity, specificity, negative predictive value and positive predictive values were all 100 %.

The cost of reagents for the ESBL NDP test per sample was US\$ 7.3, with the most expensive reagent being tazobactam salt (US\$ 6.6) which represents 90.4 % of the global cost of the test.

As shown in Table 3, depending on the number of samples processed at the same time, the time to get results in the ESBL NDP test varied from 37 ± 2 min to 45 ± 1 min.

Table 1. Percentage of antibiotic resistance among *Enterobacteriaceae* strains

| | ESBL | No ESBL | All strains |
|-------------------------------|------|---------|-------------|
| Ampicillin | 100 | 100 | 100 |
| Amoxicillin-clavulanate | 100 | 50.0 | 92.3 |
| Piperacillin-tazobactam | 100 | 21.1 | 83.5 |
| Cefoxitin | 19.6 | 46.7 | 23.8 |
| Cefotaxime | 100 | 13.3 | 86.5 |
| Ceftazidime | 100 | 25.0 | 96.5 |
| Cefepime | 98.6 | 14.3 | 85.2 |
| Imipenem | 0.6 | 10.0 | 2.0 |
| Gentamicin | 92.0 | 23.3 | 81.3 |
| Ciprofloxacin | 86.3 | 41.3 | 79.5 |
| Trimethoprim-sulfamethoxazole | 97.4 | 63.0 | 92.3 |

Table 2. Comparison between DDST and ESBL NDP test for *Enterobacteriaceae*

| Result in ESBL NDP test | Result in DDST | | |
|-------------------------|----------------|----------|-------|
| | Positive | Negative | Total |
| Positive | 155 | 0 | 155 |
| Negative | 0 | 20* | 20 |
| Total | 155 | 20 | 175 |

*All 23 non-fermenting bacilli (not included in this table) were negative in both DDST and ESBL NDP test.

DISCUSSION

Infections with E-ESBL are a major public health issue, particularly in developing countries like Benin [9–11]. In cases of severe bacterial sepsis, effective antibiotic therapy is urgent, stressing the importance of testing for E-ESBL if the aetiology is due to GNB [14]. Since conventional tests take days to provide results, rapid phenotypic tests have recently been developed.

From this study, comparison between DDST and the ESBL NDP test showed a complete agreement with 100 % sensitivity, specificity and negative and positive predictive values (Table 2). This was in agreement with results of Poirel *et al.* who compared the ESBL NDP test, the Rapid ESBL Screen (a copy of the ESBL NDP test) and the β -Lacta test, and found that among these three tests, the ESBL NDP test has the best sensitivity and specificity (95 and 100 %, respectively) [8]. This performance was confirmed in positive blood cultures in which sensitivity and specificity were 100 % for the ESBL NDP test [7, 15].

Rapid tests are reported to be cheap without any mention of the actual cost [5, 6]. In this study, reagents were either bought from a French company through a local supplier (tazobactam and phenol red), and/or in a pharmacy in Benin (cefotaxime). Therefore, the cost mentioned here may vary from one setting to another. Using a highly purified tazobactam powder from Sigma, the cost of tazobactam represented 90.4 % of the global cost of the test. However, it may not be necessary to use such a purified and expensive tazobactam powder; a less purified powder will probably decrease the cost without affecting performance of the test. If implemented as routine tests, the cost of the test might be higher than that reported in this study since cost of other consumables (pipettes, gloves, etc) as well as laboratory costs such as electricity, water supply and personnel were not included here.

Table 3. Time to get ESBL NDP test results

| Steps | Time (min) to get results for: | | |
|------------|--------------------------------|-----------|-----------|
| | 1 sample | 3 samples | 5 samples |
| Processing | 22±2 | 25±1 | 30±1 |
| Incubation | 15 | 15 | 15 |
| Total | 37±2 | 40±1 | 45±1 |

The time to get results ranged from 37 to 45 min depending on the number of samples processed at the same time. This time was similar to the 30 min reported by Dortet *et al.* in 2015 but higher than the 15 min described for the β -Lacta test [7]. However, after 15 min, performance of the β -Lacta test was found to be low and authors suggested increasing the time to 2 h. In addition, the concurrent rapid identification test used takes at least 3 h before getting results and rapid tests for detecting E-ESBL may then lose the advantage of being rapid [7, 15].

The main limitation of this study was that we were not able to identify bacteria with a rapid test at the same time as the NDP ESBL test. In their studies, Dortet *et al.* and Compain *et al.* used matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) technology (Microflex-Bruker Daltonics) to identify the GNB simultaneously with the rapid test for detecting E-ESBL which took about 3 h [7, 15]. MALDI-TOF MS requires expensive equipment rarely available in resource-limited countries. Therefore, for these settings, the usefulness of rapid tests for detecting E-ESBL is hampered by the lack of rapid identification tests. In the absence of this identification test, one can use a presumptive identification of *Enterobacteriaceae* by observing the motility of bacilli and their shape on Gram staining. *Enterobacteriaceae* are short rod-shaped GNB and are either motile by peritrichous flagella or non-motile. Non-fermenting bacilli are either long and thin rods, motile by monotrichous flagella (e.g. *Pseudomonas* spp.) or non-motile coccobacilli (e.g. *Acinetobacter* spp.), [16]. However, this presumptive identification requires experience/expertise and may not be reliable, stressing the necessity to develop a rapid and affordable identification test of *Enterobacteriaceae* from positive blood cultures. Nevertheless in our study, the proportion of non-fermenting bacilli was 11.5%, with 65 % of them susceptible to ceftazidime. Since all samples with these bacilli showed a negative ESBL NDP test, treating the patients harbouring these bacteria with ceftazidime before the availability of an antibiogram with conventional methods will be efficient in most cases.

In this study, the ESBL NDP test was implemented in Benin, a low-resource country, by reading the protocol as published, with results showing complete agreement with the conventional method. This showed the robustness of the test, which can be set up in clinical microbiology laboratories in various environments. However, it is worth noting that this result was obtained in our setting which has the following characteristics: most of blood cultures are from newborn patients, and there is a high prevalence of *Klebsiella pneumoniae* (68.9 %) and E-ESBL (84.5 %).

Finally, the impact of rapid tests on antibiotic prescription as well as on routine management of patients with severe sepsis requires further evaluation.

In conclusion, the ESBL NDP test is rapid, relatively affordable and performed well in this study. It is urgent to develop a rapid identification test to increase the usefulness

of the ESBL NDP test in routine management of severe sepsis.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

This work was approved by the Institutional Review Board (Centre National Hospitalier et Universitaire Hubert Koutoukou Maga de Cotonou).

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